BIOCHEMOTHERAPEUTIC STRATEGIES
AND
MINIMALLY INVASIVE BALLOON CATHETER TECHNIQUES
IN
REGIONAL PERFUSION

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Biochemotherapeutische strategieën en minimaal invasieve
balloncatheter technieken in regionale perfusie

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Chapter 1

General Introduction and Aims of the Thesis

REGIONAL PERFUSION

To date the primary treatment for most solid tumours is surgical resection. In order to enhance the efficacy of surgical treatment, this modality is often combined in an adjuvant or neo-adjuvant setting with other treatment forms like radiotherapy, or systemic therapy with anti-tumour agents. In case of locally advanced malignancies surgical resection is often not feasible and one has to reside to other treatment forms to achieve palliation, or in best case to convert an unresectable tumour to a resectable one. This may also apply when the sole disease manifestation is formed by metastases confined to one organ or body region. In such cases several forms of loco-regional therapy are utilized. Radiotherapy, laser coagulation and radio-frequency ablation are examples of treatment modalities aimed at obtaining loco-regional tumour control.

Within this context regional chemotherapy is theoretically an attractive concept. It allows higher concentrations of cytotoxic drugs to be delivered to a localised target region, while at the same time reducing systemic concentrations, thereby limiting systemic side effects. Regional perfusion of organs and body regions with anti-cancer drugs for treatment of non-resectable malignancies is not a new treatment strategy. Almost half a century ago regional perfusion of the liver, pelvis, abdomen and extremities with chemotherapeutic agents was already performed. Due to disappointing results and invasiveness of the procedures these therapeutic approaches were generally abandoned, except for the isolated limb perfusion (ILP) setting. Excellent results of ILP with tumour necrosis factor-alpha (TNF) and melphalan for treatment of sarcoma and melanoma in-transit-metastases demonstrated that with the introduction of new and potent anti-cancer drugs, which often demonstrate high systemic toxicity at effective concentrations, a re-evaluation becomes necessary of the role of various regional perfusion modalities in the treatment of advanced malignancies.

BALLOON CATHETER METHODOLOGY

Until recently the major problem with regional chemotherapy was the extent of surgery required for performing the procedures. When one considers the poor prognosis and very low probability of a cure in patients with non-resectable tumours, it is evident that keeping progress of the malignant disease under control is a more realistic goal than aiming for cure. Very large surgical procedures are under these circumstances uncalled for and developing alternatives to "major surgery" is mandatory for these methods to have realistic applicability. If regional perfusion is to become a treatment option applicable on a large scale, the extent, complications and costs of the interventions must be acceptable and the procedure should be repeatable and yield good response rates. Developments in balloon catheter mediated regional perfusion may allow relative, or complete vascular isolation of the abdomen, pelvis or liver with only minimally invasive surgery or even percutaneous techniques.

TUMOUR NECROSIS FACTOR-ALPHA

TNF is a cytokine with an interesting potential in the treatment of cancer. High concentrations of this polypeptide can induce tumour necrosis with acute softening of the tumour brought about by selective destruction of the tumours microvasculature, causing acute hemorrhagic necrosis of the tumour. Pre-clinical *in vivo* studies have demonstrated synergistic anti-tumour effects between high dose TNF and cytostatic agents melphalan and doxorubicine in rat extremity sarcomas. TNF results in augmented intra-tumoral concentrations of these co-administered agents, probably due to the increased permeability of the tumour vasculature caused by specific destruction of the tumour endothelium. Hypoxia and and hyperthermia seems to augment the anti-tumour effects of these agents.

Because of its general toxicity TNF cannot be given in adequate doses intravenously. However, when tumours are exposed to high concentrations, such as in ILP, in combination with melphalan, it is very effective in humans for treatment of soft tissue sarcoma with complete response rates of up to 90 %. This has resulted in approval and registration of TNF in Europe, and in attempts to extrapolate the success of ILP with these agents to other regional perfusion settings like those of liver, pelvis and abdomen. In these settings more than is the case in ILP, regional toxicity and systemic leakage will dictate the maximum dose of TNF that can be used. Experimental data suggest that anti tumour effects in animal systems are only observed at about 50-fold higher TNF dose levels than can be administered in humans. This fifty-fold gap, however, is partially based on a 4-5 fold greater avidity for human TNF in humans than exists in murine tumour systems. Therefore it can be stated that in the human setting one should strive at an about 10-fold increase in TNF concentrations, which is easily achieved in the ILP setting. Accordingly, clinical results with TNF-isolated limb perfusions suggest that only a 5-10 fold increase in TNF levels may be necessary to obtain TNF-mediated anti tumour effects in humans and that the TNF dose in ILP may be somewhat reduced.

A crucial point in cancer therapy is to use the right drug for the right tumour. Results of clinical isolated hepatic perfusion (IHP) and ILP studies with TNF suggest that the beneficial effect of adding this cytokine to these procedures may depend on type or characteristics of the treated tumour. Hypervascular tumours seem to respond very well to the combination of TNF and melphalan in contrast to hypovascular tumours. Possibly the stromal component of the tumour determines if a TNF-mediated effect can come about.

AIMS OF THE THESIS

With the coming at hand of potent, but highly toxic anti-cancer drugs, regional perfusion for non-resectable malignancies of liver, abdomen and pelvis came under renewed interest. In contrast to ILP, vascular isolation of these organs or body regions necessitates extensive surgery. As regional perfusion only has a clinical

perspective if these procedures can be performed with minimal invasive and cost-effective techniques, we investigated the feasibility of isolated hypoxic hepatic perfusion (IHHP), hypoxic abdominal perfusion (HAP) and hypoxic pelvic perfusion (HPP) using balloon catheter techniques, as treatment for patients with unresectable liver metastases or locally advanced pancreatic and pelvic malignancies.

Within this context we studied the possibility of adding TNF to these settings, as this cytokine has demonstrated to dramatically enhance the efficacy of ILP with melphalan when treating soft tissue sarcomas. Hereto we determined the bio-distribution of this agent during these balloon catheter mediated procedures. We investigated if TNF-mediated anti-tumour effects, observed in ILP, can also come about in other perfusion settings, and studied if this anti-tumour effect is dependent of tumour type and tumour characteristics like density of the microvasculature.
To these ends several experimental set-ups and clinical studies were designed.

1. In an ILP rat model we previously observed synergistic anti-tumour effects of TNF and melphalan on BN-175 soft-tissue sarcoma extremity tumours, closely mimicking clinical observations. We investigated if similar synergy in anti-tumour effects could be achieved by treating rats with experimental BN-175 soft tissue sarcoma liver tumours by isolated hepatic perfusion (IHP) with these agents.

2. In aforementioned IHP model we investigated if the degree of tumour vascularization determines if TNF augmented anti tumour effects occur, and investigated if this correlated with tissue accumulation of melphalan. Hereto we performed IHP in rats with highly vascularized BN-175 liposarcoma liver tumours and in rats with lesser vascularized ROS-1 osteosarcoma and CC531 colon carcinoma liver tumours.

3. Like ILP, theoretically an IHP can be performed without systemic leakage, thus enabling a wash-out of agents like TNF, which demonstrate severe systemic toxicity. In an experimental pig model we investigated the feasibility of isolated hypoxic hepatic perfusion (IHHP) using balloon catheter technology, and studied the feasibility of addition of TNF to this setting by studying the distribution of TNF, melphalan and mitomycin C (MMC) over the hepatic and systemic blood compartments.

4. For understandable anatomical reasons complete vascular isolation of pelvis and abdomen is not possible, when using balloon catheter techniques. Systemic leakage is therefore inherent to these procedures. In an experimental study in pigs we performed hypoxic abdominal (HAP) and hypoxic pelvic perfusion (HPP) using balloon catheter techniques. We studied the feasibility of these procedures, and more specifically investigated the possibility of adding TNF to these settings in the clinic by determining the

distribution of TNF, melphalan and MMC over regional and systemic blood compartment.

5. In a clinical phase I-II study we studied the feasibility of isolated hypoxic hepatic perfusion (IHHP) with melphalan in patients with non-resectable liver metastases using two different balloon catheter techniques, resulting in orthograde or retrograde hepatic flow. We assessed the amount of leakage of anti-tumour agents to the systemic compartment occurring with either technique, studied procedure and agents associated toxicity and determined tumour response and time to disease progression in treated patients.

6. In a clinical phase I-II study we investigated the feasibility of HAP with MMC and melphalan using balloon catheter techniques, in patients with locally advanced pancreas carcinoma. Hereto we investigated the bio-distribution of perfused agents during perfusion. We assessed procedure and agents associated toxicity, and the efficacy of the procedure regarding tumour response, median survival and pain reduction in patients with advanced pancreatic cancer.

7. In a clinical phase I-II study we investigated the feasibility of HPP with MMC and melphalan using balloon catheter techniques in patients with various types of locally advanced pelvic tumours. Again, we investigated the bio-distribution of perfused agents during perfusion. We assessed procedure and agents associated toxicity, and the efficacy of the procedure regarding tumour response, median survival and pain reduction in patients with non-resectable tumours of the pelvic region.

Chapter 2

TUMOUR NECROSIS FACTOR-α AUGMENTS TUMOUR EFFECTS IN ISOLATED HEPATIC PERFUSION WITH MELPHALAN IN A RAT SARCOMA MODEL

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SUMMARY

Isolated hepatic perfusion (IHP) is an attractive approach to treating non-resectable liver tumours, because the effects of systemic chemotherapy are poor and its application is hampered by severe general toxicity. In clinical and experimental settings, the efficacy of isolated limb perfusion (ILP) with tumour necrosis factor-α (TNF) in combination with melphalan to treat melanoma in transit and soft-tissue sarcoma has been well established. In an ILP model in rats, the authors previously observed synergistic anti-tumour effects of TNF and melphalan on BN 175 soft-tissue sarcoma extremity tumours. The aim of the current study was to determine whether similar synergy in anti-tumour effects could be achieved by treating experimental BN 175 soft-tissue sarcoma liver tumours by IHP using these agents. The authors found that IHP with TNF and melphalan resulted in a dramatic increase in regional concentrations of perfused agents with virtually no concomitant systemic leakage. Isolated hepatic perfusion with only carrier solution resulted in a significantly diminished growth rate of BN 175 liver tumours compared with the growth rate of tumours in nonperfused rats. Perfusion with melphalan alone resulted in minimal anti-tumour effects. Perfusion with only TNF had a slight growth-stimulatory effect on the BN 175 liver tumours, but no negative effects on tumour growth were observed. When TNF was added to melphalan, a dramatic anti-tumour effect was observed. Thus, as in the rat ILP setting, the anti-tumour effect is augmented when TNF is added to IHP with melphalan to treat BN 175 soft-tissue sarcoma tumour-bearing rats. Strikingly, the tumour response was potentiated at relatively low concentrations of TNF compared with concentrations that elicited synergy with melphalan in ILP.

INTRODUCTION

The clinical success of adding high-dose tumour necrosis factor-α (TNF) to isolated limb perfusion (ILP) with melphalan has renewed the interest in this cytokine as an anti-tumour agent. Systemic administration of TNFα in effective doses is restricted by severe general toxicity.[1,2] Isolated limb perfusion, however, allows high-dose TNF to be added in combination with melphalan to treat melanoma-in-transit metastases and soft-tissue sarcoma and has resulted in tumour responses in more than 80% of cases.[3-5] These promising results have prompted us to investigate the possible application of this combination of agents in isolated perfusion settings of organs such as the liver and kidney.[6-9]

In clinical and experimental settings, isolated hepatic perfusion (IHP) with high-dose melphalan has resulted in significant anti-tumour effects.[10-12] Clinical experience with adding TNF to IHP with melphalan, however, is limited.[7,13-16] Although first reports regarding tumour responses have been promising, it is clear that, because the liver is a vital organ and much more responsive to TNF, more than is the case in ILP, regional toxicity will dictate if adequate doses of TNF can be administered in IHP.

To gain insight into the mechanisms by which TNF elicits its anti-tumour effects and to determine by which agents and manipulations TNF efficacy can be enhanced and its toxicity reduced, a preclinical model IHP is essential. Therefore, we developed an IHP rat model using the highly vascularized BN 175 soft-tissue sarcoma to study the applicability and efficacy of TNF in this setting. This tumour was chosen because previous research showed synergistic anti-tumour effects between TNF and melphalan in a rat ILP model, closely mimicking clinical observations regarding tumour responses and histopathologic findings.[17-19]

In this study, we first defined the IHP rat model regarding the degree of isolation of the hepatic vascular compartment during perfusion and characterized the effect of the IHP procedure itself on growth of experimental BN 175 liver tumours. Thereafter, we tried to determine whether IHP with TNF and melphalan in rats results in similar synergistic anti-tumour effects between these agents, as we previously observed in ILP when treating BN 175 soft-tissue sarcoma liver tumours.

MATERIALS AND METHODS

Animals

We used male inbred BN strain rats that weighed 250 to 300 g and were obtained from Harlan-CPB (Austerlitz, The Netherlands). The rats were fed a standard laboratory diet. All animals were housed under standard conditions of light and accommodation. The experimental protocols adhered to the rules outlined in the Dutch Animal Experimentation Act of 1977 and the *Guidelines on the Protection of Experimental Animals* published by the Council of the European Community in 1986.

The protocol was approved by the Committee on Animal Research of Erasmus University, Rotterdam, The Netherlands.

Tumour model

BN 175 soft-tissue sarcoma (transplantable to BN rats) was used. BN 175 is a rapidly growing and metastasizing tumour and is highly vascularized. The tumour is nonimmunogenic and can be maintained in tissue culture. From culture, new tumours were produced in the rats by subcutaneous inoculation in the flank. Tumours were subsequently passaged serially.

Small viable tumour fragments of 1 or 2 mm were implanted under the liver capsule in the left liver lobe with a 19-gauge Luer lock needle in a standardized manner. Isolated hepatic perfusion was performed 6 days after implantation of BN 175 soft-tissue sarcoma, at which time the tumours had reached a diameter of approximately 6 mm. During follow-up, tumour diameters were measured using calipers through a small midline incision. Tumour volume was calculated by using the equation $0.4\ (A^2 \times B)$, where B is the largest tumour diameter measured and A is the diameter perpendicular to B. Animals were killed when tumour diameter exceeded 20 mm or when abdominal adhesions made further assessment of tumour size impossible.

Drugs

Melphalan (Alkeran, 50 mg per vial; Burroughs Wellcome, Beckenham, U.K.) was diluted in 10 ml diluent solvent. Further dilutions were made in 0.9% NaCl to yield a concentration of 0.2 mg/ml and stored at -20 °C for further use.
Recombinant human TNF was provided by Boehringer (Ingelheim, Germany) with a specific activity of 5.8×10^7 U/mg as determined in the murine L-M cell assay.[20] Endotoxin levels were less than 1.25 EU/mg protein.

Perfusion system

The perfusion circuit consisted of arterial and portal inflow limbs, a venous outflow limb, and a collection reservoir/oxygenator. The circuit was primed with 30 ml Haemaccel (Behring Pharma, Amsterdam, The Netherlands) containing 50 IU heparin. The perfusate was oxygenized in the reservoir with a mixture of oxygen and carbon dioxide (95% : 5%) and maintained at 38 - 39 °C using a heat exchanger connected to a warm water bath. A temperature probe was positioned in the lumen of the portal catheter 5 cm from the catheter tip. Arterial and portal flow was maintained with two low-flow roller pumps (Watson Marlow type 505 U, Falmouth, U.K.) set at 2.5 ml/min and 10 ml/min, respectively. Rats were perfused for 10 min with Haemaccel and dissolved agents. Afterward, agents were washed out with oxygenized Haemaccel for 2 min.

Surgical procedure

The surgical procedure was a modification of the IHP technique described by de Brauw *et al*.[21] Anesthesia was induced and maintained with ether. During the

surgical procedure, which on average lasted 60 to 80 min, rats were maintained at a constant temperature with a warmed mattress. A midline laparotomy was performed and the hepatic ligament was exposed. The pyloric side branch of the portal vein and the gastroduodenal side branch of the common hepatic artery were cannulated, positioning the tips of the Silastic cannulas (0.025-inch outer diameter, 0.012-inch inner diameter; Dow Corning; Midland, MI, U.S.A.) in the hepatic artery and portal vein. The femoral vein was exposed through an inguinal incision. To collect hepatic venous outflow, a silicon cannula (0.025-inch inner diameter and 0.047-inch outer diameter; Dow Corning) was introduced femorally and inserted in a retrograde manner in the caval vein with the tip positioned at the level of the hepatic veins.

The hepatic vascular bed was isolated by clamping the hepatic artery and the portal vein. The venous outflow limb was isolated by clamping the suprahepatic caval vein and by applying a temporary ligature around the infrahepatic caval vein containing the cannula, cranial to the right adrenal vein. During isolation, the mesenteric artery was clamped to reduce splanchnic blood pressure and the risk for translocation of intestinal bacteria.

After the IHP procedure, clamps on the caval vein, portal vein, hepatic artery, and mesenteric artery were released. The gastroduodenal artery, pyloric vein, and femoral vein were ligated, and the gastroduodenal and pyloric cannulas were removed.

Assessment of tumour necrosis factor concentrations in perfusate and systemic blood compartment during isolated hepatic perfusion

To validate the leakage-free quality of the IHP model, TNF concentrations in regional and systemic blood compartments during and after perfusion were determined. Hereto, three BN-strain rats underwent IHP with 20 µg TNF and 200 µg melphalan added to the perfusate. Samples were obtained from the perfusate 0, 5, and 10 min after the start of perfusion and drawn from the iliac artery at 0, 5, 10, 12.5, and 15 min after the start of perfusion. Samples were centrifuged at 2600 rpm for 6 min, after which the obtained plasma–carrier solution was stored at -70 °C until analysis. Plasma and perfusate TNF concentrations were determined using an enzyme-linked immunosorbent assay for rhTNF, as described by Engelberts *et al*.[22] The detection limit for human TNF was 20 pg/ml.

Treatment schedule

BN strain rats underwent IHP 6 days after implantation of the tumour if the tumour diameter was approximately 6 mm. Five rats served as untreated controls. Rats were perfused in random order with 200 µg melphalan (n = 7), 20 µg TNF (n = 8), a combination of 20 µg TNF and 200 µg melphalan (n = 8), or they underwent a sham perfusion with Haemaccel (n = 5). The administered melphalan dose was extrapolated from effective doses in ILP with TNF and melphalan when treating BN 175 soft-tissue sarcoma extremity tumours in rats. The administered TNF dose was the maximum tolerated dose of TNF for IHP in BN rats.

Statistics

Differences in mean tumour volumes of treatment groups at day 10 after IHP were evaluated for statistical significance using the Mann–Whitney U-test in SPSS 8.0 (SPSS; Chicago, IL, U.S.A.) for Windows software. Probability values less than 0.05 were considered significant.

RESULTS

More than 85% of the IHPs were technically successful. All successfully perfused animals survived after the IHP procedures until they had to be killed because of tumour size or abdominal adhesions. Isolated hepatic perfusion with carrier solution was well taken by perfused rats and had no apparent effect on their weights. Perfusing with either melphalan or TNF alone or with a combination of both agents in some cases resulted in transient weight reduction or stagnation. However, weight reduction was never more than 10% (data not shown).

Distribution of tumour necrosis factor during isolated hepatic perfusion over hepatic and systemic blood compartments

To assess the leakage quality of this IHP rat model, we determined the TNF plasma concentrations in the regional and systemic compartment during IHP. *Figure 1* shows the mean regional and systemic TNF concentrations during and after IHP of three rats. Throughout isolation, TNF concentrations in the perfusate remained stable at approximately 550 ng/ml. We observed virtually no concomitant leakage of TNF to the systemic compartment. The efficacy of the washout procedure was apparent from the fact that, after isolation was terminated, only a minor transient elevation of systemic TNF levels was observed (maximum, 60 ng/ml).

Tumour response after isolated hepatic perfusion

Figure 2 shows the growth curves of BN 175 soft-tissue sarcoma liver tumours in rats having undergone IHP with melphalan, TNF, or both; in rats having undergone a sham perfusion with carrier solution; and in nonperfused control animals. Strikingly, IHP with only carrier solution resulted in a significantly diminished growth rate of tumours compared with tumours in nonperfused animals.

Isolated hepatic perfusion with 200 µg melphalan (n = 7) significantly reduced tumour growth rates compared with sham-treated animals (n = 5; $P < 0.02$), but IHP failed to reduce the mean tumour volume (*Fig. 2*) as all rats had progressive tumour growth. Perfusing with 20 µg/ml TNF alone (n = 8) resulted in a slight growth-stimulatory effect on BN 175 liver tumours, compared with tumours in sham-treated rats ($P < 0.01$; *Fig. 2*). No negative effect on tumour growth was observed in any animal. When IHP was performed with a combination of 20 µg TNF and 200 µg melphalan (n = 8), a dramatically enhanced tumour response was observed in all animals, resulting in a significant reduction of mean tumour volume compared with mean tumour volumes in rats perfused with either TNF or melphalan alone

($P < 0.005$ and $P < 0.01$, respectively). At day 10 after IHP, the mean tumour volume was reduced by more than 50% of its value before IHP. No animals showed tumour progression, whereas four of eight animals had a reduction in tumour volume of more than 90%. At day 14, three of eight animals had progression of tumour growth, whereas other animals showed decreased tumour volume. Five of eight animals treated with TNF and melphalan were killed because of multiple abdominal adhesions. The remaining animals all showed regrowth of tumours when followed for a longer time (data not shown).

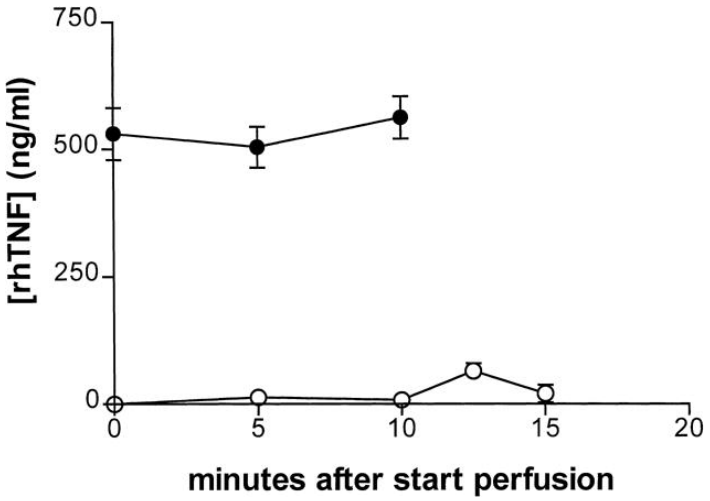

Figure 1 *Tumour necrosis factor plasma and perfusate concentrations in regional (●) and systemic (o) blood compartments during and after IHP with 20 µg TNF and 200 µg melphalan in rats. Rats were perfused for 10 min; afterwards, agents were washed out with carrier solution for 2 min. Mean values are given as ± SEM (n = 3).*

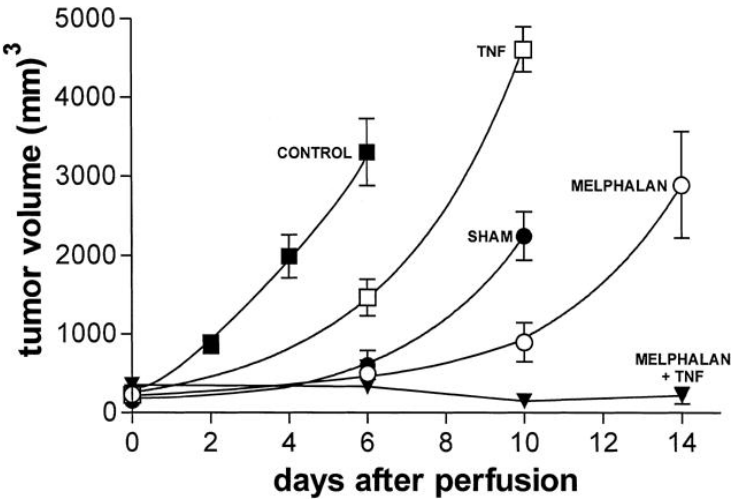

Figure 2 *Mean tumour volumes of BN 175 soft-tissue sarcoma liver tumours in rats having undergone isolated hepatic perfusion and in nonperfused control rats (■, n = 5). Rats were perfused with 20 µg TNF (□, n = 8), 200 µg melphalan (o, n = 7), a combination of 20 µg TNF and 200 µg melphalan (▼, n = 8) or they underwent a sham perfusion (●, n = 5). Mean tumour volumes are given as ± SEM.*

DISCUSSION

In the current study, we discovered a leakage-free IHP rat model. We observed that IHP without addition of anti-tumour agents had an anti-tumour growth effect in BN 175 rats bearing soft-tissue sarcomas. When IHP was performed with TNF or melphalan alone, we observed no tumour responses. However, perfusion with a combination of TNF and melphalan resulted in dramatically enhanced anti-tumour effects. Strikingly, this potentiation by TNF was observed at lower TNF concentrations than those that are necessary to elicit synergistic anti-tumour effects between these agents in ILP, when treating BN 175 soft-tissue sarcoma extremity tumours.

Regional chemotherapy of liver tumours yields higher response rates than systemic therapy.[23-25] Following the success of adding TNF to ILP with melphalan to treat soft-tissue sarcoma of the extremities,[3,4] we wanted to investigate the application of this cytokine in regional chemotherapy settings of the liver.

Hepatic artery infusion exploits the high tissue extraction ratios of many chemotherapetic agents, leading to higher tumour concentrations and fewer systemic side effects.[26,27] However for an agent such as TNF, which at high concentrations has relatively little hepatic uptake,[8] subsequent systemic exposure is potentially fatal. In addition to hepatic artery infusion, IHP has been developed as a treatment modality that not only maximizes drug concentrations in the target organ but also shields the organism from systemic toxicity.[28-30] Although TNF can be used in very high doses in the clinical ILP setting, theoretically similar doses could be used in IHP, as in both cases a complete wash out of agents can be achieved, thus limiting systemic exposure. Furthermore, temporary isolation of the hepatic vascular bed during IHP allows perfusate parameters such as temperature, oxygenation, and pH to be modulated, thus creating optimal conditions for the anti-tumour agents to have an effect.[19] Previous research showed that IHP in rats leads to significantly higher tumour concentrations of melphalan compared with hepatic artery infusion.[12] In the current study, we perfused over both arterial and portal hepatic inflow limbs. Although hepatic metastases derive their blood supply mainly from the hepatic artery, connections with the portal circulation do exist at the periphery of the tumour.[31] It seems rational, therefore, to perfuse both hepatic circulations if one aims its therapy at the viable, vascularized rim of the tumour. In addition, in the clinical situation, nonvascularized "micro-metastases" can be expected to depend completely on the portal circulation.

Tumour necrosis factor *in vivo* can induce tumour necrosis with acute softening of the tumour, believed to be brought about by a selective destruction of the tumour microvasculature, causing acute hemorrhagic necrosis of the tumour.[3,18,32,33] This effect seems to be dose dependent, because low-dose TNF has been reported to have an angioproliferative effect, whereas higher concentrations cause destruction of newly formed vessels.[34] Similar to clinical results, ILP in rats with high-dose

melphalan and TNF yields tumour responses of BN 175 soft-tissue sarcoma extremity tumours in more than 80% of animals treated.[17,19] These are believed to be predominantly indirect effects, because in previous in vitro studies no direct cytotoxic effect and no synergistic potentiation by TNF of melphalan was found using the BN 175 tumour cells.[17]

High concentrations of TNF are necessary only for a short period, because in clinical ILP an immediate increase in vascular permeability was observed, resulting in an increased accumulation of the drugs.[35] Similarly, experimental ILP in rats with TNF in combination with melphalan or doxorubicin clearly resulted in an enhanced accumulation of these agents in BN 175 soft-tissue sarcoma extremity tumours, corresponding with observed augmented tumour responses.[36,37] Having observed similar augmentation of the anti-tumour effect on BN 175 soft-tissue sarcoma after the addition of TNF to melphalan in IHP, as in ILP, we could postulate that endothelium-mediated TNF effects are responsible in both settings.

A recent report by Alexander *et al*,[38] however, questions the role of TNF in enhancing capillary endothelial permeability in tumour-associated vasculature in hyperthermic IHP, because the addition of TNF did not affect melphalan concentrations in liver tumours. Apart from the possibility that the hyperthermia-related capillary leakage may have masked a TNF effect, these contradicting observations may reflect the importance of tumour characteristics, such as for instance the degree of tumour vascularization, in determining if TNF-mediated effects can occur. Kuppen *et al*[39] showed that although IHP in patients resulted in relatively high levels of TNF in liver tissue compared with systemic administration, TNF did not accumulate preferentially in the tissue of colorectal metastases. Interestingly, in a recent clinical IHP study using TNF and melphalan, markedly better results were observed in tumours of mesenchymal origin than in colorectal metastases.[16]

It is clear that isolated perfusion of organs is accompanied by a higher risk of regional toxic side effects than ILP. In IHP, liver toxicity rather than systemic toxicity is dose limiting.[12,21] This has been shown to be particularly true when perfusing with TNF.[7,13,14] In an isolated kidney perfusion model in the rat, we found that the maximum tolerated dose of TNF (0.2 µg/ml) was ineffective, which clearly shows the restriction of TNF dose in perfusion settings as a result of local toxicity.[9] The current IHP model, however, seems to allow above-threshold levels of TNF regionally to elicit augmentation of anti-tumour effects.

In our experiments, effective treatment of BN 175 liver tumours with IHP was achieved at TNF concentrations (550 ng/ml) approximately four times less than the minimal required dose for treating this tumour in ILP (2 µg/ml).[19] Possibly, the IHP procedure itself also contributes to the anti-tumour effects observed. This could not only explain the observed reduction in tumour growth in sham-treated rats but may also be in accordance with reports of tumour responses after IHP despite low tumour concentrations of perfused agents.[11,39] Explanations could be the secondary release

of cytokines by the liver, which is associated with IHP,[40-42] or the mild hyperthermia of the perfusate, because hyperthermic IHP alone has been shown to have a tumouricidal effect.[43] Other mechanisms could be (temporary) relative ischemia during the procedure or transient changes in local hemodynamics during IHP.

With the dawning of minimally invasive IHP techniques,[8,44] the reduction in procedure-associated risks enhances the clinical application potential of TNF in this setting. An efficient preclinical IHP model is very useful to determine by what means the agents-associated risks can be reduced and their efficacy improved. Next to investigating the possibility of dose reduction, it may be of use for investigating the application potential of TNF mutants, which have shown to be less toxic than wild-type TNF but also have retained their anti-tumour effects.[45] An IHP model may also be useful in addressing questions concerning the concomitant systemic administration of agents during IHP that reduce the toxic side effects of TNF.[46,47]

In this study, we found that, as in ILP, adding TNF to IHP with melphalan to treat hepatic soft-tissue sarcoma dramatically augments anti-tumour effects. Thus, as is the case for the ILP setting, we have a preclinical rat model to study the prerequisites for optimal IHP with TNF and melphalan.

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Chapter 3

DEGREE OF TUMOUR VASCULARITY CORRELATES WITH DRUG ACCUMULATION AND TUMOUR RESPONSE UPON TNF BASED ISOLATED HEPATIC PERFUSION

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SUMMARY

Isolated hepatic perfusion (IHP) with melphalan with or without tumour necrosis factor alpha (TNF) is currently performed in clinical trials in patients with hepatic metastases. Previous studies led to the hypothesis that the use of TNF in isolated limb perfusion causes specific destruction of tumour endothelial cells and thereby induces an increased permeability of tumour vasculature. However, whether TNF contributes to the therapeutic efficacy in IHP still remains unclear. In an *in vivo* rat liver metastases model we studied three different tumours: colon carcinoma CC531, ROS-1 osteosarcoma and BN-175 soft-tissue sarcoma which exhibit different degrees of vascularisation. IHP was performed with melphalan with or without the addition of TNF. IHP with melphalan alone resulted, in all tumour types, in a decreased growth rate. However in the BN-175 tumour addition of TNF resulted in a strong synergistic effect. In the majority of the BN-175 tumour-bearing rats, a complete response was achieved. *In vitro* cytoxicity studies showed no sensitivity (CC531 and BN-175) or only minor sensitivity (ROS-1) to TNF ruling out a direct interaction of TNF with tumour cells. The response rate in BN-175 tumour-bearing rats when TNF was coadministrated with melphalan was strongly correlated with drug accumulation in tumour tissue, as only in these rats a five-fold increased melphalan concentration was observed. Secondly, immunohistochemical analysis of microvascular density (MVD) of the tumour showed a significantly higher MVD for BN-175 tumour compared to CC531 and ROS-1. These results indicate a direct relation between vascularity of the tumour and TNF mediated effects. Assessment of the tumour vasculature of liver metastases would be a way of establishing an indication for the utility of TNF in this setting.

INTRODUCTION

Tumour necrosis factor alpha (TNF) is a cytokine with an interesting potential in the treatment of cancer.[1] When administered systemically it is accompanied with severe toxicity; however, especially when TNF in combination with chemotherapy is used locoregionally without systemic exposure, it has very potent antitumour effects. Clinical trials of isolated limb perfusion (ILP) with recombinant human TNF and melphalan resulted in high complete response rates of 75 - 90% in patients with in-transit melanoma and unresectable sarcoma of the extremities.[2,3,4] This is in contrast to ILP with melphalan alone, which is relatively effective against small in-transit melanoma metastases,[5] but achieves very poor results against large tumours such as soft-tissue sarcomas.[6,7,8]

In order to elucidate the mechanism of TNF, several studies have been performed. In our preclinical ILP model, we observed drastic alterations in tumour microvasculature integrity.[9] Rüegg *et al*[10] demonstrated elegantly that TNF in combination with IFN-γ induced functional downregulation of $\alpha v\beta 3$, resulting in detachment of the endothelial cells of the tumour vasculature. Moreover, angiographic studies performed in patients pre- and post-TNF perfusion showed selective destruction of tumour-associated vasculature and histologic studies demonstrated haemorrhagic necrosis of the tumour.[11] Recently, we demonstrated, what we consider a key explanation for the potent synergy between TNF and chemotherapy, an up to six-fold increased intratumoural melphalan or doxorubicin concentration in rat sarcomas after ILP when high-dose TNF was coadministrated.[12,13] These findings led to the hypothesis that TNF causes specific destruction of tumour endothelial cells and thereby induces an increased permeability of tumour vasculature.

As a result of the favourable experience with the ILP system, other isolated perfusion settings have been developed.[14,15] Especially, the liver offers superb opportunities for isolated perfusion. Irresectable liver metastases are a significant clinical problem. Isolated hepatic perfusion (IHP) with melphalan with or without TNF is technically feasible and is currently performed in clinical trials in patients with hepatic metastases.[16-18] Whether TNF contributes to the therapeutic efficacy in IHP still remains unclear.

Based on our findings in the ILP studies, it is indicated to study whether TNF can improve tumour response in different tumours after IHP and, if so, to investigate the capability of TNF to augment drug accumulation in this perfusion setting. By addressing this issue, the usefulness of TNF in IHP might become clear. Since the tumour-associated vasculature is the target of TNF, we expect that tumour microvessel density (MVD) is a predictor of the potentiating effect of TNF during isolated perfusions. Here we present data that indicate that the antitumour effect of TNF is correlated with the tumour microvessel density.

MATERIALS AND METHODS

Rat liver metastases model

We used male inbred WAG/RIJ or Brown-Norway (BN) strain rats, weighing 250 - 300 g, obtained from Harlan-CPB (Austerlitz, The Netherlands). The rats were fed a standard laboratory diet. All animals were housed under standard conditions of light and accommodation. The protocol was approved by the committee for animal research of the Erasmus University, Rotterdam, The Netherlands. The experimental protocols adhered to the rules outlined in the Dutch Animal Experimentation Act of 1977 and the published Guidelines of the UKCCCR for the Welfare of Animals in Experimental Neoplasia.[19]

Three different tumours were used in this study. The weakly immunogenic colon carcinoma CC531 is a 1,2-dimethylhydrazine-induced, moderately differentiated adenocarcinoma transplantable in syngeneic WAG/RIJ rats. The estimated doubling *in vivo* is about 6 - 8 days. The spontaneously originated nonimmunogenic osteosarcoma ROS-1 is also transplantable in the WAG-RIJ rat and in the liver metastases model it has a mean doubling time of about 4 - 5 days. The spontaneously originated nonimmunogenic soft-tissue sarcoma BN-175 is the fastest growing tumour of the tumours tested, with an estimated doubling time *in vivo* of about 2 - 3 days and is transplantable in syngeneic BN rats. Following a standardised protocol, small viable tumour fragments of CC531, ROS-1 or BN-175 tumour fragments of 1 x 2 mm^2 were implanted under the liver capsule, one on the left and one on the right side of the left liver lobe, using a 19 G Luerlock needle. Experiments started at a fixed tumour diameter between 5 and 6 mm. When tumours reached a size of 20 mm in diameter or animals showed obvious signs of discomfort the animals were killed.

Drugs

Recombinant human TNF (4.9 - 5.8 x 10^7 U mg^{-1}) was provided as a kind gift by Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany. Melphalan (L-pam, Alkeran, Wellcome Ltd, London, UK) was obtained as a sterile powder (100 mg) that was dissolved aseptically using solvent and diluent provided by Burroughs Wellcome (London, UK).

Isolated hepatic perfusion

This rat isolated liver perfusion model has been described in detail earlier by van IJken *et al*.[15] A schematic representation is shown in *Figure 1*. Anaesthesia was induced and maintained with ether (Merck, Darmstadt, Germany). During the surgical procedure, with an average duration of 60 - 75 min, rats were kept at a constant temperature using a warmed mattress. A mid-line laparotomy was performed and the hepatic ligament exposed. The gastroduodenal side branch of the common hepatic artery was cannulated, positioning the tips of the cannula (0.025 outer diameter (OD), 0.012 in inner diameter (ID) (Dow Corning, MI, USA)) in the proper hepatic artery. Through a small inguinal incision the femoral vein was

exposed. To collect hepatic venous outflow a silicon cannula (0.047 OD, 0.025 in ID) (Dow Corning, MI, USA) was introduced in the femoral vein and moved up into the caval vein positioning the tip of the cannula at the level of the hepatic veins.

Isolation of the hepatic vascular bed was obtained by temporarily ligating the common hepatic artery and the portal vein. The venous outflow limb was isolated by temporarily clamping the supra-hepatic caval vein and by applying a temporary ligature around the infra-hepatic caval vein containing the cannule, cranial to the right adrenal vein. The mesenteric artery was temporarily clamped in order to reduce splanchnic blood pressure. The circuit was primed with 10 ml Haemaccel (Behring Pharma, Amsterdam, The Netherlands). Arterial flow of 5 ml min^{-1} was maintained with a low-flow roller pump (Watson Marlow type 505 U, Falmouth, UK). Rats were perfused for ten min with oxygenated Haemaccel in which melphalan and/or TNF was dissolved. This short perfusion time was used as we observed rapid clearance of melphalan from the perfusate in this time frame. Secondly, perfusion of the liver beyond 10 min may increase the risk for tissue damage to the liver, but also to the gut as blood flow to the gut is impaired during the perfusion. Afterwards a washout was performed by perfusing with 10 ml of oxygenated Haemaccel. Heparin (50 IU) (Heparine Leo, The Netherlands) was added to the perfusate. The perfusate was oxygenated in a reservoir with a mixture of O_2/CO_2 (95% : 5%) and was kept at 38 - 39 °C by means of a heat exchanger and a warm water bath. A temperature probe was positioned in the lumen of the arterial catheter, 5 cm from the catheter tip.

Following the washout procedure, the clamps on caval vein, portal vein, hepatic artery and mesenteric artery were released. The gastroduodenal artery and femoral vein were ligated and the gastroduodenal and femoral cannulas were removed.

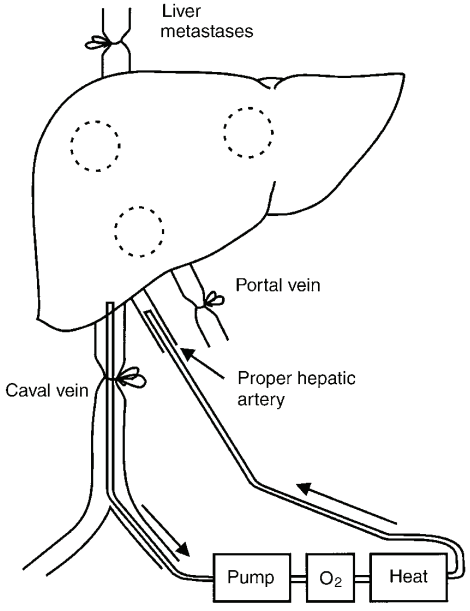


Figure 1 *Schematic representation of an IHP*

In vivo antitumour efficacy study

Treatment started at a fixed tumour size of 5 - 6 mm in diameter. Rats were perfused in random order. In a pilot dose finding study performed for each tumour type the melphalan dose inflicting a *partial tumour response* was chosen for this study. So in the case of additive or synergistic effect of TNF on melphalan this could still be demonstrated in the growth curves of the tumours. All animals underwent IHP only once. CC531-bearing rats were treated with 50 µg melphalan (n = 6), 20 µg TNF (n = 6) or a combination of 50 µg melphalan and 20 µg TNF (n = 6). ROS-1-bearing rats were perfused with 50 µg melphalan (n = 6), 20 µg TNF (n = 8), or a combination of 50 µg melphalan and 20 µg TNF (n = 6). In the BN-175-bearing rats perfusions were carried out with 200 µg melphalan (n = 6), 20 µg TNF (n = 6), or a combination of 200 µg melphalan and 20 µg TNF (n = 6). After IHP tumour size was measured via a small midline laparotomy every fourth day. Tumour volume was calculated by using the following formula: tumour volume = A^2 x B x 0.4, where *B* is the largest diameter and *A* the diameter perpendicular to B, measured with a standardised calliper. In every treatment group, sham perfused rats (n = 6) and untreated control rats (n = 5) were included.

In vitro cytotoxicity assay

CC531 and BN-175 cells were grown in RPMI 1640 and ROS-1 cells in modified Eagle's medium (Gibco BRL, Paisley, UK) supplemented with 10% foetal calf serum (Harlan/Sera-Lab, UK), 1% penicillin (5000 IU ml^{-1}), 1% streptomycin (5000 IU ml^{-1}) and 1% L-glutamine (200 mM) (all Gibco BRL) in a humidified incubator at 37 °C and 5% CO_2. Before usage, the cells were trypsinised (1 min, 37 °C), centrifuged (5 min, 700 g), resuspended and the viability measured by trypan blue exclusion. For *in vitro* testing of proliferation inhibition, 1.0 x 10^4 viable cells were seeded in flat-bottomed 96-well microtiter plates (Costar, USA). After 24 h the cells were incubated with different concentrations of TNF for 72 h ranging from 0 to 10 µg ml^{-1}. Afterwards, cells were washed with PBS and fixed for 1 h with 10% trichloroacetic acid at 4 °C. Growth of tumour cells was measured using the sulpharhodamine-B assay according to the method of Skehan *et al*.[20] Tumour cell proliferation was measured using the formula: tumour growth = (test well/control) x 100%. Five independent tests were performed for each point on the line.

Measurement of melphalan in tissue

After 5 min of the restoration of the circulation, the perfused tumour and part of the liver were excised. The tissues were immediately frozen in liquid nitrogen to stop metabolism of melphalan and stored at -80 °C. Tumour and liver tissues were homogenised in 2 ml acetonitrile (Pro 200 homogenizer, Pro Scientific, CT, USA) and centrifuged at 2500 g. Melphalan was measured in the supernatant by gas chromatography–mass spectrometry (GC–MS). *p*-[Bis(2-chloroethyl)amino]-phenylacetic acid methyl ester was used as an internal standard. Samples were extracted over trifunctional C18 silica columns. After elution with methanol and evaporation, the compounds were derivatised with trifluoroacetic anhydride and diazomethane in ether. The stable derivates were separated on a methyl phenyl

siloxane GC capillary column and measured selectively by single-ion monitoring GC–MS in the positive EI mode described earlier by Tjaden and Bruijn.[21]

Assessment of tumour microvessel density by immunohistochemistry

Cryosections of tumours were fixed for 15 min with 4% formaldehyde. After rinsing with PBS, sections were incubated for 1 h with 1 : 10 PBS diluted, mouse-anti-rat-endothelial cell antibody (RECA-1, Instruchemie, Hilversum, The Netherlands). For the negative control an aspecific mouse IgG was used (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Thereafter, sections were rinsed with PBS and incubated for 1 h with 1 : 100 diluted, in 5% normal rat serum in PBS, goat-anti-mouse peroxidase-labelled antibody (DAKO, Carpinteria, CA, USA). After rinsing with PBS, positive cells were revealed by immunoperoxidase reaction with DAB solution (DAB-kit, DAKO) and counterstained with haematoxylin. For microvessel quantification two independent persons performed a blinded analysis. Positive cells were counted in three different high-power fields (magnification x 160) in each slide according to the method of Bosari *et al*.[22] In total, three slides per tumour and three tumours per tumour type were evaluated.

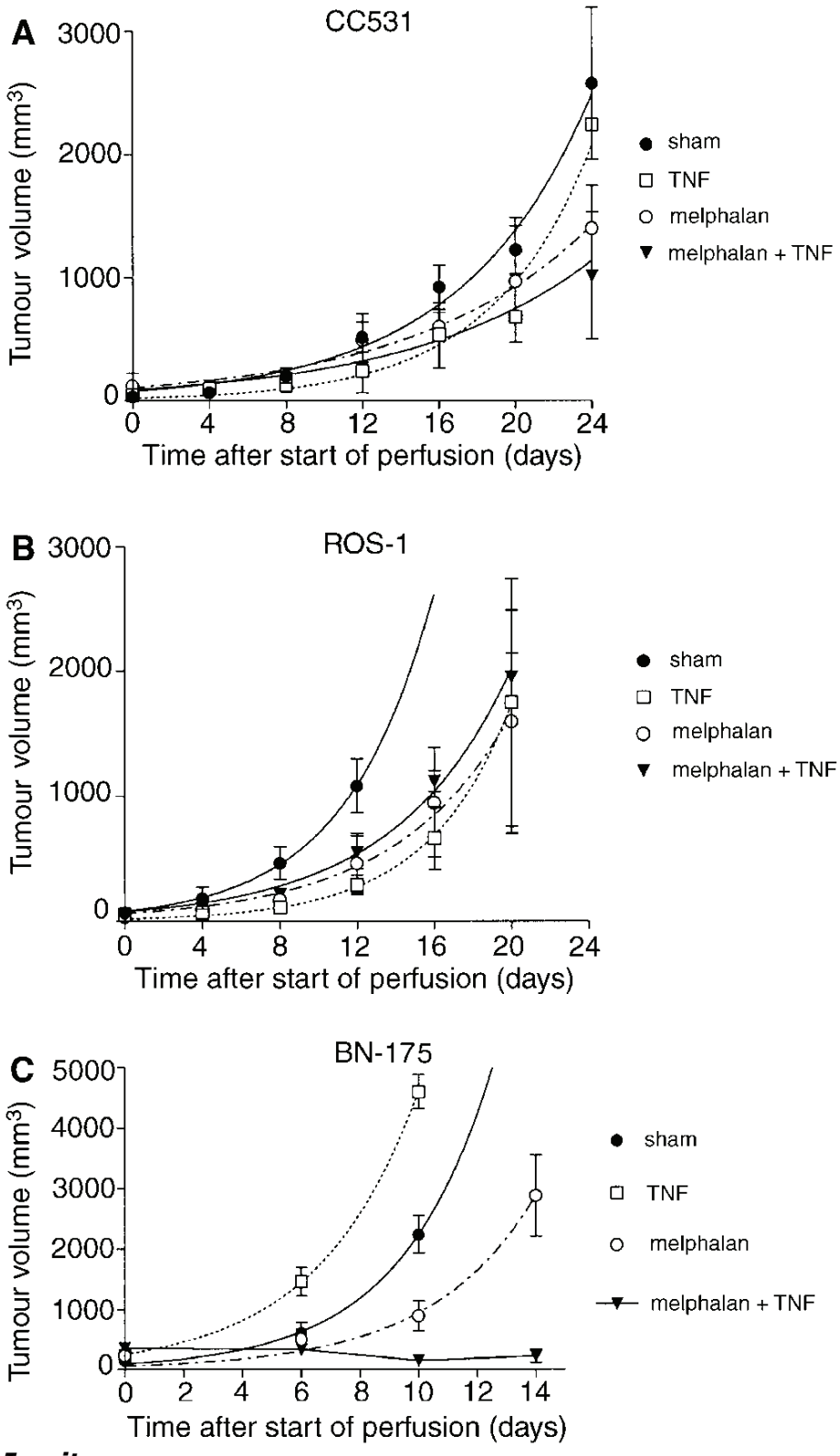
Statistical analysis

In vitro bioassays and *in vivo* tumour response results were evaluated for statistical significance with the Mann–Whitney U-tests with SPSS8.0 for Windows 98. Mann–Whitney *U*-test was used to compare melphalan concentrations in different groups and Kruskal–Wallis test to compare number of positive cells in different tumours. A significance level of $P < 0.05$ was used in all analyses.

RESULTS

Tumour response after isolated hepatic perfusion

The antitumour efficacy of IHP with melphalan with or without TNF was evaluated for the CC531, ROS-1 and BN-175 tumour starting at an equal size of 5 - 6 mm in diameter. In all groups, sham IHPs with only perfusion medium were performed. The graphs in *Figure 2* show the growth curves of CC531 tumour (a), ROS-1 (b) and BN-175 (c) after IHP with melphalan, TNF, both or after sham perfused rats. Perfusion with melphalan alone significantly reduced tumour growth rates compared with sham perfused animals in all tumour types. When IHP was performed in BN-175-bearing rats with the combination of melphalan and TNF, a dramatically enhanced tumour response was observed in all animals. This is a significant reduction of mean tumour volume compared with rats perfused with either TNF only or melphalan alone ($P < 0.005$ and < 0.01, respectively). In the CC531 or ROS-1 tumours, synergy between TNF and melphalan was not observed.



In vitro

Figure 2 *Growth curves of in vivo tumours after IHP. Each group contained at least six animals. Mean values (± s.e.m.) are shown;*
A. CC531
B. ROS-1
C. BN-175

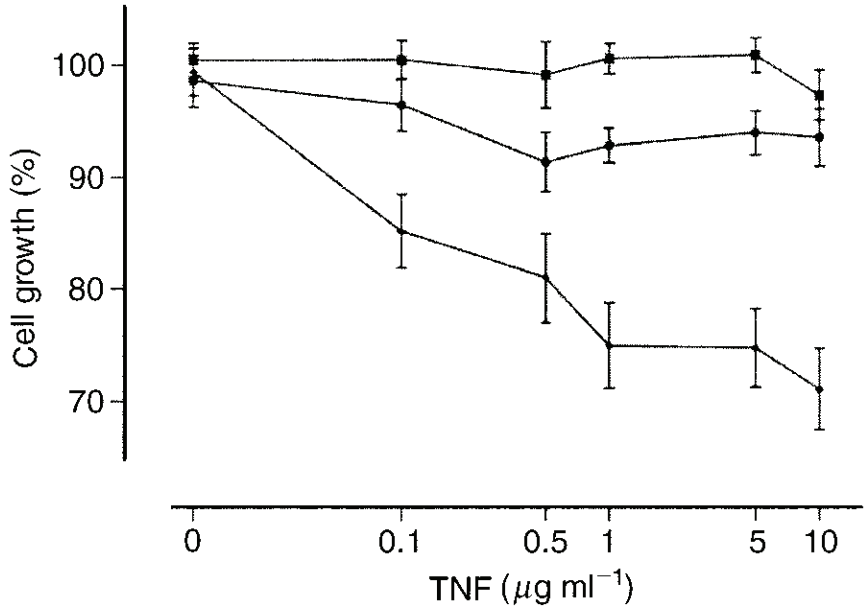


Figure 3 *In vitro growth curves of tumour cells upon exposure to TNF; CC531 (●), ROS-1 (♦), BN-175 (■). Six independent assays were performed in duplicate for each point on the line. Mean values (± s.e.m.) are shown.*

Cytotoxicity assay

The effect of TNF on the growth of tumour cells *in vitro* was determined to evaluate whether the synergistic effect of TNF could be related to direct tumour cell toxicity. The calculated concentration of TNF in the perfusate during IHP *in vivo* is about 1.5 μg ml^{-1}. So *in vitro* tumour cells were exposed to a range of TNF concentrations varying from 0 to 10 μg ml^{-1}. The growth curves are shown in *Figure 3*. It is demonstrated that the BN-175 and the CC531 tumour cell line did not show significant sensitivity to TNF. Only the ROS-1 tumour cells were moderately sensitive to TNF, a growth inhibition of up to 30% at 10 μg ml^{-1} was observed.

Melphalan concentration in tumour and liver tissue

In this perfusion setting, in which the dose of TNF is 20% of the dose used in ILP, an enhanced drug accumulation in tumour tissue might take place as well, as observed after TNF based ILP. In order to investigate this mechanism, melphalan concentrations were measured in tumour and liver tissues after IHP with melphalan with and without TNF. In the CC531 and ROS-1, tumours, melphalan concentration did not increase significantly after IHP with melphalan and TNF (*Figures 4A, 4B*). After IHP with melphalan alone in the BN175 tumour-bearing rats the melphalan concentration in tumour and liver tissue was equal (*Figure 4C*). After IHP with TNF however a more than 5-fold increase of melphalan in tumour tissue is measured compared to tumour tissue after IHP without TNF; ($P < 0.05$). So an augmented drug accumulation can also be achieved in the IHP setting when TNF is coadministered.

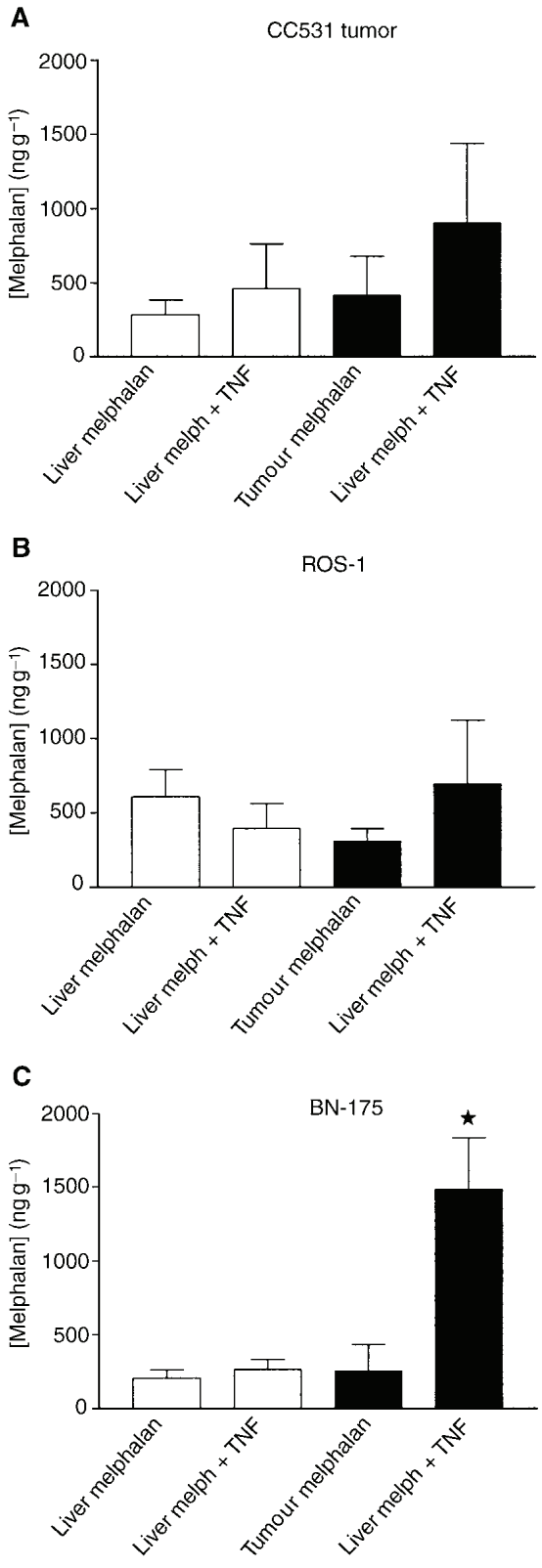


Figure 4 *Melphalan concentrations in liver and tumour tissue after IHP with melphalan with or without TNF. Six IHPs were performed per tumour type. Mean values (± s.d.) are shown. (* = P < 0.05 vs tumour melphalan concentration after IHP with melphalan alone)*
A. CC531
B. ROS-1
C. BN-175

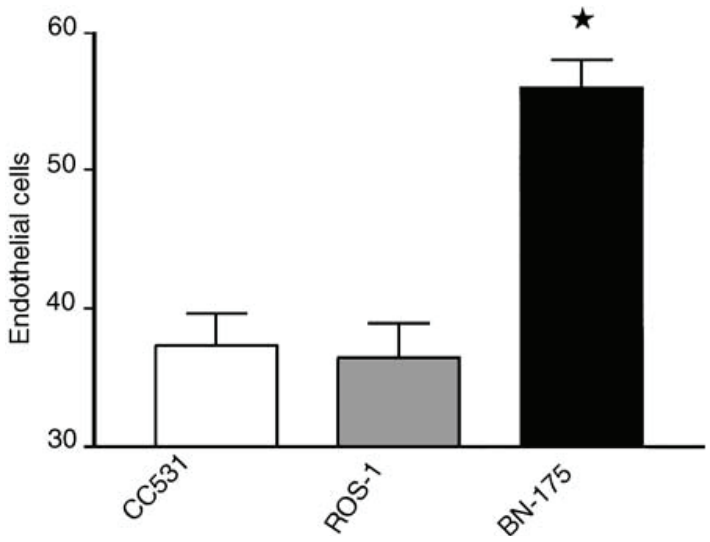


Figure 5 *Microvessel count of CC531, ROS-1 and BN-175 tumours. Mean values (± s.e.m.) are shown (* = P < 0.001 vs CC531 and vs ROS-1).*

Assessment of tumour microvessel density

We already hypothesised that TNF by increasing leakage of tumour vessels enhances intratumoural concentrations of chemotherapeutics. The increased uptake of melphalan might therefore be correlated with the microvessel density (MVD) of the tumour. Quantification of the MVD was performed by immunohistochemical staining of endothelial cells. The microvessel count of the colon carcinoma CC531 and the osteosarcoma ROS-1 was equal (*Figure 5*). The soft-tissue sarcoma BN-175 however showed a significantly higher MVD than CC531 and ROS-1. These results indicate a relations between vascularity of the tumour and TNF-mediated effects.

DISCUSSION

In the present study, we demonstrated that addition of TNF to IHP with melphalan results in strongly improved response rates in a tumour with high vascular density. *In vitro*, no or only minor sensitivity of tumour cells to TNF was found. Even in ROS-1 tumours, which are moderately sensitive to TNF *in vitro*, IHP with TNF alone showed no tumour response. These data indicate strongly that *in vivo* indirect mechanisms mediated by TNF in combination with melphalan determine antitumour effects in IHP. Our data support the notion that this indirect mechanism is the selective destructive effect of TNF on the tumour-associated vessels, thereby increasing vascular permeability.[9,10] To investigate this hypothesis, the melphalan uptake in liver and tumour tissue was measured after IHP with or without TNF. Tumour melphalan concentrations were increased in all tumours but varied significantly in a tumour-type-dependent way. Moreover, enhanced uptake of melphalan by healthy liver was not observed. With TNF alone, at the most some tumour growth was observed. Only the combination of TNF and melphalan resulted

in a complete tumour response in the BN175 tumour. To elucidate this tumour-type-dependent response, the MVD of the tumours was determined. We expected a higher tumour vascularity in this tumour. Indeed a significantly higher MVD compared to the CC531 and ROS-1 tumours could be demonstrated. This indicates that TNF has specific tumour vascular mediating capacity in this perfusion model, which results in enhanced tumour responses in highly vascularised tumours. As a result of our findings in ILP and now also in IHP, we know that TNF is able to augment the accumulation of melphalan. The presence or lack of TNF-mediated synergy appeared to be independent of tumour size as also in smaller (diameter 3 - 4 mm) or bigger (7 - 8 mm) tumours comparable tumour responses were observed (data not shown). We are of the opinion that this observation is essential in understanding and explaining the impressive responses demonstrated.

Changes in vascular permeability in patients who underwent IHP with TNF was studied by Alexander *et al.*[16,17] Vascular permeability was measured by diffusion of radiolabelled ^{131}I albumin in liver and tumour tissue. A significant increase of the ^{131}I albumin postperfusion could be demonstrated compared to levels ^{131}I albumin measured before perfusion. However, this rise was equal in tumours perfused with or without TNF. A TNF independent mechanism of the increased endothelial permeability was suggested by the authors. However, in the present study, we demonstrated that TNF is effective in increasing vascular permeability for melphalan selectively in tumour tissue. A more important finding, however, is that this effect could only be found in the highly vascularised BN-175 tumour. The results of Alexander *et al* reported on intratumoural ^{131}I albumin concentrations were mainly based on colorectal carcinoma liver metastases. In hypovascular rat colon carcinoma, we also could not find an increase of melphalan intratumourly. We therefore hypothesize that the usual hypovascularity of colorectal metastases in patients explains the lack of TNF-benefit in the experience as described by Alexander in patients, which correlates closely to our observations in our hypovascular colon cancer liver metastases model in rats.

IHP with melphalan and TNF performed in patients with metastases of ocular melanoma or leiomyosarcoma showed overall response rates of 50 - 52%.[23,24] Both tumour types are highly vascularised. A prolonged duration of response was found in melanoma patients: 14 months after IHP with TNF *vs* 6 months after IHP without TNF.[24] After IHP with melphalan with or without TNF in patients with colorectal liver metastases the mean duration of response was in both groups 8 - 10 months.[16,17,25] The data we now present and the first reports of IHP in melanoma and sarcoma liver metastases strongly indicate that in these patients TNF has therapeutic potential in IHP. In patients with colorectal liver metastases however, IHP with melphalan alone may well be just as effective as combined with TNF. Assessment of the degree of tumour vasculature of liver metastases would be a way of establishing an indication for the utility of TNF in this setting.

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Chapter 4

ISOLATED HYPOXIC HEPATIC PERFUSION WITH TUMOUR NECROSIS FACTOR-ALPHA, MELPHALAN, AND MITOMYCIN C USING BALLOON CATHETER TECHNIQUES: A PHARMACOKINETIC STUDY IN PIGS

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ABSTRACT

Objective
To validate the methodology of isolated hypoxic hepatic perfusion (IHHP) using balloon catheter techniques and to gain insight into the distribution of tumour necrosis factor-alpha (TNF), melphalan, and mitomycin C (MMC) through the regional and systemic blood compartments when applying these techniques.

Summary Background Data
There is no standard treatment for unresectable liver tumours. Clinical results of isolated limb perfusion with high-dose TNF and melphalan for the treatment of melanoma and sarcoma have been promising, and attempts have been made to extrapolate this success to the isolated liver perfusion setting. The magnitude and toxicity of the surgical procedure, however, have limited clinical applicability.

Methods
Pigs underwent IHHP with TNF, melphalan, and MMC using balloon catheters or served as controls, receiving equivalent dosages of these agents intravenously. After a 20-minute perfusion, a washout procedure was performed for 10 minutes, after which isolation was terminated. Throughout the procedure and afterward, blood samples were obtained from the hepatic and systemic blood compartments and concentrations of perfused agents were determined.

Results
During perfusion, locoregional plasma drug concentrations were 20- to 40-fold higher than systemic concentrations. Compared with systemic concentrations after intravenous administration, regional concentrations during IHHP were up to 10-fold higher. Regional MMC and melphalan levels steadily declined during perfusion, indicating rapid uptake by the liver tissue; minimal systemic concentrations indicated virtually no leakage to the systemic blood compartment. During isolation, concentrations of TNF in the perfusate declined only slightly, indicating limited uptake by the liver tissue; no leakage of TNF to the systemic circulation was observed. After termination of isolation, systemic TNF levels showed only a minor transient elevation, indicating that the washout procedure at the end of the perfusions was fully effective.

Conclusions
Complete isolation of the hepatic vascular bed can be accomplished when performing IHHP using this balloon catheter technique. Thus, as in extremities, an ideal leakage-free perfusion of the liver can now be performed, and repeated, without major surgery. The effective washout allows the addition of TNF in this setting.

INTRODUCTION

The importance of developing new treatment modalities for primary and secondary liver tumours is evident. Hepatocellular carcinoma is one of the most common malignant tumours in the world today; it develops in approximately 1 million persons each year.[1] Recent advances in early detection have improved the prognosis.[2] In general, however, tumours are not resectable at the time of diagnosis, and the prognosis of hepatocellular carcinoma remains poor.[3] The liver is a major site of metastatic spread of primary colorectal cancer, and it is the sole site of initial tumour recurrence in as many as 30% of patients.[4] Patients with resectable liver metastases can be cured by surgery, with reported 5-year survival figures ranging from 16% to 45%.[5-7] However, in 75% of patients with colorectal cancer metastases confined to the liver, these metastases are considered unresectable. Because the main determinant for duration of survival in these numerous patients is local tumour progression, new treatment modalities and schedules must be aimed at achieving local tumour control.

For most chemotherapeutic agents, steep dose-response curves can be demonstrated. Therefore, high drug concentrations are important for eradicating both sensitive and resistant tumour cells.[4,8] Systemic chemotherapy results in relatively low response rates both for colorectal liver metastases and primary liver tumours and has minimal effects on survival.[9]

The main principle of isolated regional chemotherapy is to achieve higher regional drug concentrations and thus higher exposure of tumour tissue to the agents, resulting in increased response rates, while shielding the organism from the systemic toxicity because of the much lower concentrations in the systemic circulation. These principles are even more important for the potential application of tumour necrosis factor-alpha (TNF). The toxicity of this cytokine has made systemic application of adequate doses with antitumour effect impossible.[10,11] However, in a leakage-free isolated perfusion setting, it can be safely used with remarkable results. Response rates of > 80% have been observed in the treatment of unresectable extremity soft-tissue sarcomas using isolated limb perfusion with TNF plus melphalan.[12,13]

In the wake of these promising clinical results, efforts have been made to translate this success to other regional perfusion settings. The liver is one of the most obvious targets, and successful isolated perfusions of the liver with cytostatic drugs, both in animal models and in humans, have been reported.[14-16] The applicability of TNF in the setting of leakage-free isolated liver perfusion has been reported by teams from the National Cancer Institute and by us.[17-19] Major drawbacks of clinical application, however, are the magnitude and associated toxicity of the surgical procedure and the complexity and costs of using a venous bypass system as well as a heart-lung machine operated by a team of perfusionists.[19] For this concept to become practical in a clinical setting, less invasive alternatives must be developed. Thus, if isolated

liver perfusion is to become a treatment option applicable on a large scale, the extent, complications, and costs of the intervention must be acceptable, and the procedure should be repeatable and yield good response rates. Several groups have reported on regional arterial infusion of chemotherapeutic drugs in combination with complete or incomplete hepatic venous isolation; however, these procedures were combined with hemofiltration systems.[20-22]

New balloon catheter techniques allow relative vascular isolation of the abdomen and pelvis with only minimally invasive surgery.[23-25] Several authors have published articles on these methods and have used aortic hypoxic perfusion chemotherapy for unresectable tumours confined to the pelvis or abdomen.[26-28] Hypoxia causes dividing cells to halt their progression through the cell cycle by allowing them to progress to and then remain in the G_1-like susceptibility state.[29] Drugs whose cytotoxic action is particularly potentiated by hypoxia, such as mitomycin C (MMC) and melphalan, have been reported to be particularly effective when administered by aortic stop-flow infusion.[26,27,30]

With the use of a caval double-balloon catheter for complete venous isolation of the liver, in combination with an aortic occlusion catheter and a port catheter placed in the gastroduodenal artery, it has become a relatively simple procedure to perform an isolated liver perfusion. We tested the feasibility and quality of this approach and report here on this methodology and its validation by studying the distribution of TNF, melphalan, and MMC through the regional and systemic blood compartments in pigs.

MATERIALS AND METHODS

Animals

Dutch Yorkshire pigs weighing 30 to 40 kg were used. Animals were treated in compliance with the guidelines on animal welfare of the Erasmus University, Rotterdam. The study protocol was approved by the Ethics Committee on Experimental Studies in Animals of the Erasmus University, Rotterdam.

Drugs

Recombinant human (4.9 to 5.8 × 10^7 units/mg) was provided as a kind gift by Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany. Melphalan (L-pam, Alkeran, Wellcome Ltd., London, UK) was obtained as a sterile powder (100 mg) that was dissolved aseptically using solvent and diluent provided by Burroughs Wellcome (London, UK). MMC (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) was obtained as a sterile powder (40 mg) that was dissolved aseptically in 40 ml saline 0.9%.

Perfusion set

Perfusion sets were kindly provided by PfM GmbH (Cologne, Germany) and consisted of a double-balloon catheter (12F, balloon capacity 25 ml, distance between balloons

4 cm) for venous isolation of the liver, an aortic occlusion balloon catheter (12F, balloon capacity 25 ml) for compensating the decrease in cardiac preload during the procedure, and a tubing set with a volume of 220 ml containing a bubble trap. In the perfusion circuit, flow was maintained by a roller pump, and the temperature of the perfusate was regulated by a heat exchanger (Cardioplegia heat exchanger, CSC14 Sorin, Biomedica, Italy). Drugs were infused through a sideline into the perfusion circuit.

Surgical procedure

Figure 1 illustrates the methodology of an isolated hypoxic hepatic perfusion (IHHP) with balloon catheters. It shows the position of the occlusion catheters, the inflow catheter in the gastroduodenal artery, and the other components of the perfusion circuit. The IHHP procedure was carried out as follows. General anesthesia was induced in pigs by standard methods and maintained with pancuronium and fentanyl. An arterial line was introduced into a carotid artery and a double-lumen central venous catheter was placed in an external jugular vein on either side. After a midline laparotomy was performed, the gastroduodenal or common hepatic artery was cannulated with the arterial infusion catheter (7F). Pigs were subsequently heparinized with 2 mg/kg heparin. After formal surgical exposure of the femoral vessels, the aortic occlusion catheter was introduced into the common femoral artery. Subsequently the double-balloon catheter was introduced into the common femoral vein and retrogradely moved up into the caval vein, positioning the balloons above and below the hepatic veins. To compensate for the decrease in cardiac preload, the aorta was clamped or the aortic occlusion balloon was inflated. Caval balloons were then inflated and temporary isolation of the hepatic vascular bed was obtained by clamping the hepatic artery and subsequently the portal vein. After connecting the double-balloon catheter and arterial cannula to the perfusion system primed with 220 ml Haemaccel (Behring Pharma, Amsterdam, The Netherlands), the perfusate was circulated by means of a roller pump with a constant flow of 200 ml/minute. The temperature of the perfusate was kept at 38.5 °C by means of a heat exchanger. Stable perfusion was assessed by monitoring the blood level in the bubble trap.

Drugs were rapidly administered as a bolus infusion into the perfusate. After 20 minutes of perfusion, the remaining agents were washed out of the hepatic vascular bed by perfusing the isolated circuit with Haemaccel for 10 minutes and collecting the venous effluent. Isolation was then terminated by releasing the temporary clamps on the portal vein and the hepatic artery, and the caval balloons and the aortic balloon were deflated. The total time of clamping the portal vein did not exceed 35 to 40 minutes.

Control animals were given an equivalent amount of drugs as a bolus injection through a central venous line placed in the external jugular vein. Samples were obtained from a carotid arterial line.

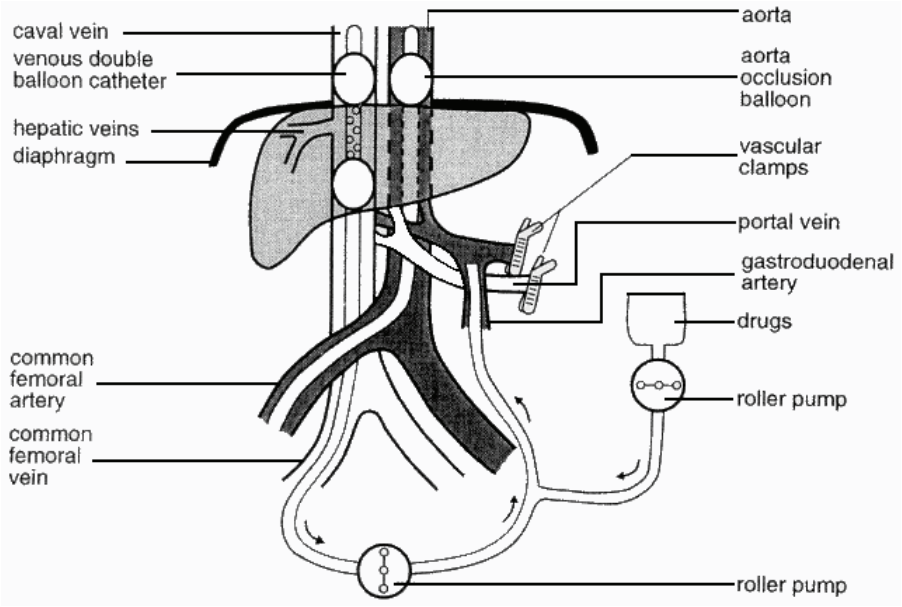

Figure 1 *An isolated hypoxic hepatic perfusion with balloon catheter techniques.*

Blood sampling

Baseline blood samples were obtained before administration of the drugs into the regional circuit. Throughout and after the procedure, blood samples were simultaneously drawn from the hepatic blood compartment through a sampling port on the caval double-balloon catheter and from the systemic blood compartment through the central venous catheter at 5, 10, 15, 20 (before washout started), 30 (before termination of isolation), 35, 55, and 85 minutes after infusion of the agents. Blood samples of animals that received the drugs as an intravenous bolus infusion were drawn at 5, 10, 15, 20, 25, 35, 55, 85, 150, and 180 minutes after administration. Blood samples were collected in glass tubes containing EDTA and immediately stored in the dark on ice. Samples were centrifuged at 2600 r.p.m. for 6 minutes at 4 °C, and the obtained plasma was stored at -70 °C until analysis.

Treatment schedule

Five pigs underwent IHHP and five pigs received the drugs as an intravenous bolus infusion. Of the group that underwent IHHP, two pigs were given MMC (0.25 mg/kg) and melphalan (0.25 mg/kg) alone, and three pigs were given the same amount of MMC and melphalan in combination with TNF (0.02 mg/kg). Of the pigs that received an intravenous bolus, two were given MMC (0.25 mg/kg) and melphalan (0.25 mg/kg) alone, and three were given the same amount of MMC and melphalan in combination with TNF (0.02 mg/kg).

Bioanalysis

Plasma TNF concentrations were determined using ELISA for rhTNF as described by Engelberts et al.[31] In short, a 96-well Immuno-Maxisorp plate was coated with murine antihuman TNF mAb 61E71. A standard titration curve was obtained by making serial dilutions of a known sample of human rhTNF in normal porcine serum.

The plates were incubated with a polyclonal rabbit antihuman TNF antiserum, followed by addition of an enzyme-labeled antirabbit reagent and enzyme reaction. The detection limit for human TNF was 20 pg/ml.

Melphalan in plasma was measured by gas chromatography/mass spectrometry. P-[Bis(2-chloroethyl)amino]-phenylacetic acid methyl ester was used as an internal standard. Samples were extracted over trifunctional C18 silica columns. After elution with methanol and evaporation, the compounds were derivatized with trifluoroacetic anhydride and diazomethane in ether. The stable derivatives were separated on a methyl phenyl siloxane GC capillary column and measured selectively by single ion monitoring mass spectrometry in the positive EI mode. The detection limit for melphalan is 10 pg/ml. Details of this assay were described by us.[32]

Concentrations of MMC were analyzed by high-performance liquid chromatography with ultraviolet detection. Porfiromycin was used as an internal standard. Samples were extracted over XAD-2 columns. After elution with methanol and evaporation, the dry residue was diluted in the mobile phase, which consisted of methanol and water (40/60). The sample was injected and separated over a C18 column and detected at 362 nm. The detection limit for MMC is 10 pg/ml. Details of the assay are described elsewhere.[33]

Calculation of area under the curve

Areas under the plasma concentration *versus* time curve (AUCs) were calculated applying the trapezoid rule using GraphPad Prism software. AUCs were calculated from 5 to 20 minutes after the start of perfusion or from 5 to 20 minutes after administration of the intravenous bolus.

RESULTS

All pigs survived the procedures. Data were obtained from five subsequent procedures. Apart from transient tachycardia in a minority of pigs, stable hemodynamics were observed throughout the procedures. After deflating the balloons, a short period of hypotension was observed, easily managed by fluid challenge. The addition of TNF to MMC and melphalan did not alter the plasma concentration *versus* time profile of MMC and melphalan during and after the procedure (data not shown). Therefore, data from pigs perfused with melphalan and MMC alone and from pigs perfused with melphalan and MMC plus TNF were pooled.

Plasma concentrations

Figure 2 shows the mean systemic plasma concentrations of MMC, melphalan, and TNF after administration of these agents as an intravenous bolus. A typical one-phase exponential decay curve was observed for TNF and MMC with an elimination half-life of 31 minutes for TNF and 7.2 minutes for MMC. Two-phase exponential decay was observed for melphalan with a λ_1 of 2.8 and a λ_2 of 161 minutes.

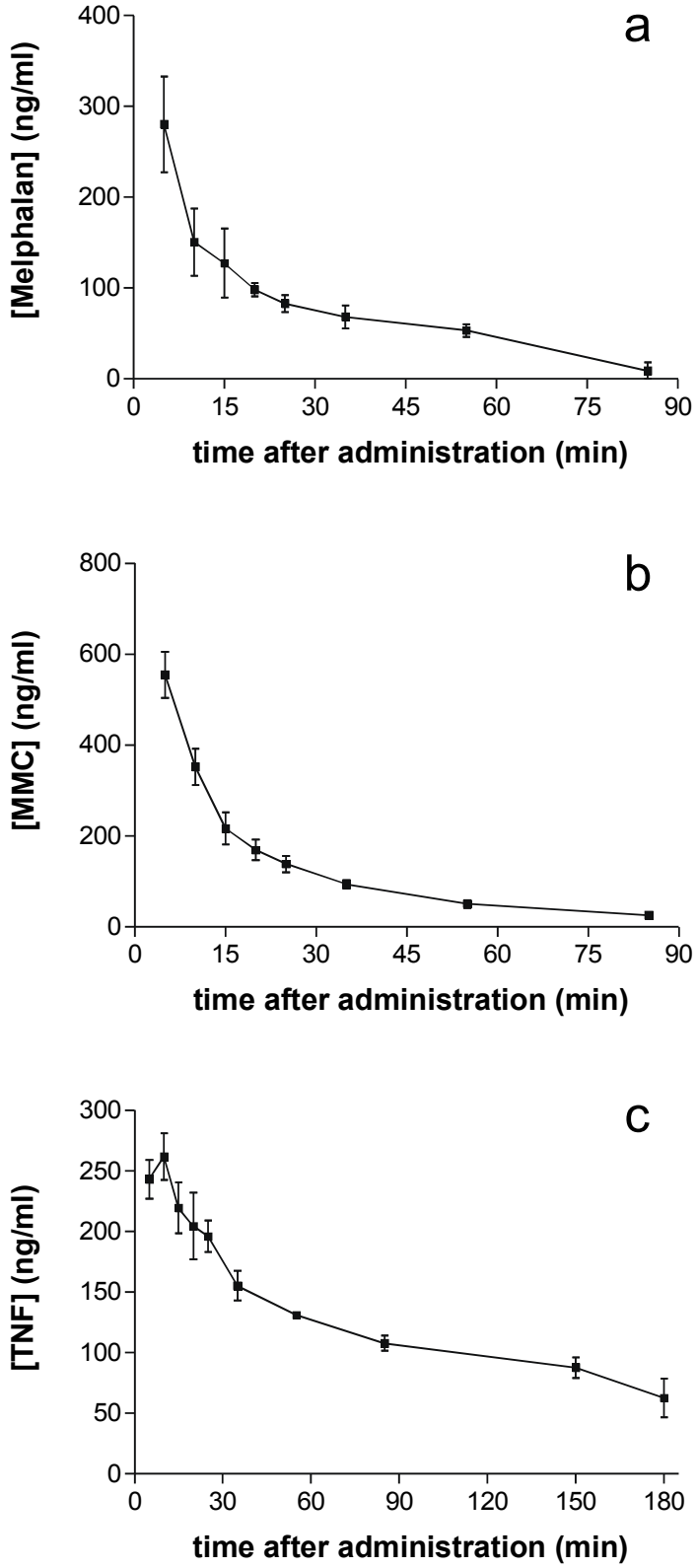

Figure 2 *Plasma concentration curves after intravenous bolus injections of melphalan (0.25 mg/kg) (A), mitomycin C (0.25 mg/kg) (B), and tumour necrosis factor-alpha (0.02 mg/kg) (C). For mitomycin C and melphalan, mean plasma concentrations of five pigs ± SEM are shown. For tumour necrosis factor-alpha, mean plasma concentrations of three pigs ± SEM are shown.*

During perfusion, concentrations of MMC and melphalan were markedly greater in the isolated hepatic vascular compartment than in the systemic compartment (*Fig. 3*). After 5 minutes of perfusion, mean MMC peak levels were 38-fold higher than systemic peak MMC plasma concentrations (2222 ng/ml *vs.* 59 ng/ml). Mean melphalan peak levels at 5 minutes were 33-fold higher than mean systemic plasma concentrations (3028 ng/ml *vs.* 93 ng/ml). During the 20 minutes of perfusion, MMC and melphalan plasma levels in the isolated hepatic vascular compartment steadily declined. However, systemic levels of melphalan and MMC remained at < 93 ng/ml and 59 ng/ml, respectively, throughout the procedure, indicating no significant leakage from the isolated circuit.

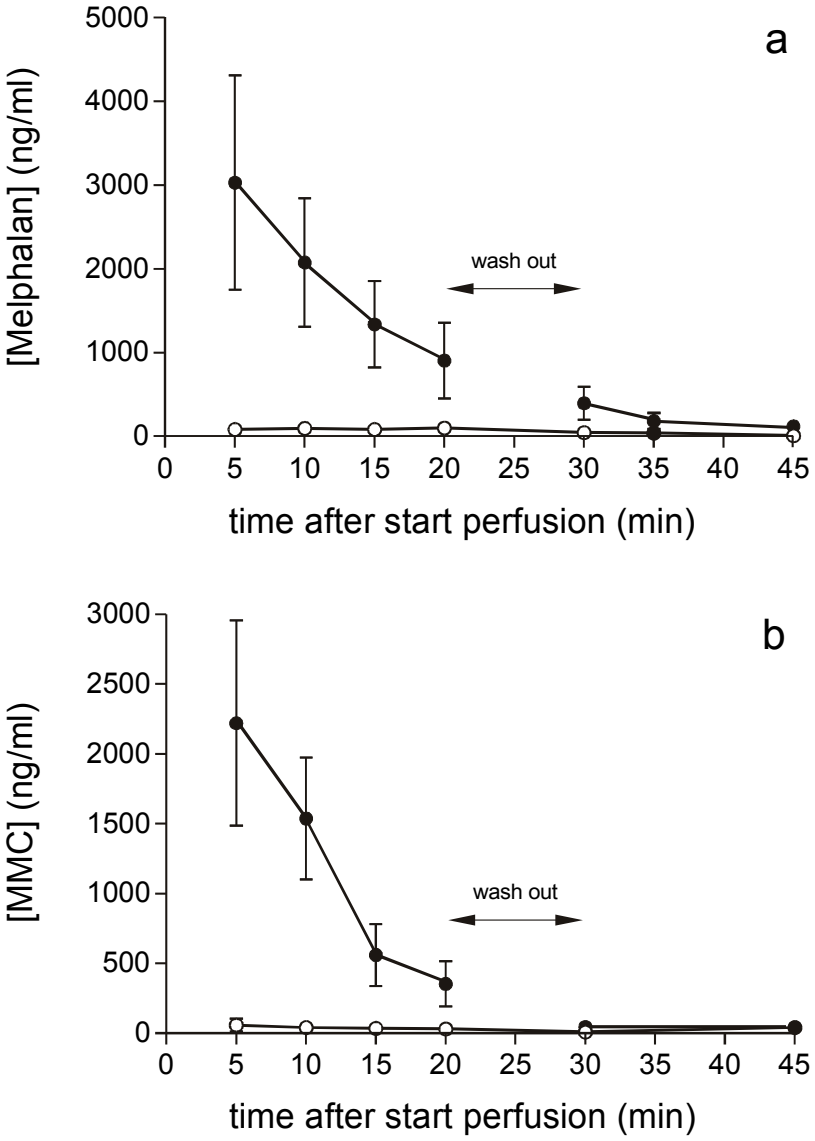

Figure 3 *Regional (black circles) and systemic (white circles) plasma concentration curves throughout and after 20 minutes of isolated hypoxic hepatic perfusion with melphalan (0.25 mg/kg) (A) and mitomycin C (0.25 mg/kg) (B). Perfusion was followed by a 10-minute washout. Mean plasma concentrations of five pigs ± SEM are shown.*

The mean plasma concentration of TNF at 5 minutes was 840 ng/ml; it remained virtually stable throughout perfusion, not exhibiting the regional concentration decline observed for MMC and melphalan (*Fig. 4*). Systemic TNF concentrations remained at < 22 ng/ml throughout the 20 minutes of perfusion. As a result of the washout procedure, regional TNF concentrations declined from 648 ng/ml to 99 ng/ml. Five minutes after termination of isolation, systemic TNF levels showed only a small rise to 50 ng/ml.

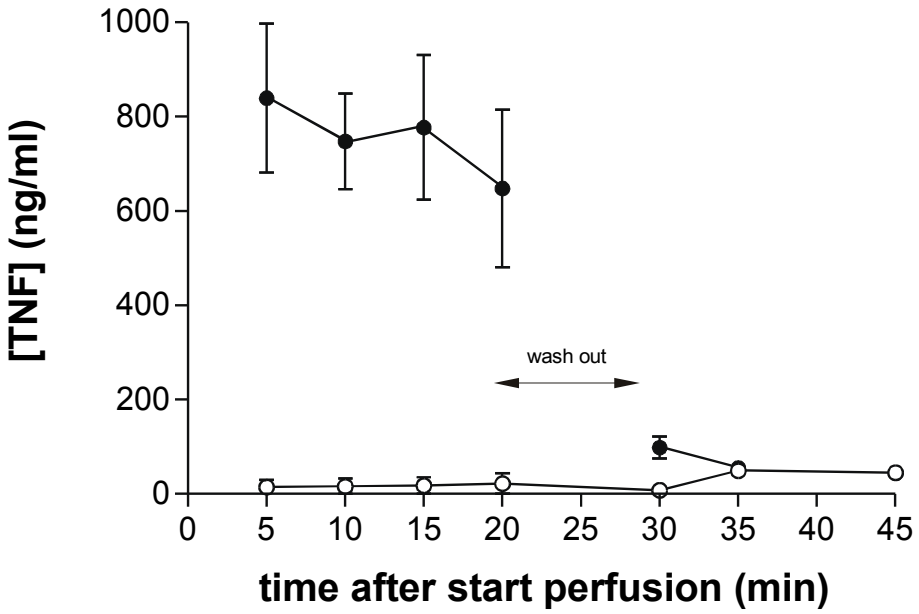

Figure 4 *Regional (black circles) and systemic (white circles) plasma concentration curves throughout and after 20 minutes of isolated hypoxic hepatic perfusion with tumour necrosis factor-alpha (0.02 mg/kg). Perfusion was followed by a 10-minute washout. Mean plasma concentrations of three pigs ± SEM are shown.*

Areas under the curve

Table 1 summarizes the regional *versus* systemic ratios for TNF, MMC, and melphalan. AUCs were calculated using values of drug concentrations from 5 to 20 minutes. Much higher AUCs were found during IHHP for the isolated hepatic vascular compartment than for the systemic compartment, or as compared with plasma levels after administration of an intravenous bolus. Regional drug concentration *versus* time advantages of 20- to 40-fold were observed during IHHP (melphalan, 20.4; MMC, 27.9; TNF, 43.3). When regional AUCs were compared with systemic AUCs after systemic bolus administration of equivalent doses, regional advantages varied from 3.2 to 11.5 (TNF, 3.2; MMC, 3.6; melphalan, 11.5).

Table 1 area under the concentration *versus* time curve and ratios during isolated hypoxic hepatic perfusion in pigs

AUC (ng x min/ml)	Melphalan	MMC	TNF
Regional*	26860	16940	11340
Systemic†	1319	608	262
Intravenous‡	2339	4657	3528
AUC ratios			
Regional/systemic	20.4	27.9	43.3
Regional/intravenoous	11.5	3.6	3.2

* *Regional is regional plasma concentration in isolated hepatic vasculair blood-compartment during IHHP*
† *Systemic is systemic plasma concentration during IHHP*
‡ *Intravenous is systemic drug concentrations after intravenous bolus administration*
AUC was calculated between t = 5 and t = 20 minutes after perfusion

DISCUSSION

We have demonstrated in this series of experiments in pigs the feasibility and safety of a leakage-free IHHP using balloon catheter methodology. Regionally, tissue exposure to perfused drugs was 20 to 40 times greater than systemic exposure. Because of the leakage-free quality of the method, these ratios are just as good as those observed with classic surgical isolated liver perfusions.[17,18,34] Regional MMC and melphalan levels declined rapidly during perfusion, indicating a fast uptake by the liver tissue; concomitant minimal systemic concentrations indicated virtually no leakage to the systemic blood compartment. This holds true not only for the parent drugs, but also for possible toxic metabolites and degradation products.[32] During isolation, concentrations of TNF in the perfusate declined only slightly, indicating limited uptake by the liver tissue; no leakage of TNF was observed. After termination of isolation, systemic TNF levels showed only a minor transient elevation, indicating that the washout procedure at the end of the perfusion was fully effective.

One could argue that regional/intravenous AUC ratios (see *Table 1*) become less favorable if they are calculated over the time period from 0 to 180 minutes instead of over the time period of isolation. For instance, calculated regional/intravenous ratios for melphalan, MMC, and TNF would become 7, 13, and 1, respectively. However, the high concentrations of TNF that are maintained during isolation have a different effect:[35] the high concentrations have been shown to have vascular toxicity but are necessary to obtain an optimal effect,[36] thus promoting enhanced uptake of MMC and melphalan. It is important that high concentrations are achieved for a short period, because in previous studies by us in patients with isolated limb perfusion, immediate TNF-induced vascular leakage was observed.[37]

Isolated hepatic perfusion has been developed as a treatment modality that maximizes drug concentrations in the target organ while shielding the organism from systemic toxicity.[14-16] It is a means to improve the selectivity of administration of antitumour agents to the liver, as compared with hepatic artery infusion (HAI). When

MMC was administered by IHP, a fourfold higher dose could be safely administered; this resulted in a tumour tissue concentration five times greater than that of HAI.[38] Similar results were obtained with melphalan.[39] The relative pharmacologic advantage of IHHP for MMC in our experiments may seem relatively small when compared with HAI with MMC. However, in IHHP the dose of MMC can be increased and the exposure time can be prolonged, whereas these parameters cannot be further exploited with HAI. It is clear from experimental data that in this setting liver toxicity rather than systemic toxicity is dose-limiting.[34,38] In earlier experiments, we performed classic isolated hepatic perfusion on pigs with melphalan and TNF for 90 minutes. In these experiments a transient elevation of liver enzyme levels reflected acute but overall moderate toxicity; no late toxicity was observed.[17]

Clinical experience with isolated hepatic perfusion is limited. Aigner[40] treated patients using classic surgical isolated hepatic perfusion chemotherapy, reporting 5-year survival rates of > 10%. However, classic procedures have been associated with significant mortality and morbidity rates of > 10%.[16,41,42]

The development of balloon catheter-mediated IHHP represents a significant improvement over the classic procedure in four ways. First, it limits the scale of the procedure and thus reduces the associated complications and high costs. IHHP, using described techniques, takes about 2 hours; the classic procedure takes an average of > 6 hours,[17] which in patients was found to be associated with a high morbidity rate.[18,19] Moreover, IHHP is less costly in terms of personnel (no perfusionists required) and material (no heart-lung machine or portocaval venous bypass). Second, IHHP is a technique that in principle allows for repeated isolated perfusions when the arterial infusion catheter is left in place after the first perfusion and connected to a subcutaneous port. This is of particular importance because multiple treatments are needed to keep the disease in the liver under control. Third, IHHP makes use of hypoxia, which renders tumour cells more sensitive to cytostatic agents in general and enhances in particular the antitumour effects of drugs such as MMC and melphalan.[29,30]

Finally, by limiting the extent of the surgical intervention, while still achieving full isolation and thus allowing complete washout of perfused agents after perfusion, IHHP in theory makes possible the introduction of drugs such as TNF. This is important because the combination of TNF and melphalan has been shown to exert highly synergistic antitumour effects against otherwise resistant tumours such as soft-tissue sarcomas and melanoma in the setting of isolated limb perfusion. Therefore, IHHP facilitates the translation of the successful experience with TNF in isolated limb perfusion into its application in isolated hepatic perfusion.

The pharmacokinetic profiles of TNF, MMC, and melphalan in balloon catheter-mediated isolated pelvic and abdominal perfusions have been virtually identical in our experience in pigs[43] and in humans (manuscript in preparation). Therefore, we are confident that a similar correlation will exist between the pharmacokinetic

profiles of these agents during IHHP in pigs and those that will be obtained in patients in a phase I-II study we have recently started in our clinic.

A crucial point in cancer therapy is to use the right drug in the right patient. A promising substance with important *in vitro* and *in vivo* antitumour effects is TNF.[18,44] Because of its general toxicity, however, TNF cannot be given in adequate doses intravenously.[10,11] When administered at adequate concentrations, such as in isolated limb perfusions, it is effective in humans.[12,13,45,46] High doses of TNF can induce tumour necrosis with acute softening of the tumour brought about by selective destruction of the tumour's microvasculature, causing acute hemorrhagic necrosis of the tumour.[12,35,47,48] TNF can be used in high doses in the extremity perfusion setting because leakage to the systemic circulation is usually limited to 0% to 10%, and in theory similar doses could be used in IHHP because a complete washout of agents can be achieved. Hepatic toxicity, however, is expected to be the dose-limiting factor. This was indicated by the experience of Fraker and Alexander[18,49] at the National Cancer Institute with classic isolated hepatic perfusions: responses were achieved in about 75% of the patients, which corresponds with our limited clinical experience with classic isolated hepatic perfusion with TNF and melphalan.[19]

In conclusion, we have demonstrated that IHHP has favorable pharmacokinetic characteristics for both TNF and cytostatic agents that equal those that can be achieved by the major procedure of a classic surgical isolated liver perfusion. Moreover, the balloon catheter procedure can be repeated, which is of eminent importance in prolonging control of metastatic disease in the liver. IHHP may provide a new chance to reintroduce TNF into the clinical setting in an attempt to translate its success in combination with chemotherapy in isolated limb perfusions into the treatment of the much more common problem of managing unresectable malignant disease to the liver.

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Chapter 5

BALLOON CATHETER HYPOXIC ABDOMINAL AND PELVIC PERFUSION WITH TUMOUR NECROSIS FACTOR-ALPHA, MELPHALAN AND MITOMYCIN C: A PHARMACOKINETIC STUDY IN PIGS

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SUMMARY

Background

Addition of tumour necrosis factor-alpha (TNF) to hypoxic abdominal perfusion (HAP) and hypoxic pelvic perfusion (HPP) with chemotherapeutic agents for treatment of un-resectable malignancies may lead to similar enhanced anti-tumour effects as are observed when TNF is added to isolated limb perfusions (ILP) with melphalan. Here, we validate the methodology of HAP and HPP using balloon catheter techniques, and investigate the distribution of TNF, melphalan and mitomycin C (MMC) over the regional and systemic blood compartments when applying these techniques.

Materials and methods

Twelve pigs underwent HAP or HPP with TNF, melphalan and MMC for 20 min. Throughout and after the procedures blood samples were obtained from hepatic, portal and systemic blood compartments and plasma concentrations of perfused agents were determined.

Results

We demonstrated that HAP and HPP result in temporary loco-regional concentration advantages of all perfused agents, although from start of perfusion significant systemic leakage occurred.

Conclusion

On basis of these results it seems that the advantage in terms of regional plasma concentration of TNF may be insufficient for TNF-mediated effects to occur, making future addition of this cytokine to these procedures in the clinical setting questionable. The observed regional concentration advantages of MMC and melphalan, however, warrant further studies on clinical application of these agents in both settings.

INTRODUCTION

Locally advanced abdominal and pelvic malignancies remain a major clinical problem.[1,2] A multidisciplinary approach will be necessary to convert these un-resectable tumours into respectable ones. Neo-adjuvant chemotherapy, sometimes in combination with radiotherapy, aims at achieving this. However, systemic treatment has very little effect on many tumours in these locations.[3]

As most chemotherapeutic drugs exhibit steep dose-response relationships, regional chemotherapy is an attractive concept, allowing higher concentrations of cytotoxic drugs to be delivered to the target region, resulting in increased tumour response rates, while shielding the organism from systemic toxicity. Several modalities have been utilized for regional chemotherapy of pelvis and abdomen e.g. celiac axis infusion, hypo gastric artery infusion, aortic stop-flow and isolated hypoxic perfusion. In addition to a first-pass effect, resulting in a higher regional drug concentration the latter two modalities also offer the theoretical advantage of an increased drug exposure time and regional induction of hypoxia.

Tumour necrosis factor-alpha (TNF) is a pro-inflammatory cytokine, which has demonstrated important *in vitro* and *in vivo* anti-tumour effects.[4,5] Disappointingly its toxicity has made systemic application of adequate doses with anti-tumour effect impossible.[6,7] However, in a leakage free isolated perfusion setting it can be safely used with remarkable results. Response rates greater than 80% have been observed in the treatment of un-resectable extremity soft tissue sarcomas and melanoma by isolated limb perfusion (ILP) with TNF in addition to melphalan.[8,9] These outstanding clinical results have resulted in approval and registration of TNF in Europe.[10] A new challenge now lies in attempting to extrapolate the success of ILP with these agents to other regional perfusion settings like liver, kidney, pelvis and abdomen.[11–13]

Regional chemotherapy is most effective when complete vascular isolation can be accomplished, as is the case in ILP and isolated liver perfusion.[14,15] Because of obvious anatomical difficulties in achieving complete vascular isolation of the abdominal or pelvic region, isolated perfusion for malignancies in this area is difficult to achieve. As a result systemic leakage of perfused agents is inherent to these procedures. Nevertheless, significant regional concentration advantages of chemotherapeutic drugs during regional abdominal and pelvic perfusion have been reported.[16-22]

An innovative method for obtaining relative vascular isolation of abdomen or pelvis with minimal invasive surgery was introduced by Aigner *et al*.[23–25] Reports on effectiveness of hypoxic perfusion regarding tumour responses have, however, not been conclusive.[20,26,27] This may be due to the variety of anti-tumour agents used and the different tumours treated, but some have also ascribed these different results to misuse of the technique. Recently, we demonstrated in pigs that isolated hypoxic hepatic perfusion (IHHP) with TNF, melphalan and mitomycin C (MMC) using

balloon catheters results in dramatic regional concentration advantages of these agents with only minimal systemic exposure.[13] On the basis of these results we initiated a phase I–II study on IHHP in patients with un-resectable liver tumours.[28]

In this pre-clinical study, we investigated the application potential of TNF in balloon catheter mediated hypoxic abdominal perfusion (HAP) and hypoxic pelvic perfusion (HPP) by studying the distribution of TNF, melphalan and MMC over the regional and systemic blood compartments in pigs during and after these procedures.

MATERIALS AND METHODS

Animals

Dutch Yorkshire pigs weighing 30 – 40 kg were used. Animals were treated in compliance with the guidelines on animal welfare of the Erasmus University Rotterdam. The study protocol was approved by the ethics committee on experimental studies in animals of the Erasmus University Rotterdam.

Drugs

Recombinant human TNF-alpha (TNF, 4.9 – 5.8 x 10^7 units/mg) was provided as a kind gift by Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany. Melphalan (L-pam, Alkeran, Wellcome Ltd., London, UK) was obtained as a sterile powder (100 mg) that was dissolved aseptically using solvent and diluent provided by Burroughs Wellcome (London, England). MMC (Kyowa Hakko Kogyo Co Ltd, Tokyo, Japan) was obtained as a sterile powder (40 mg) that was dissolved aseptically in 40 ml saline 0.9%.

Perfusion set

Perfusion sets were kindly provided by PfM, GmbH (Cologne, Germany) and consisted of an aorta-occlusion balloon catheter and a caval vein occlusion balloon catheter (12F, balloon capacity 25 ml) and a tubing set with a volume of 220 ml containing a bubble trap. In the perfusion circuit, flow was maintained by a roller pump, while drugs were infused through a side-line into the perfusion circuit.

Surgical procedure

Fig. 1 shows the methodology of HAP and HPP with balloon catheters. It illustrates the position of the occlusion catheters in aorta and caval vein and other components of the perfusion circuit. The procedures of HAP and HPP were carried out as follows: general anaesthesia was induced in pigs by standard methods and maintained with pavulon and fentanyl. An arterial line was introduced into a carotid artery and a double lumen central venous catheter was placed in an external jugular vein on either side. After a mid-line laparotomy was performed the pyloric vein was cannulated. Pigs were subsequently heparinized with 2 mg/kg heparin. After formal surgical exposure of the femoral vessels arteriotomy and venotomy were performed, whereafter the aorta-occlusion catheter and the caval vein occlusion catheter were

introduced in the common femoral vessels and retro-gradely moved up into the aorta and caval vein. For HAP balloons were positioned at the level of the diaphragm, above the hepatic veins. When performing HPP balloons were positioned just above the bifurcations of the major abdominal vessels. Temporary relative isolation of the abdominal vascular bed was achieved by inflating the balloons and pneumatic tourniquets around both upper thighs of the hind extremities. In order to compensate for the decrease in cardiac preload the aorta occlusion balloon was inflated prior to inflating the caval balloon. After connecting the catheters to the perfusion system primed with 220 ml Haemaccel (Behring Pharma, Amsterdam, The Netherlands) the perfusate was circulated by means of a roller pump with a constant flow of 200 ml/min. Stable perfusion was assessed by monitoring the blood level in the bubble trap. Hereafter, drugs were rapidly administered as a bolus infusion into the perfusate during approximately half a minute. After twenty minutes of perfusion isolation was terminated by subsequently deflating caval and aortic balloons.

Blood sampling

Baseline blood samples were obtained before administration of the drugs into the regional circuit. Isolation was terminated 20 min after infusion of the agents. Throughout and after the procedure blood samples were simultaneously drawn from the abdominal or pelvic blood compartments through a sampling port on the venous balloon catheter, from the portal circulation through the cannulated pyloric vein and from the systemic blood compartment through the central venous catheter at 5, 10, 15, 20, 25, 35, 55 and 85 min after infusion of the agents.

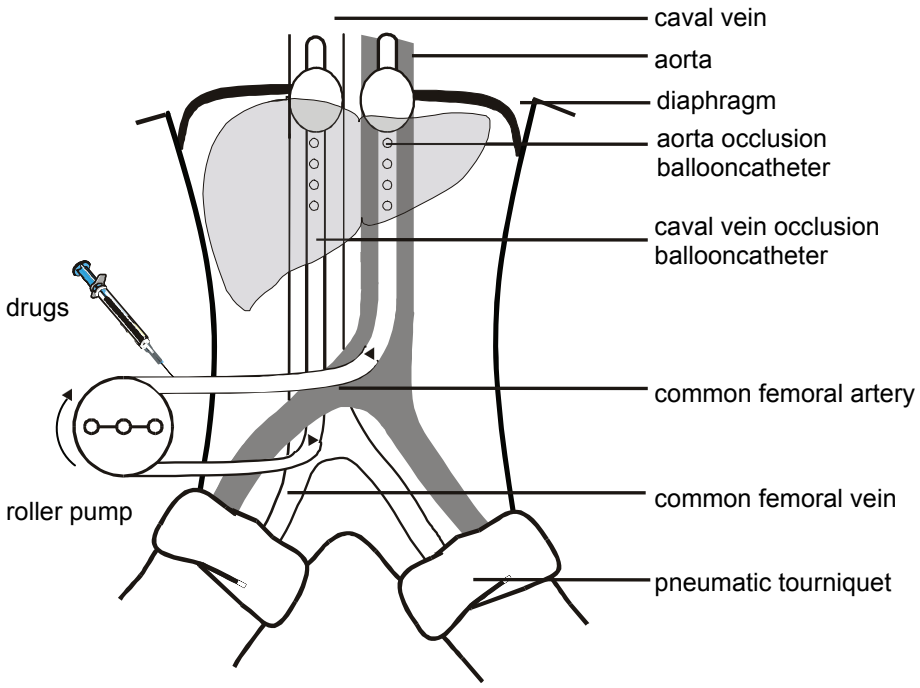

Figure 1 *Represents a schematic representation of the hypoxic abdominal perfusion. In case of hypoxic pelvic perfusion balloons were positioned at the level of the bifurcation of the major abdominal vessels.*

Blood samples were collected in glass tubes containing EDTA and immediately stored in the dark on ice. Samples were centrifuged at 2600 RPM for 6 min at 4 °C, whereafter the obtained plasma was stored at -70 °C until analysis.

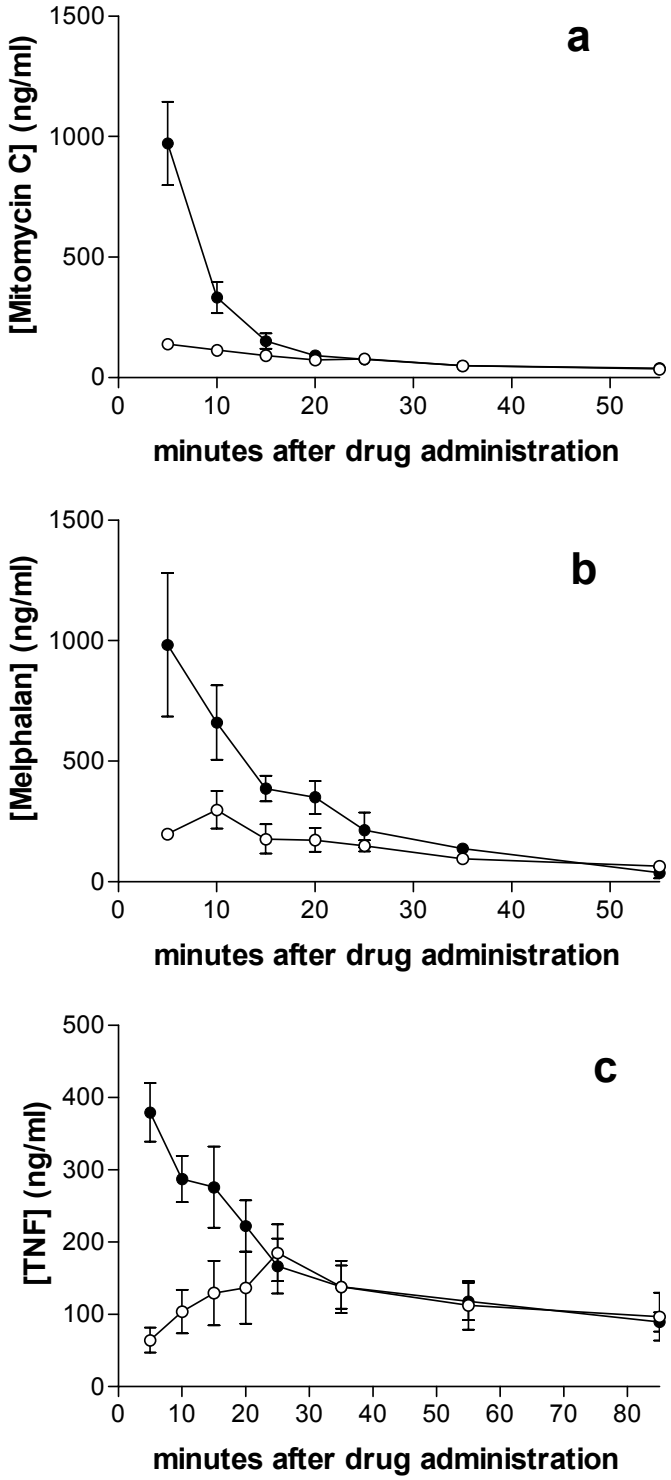

Figure 2 *Regional (black circles) and systemic (white circles) plasma concentrations of melphalan (a), MMC (b) and TNF (c) throughout and after 20 min of hypoxic abdominal perfusion. Pigs were perfused with 0.25 mg/kg melphalan (n = 6), 0.25 mg/kg MMC (n = 6) and 0.02 mg/kg TNF (n = 3). Mean plasma concentrations ± SEM are shown.*

Treatment schedule

Six pigs underwent HAP and six pigs underwent HPP. Three pigs of each group were administered MMC (0.25 mg/kg) and melphalan (0.25 mg/kg) alone and three pigs were administered the same amount of MMC and melphalan in combination with TNF (0.02 mg/kg) (*Figs. 2 and 3*).

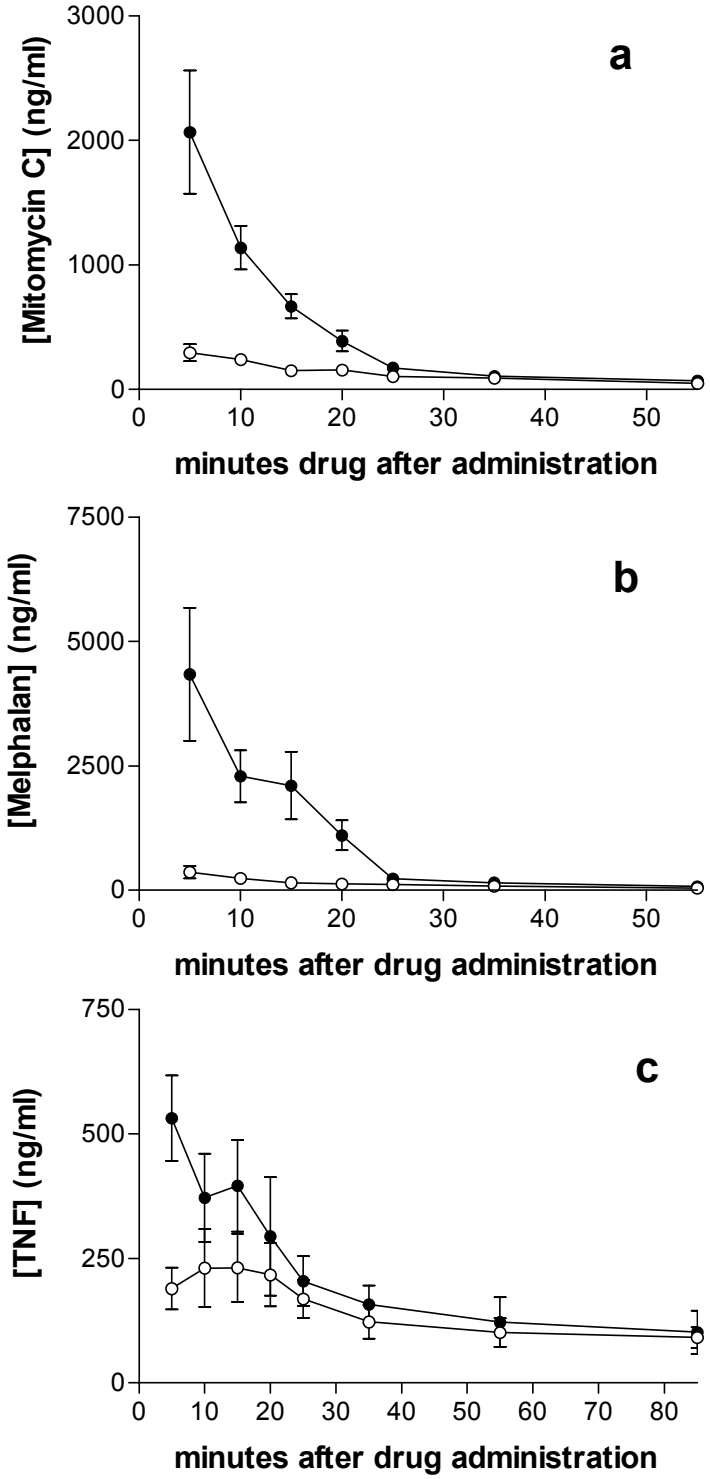

Figure 3 *Regional (black circles) and systemic (white circles) plasma concentrations of melphalan (a), MMC (b) and TNF (c) throughout and after 20 min of hypoxic pelvic perfusion. Pigs were perfused with 0.25 mg/kg melphalan (n = 6), 0.25 mg/kg MMC (n = 6) and 0.02 mg/kg TNF (n = 3): Mean plasma concentrations ± SEM are shown.*

TNF bio-analysis

Plasma TNF concentrations were determined by an ELISA for rhTNF as described by Engelberts *et al*.[29] In short, a 96-well Immuno-Maxisorp plate was coated with murine anti-human TNF mAb 61E71. A standard titration curve was obtained by making serial dilutions of a known sample of human rhTNF in normal porcine serum. The plates were incubated with a poly-clonal rabbit anti-human TNF antiserum, followed by addition of an enzyme-labeled anti-rabbit reagent and enzyme reaction. The detection limit for human TNF was 20 pg/ml.

Melphalan bioanalysis

Melphalan in plasma was measured by gas chromatography- mass spectrometry. *p*-[Bis(2-chloroethyl) amino]-phenylacetic acid methyl ester was used as an internal standard. Samples were extracted over trifunctional C18 silica columns. After elution with methanol and evaporation, the compounds were derivatised with trifluoroacetic anhydride and diazomethane in ether. The stable derivatives were separated on a methyl phenyl siloxane GC capillary column and measured selectively by single ion monitoring mass spectrometry in the positive EI mode. The detection limit for melphalan is 10 pg/ml. Details of this assay have recently been described by us.[30]

Mitomycin C bioanalysis

Mitomycin C was analysed by high performance liquid chromatography with ultraviolet detection (HPLC-UV). Porfiromycin was used as an internal standard. Samples were extracted over XAD-2 columns. After elution with methanol and evaporation, the dry residue was diluted in the mobile phase, which consisted of methanol and water (40/60). The sample was injected and separated over a C18 column and detected at 362 nm. The detection limit for MMC is 10 pg/ml. Details of the assay are described elsewhere.[31]

Calculation of area under the curve (AUC)

Areas under the plasma concentration *vs* time curve were calculated applying the trapezoid rule using GraphPad Prism version 3.0 for Windows 98 software. AUCs of TNF were calculated from 5 to 85 min after start of perfusion. AUCs of melphalan and MMC were calculated from 5 to 55 min after start of perfusion.

RESULTS

Apart from transient tachycardia in a minority of pigs, stable hemodynamics were observed in all experiments throughout the procedures. After deflating the balloons, a short period of hypotension was observed, easily managed by fluid challenge. All pigs survived the procedures. The addition of TNF to MMC and melphalan did not alter the plasma concentration *vs* time profile of MMC and melphalan during and after the procedure (data not shown). Therefore, data from pigs perfused with MMC and melphalan alone and from pigs perfused with MMC and melphalan in combination with TNF were pooled.

During HAP portal plasma concentrations of perfused agents did not significantly differ from loco-regional plasma concentrations and during HPP portal plasma concentrations of perfused agents did not significantly differ from systemic concentrations. Therefore, data concerning portal concentrations are omitted.

Pharmacokinetics of MMC and melphalan during HAP and HPP

HAP resulted in significantly higher loco-regional concentration advantages of MMC and melphalan compared to concomitant systemic concentrations. However, as soon as 5 min after starting perfusion systemic leakage was evident. After 5 min of perfusion mean loco-regional peak levels were 971 and 983 ng/ml, respectively, compared to mean systemic peak levels of MMC and melphalan at 5 min of 139 and 298 ng/ml, respectively. During perfusion, MMC and melphalan plasma concentrations rapidly declined, with systemic and loco-regional plasma levels reaching almost equilibrium just before deflating the balloons.

During HPP loco-regional concentration advantages of MMC and melphalan were more profound, although systemic leakage was evident for both agents 5 min after start of perfusion. MMC and melphalan mean peak levels at 5 min were 2069 and 4339 ng/ml, respectively, compared to mean systemic peak levels of 297 and 367 ng/ml, respectively. Again levels of MMC and melphalan declined during perfusion, accompanied by measurable systemic levels of both agents, proving leakage to be contributing to the decline.

Pharmacokinetics of TNF during HAP and HPP

Mean loco-regional plasma concentration advantages of TNF during HAP were markedly less favorable than those for MMC and melphalan. Loco-regional peak levels at 5 min were 380 ng/ml. During perfusion systemic levels showed a persistent rise, indicative of leakage, with systemic levels at the end of the procedure of 141 ng/ml. HPP resulted in a similar pharmacokinetic profile with peak levels of TNF at 5 min of 532 ng/ml and systemic peak levels at 15 min of 231 ng/ml. As was seen for MMC and melphalan, leakage was less profound in HPP compared to HAP.

Comparison of regional *vs* systemic area under the curve

Tables 1 and 2 summarise the regional *vs* systemic plasma concentration ratios in both settings for TNF, melphalan and MMC. AUCs were calculated using values of drug concentrations from 5 to 85 min after start of treatment for TNF and from 5 to 55 min for MMC and melphalan. HAP was associated with a Regional/Systemic AUC Ratio of only about 2 for both melphalan and MMC and of only 1.28 for TNF reflecting the quick and total leakage to the systemic compartment that is associated with HAP. HPP was associated with better Regional/Systemic AUC Ratios, being 7.0 for melphalan and 3.2 for MMC and 1.4 for TNF, again reflective of important leakage to the systemic compartment.

Table 1 Area under concentration versus time curves and ratio's during hypoxic abdominal perfusion in pigs

	AUC (ng x min/ml)		Regional/Systemic AUC ratio
Drug	**Regional**	**Systemic**	
melphalan	13500	6945	1.9
MMC	7007	3428	2.0
TNF	12490	9742	1.28

Regional is plasma concentration in abdominal vascular compartment during hypoxic abdominal perfusion. Systemic is systemic plasma concentration during hypoxic abdominal perfusion. AUC for melphalan and MMC was calculated between t = 5 and t = 55 min after start of perfusion. AUC for TNF was calculated between t = 5 and t = 85 min after start of perfusion.

Table 2 Area under concentration versus time curves and ratio's during hypoxic pelvic perfusion in pigs

	AUC (ng x min/ml)		Regional/Systemic AUC ratio
Drug	**Regional**	**Systemic**	
melphalan	43210	6132	7.0
MMC	19750	6140	3.2
TNF	15140	10900	1.4

Regional is plasma concentration in pelvic vascular compartment during hypoxic pelvic perfusion. Systemic is systemic plasma concentration during hypoxic pelvic perfusion. AUC for melphalan and MMC was calculated between t = 5 and t = 55 min after start of perfusion. AUC for TNF was calculated between t = 5 and t = 85 min after start of perfusion.

DISCUSSION

In this study, we demonstrated that HAP and HPP using described minimally invasive balloon catheter methodology resulted in regional augmented plasma concentrations of MMC, melphalan and TNF. The concentration advantages of MMC and melphalan during these procedures were in accordance with reports by others.[16–22] Peak loco-regional concentrations of all perfused agents were lower in case of HAP than in HPP, probably reflecting the larger blood compartment over which agents were distributed, the larger collateral circulation in case of HAP and the higher tissue clearance of the abdominal compartment. Apart from significant systemic leakage, the rapid loco-regional decline in MMC and melphalan concentrations should also be attributed to tissue uptake, as in the leakage free IHHP setting a similar decline in regional drug levels was observed.[13] This tissue uptake holds true not only for the parent drugs, but also for possible toxic metabolites and degradation products.[30]

Concentrations of TNF in the perfusate declined relatively faster, compared to the other perfused agents. Possibly, the minimal uptake of this, relatively large cytokine

by loco-regional tissue results in a more rapid distribution of this compound over the regional and systemic blood compartments. Comparing the AUCs of the regional and systemic concentration *vs* time curve of MMC, melphalan and TNF during HPP and HAP gives insight into the effect of these procedures on regional and systemic tissue exposure to these agents. However, one must bear in mind that for an agent like TNF peak levels of short duration may be sufficient to elicit anti-tumour effects.[32,33] In HPP there was a marked augmented loco-regional tissue exposure to MMC and melphalan during and after perfusion. For HAP the regional concentration advantages were less profound. However, when calculating the AUCs for both procedures the concentrations of perfused agents during the first 5 min of perfusion where not taken into account. During the first 5 min loco-regional concentrations must have been higher than those measured at later time points. These initially higher concentrations may have resulted in a markedly enhanced tissue uptake of the drugs, as similarly, arterial infusion settings result in elevated tissue uptake of infused drugs due to a first-pass effect.[34]

As mentioned, complete vascular isolation of abdominal and pelvic vascular beds is difficult to achieve. Although it has been demonstrated that an almost complete isolation of the abdomen can be achieved after ligation of all non-gastro-intestinal branches of the abdominal aorta and caval vein,[18] such an approach does not seem feasible in a palliative treatment setting. If regional abdominal and pelvic perfusion, with anti-tumour agents are to be applied on a wide scale, magnitude and morbidity of the surgical intervention must be limited, while the procedure should be repeatable and yield good response rates.

Several authors have published on HAP and HPP using balloon catheter techniques and used these modalities for treating a number of malignant conditions not treatable by conventional means.[16,17,20,21,23–25,35] These have included pelvic recurrences of colorectal carcinoma, advanced primary gastric and pancreatic carcinomas, and locally extensive ovarian malignancies.

For most chemotherapeutic agents steep dose-response curves can be demonstrated. Therefore, high drug concentrations are important for eradicating both sensitive and resistant tumour cells.[34,36] Drugs whose cytotoxic action is particularly potentiated by hypoxia, such as MMC,[37,38] have been reported to be particularly effective when administered by regional hypoxic perfusion.[16,35] Hypoxia causes dividing cells to halt their progression through the cell cycle, by allowing them to progress to and then remain in the G1-like susceptibility state.[37]

A crucial point in cancer therapy is to use the right drug in the right patient. A very promising substance with important *in vitro* and *in vivo* anti-tumour effects is TNF.[4,5] Because of its severe general toxicity, however, TNF cannot be given in adequate doses intravenously.[6,7] When administered at adequate concentrations, such as in ILPs, it is very effective in humans.[8,9,39,40] Moreover, hypoxia has also been demonstrated to have a potentiating effect on the anti-tumour effects of TNF in ILPs

of only 20 min duration.[33] High dose TNF has the ability to induce tumour necrosis with acute softening of the tumour brought about by a selective destruction of the tumour micro-vasculature, causing acute hemorrhagic necrosis of the tumour.[8,32,41,42] As the main TNF anti-tumour effect is mediated by the tumour stroma, theoretically it could be of importance in the treatment of a wide variety of solid tumours. While TNF can be used in very high doses in the extremity perfusion setting, since leakage to the systemic circulation is usually limited to 0 – 10%, application of this agent in a system which is not completely leakage free, as is the case for HAP and HPP, constitutes a major hazard.

In earlier experimental and clinical studies concerning balloon catheter mediated IHHP, we demonstrated the pig to be an excellent model for studying the distribution of perfused agents.[13,28] The pharmacokinetic profiles of MMC and melphalan during HAP and HPP in this study in pigs are also virtually identical to those observed in clinical studies by us (manuscript in preparation) and others.[20] Therefore, we feel confident that observations regarding the distribution of TNF over the regional and systemic compartments during HAP and HPP in this porcine perfusion model can be extrapolated to the clinical setting.

In these experiments, we observed minimal and short lasting loco-regional concentration advantages of TNF during HAP and HPP. Due to systemic leakage the concentrations of TNF regionally and systemically had virtually reached equilibrium after 20 min of perfusion, making a wash-out procedure of the drug, as is performed in ILP and IHHP, obsolete. As in both HAP and HPP all TNF has entered into the systemic circulation at the end of the procedures, one can expect the maximal tolerable dose (MTD) for TNF in these procedures to be equivalent to the MTD when TNF is administered intravenously.

Earlier, we demonstrated in an ILP rat model that a decrease in perfused dose of TNF may be feasible.[33] Although clinical results of TNF ILPs, accordingly, suggest that only 5 - 10 fold increases in TNF-drug levels are necessary to obtain TNF-mediated anti-tumour effects in humans, as the publication by Hill and coworkers[5] has demonstrated, it does not seem likely, on basis of these experiments, that these concentration advantages for TNF can be achieved by HAP or HPP using minimally invasive techniques.

In conclusion, we have demonstrated that HAP and HPP result in temporary concentration advantages of all perfused drugs. In terms of tissue exposure these advantages are more profound for MMC and melphalan than for TNF. On basis of these results it seems unlikely that the advantage in terms of regional concentration of the TNF reached by HAP and HPP is sufficient for TNF effects to occur, thus making future addition of TNF to clinical procedures unlikely. The observed concentration advantages of MMC and melphalan in both settings warrant further clinical studies. With the prospect of further sophistication of balloon catheter technology this procedure may be carried out by the intervention-radiologist.[35] In such a minimally

invasive setting HAP and HPP will be methodologies that in principle allows for repeated perfusions. This is of particular importance when multiple treatment events are needed to keep the disease under control.

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Chapter 6

ISOLATED HYPOXIC HEPATIC PERFUSION WITH ORTHOGRADE OR RETROGRADE FLOW IN PATIENTS WITH IRRESECTABLE LIVER METASTASES USING PERCUTANEOUS BALLOON CATHETER TECHNIQUES: A PHASE I AND II STUDY

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SUMMARY

Background
Isolated hepatic perfusion for irresectable metastases confined to the liver has reported response rates of 50% to 75%. Magnitude, costs, and nonrepeatability of the procedure are its major drawbacks. We developed a less invasive, less costly, and potentially repeatable balloon catheter–mediated isolated hypoxic hepatic perfusion (IHHP) technique.

Methods
In this phase I and II study, 18 consecutive patients with irresectable colorectal or ocular melanoma hepatic metastases were included. Two different perfusion methods were used, both with inflow via the hepatic artery, using melphalan 1 mg/kg. In the first eight patients, the portal vein was occluded, and outflow was via the hepatic veins into an intracaval double-balloon catheter. This orthograde IHHP had on average 56% leakage. In next 10 patients, we performed a retrograde outflow IHHP with a triple balloon blocking outflow into the caval vein and allowing outflow via the portal vein. The retrograde IHHP still had 35% leakage on average.

Results
Although local drug concentrations were high with retrograde IHHP, systemic toxicity was still moderate to severe. Partial responses were seen in 12% and stable disease in 81% of patients. The median time to local progression was 4.8 months.

Conclusions
We have abandoned occlusion balloon methodology for IHHP because it failed to obtain leakage control. We are presently conducting a study using a simplified surgical retrograde IHHP method, in which leakage is fully controlled, which translates into high response rates.

INTRODUCTION

Approximately 50% to 60% of colorectal cancer patients will develop liver metastases during follow-up. In nearly a quarter of these patients, the liver is the only site of disease.[1] If hepatic metastases of colorectal cancer are resectable, 5-year survival rates are reported between 25% and 45%, depending on several prognostic factors.[2] Patients with irresectable hepatic metastases have a 0% to 2% 5-year survival rate. Therefore, aggressive, selective treatment of the liver seems justified because control of hepatic metastases translates into improved overall survival. There is no standard treatment for unresectable hepatic metastases confined to the liver, so novel treatment modalities have to be developed.

Although response rates with novel systemic chemotherapeutic agents such as oxaliplatin and irinotecan in combination with 5-fluorouracil are promising, overall survival remains poor.[3-5] To improve responses and survival, locoregional chemotherapeutic regimens have been developed, such as hepatic artery infusion (HAI), chemoembolization, and isolated hepatic perfusion (IHP). For most chemotherapeutic agents, a steep dose-response curve can be demonstrated, and exposure of the liver metastases to higher drug concentrations by means of locoregional treatment might result in improved control of hepatic metastases. HAI exploits the first-pass effect of the liver, resulting in high local, but low systemic, drug exposure. Several repeated HAI regimens produced higher response rates compared with systemic chemotherapy, with a 2-year survival of 50% to 60%.[6-11]

In animal studies, Marinelli *et al*.[12,13] demonstrated that significantly higher intrahepatic concentrations can be reached by IHP compared with HAI. In a leakage-free perfusion setting, IHP shields the systemic compartment to drug exposure, and in combination with a washout procedure, it protects against systemic toxicity. Classic surgical IHP (SIHP) with melphalan or mitomycin C has been studied in animal models and has resulted in high response rates.[14-16] Clinical studies using melphalan with or without tumour necrosis factor (TNF)-α have shown promising results.[17-24] The phase II trial performed by the National Cancer Institute of SIHP with melphalan and TNF demonstrated an overall response rate of 75%.[18]

SIHP is a major, complex, expensive, and time-consuming operation. These features in combination with potential toxicity are major drawbacks toward wide clinical application. Moreover, this procedure can be performed only once. To address these problems, we developed a leakage-free isolated hypoxic hepatic perfusion (IHHP) technique with balloon catheters in pigs.[25] With melphalan and TNF, it was demonstrated that isolated perfusion with balloon catheters was feasible and showed minimal systemic leakage. On the basis of these favorable pharmacokinetic results, a phase I and II study with melphalan was developed for patients with irresectable liver metastases. In this report, we present the results of the first 18 patients who underwent IHHP with balloon catheter techniques with orthograde or retrograde flow through the liver.

MATERIALS AND METHODS

Patient selection criteria

In all patients, a radical resection of the primary tumour was performed before the patient entered the study protocol. The liver metastases were considered irresectable on the basis of multiple lesions in multiple segments of the liver or location near vascular structures. Tumour involvement had to be < 50% of the total liver volume to prevent massive necrosis in case of a response. Absence of extrahepatic tumour growth was evaluated by computed tomographic (CT) scan of the thorax and abdomen. All patients had a Karnofsky performance score of at least 90, liver enzymes (ALT, AST, and AF) not higher than five times the normal values, and bilirubin not higher than two times the normal values. Exclusion criteria included age younger than 18 or older than 75 years; portal hypertension; significant central nervous system disease; significant cardiovascular, pulmonary, or renal disease; uncontrolled infections; presence of organ grafts; and chemotherapy or radiotherapy within 4 weeks before the IHHP. Angiography was routinely performed to exclude aberrant hepatic arteries or to visualize other anatomical anomalies. Patients with severe arteriosclerosis of the aortic-iliac-femoral vessels that made placement of balloon catheters impossible were also excluded. All IHHPs were performed at the Daniel den Hoed Cancer Center. The study protocol was approved by the Medical Ethical Committee of the Erasmus University Medical Centre, and written, informed consent was obtained from all patients.

Perfusion circuit

Perfusion sets (PfM, GmbH, Cologne, Germany) consisted of a double-balloon catheter (12F; balloon capacity, 25 ml; distance between balloons, 4 - 5 cm) for venous isolation of the liver. An aortic occlusion balloon catheter (12F; balloon capacity, 25 ml) for compensating for the decrease of cardiac preload during the procedure and a tubing set with a volume of 220 ml containing a bubble trap were used. All IHHPs were performed with inflow via the hepatic artery. In the first eight patients, a predominantly open technique was used to cannulate the proper hepatic artery via the gastroduodenal artery with a 8F catheter (*Fig. 1*). From patient 9 to 18, we used a percutaneous 5F stopflow occlusion catheter (PfM) introduced before surgery via the groin by using the Seldinger technique (Table 1; Fig. 2). Except for patient 12 and patient 15, who had a double hepatic artery, the balloon was positioned in the proper hepatic artery.

The first eight patients were perfused with a double-balloon catheter in the caval vein, with occlusion of the portal vein and outflow via the side holes in the caval vein catheter (*Fig. 1*). To improve leakage control, a triple-balloon occlusion catheter was developed (PfM), and the outflow was diverted to the portal vein, creating a retrograde flow. This triple balloon was used from patients 9 to 18. It occludes the retrohepatic caval vein in a section of 18 cm, versus 12 cm with the double-balloon catheter (*Fig. 2*). In the perfusion circuit, flow was maintained by a roller pump, and pressure was measured via a side-line.

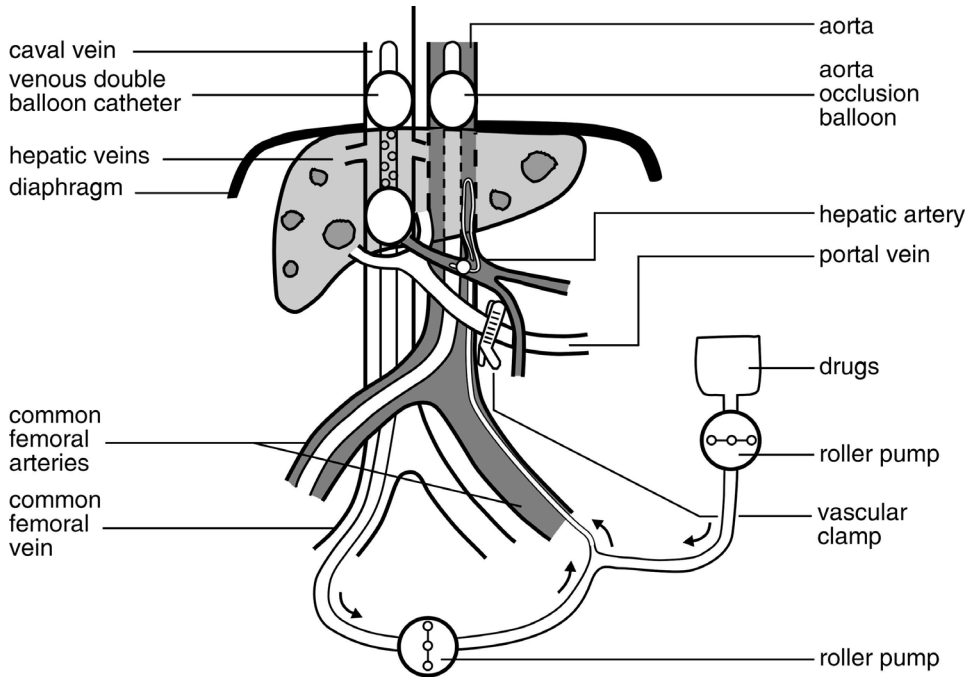

Figure 1 *Schematic representation of isolated hypoxic hepatic perfusion with a percutaneous catheter in the hepatic artery (inflow) and double-balloon catheter in the caval vein (outflow). Orthograde flow method.*

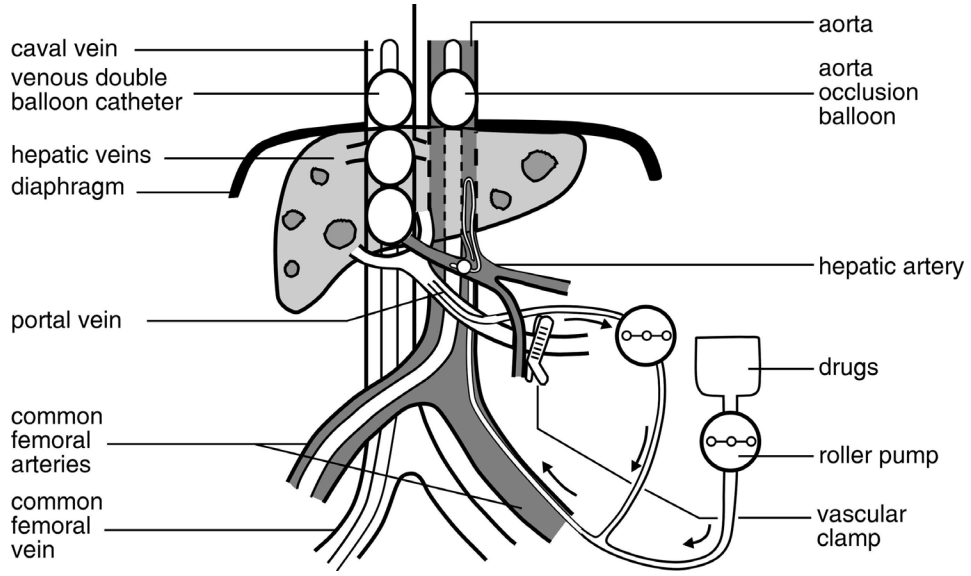

Figure 2 *Schematic representation of isolated hypoxic hepatic perfusion with a percutaneous catheter in the hepatic artery (inflow), a triple-balloon occlusion catheter in the caval vein, and an outflow catheter in the portal vein. Retrograde flow method.*

Drugs

Melphalan 1 mg/kg (Alkeran; Wellcome Ltd., London, UK) was used in all patients and infused through a side-line into the perfusion circuit.

Surgical procedure of the orthograde flow IHHP

A small right subcostal incision was made, and cannulation of the gastroduodenal or hepatic artery was established. A cholecystectomy was routinely performed in only the first five patients. When percutaneous techniques were used before surgery, palpation confirmed the position of the balloon in the proper hepatic artery. Surgical exposure of the femoral artery and vein in the groin was made, and cannulation of the artery was performed with an aortic occlusion catheter positioned under radiographic control just above the celiac axis. Patients were subsequently heparinized with heparin 2 mg/kg. Cannulation of the femoral vein was then performed with the caval double-balloon catheter positioned under radiographic control. The proximal balloon was placed at the level of the diaphragm, and the distal balloon was placed just below the liver; this was confirmed by palpation. Between the two caval balloons, 20 ml of contrast solution was rapidly injected to visualize the hepatic veins at their confluence into the retrohepatic caval vein. After clamping of the portal vein and connecting the hepatic artery catheter and the caval balloon catheter to the perfusion circuit primed with 220 ml of Hemaccel (Behring Pharma, Amsterdam, The Netherlands), the orthograde isolated perfusion was performed (*Fig. 1*; *Table 1*; patients 1 - 8).

Surgical procedure of the retrograde flow IHHP

Before the operation, the hepatic artery was cannulated via the groin as described previously. Via the abdominal incision, the portal vein was dissected. The femoral artery and vein were cannulated, and the occlusion balloons were positioned both in the vena cava and the aorta. The portal vein was then cannulated with a 14F catheter for outflow (*Table 1*; patients 9 - 18). Patients were subsequently heparinized with heparin 2 mg/kg. After connection to the perfusion circuit, a retrograde perfusion was commenced through the portal veins. The retrograde perfusion setup is depicted in *Fig. 2*.

The perfusate was circulated by a constant flow (*Table 1*). Stable perfusion was monitored by pressure measurement and the perfusate level in the bubble trap. Methylene blue was injected into the arterial catheter to check homogeneous distribution over both lobes of the liver. Then melphalan was infused into the circuit, and the perfusion was conducted for 20 minutes. After 20 minutes, a washout procedure was performed by using 1 l of Hemaccel to collect the venous effluent. Total liver ischemia time never exceeded 60 minutes. The isolation was terminated by deflating the caval balloon followed by the aortic balloon and releasing the ligature of the portal vein (orthograde IHHP), or by decannulation and closing the venotomy of the portal vein (retrograde IHHP).

Leakage monitoring

During IHHP, potential drug leakage was monitored by using a radioactive tracer. A small calibration dose of human serum albumin radiolabeled with iodine-131 was injected into the systemic circulation before the perfusion, and a 10-fold higher dose of the same isotope was injected into the IHP circuit. Continuous monitoring was

performed with a precordial scintillation probe. Systemic leakage is expressed quantitatively as a percentage (100% leakage represents a homogeneous distribution of the isotope in the body).[21]

Blood sampling

Before, during, and after the perfusion, blood samples were taken and collected to study the pharmacokinetics of melphalan and the hematological, renal, hepatic, and gastrointestinal toxic side effects. Toxicity was graded according to the standard World Health Organization (WHO) common toxicity criteria.[26]

Measurement of melphalan concentrations

Melphalan was measured in plasma by gas chromatography- mass spectrometry. P-[Bis(2-chloroethyl)- amino]-phenylacetic acid methyl ester was used as an internal standard. Samples were extracted over trifunctional C18 silica columns. After elution with methanol and evaporation, the compounds were derivatized with trifluoroacetic anhydride and diazomethane in ether. The stable derivates were separated on a methyl phenyl siloxane gas chromatography capillary column and measured selectively by single ion monitoring gas chromatography- mass spectrometry in the positive EI mode described previously by Tjaden and de Bruijn.[27]

Assessment of tumour response

Tumour response was assessed by comparing preperfusion CT and magnetic resonance imaging scans of the liver with scans made at 6 to 8 weeks after IHHP. The tumour marker carcinoembryonic antigen (CEA) was monitored (when indicated) before surgery and 6 to 8 weeks after perfusion but was not used for response assessment. Clinical responses were assessed by standardized WHO criteria:[26] complete remission, regression of all measurable disease in the liver for > 4 weeks; partial remission (PR), regression of the tumour size by > 50% for > 4 weeks; stable disease, regression < 50% of the tumour in the liver or progression < 25% for > 4 weeks; and progressive disease, progression > 25%.

RESULTS

Patient characteristics

In total, 18 patients were included in the protocol: 7 women and 11 men with a mean age of 63.2 years (range, 39 - 71 years). Seventeen patients had irresectable colorectal liver metastases, and one patient had ocular melanoma hepatic metastases.

Leakage control

In the first eight IHHPs with a double-balloon intracaval catheter, a mean leakage of 56% (range, 20% - 100%) was measured. Repeated adaptations to the catheter design in terms of balloon size and interballoon distance were performed. This led to a change of concept and the design of a triple-balloon caval vein occlusion catheter

with outflow via the portal cannula and a retrograde flow direction (patients 9 - 18). Leakage then decreased to an average of 35% (range, 5% - 85%).

Toxicity study

Regional toxicity consisted mainly of a transient increase of liver enzymes during the first week after IHHP: 83% of the patients had WHO grade 2 or 3 toxicity (*Table 1*). No coagulopathy was observed. In one patient we were confronted with severe hepatic toxicity (grade 4). Unfortunately, this patient died within 30 days of the operation (discussed in detail in Complications).

Because of the leakage of melphalan during the perfusion, most patients were treated with granulocyte colony-stimulating factor (Neupogen; Amgen B.V., Breda, The Netherlands) in an attempt to prevent severe leucopenia. Systemic toxicity consisted mainly of leucopenia (WHO grade 1 - 3 in 44% and severe grade 4 leucopenia in 27%) for 10 to 20 days after perfusion. In most patients with relatively less leakage, no or only mild leucopenia was observed. No renal or gastrointestinal toxicity was observed.

Melphalan pharmacokinetics

Figure 3 shows a drug concentration-versus-time curve in the isolated circuit and in the systemic circulation. It shows melphalan concentrations during a retrograde IHHP (patient 9). Very high regional and negligibly low systemic melphalan concentrations were observed. After surgery, this patient had only mild hepatic toxicity and no signs of systemic toxicity. The area under the concentrations-versus-time curve calculation showed a regional concentration advantage, with an area under the curve regional/systemic ratio of 28.2.

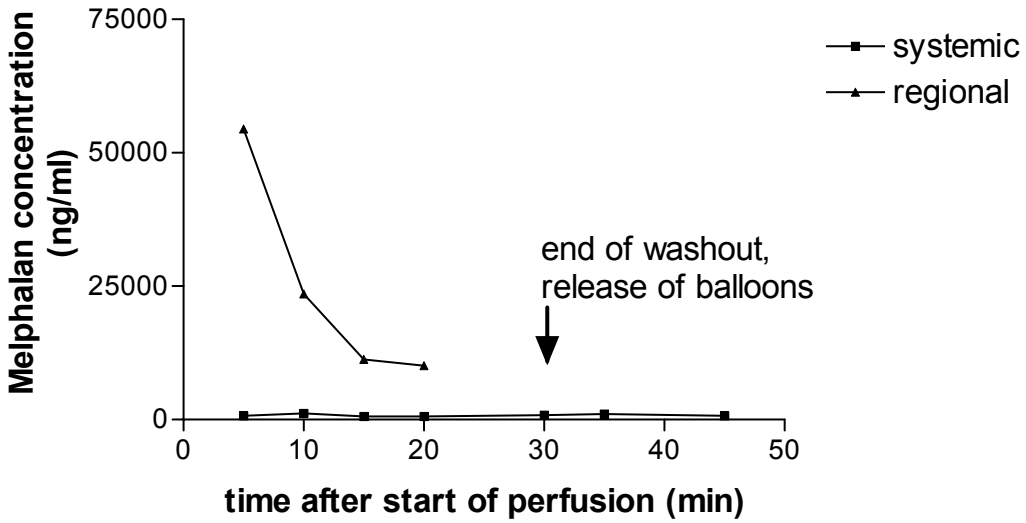

Figure 3 *Pharmacokinetics of melphalan during isolated hypoxic hepatic perfusion (patient 9, with 15% leakage during perfusion). The area under the concentration-versus-time curve (AUC) was calculated between 5 and 20 minutes: regional 335,500 ng x min/ml versus systemic 11,870 ng x min/ml. The AUC ratio (regional/systemic) was 28.2.*

Table 1 Characteristics of 18 patients with irresectable liver metastases treated by IHHP with melphalan

Patient No.	Sex	Age (y)	Tumour type	Balloon (vena cava)	Outflow (site)	Leakage (%)	Toxicity				Response			Time until PD at liver (mo)
							Heamatological	Renal	Hepatic	GI	CEA before	CEA at 6 wk	CT	
1	M	67	Colorectal	Double	Caval	50	3	0	3	0	-	-	SD	4
2	M	70	Colorectal	Double	Caval	60	4	0	2	0	74	10	SD	3
3	F	67	Colorectal	Double	Caval	20	4	0	3	0	211	171	SD	3
4	F	58	Colorectal	Double	Caval	100	3	0	3	0	9980	2260	SD	3
5	M	69	Colorectal	Double	Caval	50	3	0	2	0	13.7	5.9	SD	5
6	M	66	Colorectal	Double	Caval	33	1	0	1	0	29.7	30.2	SD	13
7	M	68	Colorectal	Double	Caval	40	4	0	3	0	45.9	27.6	PD	5
8	M	39	Colorectal	Double	Caval	100	4	0	2	0	12.0	20.7	SD	3
9	F	68	Colorectal	Triple	Portal	15	0	0	1	0	6.33	2.74	PR	5
10	M	71	Colorectal	Triple	Portal	30	4	0	2	0	15.73	21.5	SD	7
11	F	64	Colorectal	Triple	Portal	17	0	0	3	0	81.68	58.75	SD	3
12	F	69	Ocular melanoma	Triple	Portal	5	0	0	4	0	NA	NA	NA	NA
13	M	65	Colorectal	Triple	Portal	35	3	0	3	0	480	87.74	SD	3
14	M	51	Colorectal	Triple	Portal	85	1	0	3	0	5.12	2.0	PR	5
15	F	64	Colorectal	Triple	Portal	55	0	0	3	0	2330	NA	SD	4
16	M	71	Colorectal	Triple	Portal	65	3	1	3	0	54	NA	Abscess	NA
17	F	52	Colorectal	Triple	Portal	16	0	0	2	0	43.5	15.5	SD	4
18	M	59	Colorectal	Triple	Portal	50	3	0	3	0	14.27	NA	SD	6

		Grade 4 Toxicity				Mortality	Response		
Mean	63.2	5/18	0/18	1/18	0/18	0/18	SD	81%	4.8
Median	66.5	27%	0%	5%	0%	5%	PR	12%	4.0

IHHP *isolated hypoxic hepatic perfusion*
GI *gastrointestinal*
CEA *carcinoembryonic antigen*
CT *computed tomography*
PD *progressive disease*
SD *stable disease*
PR *partial response*
NA *not available*

Complications

Patient 3 developed paralysis of both legs a few days after the procedure. During the clinical observation period, the symptoms decreased, and after approximately 3 months, the paralysis had disappeared completely. This temporary neurological feature was probably caused by perioperative ischemia of spinal marrow by occlusion of the Adamkiewicz artery. This arteria radicularis magna supplies part of the spinal marrow and was probably occluded by the aortic balloon catheter.

One patient (patient 20) developed liver abscesses 2 weeks after IHHP. He underwent the perfusion with 65% leakage and developed grade 3 hematological and hepatic toxicity. Then a period with fever occurred, and CT scan demonstrated multiple abscesses in the liver. These abscesses were located at the former sites of the colorectal metastases. Moreover, an abscess was apparent at the blind end of the rectum as a result of the Hartmann procedure performed for his primary tumour several months before. This was the possible focus for the bacteremia causing the infected necrotic masses in the liver. After 2 months with multiple percutaneous draining periods of the liver abscesses and antibiotic treatment, he finally developed aspiration pneumonia and died of respiratory failure.

One patient died within 30 days of the operation, resulting in a mortality during this phase I and II study of 5%. This patient (patient 12) presented with ocular melanoma metastases and had an uneventful IHHP with only 5% leakage. After surgery, she developed severe dyspnea and grade 4 hepatic toxicity. Mechanical ventilation was indicated because of respiratory failure. Hepatic dysfunction increased rapidly, and 8 days after surgery, the patient died. Autopsy results showed pneumonia. There were no signs of pulmonary (thrombus or tumour) embolism. An ischemic liver was found with almost total necrosis of the melanoma metastases. Surprisingly, almost 70% of the liver was replaced by tumour, although a CT scan 4 weeks before perfusion showed an estimated tumour involvement of < 40%. We assume that the metastases must have grown very rapidly in the last weeks before IHHP and the remnant of normal liver tissue was not enough to survive the hepatic toxicity caused by the IHHP.

Tumour response and patient survival

Stable disease was demonstrated in 81% (13 of 16) of assessable patients after 6 to 8 weeks (*Table 1*). Patients 12 and 16 were not assessable with respect to tumour response. In 12% of patients (2 of 16), a PR was seen. Two patients (12%) developed progressive disease after IHPP. In both patients who had a PR, CEA levels decreased to normal (< 5 µg/ml) levels. CEA levels decreased in at least eight of the stable disease patients, but none had reached normal levels. Progressive disease at the liver occurred with a mean interval after IHHP of 4.8 months (range, 3 - 13 months). Seven patients developed systemic metastases. Five developed pulmonary metastases 3 to 7 months after IHHP. One patient had metastatic lesions in the sacral bone at the same time of liver metastasis progression at 4 months after perfusion. In one patient, peritoneal carcinomatosis was detected 5 months after

IHHP. In one patient, a local recurrence at the rectum was detected. Median patient survival was 11.1 months (range, 0 - 32 months).

DISCUSSION

In the last decade, isolated liver perfusions have been performed by a few centers worldwide, and the antitumour effect showed promising response rates (up to 75%) and a potentially prolonged mean survival of 16 to 24 months.[17,20] The major drawbacks of the technique are the magnitude, the costs, and the nonrepeatability of the surgical procedure. In the open procedure, the entire liver has to be mobilized, and all lumbar veins have to be ligated to guarantee a leakage-free perfusion. Classic SIHP also uses a venovenous bypass and a heart-lung machine, which is a time-consuming procedure that necessitates a specialized perfusion team. The mean SIHP operation time is 8.6 hours, with a mean packed RBC transfusion of 5.7 units. The main goal of our study was to develop a less invasive, less costly, and potentially repeatable percutaneous technique that would allow safe perfusion in a much shorter time and that could be repeated and performed without a heart-lung machine or venovenous bypass. Moreover, IHHP makes use of hypoxia, which renders tumour cells more sensitive to cytostatic agents in general and which particularly enhances the antitumour effects of drugs such as melphalan.[28,29] In pigs we previously demonstrated a leakage-free IHHP technique with an open double-balloon catheter in the caval vein.[25] The same technique in patients resulted in major leakage in this study. This occurred despite positioning the two balloons directly above and below the orifices of the hepatic veins and trying to occlude the lumbar veins. By replacing the open double-balloon catheter by a triple-balloon occlusion catheter, which should occlude the adrenal vein and all lumbar veins, but also the hepatic veins, more successful hepatic perfusion could be performed with inflow via the hepatic artery and outflow via the portal vein. The mean operating room time was reduced to 3 hours, and the mean transfusion was one RBC unit.

The mean leakage decreased from 56% in the orthograde IHHP to 35% in the retrograde setting. We anticipated a decrease in leakage along the learning curve, but unless increasing experience and technical modifications occur with IHHP, it is still not possible to perform it leakage free in this setting. We assume that diaphragmatic, lumbar, and adrenal veins are the main cause for the type of leakage we observed. Veins around the common bile duct in the hepatic ligament could also be a potential cause, but temporary ligation of the ligament during perfusion was performed routinely, because we started with the retrograde perfusion, and leakage remained. Leakage started directly after the start of the perfusion and remained at a constant level during the procedure. Because of this persisting leakage problem and subsequent dose-limiting systemic toxicity, we were not able to escalate to higher melphalan dosages. Higher local melphalan concentrations seem to be a prerequisite for improving tumour responses. Vahrmeijer *et al*.[20] reported a correlation between

high-dose melphalan SIHP (3 mg/kg) for colorectal liver metastases and patient survival.

Leakage-free perfusion is of major importance for the potential application of TNF-α, a cytokine with significant antitumour effects at high concentrations. The adequate concentration for TNF to induce its synergistic antitumour effect is too high for intravenous administration. The use of TNF has led to excellent clinical responses after isolated limb perfusion with melphalan and TNF for irresectable soft tissue sarcomas and melanomas.[30,31] In isolated limb perfusion, TNF proved to be very effective and safe. These perfusions are performed with minimal systemic leakage of 0% to 10%.[30,31] Whether TNF contributes to therapeutic efficacy in IHP remains unclear. We recently demonstrated in a preclinical rat liver metastasis model that increased intratumoural melphalan uptake is strongly correlated with the microvessel density of the tumour.[32] Only hypervascularized tumours showed improved melphalan uptake in tumour tissue and synergistic antitumour effects after IHP with melphalan and TNF. Because colon carcinoma metastases are hypovascular, IHP with melphalan alone might be just as effective as it is combined with TNF. This was demonstrated in our colorectal liver metastasis model, which showed no increased intratumoural melphalan concentrations and a lack of therapeutic efficacy compared with IHP with melphalan alone. Results from the National Cancer Institute showed the same duration of response after SIHP for colorectal metastases with or without TNF.[18,33,34] However, in patients with highly vascularized ocular melanoma metastases, the addition of TNF in SIHP yielded to a prolonged response compared with SIHP with melphalan alone: 14 *vs*. 6 months, respectively.[35] These clinical results seem to confirm the hypothesis about the indication for the utility of TNF in IHP. Because the minimally invasive IHHP methodology we report here is not without leakage, the addition of TNF in this setting seems impossible.

Savier *et al*.[36] recently reported a phase I study with four patients repeatedly treated by 10 courses of melphalan-based SIHP and percutaneous IHP. At percutaneous IHP, the hepatic artery was used for inflow of the perfusate, and an open double-caval balloon catheter was used for outflow. The portal vein was occluded by a percutaneous balloon catheter to complete isolation. This group was also confronted with major leakage starting as soon as the perfusion commenced. This was measured after surgery by systemic melphalan levels. Severe (grade 3 - 4) systemic toxicity (hematological) was observed after perfusion in this study. Because of lower melphalan doses varying from 15 to 45 mg, no severe hepatic toxicity was observed. Others have used percutaneous IHP techniques combined with extracorporal charcoal hemoperfusion filters for the venous effluent, predominantly in patients with hepatocellular carcinoma.[37-39] This technique is completely different from the balloon catheter technique described in our study, but results look promising, although toxicity is also significant. Systemic toxicity not only might be related to the surgical technique or catheter, but also might be influenced by the drug used. Our study with 18 consecutive patients in this phase I and II study showed advantages compared with SIHP regarding magnitude and operating time, although morbidity and mortality

were still significant. Despite technical modifications and increasing experience, leakage was still observed, and regional and systemic toxicity remained.

With respect to regional toxicity, we emphasize that at least 50% of functional liver tissue should be present. Especially in fast-growing melanoma metastases, liver imaging and laboratory investigation should be performed shortly before the operation. The only fatal complication occurred after a technically uncomplicated IHHP with less leakage but was due to hepatic failure, on the basis of massive liver replacement by fast-growing metastases.

Systemic toxicity is directly correlated with leakage of cytostatic agents during perfusion and an effective washout procedure. Vahrmeijer *et al*.[20] could escalate up to 3 mg/kg of melphalan safely provided that leakage was minimal. In our series, leakage < 20% prevented leucopenia almost completely. In a leakage-free perfusion setting, hepatic toxicity will be the dose-limiting factor.

In conclusion, balloon catheter–mediated IHHP failed because no good leakage control was achieved by either the orthograde or the retrograde method. We therefore have abandoned this program. Instead we have used the experience to develop a surgically much simplified method to perform a retrograde IHHP with fully controlled leakage and, thus, improved local drug concentrations; improved washout at the end of the perfusion; and much improved toxicity and response rates. Finally, we emphasize that systemic or locoregional maintenance therapy after IHHP also has to be considered to control the liver metastases. Continuing locoregional treatment by HAI after an IHP procedure is technically feasible and seems to prolong the duration of the response and survival.[18]

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Chapter 7

BALLOON CATHETER HYPOXIC ABDOMINAL PERFUSION WITH MITOMYCIN C AND MELPHALAN FOR LOCALLY ADVANCED PANCREATIC CANCER: A PHASE I-II TRIAL

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SUMMARY

Background
Developments in balloon catheter methodology have made hypoxic abdominal perfusion (HAP) with anti-tumour agents possible with only minimal invasive surgery. The initial reports on this modality and celiac axis stop-flow infusion for treatment of pancreatic cancer were very promising in terms of tumour response, median survival and pain reduction. Recent reports, however, have not been able to confirm these results and some have disputed the efficacy of these currently still applied treatment modalities.

Methods
Twenty-one patients with advanced pancreatic carcinoma were included in a phase I–II trial of HAP with MMC and melphalan followed by celiac axis infusion (CAI) with the same agents six weeks later. Tumour response was assessed by abdominal-CT and by determining tumour markers. Effect on pain reduction was assessed by evaluation of pain registration forms.

Results
HAP resulted in augmented regional drug concentrations. One patient died after CAI due to acute mesenterial ischaemia. One agent-toxicity related death was observed in the phase-I study. Significant hematological toxicity was observed after HAP and CAI at MTD. No patients were considered resectable after treatment. Median survival after HAP was 6 months (range 1 - 29). Pain reduction was experienced by only 5/18 patients and was short-lived.

Conclusion
In contrast to earlier reports HAP and CAI with MMC and melphalan did not demonstrate any benefit in terms of tumour response, median survival and pain reduction, compared to less invasive treatment options. As this treatment was associated with significant toxic side-effects and even one procedure related death, we do not consider this a therapeutic option in patients with advanced pancreatic cancer.

INTRODUCTION

Locally advanced pancreatic carcinoma remains associated with a dismal prognosis.[1,2] Although survival rates have improved somewhat over the last 20 years the relative 5-year survival today is only 5%. The current treatment for pancreatic carcinoma consists of a resection. However, only 10 - 20% of patients with pancreatic cancer have disease suitable for resection by the time the diagnosis is made. About 50% of these patients turn out to be non-resectable upon laparotomy. The median survival of patients after surgical treatment with curative intent is about 12 - 17 months. Patients with palliative surgery have an average survival of 6 months.[1–7] Despite this grim outcome, considerable progress has been made in the reduction of surgical morbidity and mortality and in increase in overall survival with the use of combined treatment modalities.[2,8–11]

Several authors have examined the patterns of failure after curative resection of the pancreas. Griffin *et al*. reported metastatic disease outside of the abdomen occurred as a component of failure in only 27% of patients,[12] and patients with recurrence after resection, all demonstrated intraabdominal disease. The local resection bed, liver and peritoneal surfaces accounted for 73, 63 and 42%, respectively, of these recurrences. Another study of 558 autopsies of patients with pancreatic cancer showed that in 46% of cases disease was confined to the peritoneal cavity.[13] New treatment modalities impacting upon these failure patterns should, therefore, be directed at the tumour bed and area of resection as well as the whole abdominal cavity. Hypoxic abdominal perfusion (HAP) combined with a selective stop-flow infusion via the celiac axis (CAI) as a second procedure has been designed to cover just these two elements of treatment design. Apart from its potential as adjuvant therapy, this therapeutic approach may of value as neo-adjuvant therapy. Patients with locally advanced pancreatic malignancy may benefit from regional chemotherapy to such an extent that secondary resection of the tumour may become possible. In cases of clearly non-resectable tumours these modalities could be applied in a palliative setting.

Over a decade ago an innovative method for obtaining relative vascular isolation of abdomen or pelvis with minimal invasive surgery was introduced by Aigner.[14–17] In several studies by different groups HAP using this balloon catheter technology was used to treat a number of malignant conditions not treatable by conventional means. These have included pelvic recurrences of colorectal carcinoma, advanced primary gastric and pancreatic carcinomas and locally extensive ovarian malignancies.[18–22] Results of the initial experiences with HAP and CAI using mitomycin C (MMC) in locally advanced pancreatic cancer reported were promising: more than 50% response rates were reported, with increased median survival. Moreover, impressive histological response rates of 92% were reported and pain relief observed in 85% of patients.[23,24]

Reports on effectiveness of hypoxic perfusion with MMC regarding tumour responses have, however, not been conclusive and some reports question the effectiveness of these methodologies.[25–27] The conflicting results may be due to the size and (tumour-) characteristics of the study groups, as well as due to the fact that different definitions and parameters for tumour responses were used.[27] Additionally, some have ascribed these different results to misuse of the methodology.[28]

If conclusions are to be made regarding the feasibility of these currently still applied modalities for treatment of advanced pancreatic cancer, these should be based on controlled clinical trials, using internationally accepted definitions of tumour response. In the current phase I–II trial regarding HAP and CAI with MMC and melphalan, we validated the methodology in our hands by determining the distribution of perfused agents over the regional and systemic compartments during and after HAP. We assessed procedure and agents associated toxicity and the efficacy of the procedure regarding tumour response, median survival and pain reduction in patients with advanced pancreatic cancer.

MATERIALS AND METHODS

Patients and methods

In a phase I–II trial, 21 patients with histologically proven locally advanced carcinoma of the pancreas were included. In the phase-II study patients with distant metastases were excluded. Hepatic-duodenal ligament metastases were not an exclusion criterion. In order to be amenable for inclusion, age had to be between 18 and 75 years and the Karnofsky performance status had to be above 80%. Patients with significant cardiovascular disease (New York Heart Association Class II/III/IV), insufficient renal function, or pregnancy were excluded. Further criteria were a leukocyte count > 2.5 10.9, no haemorrhage or active bleeding disorder, and no infections not controlled by antibiotics. Patients were not to have liver disease, although in the phase-I study patients with hepatic metastases were included. Patients were not to have a concurrent malignancy-determining prognosis. Patients having received radiation within 3 months prior to HAP, or chemotherapy within 6 weeks prior to entry into the study were excluded. The study was conducted in full accordance with the principles of the 'Declaration of Helsinki'. All patients were submitted to informed consent.

Treatment schedule

Patients underwent HAP and after a minimal interval of 6 weeks a celiac axis stop-flow infusion (CAI) using Seldinger techniques, providing encountered toxicity and clinical condition allowed for such a second procedure, and providing there was no evidence of progressive disease. If clinical condition allowed patients were evaluated by abdominal-CT approximately 6 weeks after HAP and CAI, in order to assess if a ('2nd look') laparotomy to assess resectability was feasible.

Diagnostics

Before, and approximately six weeks after HAP and CAI, an abdominal-CT and chest X-ray were performed. In the phase-II study a diagnostic laparoscopy was performed in all patients to exclude those with peritoneal metastases. In the last 14 patients of the phase-II study tumour markers (CA 19–9 and CEA) were assessed prior to and approximately 6 weeks after HAP.

Toxicity and response

Therapy-induced toxicity was graded according to the standard criteria of the National Cancer Institute Common Toxicity Grading System (NCICTGS). Tumour response was assessed by CT-scan strictly according to WHO-criteria:[29] Complete remission (CR) requires disappearance of any tumour evidence; partial remission (PR) is a decrease in product of the two greatest diameters of the tumour of 50%; No Change (NC) is defined as an increase of less than 25% or a decrease of less than 50%. Progressive disease (PC) is defined as an increase in tumour diameter of more than 25%. Appearance of new lesions also constitutes progressive disease.

HAP procedure

Fig. 1 shows the methodology of HAP with balloon catheters. It illustrates the position of the occlusion catheters in aorta and caval vein and other components of the perfusion circuit. Perfusion sets were provided by PfM, GmbH (Cologne, Germany) and consisted of an aorta occlusion balloon catheter and a caval vein occlusion balloon catheter (12F-600 mm, balloon capacity 30 ml) and a tubing set with a volume of 220 ml containing a bubble trap. In the perfusion circuit a roller pump maintained flow, while drugs were infused through a sideline into the perfusion circuit.

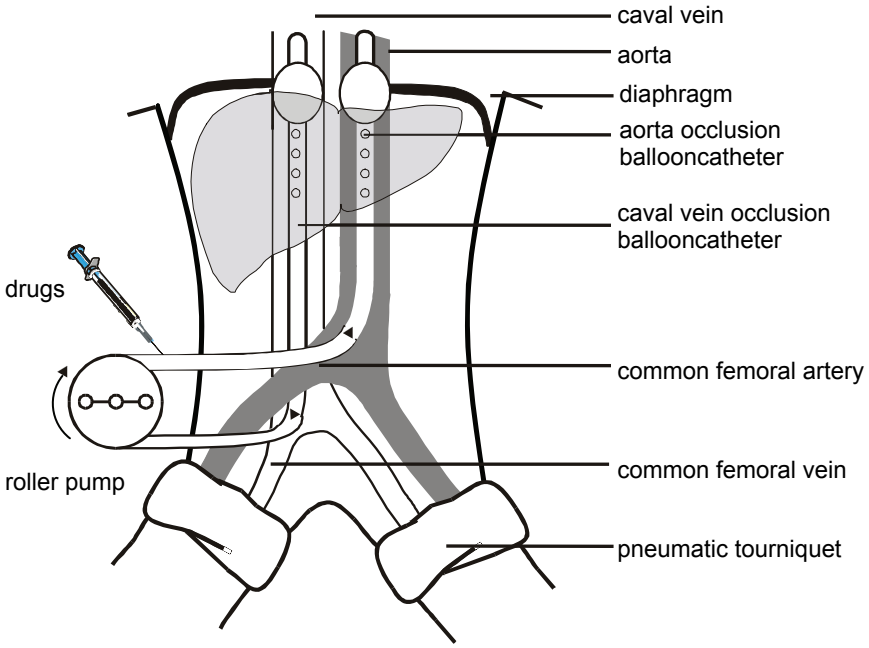

Figure 1 *Represents a schematic representation of hypoxic abdominal perfusion (HAP). Balloons were positioned at the level of the diaphragm and the lower extremities were excluded from circulation by pneumatic tourniquets.*

HAP was carried out as follows: patients were brought under general anaesthesia. Details described by us elsewhere.[30] After formal surgical exposure of the femoral vessels, patients were administered 5000 IU Heparin as a bolus injection. Arteriotomy and venotomy were performed, where after the aorta-occlusion catheter and the caval vein occlusion catheters were introduced in the common femoral vessels and retro-gradely moved up into the aorta and caval vein. Balloons were positioned in the aorta, above the celiac trunk and in the caval vein at the level of the diaphragm, above the level of the hepatic veins. Correct position was radiologically confirmed. Temporary relative isolation of the abdominal vascular bed was achieved by inflating the balloons and pneumatic tourniquets around both upper thighs of the lower extremities. In order to compensate for the decrease in cardiac preload the aorta occlusion balloon was inflated prior to inflating the caval balloon. After connecting the catheters to the perfusion system primed with 220 ml Haemaccel (Behring Pharma, Amsterdam, The Netherlands) the perfusate was circulated by means of a roller pump with a constant flow of 250 ml/min. When stable perfusion was ensured, drugs were slowly administered into the perfusion circuit during 10 min. Twenty minutes after start of perfusion, isolation was terminated by subsequently deflating caval and aortic balloons. After 10 min of hemodynamic stabilization the tourniquets of the lower extremities were sequentially released.

CAI procedure

The procedure was performed under local anaesthesia. A 7F side winder balloon catheter was retrogradely introduced into the femoral artery using standard Seldinger techniques. Patients were heparinized with 5000 IU. Under radiological guidance the celiac axis was cannulated, where after the occlusion balloon was inflated. Drugs were dissolved in 500 ml Saline and infused during 10 min, where after the balloon was kept inflated for an additional 10 min, before terminating occlusion.

Blood sampling and melphalan and MMC bio-analysis

Baseline blood samples were obtained before administration of the drugs into the perfusion circuit. Isolation was terminated 20 min after start of infusion of the agents. Throughout and after the procedure blood samples were simultaneously drawn from the abdominal blood compartments through a sampling port on the venous balloon catheter and from the systemic blood compartment through a central venous catheter at 5, 10, 15, 20, 25, 45 and 75 min after infusion of the agents. Blood samples were collected in glass tubes containing EDTA and immediately stored in the dark on ice. Samples were centrifuged at 2600 RPM for 6 min at 4 °C, where after the obtained plasma was stored at -70 °C until analysis.

Melphalan in plasma was measured by gas chromatography-mass spectrometry. MMC was analysed by high performance liquid chromatography with ultraviolet detection (HPLC-UV). Details of these assays have been described by us elsewhere.[31,32]

RESULTS

Patient and tumour characteristics

A total of 21 patients were included in the phase I-II study. The male/female ratio was 12/9. The mean age was 59 years (range 49 - 67). In 18 patients the malignancy was located in the head of the pancreas, in three patients in the corpus/tail region. Two patients had liver metastasis at time of inclusion. Both were included in the phase-I study only. Four patients were known to have metastases in the hepatic-duodenal ligament at time of inclusion. Prior to inclusion in the trial, a laparotomy without further surgery had been performed in seven patients. Six patients had undergone a surgical bypass procedure. A celiac plexus blockade had been performed in three patients.

Procedure related complications

All patients survived the procedures. The procedure was accompanied by severe hemo-dynamic changes making invasive cardiovascular monitoring necessary.[30] In two procedures, there was preliminary balloon rupture, ending the relative isolation. In both cases balloon rupture occurred within the last 5 min of perfusion. As could be assessed from the individual pharmacokinetic profiles this did not effect the distribution of the agents over the compartments as agent concentrations in both compartments had reached equilibrium prior to rupture (data not shown). In one patient postoperative haemorrhage occurred after HAP at the catheter insertion site in the groin, necessitating surgical re-intervention. One patient developed a dehiscent wound in the groin, which was treated conservatively. One patient demonstrated symptoms of transient cerebral ischaemia during the CAI procedure. One patient died as a result of an acute mesenterial ischaemia four days after CAI. At autopsy severe athero-scleroses was found.

MMC and melphalan concentrations in perfusate and systemic circulation

Fig. 2 demonstrates that HAP resulted in significantly higher loco-regional concentrations of MMC and melphalan compared to concomitant systemic concentrations. However, as soon as 5 min after starting perfusion systemic leakage was evident. After infusion of the drug over 10 min MMC and melphalan plasma concentrations rapidly declined, with systemic and loco-regional plasma levels reaching equilibrium of concentrations after approximately 15 – 20 min.

HAP related toxicity

In the phase-I study patient groups were perfused and infused with 10 mg/m^2 melphalan and subjected to dose-escalation of MMC (10/15/20 mg/m^2). Three patients were treated with 10 mg/m^2 melphalan and 10 mg/m^2 MMC. In one patient a transient grade 3 neutropenia was observed (*Table 1*). Three patients were treated with 10 mg/m^2 melphalan and 15 mg/m^2 MMC, whereafter four patients were perfused at the highest dose MMC (20 mg/m^2). Of these four patients one developed severe nausea, and subsequent diarrhea lasting for 3 weeks, necessitating parenteral nutrition (*Table 1*). Two patients developed a transient grade 4 neutropenia. One of

these patients died three weeks after HAP having acute liver failure and occlusion of here billiary stent due to tumour growth. Permission for autopsy was not obtained. A possible explanation for her liver failure may have been her severe obesities. As the dose of drugs was indirectly calculated from body weight, the dose may have been relatively high for the perfused organs. One patient developed a liver abscess requiring drainage six weeks after perfusion. Because of encountered toxicity at this dose the maximum tolerated dose (M.T.D.) was assessed as 15 mg/m^2 MMC and 10 mg/m^2 melphalan. In total 14 patients were perfused with the M.T.D. In four cases patients developed a transient grade 4 neutropenia. Two patients had a transient grade two neutropenia. In two patients a mild transient hepatic toxicity was observed (*Table 1*).

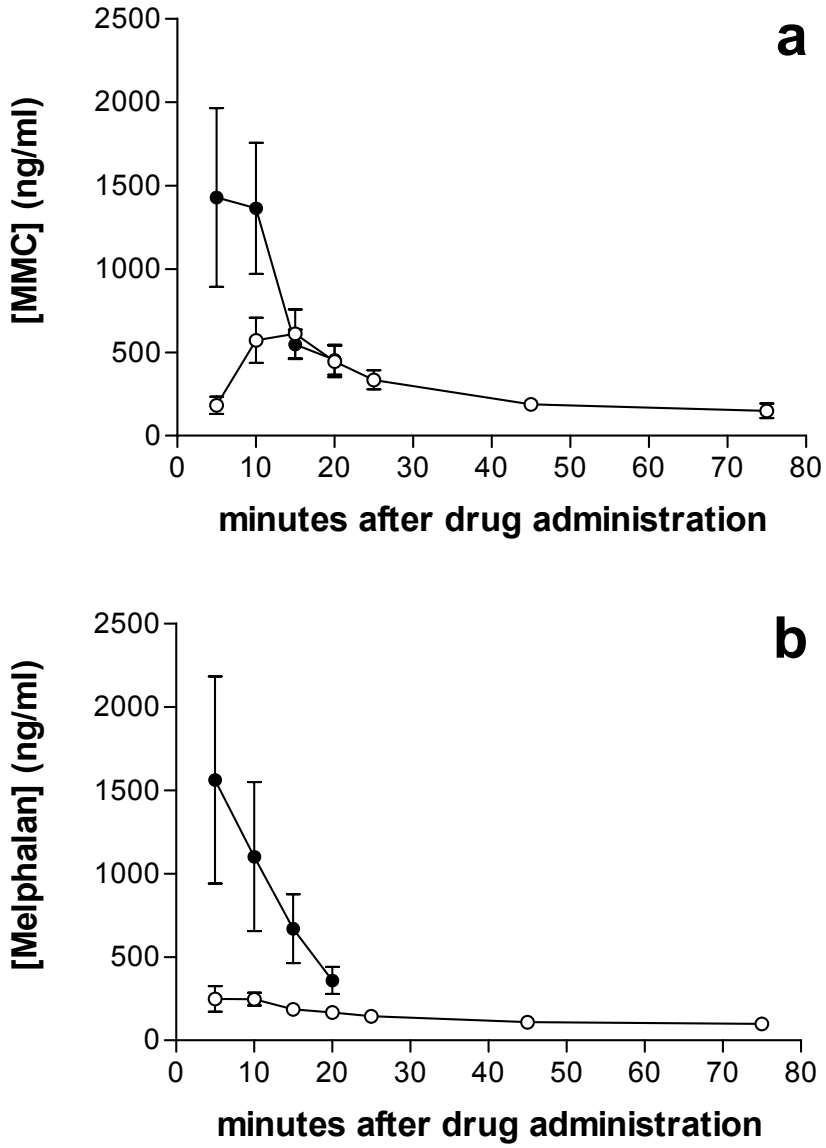

Figure 2 *Regional (black circles) and systemic (white circles) plasma concentrations of MMC (a) and melphalan (b) throughout and after 20 min of hypoxic abdominal perfusion. Data shown represent eight consecutive patients perfused with 15 mg/m^2 MMC and 10 mg/m^2 melphalan. Mean plasma concentrations ± SEM are shown.*

Table 1 Toxicity of HAP according to NCI-CTG criteria

MMC + melphalan (mg/m^2)	Pts (n)	Haematogical	Renal	Hepatic	GI	Other
10 + 10	3	1 (Gr 3)	-	-	-	-
15 + 10	14	6 (Gr 2,2,2,2,4,4)	-	2 (Gr 1,1)	-	-
20 + 10	4	2 (Gr 4,4)	-	1 (Gr 5)	3 (Gr 2,3,4)	Liver abcess

For each dosage group the total number of patients demonstrating toxicity for each NCICTG-category is shown. Individual grades of toxicity are given between brackets.

Table 2 Toxicity of CAI according to NCI-CTG criteria

MMC + melphalan (mg/m^2)	Pts (n)	Haematogical	Renal	Hepatic	GI	Alopecia
10 + 10	1	-	-	-	-	-
15 + 10	6	2 (Gr 2,4)	-	-	1 (Gr 5)	1 (Gr 2)
20 + 10	2	1 (Gr 4)	-	-	-	

For each dosage group the total number of patients demonstrating toxicity for each NCICTG-category is shown. Individual grades of toxicity are given between brackets.

CAI related toxicity

Of the 21 patients that underwent HAP, nine patients underwent CAI. Reasons for not performing CAI were clinical or radiological progression of disease and withdrawal from the study by patients. Mean time interval between HAP and celiac axis infusion (CAI) was 7.8 weeks (range 5 – 12).

Of the six patients treated with 10 mg/m^2 melphalan and 15 mg/m^2 MMC two demonstrated a grade 2 and 4 neutropenia (*Table 2*). As mentioned before, one patient was re-administered into hospital 4 days after CAI, who died of acute mesenterial ischaemia, as was confirmed at autopsy. Considering the severe athero-sclerosis found in this patient, this seems more likely a complication associated with the CAI-procedure itself, than with the administered agents. One patient developed a grade 2 allopecia. One of two patients who were infused with 10 mg/m^2 melphalan and 20 mg/m^2 MMC demonstrated a grade 4 neutropenia.

Tumour response and survival

In total 18 patients were included in the phase-II part of the study. The four patients perfused with the highest dose MMC (20 mg/m^2) were included in these results. Sixteen patients were evaluated for tumour response by an abdominal-CT, approximately 6 weeks after undergoing HAP. Two patients could not be evaluated due to clinical progression of the disease and their withdrawal from the study. In three patients (17%) there was radiological progression of local disease. Twelve patients (67%) demonstrated stable disease of the primary tumour. However, five of these radiological stable patients clearly demonstrated clinically progressive disease

or had developed distant metastases and did not undergo CAI. One patient demonstrated stable disease for 24 months. A 2nd look laparotomy was performed, which revealed hepatic metastases. A partial response was observed in one patient (5%). Disappointingly, at laparotomy a locally un-resectable tumour was found. The overall median survival after HAP was 6 months (range 1 – 29 months).

Tumour markers

Tumour markers were assessed in the last 14 consecutive patients of the phase-II study, before and approximately six weeks after HAP. As *Table 3* shows, an evident CA 19-9 reduction (> 20%) was observed in four patients. However, radiological PR was observed in only one of these patients. One had radiological and clinical stable disease. The remaining two patients had radiological stable disease, but clearly clinical disease progression. In four patients there was an evident reduction (> 20%) in CEA after HAP. One of these patients had a radiological confirmed PR without signs of clinical progression. Two patients had radiological SD with one patient demonstrating clinical progression. One patient demonstrated clinical and radiological progression of disease.

Pain response

Pain response after HAP and CAI was determined by investigating the pain score of the patient as filled out in a patient pain diary and by investigating the subscribed pain medication. Of the 18 patients in the phase-II study four did not experience pain prior to HAP. Nine patients did not experience any reduction in pain after HAP or CAI. Five patients experienced a reduction in pain during and after treatment. However, in all five patients this reduction was short-lived and within weeks pain was present at the pre-treatment level.

Table 3 Tumour marker response 6 weeks after HAP. Individual radiological and clinical responses are also shown for each patient

Patient no	CA 19-9 pre-HAP	CA 19-9 post-HAP	Ratio	CEA pre-HAP	CEA post-HAP	Ratio	Radiological response	Clinical response
8	162	32	0.19	6.80	5.10	0.75	sd	sd
9	3300	6105	1.85	28.7	50.2	1.74	pd	pd
10	2600	nd		13.9	Nd	-	pd	pd
11	413	329	0.79	2.51	3.20	1.27	sd	pd
12	262	1550	5.9	1.83	3.32	1.81	sd	pd
13	970	950	0.97	1.65	1.32	0.80	pd	pd
14	1550	nd	-	2.64	Nd	-	nd	pd
15	87	nd	-	6.90	8.44	1.22	sd	sd
16	5600	5150	0.91	23.57	31.94	1.35	sd	pd
17	160	144	0.9	5.13	4.85	0.94	sd	sd
18	460	700	1.52	3.36	2.54	0.75	sd	pd
19	1445	795	0.55	4.16	3.61	0.86	sd	pd
20	18,000	nd	-	30.40	33.5	1.10	pd	pd
21	103	54	0.52	2.47	1.09	0.44	pr	sd

DISCUSSION

The design of (neo-) adjuvant therapies for pancreatic cancer of the exocrine pancreas is made difficult by the relatively low sensitivity of this cancer to intravenous therapy. Response rates of only 20% and a median survival of 6 months have been reported for the most frequently used agent 5-FU in patients with locally advanced disease.[33] Better response rates have been be achieved with intravenously administered Gemcitabin, but these promising initial results have yet to be confirmed by larger controlled studies.[34]

For most chemotherapeutic agents steep dose-response curves can be demonstrated. Therefore, high drug concentrations are important for eradicating both sensitive and resistant tumour cells.[35–37] This is all the more the case in pancreatic cancer where the multi-drug resistance gene causes an increased efflux of drugs out of tumour cells.[33]

Theoretically, regional perfusion of the abdomen increases tumour exposure to the anti-tumour agents, whilst limiting systemic toxicity. Furthermore, the relative vascular isolation allows modulation of oxygen pressure and temperature of the perfusate. Drugs whose cytotoxic action are particularly potentiated by hypoxia, such as MMC,[38,39] have been reported to be particularly effective when administered by regional hypoxic perfusion.[20,22] Hypoxia causes dividing cells to halt their progression through the cell cycle, by allowing them to progress to and then remain in the G1-like susceptibility state.[39] Melphalan is an anti-tumour agent which is frequently used in regional perfusion settings[40-42] and which has demonstrated impressive results in isolated limb perfusion for soft tissue sarcoma.[43]

If regional perfusion with anti-tumour agents is to be applied on a wide scale, magnitude and morbidity of the surgical intervention must be limited, while the methodology should have good response rates. Complete vascular isolation of the abdominal vascular beds is difficult to achieve. Although it has been demonstrated that an almost complete isolation of the abdomen can be achieved after ligation of all non-gastro-intestinal branches of the abdominal aorta and caval vein,[44] such an approach is clearly not feasible in a mainly palliative treatment setting. Developments in balloon catheter methodology in recent years have made it possible to perform HAP with minimally invasive surgery.[22,23]

In this and previous studies,[45] we demonstrated that HAP using described balloon catheter methodology results in markedly augmented regional plasma concentrations of MMC and melphalan. Although significant systemic leakage occurred, the rapid loco-regional decline in MMC and melphalan concentrations should also be attributed to local tissue uptake, as in the leakage free isolated hepatic perfusion setting a similar decline in regional MMC and melphalan levels was observed.[46] This tissue uptake holds true not only for the parent drugs, but also for possible toxic metabolites and degradation products.[32] Loco regional concentration advantages of

perfused agents were also reported in other studies.[25,44,47,48] We have to stress that we sampled the perfusate from the venous balloon catheter and the systemic samples from a central venous line. Had the samples been taken from the arterial balloon catheter and an arterial line the concentration advantages would be far more favorable. The concentrations in the perfusate measured in the first minutes of the perfusion would reflect the initial concentration of the drugs in the carrier solution before the first-pass concentration decline due to tissue-clearance. Measured systemic concentrations would be lower when samples would have been drawn from an arterial line due to clearance by lung tissue. This explains the differences in absolute regional drug concentrations and regional *vs* systemic drug concentration ratio's when comparing with other studies.[24,25] To our opinion our method demonstrates more clearly the pharmacokinetic advantage of HAP over arterial infusion as the venous concentrations reflects the quantity of drugs to be locally reperfused instead of systemically distributed as is the case in arterial infusion.

The HAP and CAI procedures themselves were well tolerated. The hemodynamic changes during and after HAP due to simultaneous occlusion of aorta and vena cava were severe but transient.[30] Although some have postulated that with the development of balloon catheter methodology HAP could in future be performed percutaneously under local anaesthesia,[22] the hemodynamic effects associated with the procedure necessitate invasive cardiovascular monitoring.[30]

Apart from the case of acute mesenterial ischaemia causing death that occurred four days after CAI in a patient with severe arterioscleroses no major complications related to the procedures occurred. Severe hepatic and gastro-intestinal toxicity was encountered in four patients perfused with higher than MTD dosages in the dose-escalation phase possibly resulting in one treatment related death. In patients perfused with the M.T.D. of MMC and melphalan, almost 50% experienced haematological toxicity, which was mainly determined by (severe) transient neutropenias. At this dose no detrimental effect was observed on hepatic, renal and gut function, which suggests that the HAP-procedure itself does not cause any transient or permanent organ dysfunction.

Despite the fact that highly advantageous loco-regional drug concentrations were established, the outcome of this trial with respect to tumour response and pain response was disappointing. No patients were considered having respectable tumours after the loco regional induction chemotherapy. There was no major effect in pain reduction and a median survival of 6 months does not show any benefit in survival compared to less invasive therapy regimens in patients with locally advanced pancreatic cancer.[49,50]

Several authors have reported excellent clinical results following regional chemotherapy for advanced pancreatic carcinoma.[23,24,51] Aigner and Fiorentini reported response rates of up to 50% and median survival rates of 9.8 and 12 months (responders), respectively, in patients treated by HAP and CAI with MMC.

Disappointingly, these results were not confirmed by our trial and two other prospective phase I-II trials.[25,26] In these two trials of 17 patients each, Lorenz and Gebauer reported tumour responses of 0 and 18%, respectively, and median survivals of 4.2 and 4.5 months after HAP with MMC. The procedures were associated with severe, mainly gastro-intestinal, side-effects.

Discrepancies in efficacy between different studies on regional chemotherapy may be due to the different methods of assessing tumour response. Reports where tumour marker response was used as criterion had far better results regarding tumour response.[27] In our study, we observed that tumour marker response did in most cases not predict the course of the disease and progression of disease occurred although there was an observed tumour marker response.

Although HAP with MMC and melphalan resulted in disappointing tumour responses, whilst demonstrating high toxicity and morbidity, the rate of side-effects associated with the procedure itself was acceptable. The death, which occurred after CAI in a patient with severe athero-scleroses, stresses the fact that this patient category should be carefully evaluated before undergoing this treatment.[52] The application potential of other drugs using these regional perfusion techniques should be investigated. In earlier animal studies, we demonstrated that the advantage in terms of regional plasma concentration of TNF was insufficient for TNF-mediated effects to occur.[45] Due to the intravascular localization and very low tissue uptake of this highly toxic drug, systemic leakage was significant. In contrast Gemcitabin, which has demonstrated efficacy against pancreas carcinoma[53] exhibits a high total body clearance which may make this agent highly suitable for regional perfusion.[35] Ongoing trials may prove regional perfusion with this agent effective in combination with radiotherapy.[54]

In conclusion, HAP using described balloon catheter technique followed by CAI resulted in significant loco-regional plasma concentration advantages of MMC and melphalan. This higher tissue exposure did, however, not lead to enhanced tumour responses. No tumours were considered resectable after treatment and no beneficial effect on pain and survival was observed compared to less invasive therapeutic alternatives. These results are in line with other phase I-II studies where no beneficial effect of this treatment was found. If one takes into account the invasiveness of the procedure, length of hospital stay, morbidity, and even mortality which was associated with some procedures, HAP and CAI with MMC and melphalan does not seem a therapeutic option in patients with locally advanced pancreatic cancer.

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Chapter 8

BALLOON CATHETER HYPOXIC PELVIC PERFUSION WITH MITOMYCIN C AND MELPHALAN FOR LOCALLY ADVANCED TUMOURS IN THE PELVIC REGION: A PHASE I-II TRIAL

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ABSTRACT

Aims
To investigate the feasibility of hypoxic pelvic perfusion (HPP), using balloon catheter techniques as treatment modality for locally advanced pelvic malignancies.

Methods
In a phase I-II study, 16 patients with various non-resectable pelvic tumours were treated with two HPP with MMC and melphalan, followed by radiotherapy (25 Gy) and surgical resection if feasible. Toxicity and procedure related complications were documented. Tumour responses were assessed by MRI or CT. Pain reductive effects were assessed by evaluation of pain registration forms.

Results
HPP resulted in augmented regional drug concentrations with relatively low systemic levels. Some severe systemic toxicity was observed. One procedure related death occurred. Pain reduction effects were short-lived. Ten patients had radiological NC, two PD and one PR. In 11 patients surgical resection was performed, which was microscopically radical in six cases. Mean survival was 26.8 months (range 1 - 86).

Conclusion
The seemingly favorable pharmacokinetic profiles observed with HPP in this and other studies can still lead to severe systemic toxicity. In terms of survival, local (re-)recurrence and pain reduction there seems no benefit of addition of HPP to pre-operative radiotherapy. HPP with MMC and melphalan, does not seem a therapeutic option in patients with locally advanced pelvic tumours.

INTRODUCTION

Loco-regional recurrence after low anterior or abdomino-perineal resection for rectal carcinoma remains a common and at the same time arduous clinical problem.[1,2] The incidence reported in literature after surgery ranges from 7 to 50% depending on surgeon, site and stage of the primary tumour and surgical technique.[3-8] Of these patients 50% will have concomitant distant metastasis,[9] but the mortality and often grave morbidity of recurrent rectal carcinoma usually is the result of local pelvic invasion of nerves, ureters, bones, vessels and remaining rectum.[10,11] Obtaining loco-regional control of the recurrent disease is the main goal of therapy. However, for this therapy of local recurrence there is no established standard of care. Depending on the treatment of the primary tumour different modalities of treatment such as surgery, radiotherapy, hyperthermia, chemotherapy or combinations are utilized but the optimal treatment schedule remains to be established. Surgery (if feasible) in combination with radiotherapy can provide the best results with regard to survival and local control.[12,13] However, many local recurrences cannot be resected with microscopically tumour free margins. Of 163 consecutive cases at our own institution with local recurrence of rectal carcinoma after previous curative therapy only 27 patients had a local recurrence amenable to resection. In these 27 patients extended surgery in combination with high-dose radiotherapy resulted in 59% re-recurrences and a 5 year survival of 20%.[1]

Local recurrences of carcinomas of the urogenital tract carry a dismal prognosis and radical surgery can rarely be performed.[14] Unresectable primary soft tissue sarcoma's and other mesenchymal pelvic tumours are rare, but patients with such tumours present a difficult clinical problem for which no standard therapy exists.

For all these different entities balloon catheter mediated hypoxic pelvic perfusion (HPP), also referred to as 'pelvic stop-flow infusion', with anti-tumour agents may be an additional treatment option when attempting to achieve regional tumour control, alleviation of pain, or in the most favourable case; conversion of an unresectable tumour into a resectable one. However, the scientific validation of this in several institutions worldwide utilized modality remains poor as controlled clinical trials using internationally accepted definitions of tumour response and toxicity are lacking.

In this phase I-II study, we investigated the feasibility of HPP with MMC and melphalan followed by radiotherapy as neo-adjuvant treatment for locally advanced pelvic tumours.

MATERIALS AND METHODS

Patients

In a phase I-II trial patients with histological evidence of a non-resectable tumour in the pelvic region were included. Prior to inclusion computed tomography (CT) or magnetic resonance imaging (MRI) of thorax and abdomen was performed. Patients

were not to have distal metastases or a concurrent malignancy determining prognosis. Patients having received radiation within 6 months prior to HAP, or chemotherapy within 6 weeks prior to entry into the study were excluded. The study was conducted in full accordance with the principles of the 'Declaration of Helsinki'. All patients were submitted to informed consent.

Treatment schedule

Included patients were to undergo two HPP procedures with a time interval of approximately 1 month providing encountered toxicity and clinical condition allowed for a second procedure and providing there was no clinical evidence of progressive disease. If clinical condition allowed patients were re-evaluated by CT or MRI approximately 8 weeks after first HPP to assess tumour response. If resection was considered feasible patients underwent pre-operative radiotherapy with 25 Gy before operation. In the phase-I study patient groups were perfused and infused with 10 mg/m^2 melphalan and subjected to dose-escalation of MMC (10/15/20 mg/m^2).

Toxicity and response

Therapy-induced toxicity was graded according to the standard criteria of the national cancer institute common toxicity grading system (NCICTGS). Tumour response was assessed by CT-scan or MRI strictly according to WHO-criteria.[15]

Perfusion procedure

Patients were brought under general anesthesia (details described by us elsewhere).[16] After formal surgical exposure of the femoral vessels, patients were administered 5000 IU heparin as a bolus injection. Arteriotomy and venotomy were performed, where after the aorta-occlusion catheter and the caval vein occlusion catheters (12F- 600 mm, balloon capacity 30 ml, PfM, GmbH (Cologne, Germany)) were introduced in the common femoral vessels and retrogradely moved up into the aorta and caval vein. Balloons were positioned in the aorta and caval vein just proximal of their respective bifurcations. Correct position was radiologically confirmed. Temporary relative isolation of the pelvic vascular bed was achieved by inflating the balloons and pneumatic tourniquets around both upper thighs of the lower extremities. In order to compensate for the decrease in cardiac preload the aorta occlusion balloon was inflated prior to inflating the caval balloon. After connecting the catheters to the perfusion system primed with 220 ml Haemaccel (Behring Pharma, Amsterdam, The Netherlands) the perfusate was circulated with a roller pump at a constant flow of 250 ml/min. When stable perfusion was ensured, drugs were administered through a sideline into the perfusion circuit during 10 min. Twenty minutes after start of perfusion, isolation was terminated by subsequently deflating caval and aortic balloons. After 10 min of hemodynamic stabilization the tourniquets of the lower extremities were sequentially released.

Melphalan and MMC bio-analysis

Throughout and after the procedure blood samples were simultaneously drawn from the pelvic blood compartment through a sampling port on the venous balloon

catheter and from the systemic blood compartment through a central venous catheter. Details of the MMC and melphalan assays have been described by us elsewhere.[17,18]

RESULTS

Patient and treatment characteristics Sixteen patients were included in the phase I-II study. The male/female ratio was 11/5. The mean age was 59.6 years (range 42 - 75 years). All patients had locally advanced tumours of the pelvic region (*Table 1*). Ten patients underwent a second HPP procedure. Mean time interval between the two HPP procedures was 4.4 weeks (range 4 - 5 weeks). Two patients did not wish to undergo a second HPP procedure, and in one case the tumour response after the first perfusion, made surgical resection possible. Other reasons for not performing the second HPP are mentioned in 'Procedure and toxicity related complications'.

Eleven patients underwent radiotherapy after HPP. Nine patients were treated with 25 Gy 1 week prior to surgery. One patient had adjuvant radiotherapy and one patient underwent radiotherapy for palliation. In two cases the tumour was considered resectable after HPP and radiotherapy was not performed. One patient withdrew himself from further therapy, as mentioned, one patient had died due to earlier complications, and one patient demonstrated clear disease progression.

Procedure and toxicity related complications

All patients survived the HPP procedures. In one patient who was to undergo the second HPP procedure cannulation of the femoral vessels on either side was not possible. In two perfusion procedures there was a preliminary balloon rupture. Noteworthy is that both complications occurred in the same patient. Two patients developed a venous thrombosis post-operatively, originating at the catheter insertion site. One of these resulted in a fatal pulmonary embolism. One patient developed transient cardiac ischemia during the first HPP procedure and did not undergo a second. Four patients had wound infections.

HPP related toxicity

As *Table 2* demonstrates the systemic and local toxicity after HPP was severe. Haematological toxicity was significant and mainly determined by transient neutropenia's, which occurred in 50 - 100% of patients, depending on the perfused dose. Gastro-intestinal toxicity occurred in 25 - 50% of patients, depending on perfused dose and was determined by transient episodes of nausea, diarrhea and ileus. Local toxicity was determined by transient periods of bladder retention and haematuria after HPP, and more distressingly by severe local pain reactions, mainly experienced in the hip region.

Table 1 Patients characteristics and treatment outcome

Tumor type	MMC + melphalan (mg/m^2)	HPP #	CT-response	Pain response	RT	Resection margins	Survival (months)	Follow-up
Rectal recurrence	10 + 10	2	NC	Days	Pre-op	Irresectabel	10	LP
Rectal recurrence	10 + 10	2	NC	Days	Pre-op	Irradical	26	LR
Rectal recurrence	10 + 10	1	-	< 4weeks	Pre-op	Irradical	12	LR
Adenocarc unkn origin	10 + 10	2	NC	NP	Pre-op	Irradical	12	M
Vulva squam cell carc	10 + 15	2	PR	NP	Post-op	Radical	20	M
Colon carcinoma	10 + 15	1	NC	< 4weeks	No	-	15	LP
Rectal recurrence	10 + 15	2	NC	NP	Pre-op	Radical	15	LR + M
Leiomyosarcoma	10 + 15	2	-	NP	No	Radical	86	NR
Rectal recurrence	10 + 15	1	NC	< 4weeks	Pre-op	Irradical	23	LR + M
Rectal recurrence	10 + 15	2	PD	No	Palliative	Irresectabel	23	LP + M
Undiff squam cell carc	10 + 20	1	NC	No	Pre-op	Irradical	14	LR + M
Rectal recurrence	10 + 20	1	NC	No	Pre-op	Radical	74	NR
Rectal recurrence	10 + 20	2	NC	NP	No	Radical	22	LR
Anal squas cell carc	10 + 20	2	PD	Days	Palliative	-	9	LP
Bladder squas cell carc	10 + 20	2	NC	< 4weeks	-No	Radical	67	NR
Rectal recurrence	10 + 20	1	-	No	-	-	1	Pulm embolism

LP *local disease progression*
LR *local recurrence*
M *distant metastases*
NP *no pain at inclusion*
NC *no change*
PR *partial response*
PD *progressive disease*
NR *no symptomatic recurrent disease to date*

Table 2 Toxicity after first (a) and second (b) HPP according to NCI-CTG criteria

MMC + melphalan (mg/m^2)	Pts (n)	Haematological	Renal	Hepatic	GI	Alopecia	Local
a							
10 + 10	4	3 (3,3,2)	1 (1)	-	1 (2)	-	1
15 + 10	6	5 (3,3,3,2,2)	-	3 (1,1,1)	2 (3,1)	2 (2,2)	3
20 + 10	6	6 (4,3,3,3,3,1)	-	3 (2,2,2)	3 (3,3,2)	3 (2,2,2)	2
b							
10 + 10	2	1 (2)	1 (1)	-	-	-	
15 + 10	4	3 (3,2,1)	-	2 (1,1)	1 (2)	1 (3)	
20 + 10	4	4 (4,4,3,2)	-	1 (1)	1 (2)	3 (2,2,2)	1

For each dosage group the total number of patients demonstrating toxicity for each CICTG-category is shown. Individual grades of toxicity are given between brackets.

Tumour response, surgical resection and survival

As *Table 1* shows one patient demonstrated radiological PR. In 10 cases there was radiological NC, although in two of these patients clinically there was evident PD, and in one of these patients there was evident clinical PR. In three patients CT/ MRI-evaluation of response was not performed (one patient had died, one patient demonstrated evident clinical tumour response, and one patient did not wish a second CT, but had clinical disease progression).

In 13 patients a surgical resection was attempted, which could be performed in 11 cases, resulting in six microscopically radical resections. Mean survival is 26.8 months (range 1 - 86) with three patients alive to date. In six patient's loco-regional (re-)recurrence of disease occurred after resection.

Pain response

Pain response after HPP was determined by investigating until start of radiotherapy the pain score of the patient as filled out in a patient pain diary and by investigating the subscribed pain medication. Of the 11 patients that experienced pain prior to treatment, seven patients experienced a reduction of pain, after HPP (*Table 1*). However, in all cases this pain reduction was short-lived, sometimes days, with pain present at the pre-treatment level in all cases within 4 weeks. Noteworthy is that after perfusion three patient's experienced temporary, severe pain, in the hip region, in two cases even requiring readmission, which was the reason for these patients to refrain from a second HPP.

DISCUSSION

Most chemotherapeutic agents demonstrate steep dose-response curves, and, therefore, exposure of the tumour to high drug concentrations is mandatory for eradication of both sensitive and drug resistant tumour cells.[19-21] Several modalities

are utilized for regional chemotherapy of the pelvis e.g. hypo-gastric artery infusion, aortic stop-flow, and HPP. In addition to a first-pass effect, resulting in a higher regional drug concentration, HPP has the theoretical advantages of increased drug exposure time, reduced systemic exposure and regional induction of hypoxia. In some studies HPP is combined with haemofiltration to further reduce systemic exposure.[22,23]

If regional perfusion with anti-tumour agents is to be applied on a wide scale, magnitude and morbidity of the surgical intervention must be limited. Developments in balloon catheter methodology have made it possible to perform HPP using minimally invasive surgery or percutaneous techniques. [23-28] However, it does not seem likely that these interventions can in future be performed under local anaesthesia, as some papers suggest.[23,24] The hemo-dynamic effects associated with the temporary occlusion of aorta and caval vein necessitates invasive cardiovascular monitoring.1[6] Furthermore, patients with severe athero-scleroses are at risk when undergoing these interventions and that they should be carefully evaluated before undergoing this treatment.[26,29]

Drugs whose cytotoxic action are particularly potentiated by hypoxia, such as MMC,[30,31] have been reported to be particularly effective when administered by regional hypoxic perfusion.[24,32,33] Melphalan is an anti-tumour agent which is frequently used in regional perfusion settings[34-37] and which has demonstrated impressive results in isolated limb perfusion for soft tissue sarcoma and melanoma.[38]

Systemic toxicity

Because of obvious anatomical reasons, complete vascular isolation of the pelvic region, is impossible to achieve, unless extensive surgery is performed.[39] As a result systemic exposure to perfused agents is inherent to HPP.[27] Several clinical studies demonstrated that HPP results in markedly augmented regional plasma concentrations of perfused drugs with relatively low concomitant systemic levels, but in most studies toxic side-effects were not clearly defined and documented.[22,23,40-43] We found HPP to be associated with severe systemic toxicity, although the systemic drug concentrations were relatively low (*Fig. 1*). An explanation may be that tissue clearance of perfused drugs has a far greater effect on drug concentrations in the systemic than in the pelvic vascular compartment, due to drug uptake by viscera and bone marrow: The liver has demonstrated a rapid clearance of MMC and melphalan during isolated hypoxic perfusion,[44] and hypoxic abdominal perfusion with these agents using the same methodology, resulted in far lower regional plasma concentrations compared to HPP, than can be explained just by the larger volume of the perfusion compartment.[26,27] This tissue clearance holds true not only for the parent drugs, but also for possible toxic metabolites and degradation products.[18] It seems unlikely that addition of haemofiltration to the procedure will diminish the level of systemic toxicity, as this has been demonstrated to result in the eradication of less than 10% of the administered dose of drugs.[23]

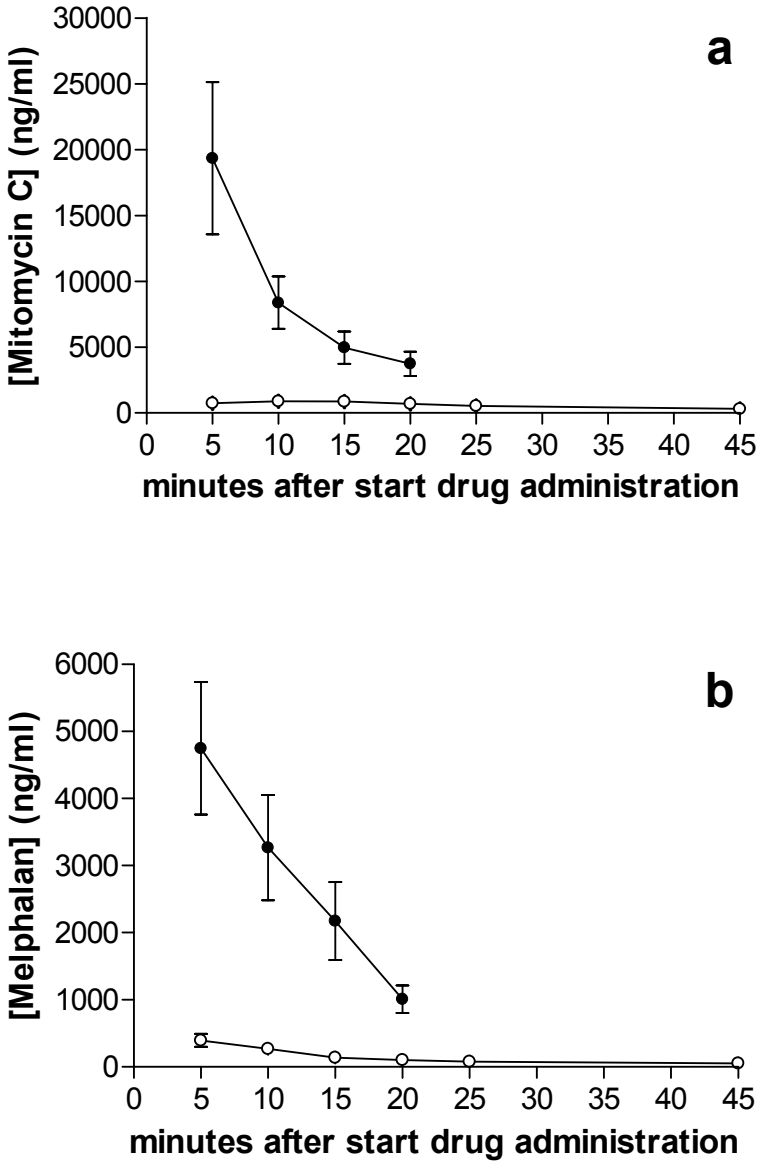

Figure 1 *Regional (black circles) and systemic (white circles) plasma concentrations of melphalan (a), MMC (b) throughout and after 20 min of hypoxic pelvic perfusion. Data shown represent four consecutive patients perfused with 10 mg/m^2 melphalan and 15 mg/m^2 MMC. Mean plasma concentrations ± SEM are shown.*

Anti-tumour effects

Most reports on HPP represent small studies, using different anti-tumour agents for treatment of various tumours. Also different definitions of tumour response are used, which makes it difficult to interpret these results regarding the anti-tumour efficacy of this treatment.[22,23,40-43,45-49] Furthermore, the assessment of tumour response by interpreting MRI and CT of the pelvic region is often made difficult by the associated tumour necrosis and earlier surgical- or radio-therapy, and in many cases is not representative of the clinical situation. Nevertheless, there seems no clear benefit of the treatment schedule of this study compared to our before mentioned results of high-dose radiotherapy combined with extensive surgery in terms of local (re-)recurrences and survival.[1]

Pain reduction

The pain reduction effects of HPP may be the result of an effect on the tumour or may be due to local neurotoxic effects of the perfused agents. In our study, the majority of patients experienced pain reduction, but in all cases this effect was short-lived, sometimes only a matter of days. Some patients experienced more pain, after the procedure, probably due to local perfusion effects on the tumour. Although others have reported pain reduction effects of longer duration,[22,49] in the majority of cases these effects were also temporary and repetitive perfusions would be required for continuative palliation of pain.

CONCLUSION

HPP using these balloon catheter techniques is a currently utilized modality in several institutions worldwide as treatment modality for various unresectable pelvic tumours, using different anti-tumour agents.[23,45-49] Thus far the scientific validation of this modality has been poor, due to marginal documentation of side-effects and the unclear definition of tumour responses. The seemingly favorable pharmacokinetic pro- files,[22,23,41-43] do not correlate with the severe degree of systemic toxicity, associated with this treatment modality. In terms of survival, local (re-)recurrence and pain reduction there seems no benefit of addition of HPP to neo-adjuvant radiotherapy. If one further takes into account the invasiveness of the procedure, length of hospital stay, morbidity, and even mortality which was associated with some procedures, HPP with MMC and melphalan to our opinion, is not a therapeutic option in patients with locally advanced pelvic tumours.

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Chapter 9

DISCUSSION

BIOCHEMOTHERAPEUTIC STRATEGIES AND THE (DIS) UTILITY OF HYPOXIC PERFUSION OF LIVER, ABDOMEN AND PELVIS USING BALLOON CATHETER TECHNIQUES

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ABSTRACT

Aims
To review the development and current status of balloon catheter mediated hypoxic perfusion of abdomen, pelvis and liver for treatment of locally advanced malignancies. Within this context we focus on the addition of tumour necrosis factor-alpha (TNF) to these minimal invasive perfusion procedures.

Methods
A literature search on these topics was carried out in PubMed for indexed articles and in all issues of Regional Cancer Treatment. The findings were related to our own experiences.

Results
Hypoxic abdominal (HAP) and hypoxic pelvic perfusion (HPP) using balloon catheters, are currently applied modalities for treatment of a wide variety of abdominal and pelvic tumours, yet scientific validation of these procedures is poor. Following the results of several Phase I–II trials, both treatments are associated with severe systemic toxicity, significant morbidity and even mortality. The degree of systemic leakage associated with these procedures prohibits addition of TNF. For leakage free liver perfusion surgery is still required, as with current balloon catheter techniques it is not possible to perform leakage free isolated hypoxic hepatic perfusion (IHHP), using either orthograde or retrograde hepatic flow. Experimental and clinical observations suggest that within any perfusion setting, the utilization of TNF is only indicated for treatment of highly vascularised tumours and not for treatment of colorectal tumours.

Conclusion
Balloon catheter technology in its present form does not provide adequate leakage control in any of these settings and is therefore associated with considerable toxicity. It is associated with poor response rates and cannot be considered in any setting as a standard of care.

INTRODUCTION

Regional perfusion with anti-tumour agents is theoretically an attractive option, to achieve in a higher tumour exposure to the agents. This may overcome drug resistance of the tumour,[1] while minimizing systemic exposure to the often highly toxic drugs. Apart from its potential as a palliative treatment modality for various tumours, regional perfusion may also be of use within a neo-adjuvant setting, as it may convert un-resectable malignancies to resectable cases.

In this review we address the developments and current status of balloon catheter mediated hypoxic perfusion of abdomen, pelvis and liver for treatment of locally advanced malignancies. We focus on the addition of tumour necrosis factor-alpha (TNF) and less toxic anticancer drugs to these minimally invasive perfusion procedures. A literature search on these topics was carried out in PubMed for indexed articles and in all issues of Regional Cancer Treatment. The findings were related to our own experiences.

History and background

Regional perfusion with anticancer drugs for the treatment of un-resectable malignancies is well established. Half a century ago, regional perfusion of the liver, pelvis, abdomen and extremities with chemotherapeutic agents was first performed.[2-4] Due to disappointing results, and the invasiveness of the procedures, these methods were generally abandoned, apart from the isolated limb perfusion (ILP). ILP with TNF and melphalan for treatment of sarcoma and melanoma in-transit-metastases, resulted in dramatic tumour responses.[5,6] The high regional TNF concentrations during ILP cannot be achieved with systemic therapy due to its severe systemic toxicity. The question now arises as to whether this success of regional immuno-chemotherapy can be extrapolated to other regional perfusion settings, as in the liver, abdomen and pelvis.

Minimal invasive perfusion procedures

Conventional surgical perfusion procedures of the liver, abdomen or pelvis are major, complex, costly, and time-consuming operations and can only be performed once. Palliation of the malignant disease under control is a more realistic goal than cure. If regional perfusion in these settings is to become a practical treatment option, the extent, complications and costs of the interventions must be acceptable, the procedures should be repeatable, and should yield good response rates.

Developments in balloon catheter technology allow relative and complete vascular isolation of the abdomen, pelvis or liver, by minimally invasive or percutaneous techniques. These procedures can be performed under regional hypoxic conditions, without the high costs of a heart-lung machine operated by perfusionists. Apart from modulation of oxygen pressure, the (relative) vascular isolation also allows perfusing under hyperthermia, to improve the efficacy of anti-tumour agents.

TNF in regional perfusion

TNF *in vivo* has the ability to induce tumour necrosis with acute softening of the tumour. This is believed to result from selective destruction of the tumour microvasculature, causing acute hemorrhagic necrosis of the tumour.[6-10] TNF has an immediate effect on the uptake of drugs in the tumour. When TNF is combined with cytostatic agents, the uptake of the perfusion drugs is selectively enhanced in the tumour tissue. This results in synergistic anti-tumour responses.[11-13]

Because of its systemic toxicity, TNF cannot be given in effective doses intravenously.[14,15] However, when adequate concentrations are achieved in combination with melphalan, such as in ILP for treatment of sarcoma and melanoma in-transit-metastases, it is highly effective in clinical cases.[5,6,16] The impressive response rates achieved with ILP, have led to the approval and registration of TNF in Europe in 1998. A challenge now lies in identifying other situations, where TNF can be utilized.[17]

Indications for use of TNF

The feasibility of adding TNF to a regional or isolated perfusion procedure is first of all determined by the 'sensitivity' of the tumour to this cytokine. Isolated hepatic perfusion (IHP) studies performed in our institution in hepatic sarcoma bearing rats with TNF and melphalan demonstrated that IHP with TNF and melphalan resulted in a dramatic increase in regional concentrations of perfused agents, with virtually no systemic leakage.[18] IHP with melphalan alone resulted in minimal anti-tumour effects. Perfusion with only TNF had a slight growth stimulatory effect on the liver tumours, in any case no negative effect on tumour growth was observed. When TNF was used in combination with melphalan, a synergistic anti-tumour effect was seen, as in isolated limb perfusions on rats with experimental sarcoma of the extremities.[11,13,19] Strikingly, augmentation of tumour response occurred at relatively low concentrations of TNF compared to concentrations eliciting synergy with melphalan in ILP.[19]

The synergy between TNF and melphalan could not be extrapolated to a colon carcinoma hepatic metastases IHP model in the rat. The difference in response rates between the two tumour models seemed to correlate with the density of the microvasculature of the tumours and the tissue uptake of melphalan.[12] Clinical studies have showed the same duration of tumour response after IHP for colorectal metastases with or without TNF.[20,21] IHP resulted in an enhanced capillary leakage in liver parenchyma and tumour, which was irrespective of addition of TNF, as this did not result in augmented melphalan concentrations in colorectal tumour.[22] Nevertheless, in patients with highly vascularized melanoma or leio-myosarcoma metastases, addition of TNF in IHP resulted in partial responses and prolonged duration of response compared to IHP with melphalan alone. Patients with liver metastases from colorectal origin showed no response.[23,24] These experimental and clinical results, seem to confirm the hypothesis that tumour (stroma) characteristics

determine the indication for use of TNF in regional perfusion settings and possibly in general.

Toxicity due to systemic TNF leakage

Regional perfusion aims for a minimal systemic exposure to perfused agents. This is particularly the case when perfusing with a highly toxic agent like TNF.[14,15] For anatomical and technical reasons, complete vascular isolation cannot be achieved in every perfusion setting. In complicated ILP, high leakage of TNF to the systemic circulation led to a 10- to more than 100-fold increased levels of TNF, IL-6 and IL-8 in comparison to patients without leakage, but the increase of the acute phase proteins was limited.[25] However, even when high TNF leakage occurred, this procedure did not lead to fatal complications. The most prominent clinical toxicity was hypotension (grade III in four patients), which was easily corrected. No pulmonary or renal toxicity was observed in any patient. It is our experience that even in the rare event of significant leakage during a TNF-based ILP post-operative toxicity is usually mild and can be easily managed by use of fluid and in some case vaso-pressors.[26]

Hypoxic abdominal perfusion and hypoxic pelvic perfusion

Regional chemotherapy is most effective when complete vascular isolation can be accomplished, as is the case in ILP and IHP. Complete vascular isolation of the abdominal or pelvic region, is impractical.[34-37] Methods that have been used for regional chemotherapy of pelvis and abdomen include celiac axis infusion, hypogastric artery infusion, aortic stop-flow and hypoxic abdominal and pelvic perfusion.[27-29] In addition to a first-pass effect, resulting in a higher regional drug concentration, the latter two methods offer the possibility of increased drug exposure time, and regional induction of hypoxia. Drugs whose cytotoxic action are potentiated by hypoxia, such as MMC,[30,31] have been reported to be particularly effective when administered by regional hypoxic perfusion.[32,33]

Several authors have published on HAP and HPP using balloon catheter techniques, and used these modalities for treating a number of malignant conditions not treatable by conventional means.[32,33,37-42] These have included pelvic recurrences of colorectal carcinoma, advanced primary gastric and pancreatic carcinomas and locally extensive gynaecological malignancies. Significant regional concentration advantages of chemotherapeutic drugs during regional abdominal and pelvic perfusion have been reported,[33-38,41,43] but it is difficult to interpret the results of different studies, regarding toxic side-effects and efficacy, as most of the reports on HAP and HPP represent small series of patients with heterogeneous tumour types using various anti-tumour agents. Toxic side-effects and tumour responses have in many studies not been clearly defined.

Hypoxic perfusion for pancreatic cancer and locally advanced pelvic tumours

Results of the initial experiences with HAP and CAI using mitomycin C (MMC) in locally advanced pancreatic cancer reported were promising: more than 50% tumour

response rates were reported. However, these reports usually included responses defined by a drop in tumour markers and it was not clear whether all these responses were objective responses documented by repeated CT or MRI-imaging according to WHO response criteria. Histological response rates of 92% were reported and pain relief observed in 85% of patients.[28,44] Others question the effectiveness of these methods.[35,37,45,46] The conflicting results may be due to the small size and varying characteristics of the study groups, and because different definitions and parameters for tumour responses were used.[45,47]

Conclusions should be based on controlled clinical trials, using internationally accepted definitions of tumour response and toxicity. In a Phase I–II study,[35] we investigated the feasibility of HAP with MMC and melphalan as treatment for pancreatic carcinoma. We demonstrated that HAP using this balloon catheter methodology resulted in augmented regional plasma concentrations of MMC and melphalan. Advantageous loco-regional drug concentrations, comparable to other studies were established but the outcome of this trial in respect of tumour response and pain response was disappointing. No patients were considered having resectable tumours after the loco-regional induction chemotherapy. There was no major effect in pain reduction and a median survival of 6 months did not show any benefit in survival compared to less invasive therapy regimens in patients with locally advanced pancreatic cancer.[48-50] Moreover, the procedures were associated with significant haematological and gastro-intestinal toxic side-effects, and with one treatment related death. The results of our study were in line with other Phase I–II trials on HAP in patients with pancreatic cancer, where also a validation of the technique by studying the distribution of the agents was performed.[37,46]

A Phase I–II study on HPP with MMC and melphalan followed by radiotherapy, as neo-adjuvant treatment for locally advanced (recurrent) tumours of the pelvic region[51] demonstrated equally dismal results, with severe agent and procedure related toxicity. No beneficial effect of this demanding treatment schedule was demonstrated, when comparing with results of surgery and high-dose radiotherapy alone.[52]

The reported rates of side-effects associated with these technical procedures themselves were acceptable.[35,37,51] Although these procedures may be performed using percutaneous techniques, it does not seem likely that these interventions can in future be performed under local anaesthesia, as some have postulated.[32] We earlier reported that the hemo-dynamic effects associated with the procedures necessitate invasive cardiovascular monitoring.[57] Furthermore, patients with severe athero-scleroses are at risk when undergoing these interventions and should be carefully evaluated before undergoing this treatment.[35,58]

In conclusion, HAP and HPP using these balloon catheter techniques are currently still used modalities in several institutions worldwide as treatment for various un-resectable tumours of abdomen and pelvis, using different anti-tumour agents.[42,53-56] However, the scientific validation of these techniques so far is poor. The seemingly

favourable pharmacokinetic profiles observed during HPP and HAP do not reflect the severe systemic toxicity associated with these treatment modalities. If one takes into account the invasiveness of the procedures, length of hospital stay, morbidity, and mortality which was associated with some procedures, HAP and CAI or HPP with MMC and melphalan to our opinion, do not seem a sensible therapeutic option in patients with locally advanced abdominal or pelvic malignancies.

Addition of TNF to HAP and HPP

Despite disappointing results regarding HAP for pancreatic carcinoma and HPP for various non-resectable tumours in the pelvis we investigated the possibility to use different anticancer drugs. We postulated that addition of TNF to HAP and HPP might augment anti-tumour effects in a similar way it has done in ILP, if the advantage in terms of regional concentration of the drug times exposure time, reached by HAP and HPP was sufficient for TNF effects to occur. This might especially be the case when using these modalities for treating TNF-'sensitive' tumours.[54] In a pharmacokinetic study in pigs we investigated the feasibility of addition of TNF to HAP and HPP.[36] We demonstrated significant leakage of this cytokine to the systemic compartment during these procedures. The concentrations of TNF regionally and systemically, had virtually reached equilibrium after 20 min of perfusion, making a wash-out procedure of the drug, as is performed in ILP and IHP, obsolete. As mentioned before, to a certain extent systemic leakage may be accepted.[26] However, due to the intravascular localization and very low tissue uptake of this drug,[59] all administered TNF has entered into the systemic circulation at the end of the procedures. Thus, one can expect the maximal tolerable dose (MTD) for TNF in HAP and HPP procedures to be equivalent to the MTD when TNF is administered intravenously. The question remains, if the temporarily loco-regional elevated TNF levels can elicit an augmentation of anti-tumour effects. Earlier, we demonstrated in an isolated limb perfusion rat model that a decrease in perfused dose of TNF may be feasible.[19] Although clinical results of TNF isolated limb perfusions, accordingly, suggest that only 5 - 10-fold increases in TNF-drug levels are necessary to obtain TNF-mediated anti-tumour effects in humans,[60] it does not seem likely, on basis of our experience, that adequate concentration advantages for TNF can be achieved by HAP or HPP, using these balloon catheter techniques. Although some studies show that only a short elevation of TNF levels may be necessary for TNF mediated effects to occur,[61] the very rapid decline in regional TNF concentrations prohibits TNF mediated effects to occur, making future addition of this cytokine to these procedures highly unlikely. In contrast an agent like Gemcitabin, which has demonstrated efficacy against pancreas carcinoma[50] exhibits a high total body clearance, which may make this agent highly suitable for HAP.[62] Ongoing trials may prove regional perfusion with this agent effective in combination with radiotherapy[63] for treatment of pancreatic cancer.

Isolated hepatic perfusion

Several forms of regional chemotherapy of the liver have been used for treatment of primary and secondary hepatic malignancies. Hepatic artery infusion (HAI), the most

widely studied form, exploits the first-pass effect of the liver, resulting in high local, but low systemic drug exposure. Repeated hepatic artery infusions regimens produced higher response rates, compared to systemic chemotherapy, but convincing evidence of improved survival is lacking.[64-67] TNF has also been exploited in clinical HAI. Although this resulted in a six times higher MTD tan when administered systemically, the transient toxicity was severe and only modest responses were demonstrated.[68]

IHP encompasses another form of regional chemotherapy, but experience with the technique has been limited to a few centers worldwide. Animal studies demonstrated that up to five times higher intrahepatic concentrations of cytostatic agents can be reached by IHP compared to HAI.[69,70] Other, theoretical, advantages of IHP over HAI are the possibility to use perfusion drugs that do not have a high first-pass hepatic extraction rate, as IHP enables a prolonged exposure of the tumour to these drugs, without concomitant systemic exposure. Drugs, which demonstrate high systemic toxicity such as TNF might be used because the liver vascular bed can be washed out after a perfusion, thus preventing systemic release of the agents. Additionally, the vascular isolation of the liver allows for a modulation of perfusion parameters. Hyperthermic and/or hypoxic conditions can be achieved during IHP, which might induce synergistic effects in combination with chemotherapeutic drugs.[71] Drugs as 5-FU, MMC, cisplatin, melphalan and TNF have been used in various IHP studies, in some cases combined with hyperthermia, but most studies have used melphalan. In animal models IHP with melphalan or MMC has resulted in high response rates.[18,72,73] Higher local melphalan concentrations seem to be a pre-requisite for improving tumour responses. Local hepatic toxicity will be the dose limiting factor using IHP as systemic toxicity will be minimal, if leakage from drugs to the systemic circulation is prevented. In a Phase I trial in 24 patients with colorectal liver metastases Vahrmeijer *et al*. demonstrated the MTD of melphalan to be 3.0 mg/kg,[74] which is twice as high as the dose used in the NCI study by Bartlett *et al*.[20] Apart from procedure related deaths toxicity was transient. In a Phase II study in 73 patients, they demonstrated a tumour response rate of 59% and a median survival of 29 months.[75]

Addition of TNF to IHP

Clinical studies of IHP using melphalan, both with or without tumour necrosis factor-alpha (TNF) have shown promising results.[20,74-78] The Phase II trial performed by the NCI of IHP with melphalan and TNF demonstrated a response rate of 77% and a median survival of 16 months, which was prolonged to 27 months if IHP was followed by HAI with 5-FU and Leucovorin.[20]

In virtually all patients, transient elevation of hepatic enzymes is observed after IHP. These elevations seem more procedure-related than drug-related and normalize within second weeks. De Vries *et al*. could not demonstrate a significant difference between liver enzyme level patterns of patients treated with melphalan and patients treated with melphalan and TNF,[78] although they did demonstrate significantly

elevated levels of IL-6 and Il-8 when TNF was added to IHP. Lans *et al*. showed that the production of secondary mediators in the liver after IHP with TNF and melphalan might result in subsequent transient hemodynamic alterations not observed with melphalan alone.[79] Most of these side effects can be minimized by a complete isolation and a thorough wash-out of in order to keep systemic levels of perfusion agents during and after IHP as low as possible.

Fraker *et al*. demonstrated that the MTD of TNF in IHP is 1.5 mg, which is below the dose used in ILP.[80] However, this does not implicate that the dose used in IHP is less effective. We demonstrated in our rat IHP model that, potentiation of tumour response occurred at relatively low concentrations of TNF compared to concentrations eliciting synergy with melphalan in ILP,[18] and in another animal study by de Wilt *et al*. that lower doses of TNF in ILP can be as effective as the high doses normally used.[19]

Balloon catheter mediated hypoxic IHP

As mentioned, if IHP is to become a treatment option applicable on a large scale, the extent, complications and costs of the interventions must be acceptable and the procedure should be repeatable and yield good response rates. In the open procedure the whole liver has to be mobilised and all lumbar veins have to be ligated to guarantee a leakage free perfusion. Moreover, this conventional approach necessitates a temporary veno-venous bypass and the utilization of a heart–lung machine, operated by a specialized perfusion team. This makes it a major, time-consuming and costly procedure.[75,81]

A new method to improve the current IHP technique is the development of a percutaneous balloon catheter technique, which makes the surgical procedure easier and faster and, in theory, would allow for repeated perfusions. Several groups have developed different modifications of this concept.[82-84] Perfusion is commenced under hypoxic conditions to create an isolated hypoxic hepatic perfusion (IHHP).

We performed IHHP with TNF, melphalan and MMC using these techniques in pigs and demonstrated that a leakage free IHHP can be performed with a small surgical procedure and that it is well tolerated in pigs. Regional drug levels were 20 - 40 times higher than after intravenous drug administration.[59] After these promising results in pigs, our group started a Phase I–II study on IHHP with melphalan in patients with un-resectable hepatic metastases of colorectal origin.[83,85] In our first patients we had no serious adverse events, but responses were marginal presumably due to substantial leakage, which was present and exceeded 30% in most patients. Like we demonstrated in pigs that percutaneous IHP using orthograde flow can be performed leakage free, others demonstrated in pigs that retrograde IHHP using percutaneous balloon catheter techniques is also technically feasible and may result in a leakage free procedure.[86]

In 10 patients perfused with this modified technique we demonstrated that the technique of retrograde venous outflow via the portal vein was feasible, but again systemic leakage was a dose-limiting problem. Systemic toxicity is directly correlated with leakage of cytostatic agents during perfusion and efficacy of the washout procedure.[85]

Savier *et al*. recently reported a Phase I study with four patients repeatedly treated by 10 courses of melphalan based surgical and percutaneous IHP.[82] At percutaneous IHP the hepatic artery was used for inflow of the perfusate and an open double caval balloon catheter was used for outflow. The portal vein was occluded by a percutaneous balloon catheter to complete isolation. This group was also confronted with major leakage starting as soon as the perfusion commenced. This was post-operatively measured by systemic melphalan levels. Severe (grade III - IV) systemic toxicity (haematological) was observed post-perfusion in this study.

Our experience with 18 consecutive patients in this Phase I–II study showed advantages compared to surgical IHP regarding magnitude and operating time, although morbidity and mortality are still significant. Despite technical modifications of our balloon catheter mediated IHHP, leakage is still observed and regional and systemic toxicity remained. These disappointing experiences with the various forms of occlusion catheters have brought us to abandon the balloon catheter program and now to perform a smaller surgical procedure maintaining the method of retrograde outflow through the portal vein: the surgical IHHP. This procedure takes 2 - 2.5 h, includes a 30 min isolated hypoxic perfusion period and an overall hypoxia of the liver of maximally 1 h.

In conclusion, balloon catheter mediated IHHP has failed, since no good leakage control was achieved by either the orthograde or the retrograde method. We, therefore, have abandoned this program and are developing a much simplified surgical method to perform a retrograde IHHP with fully controlled leakage. This may set the stage for re-introducing TNF to this procedure in future. At the same time TNF has clear limitations, because of it is dose-related hepatic toxicity. Therefore, we have looked for alternative drugs in our laboratory program and have identified histamine as a clearly active agent, that enhances the intratumoural uptake of melphalan and that displays no hepatic toxicity in tumour models in rats.[87,88]

CONCLUSIONS

In animal IHP models we demonstrated that the augmentation of anti-tumour effects by TNF, can also be achieved in other perfusion settings than ILP. However, this enhancement of anti-tumour effects by TNF is tumour type dependent, as tumour characteristics like microvasculature density determine if this effect can come about. Clinical observations accordingly suggest that the utilization of TNF is only indicated for treatment of highly vascularized mesenchymal tumours. Disappointingly, there

does not seem to be a beneficial effect when TNF is used for treatment of the frequently occurring colorectal tumours.

HAP and HPP are currently applied modalities for treatment of a wide variety of abdominal and pelvic tumours, although scientific validation of these procedures is poor. In recent Phase I–II studies no beneficial effect of HAP with MMC and melphalan for treatment of advanced pancreatic carcinoma was observed compared with less invasive therapies. Moreover, our experience with HPP for non-resectable tumours in the pelvic region was equally dismal. Both treatments are associated with severe systemic toxicity and significant morbidity and even mortality. Future addition of TNF to these procedures does not seem feasible, as in an animal study regional TNF concentration advantages during HAP and HPP were minimal and short-lived, and were accompanied by significant systemic leakage. We come to the conclusion that there is no evidence that HAP and HPP procedures should be considered as standard of care for any type of tumour, or that these procedures should be conducted outside the setting of rigorous controlled clinical trials. Because of the failure to achieve adequate leakage control we question the clinical potential of these methods.

Although IHHP in pigs with TNF, MMC and melphalan was well tolerated and resulted in a leakage free perfusion, clinical IHHP with melphalan could not be performed without dose limiting leakage using either orthograde or retrograde hepatic flow. For leakage free liver perfusion surgery is still required, as with the current balloon catheter techniques it is not possible to perform a leakage free liver perfusion. However, as there could still be a role for TNF in a leakage free IHP for treatment of highly vascularized mesenchymal metastases, further efforts will have to focus on minimizing the invasiveness of the surgical procedure. Furthermore non-hepatotoxic chemotherapy enhancing drugs need to be identified to increase the efficacy and potential of this procedure in the clinic. Recently we have identified in our tumour models histamine as a drug with that potential.

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Chapter 10

SUMMARY AND CONCLUSIONS

SAMENVATTING EN CONCLUSIES

SUMMARY

Regional perfusion with anti-cancer drugs is, theoretically, an attractive therapeutic option in the clinical management of unresectable malignancies. This concept results in higher tumour exposure to administered agents, while at the same time reducing their systemic concentrations, thereby limiting toxic side-effects.

Conventional surgical perfusion procedures of liver, abdomen or pelvis are major, complex, costly, and time-consuming operations. These features are major drawbacks towards their wide clinical application. When one considers the poor prognosis and very low probability of cure in patients with locally advanced solid tumours, it is evident that keeping progress of the malignant disease under control is a more realistic goal than aiming for cure. Very large surgical procedures are under these circumstances uncalled for and developing alternatives to "major surgery" is mandatory for a new method to have realistic applicability. Developments in balloon catheter technology may allow relative or complete vascular isolation of the abdomen, pelvis or liver, with only minimally invasive surgery. As these procedures are less time consuming than their conventional counterparts, they may be performed under regional hypoxic conditions, bypassing the need for a costly heart-lung machine.

Tumour necrosis factor-alpha (TNF) is a cytokine with an interesting potential in the treatment of cancer. Because of its general toxicity, however, it cannot be given in adequate doses intravenously. In isolated limb perfusions (ILP), high regional concentrations TNF can be achieved, without the occurrence of dose-limiting systemic side effects. When treating patients with sarcoma and melanoma in-transit-metastases with ILP using TNF and melphalan, tumour responses of up to 90% are observed. Possibly the success of this combination of agents can be extrapolated to other regional perfusion settings like those of liver, abdomen and pelvis.

Chapter one describes abovementioned background to the study. It explains that the clinical perspective of anti-cancer drugs with severe systemic side-effects, like TNF, may depend on the coming at hand of minimally invasive and relatively leakage free regional perfusion methodologies. The aims of the presented studies in this thesis were to investigate if regional or isolated hypoxic perfusion of liver, abdomen or pelvis, can be performed using minimally invasive balloon catheter techniques. Within this context the study focussed on the feasibility of adding TNF to these balloon catheter procedures. We also investigated if TNF-mediated anti-tumour effects can occur in other perfusion settings than ILP, and more specifically we studied if these TNF anti-tumour effects are dependant of tumour type and tumour characteristics.

In **chapter two** we investigated if synergy in anti tumour effects between melphalan and TNF, as was earlier observed in ILP in rats, could also be achieved in isolated hepatic perfusion (IHP). Hereto we performed IHP with combinations of these agents

in rats with BN-175 soft-tissue sarcoma liver tumours. We validated the IHP technique by demonstrating high concentrations of TNF in the perfusate, without significant systemic leakage during or after the procedure. IHP with only carrier solution resulted in a significantly diminished growth rate of liver sarcoma tumours, compared to the growth rate of tumours in non-perfused rats. Perfusing with melphalan alone resulted in minimal additional anti-tumour effects. Perfusion with only TNF had a slight growth stimulatory effect on the liver tumours, in any case no negative effect on tumour growth was observed. When TNF was used in combination with melphalan a dramatic anti-tumour effect was observed, similar as has been demonstrated in ILP in rats. Strikingly, augmentation of tumour response occurred at relatively low concentrations of TNF compared to concentrations eliciting synergy with melphalan in rat ILP.

We thus demonstrated that a leakage free IHP can be performed in rats and that TNF augments anti-tumour effects in IHP, similarly as was observed in ILP with melphalan.

In **chapter three** this IHP model was used to determine if TNF augmented anti-tumour effects occur in experimental liver tumours with varying degree of micro-vasculature density: colon carcinoma CC531, ROS-1 osteosarcoma and BN-175 soft tissue sarcoma. IHP was performed with melphalan with or without addition of TNF. IHP with melphalan alone resulted in all tumour types in a decreased growth rate. However, in the BN-175 tumour addition of TNF resulted in a strong synergistic effect. In the majority of the BN-175 tumour bearing rats a complete response was achieved. *In vitro* cytoxicity studies showed no sensitivity (CC531 and BN-175) or only minor sensitivity (ROS-1) to TNF, ruling out a direct interaction of TNF with tumour cells. The response rate in BN-175 tumour bearing rats when TNF was co-administrated with melphalan, was strongly correlated with drug accumulation in tumour tissue, as only in these rats a 5-fold increased melphalan concentration was observed. Secondly, immuno-histochemical analysis of microvascular density (MVD) of the tumour showed a significantly higher MVD for BN-175 tumour compared to CC531 and ROS-1.

These results indicate a direct relationship between vascularity of the tumour and TNF-mediated effects.

Chapter four describes an experimental study where the methodology of isolated hypoxic hepatic perfusion (IHHP) using balloon catheter techniques was validated, and the distribution of TNF, melphalan and mitomycin C (MMC) over the regional and systemic blood compartments during this procedure was investigated. Hereto pigs underwent IHHP with TNF, melphalan and MMC or served as controls, receiving equivalent dosages of these agents intravenously. At the end of a twenty minutes perfusion a wash out procedure was performed for ten minutes, where after isolation was terminated. During perfusion loco-regional plasma drug concentrations were 20 to 40-fold higher than systemic concentrations. Compared with systemic

concentrations after intravenous administration, regional concentrations during IHHP were up to 10-fold higher. Regional MMC and melphalan levels steadily declined during perfusion, indicating rapid uptake by the liver tissue, as minimal concomitant systemic concentrations indicated virtually no leakage to the systemic blood compartment. During isolation concentrations of TNF in the perfusate declined very little, due to limited uptake by the liver tissue and no leakage of TNF to the systemic circulation. After termination of isolation, systemic TNF levels showed only a minor transient elevation, demonstrating that the wash out procedure at the end of the perfusions was fully effective.

On basis of these results we concluded that complete isolation of the hepatic vascular bed can be accomplished without major surgery, when performing IHHP with this balloon catheter technique. These results led to the start of a clinical phase I-II IHHP study.

In **chapter five** we describe the results of an experimental study in pigs on hypoxic abdominal (HAP) and hypoxic pelvic perfusion (HPP) using similar balloon catheter techniques. For understandable anatomical reasons systemic leakage is inherent to these procedures, which may prevent utilization of TNF in these settings. We studied the feasibility of these procedures and more specifically investigated the possibility of adding TNF to these settings in the clinic by determining the distribution of TNF, melphalan and MMC over regional and systemic blood compartment. Pigs underwent HAP or HPP with TNF, melphalan and MMC for twenty minutes. We demonstrated that HAP and HPP resulted in temporary loco-regional concentration advantages of all perfused agents, although from start of perfusion significant systemic leakage occurred.

On basis of these results it seems that the advantage in terms of regional plasma concentrations of TNF during HAP and HPP is insufficient for TNF-mediated effects to occur, making future addition of this cytokine to these procedures in the clinical setting questionable. The observed regional concentration advantages of MMC and melphalan, however, warranted further studies on clinical application of these agents in both settings.

Chapter six describes the feasibility of isolated hypoxic hepatic perfusion (IHHP) in patients with unresectable liver metastases using two different balloon catheter techniques, resulting in orthograde or retrograde hepatic flow. We assessed the amount of leakage of anti-tumour agents to the systemic compartment, occurring with either technique, studied procedure and agents associated toxicity and determined tumour response and time to disease progression in treated patients. In this phase I-II study 18 consecutive patients with unresectable colorectal or ocular melanoma hepatic metastases were included. Both perfusion techniques had inflow via the hepatic artery, using melphalan at a dose of 1 mg/kg. In the first eight patients the portal vein was occluded and outflow was via the hepatic veins into an intra-caval double balloon catheter. This orthograde IHHP had on average a 56%

leakage. In the next 10 patients we performed a retrograde outflow-IHHP with a triple balloon blocking outflow into the caval vein and allowing outflow via the portal vein. The retrograde IHHP still had a 35% leakage on average. Although local drug concentrations were high with retrograde IHHP, systemic toxicity was still moderate to severe. Partial responses were seen in 12 % and stable disease in 81% of patients. Median time to local progression was 4.8 months.

On basis of these results we abandoned the occlusion balloon methodology for IHHP, as it failed to obtain leakage control.

In **chapter seven** we describe the results of a clinical study on the feasibility of HAP with MMC and melphalan using balloon catheter techniques in patients with locally advanced pancreatic carcinoma. 21 patients with advanced pancreatic carcinoma were included in a phase I-II trial of HAP with MMC and melphalan, followed by celiac axis infusion (CAI) with the same agents six weeks later. Tumour response was assessed by abdominal-CT and by determining tumour markers. Effect on pain reduction was assessed by evaluation of pain registration forms. HAP resulted in augmented regional drug concentrations. One patient died after CAI due to acute mesenterial ischaemia. One possibly agent-toxicity related death was observed in the phase-I study. Significant hematological toxicity was observed after HAP and CAI at the maximum tolerable dose (MTD). No patients were considered resectable after treatment. Median survival after HAP was 6 months (range 1-29). Pain reduction was experienced by only 5/18 patients and was short lived.

We concluded that, in contrast to earlier reports, HAP and CAI with MMC and melphalan does not demonstrate any benefit in terms of tumour response, median survival and pain reduction, compared to less invasive treatment options. As this treatment is associated with significant toxic side-effects and even one procedure related death we do not consider this a therapeutic option in patients with advanced pancreatic cancer.

In **chapter eight** we described the results of a phase I-II study on HPP with MMC and melphalan, using balloon catheter techniques in patients with various types of locally advanced pelvic tumours. Again, we investigated the bio-distribution of agents during perfusion and studied procedure and agents associated toxicity. We assessed the efficacy of the procedure regarding tumour response, pain reduction, local recurrence and median survival. HPP resulted in augmented regional drug concentrations with relatively low concomitant systemic levels. Nevertheless, severe systemic toxicity was observed. One procedure related death occurred. Pain reductive effects were short-lived. 10 patients had radiological NC, 2 PD and 1 PR. In 11 patients surgical resection was performed, which was microscopically radical in 6 cases. Mean survival is 26.8 months (range 1-86) with three patients alive when the study was ended. In six patients there was loco-regional recurrence of disease at time of death.

In terms of survival, local (re-)recurrence and pain reduction there seems no benefit of addition of HPP to pre-operative radiotherapy. If one takes into account the invasiveness of the procedure, length of hospital stay, morbidity, and even mortality, HPP with MMC and melphalan to our opinion, does not seem a therapeutic option in patients with locally advanced pelvic malignancies.

In **chapter 9** the results of the presented studies and other studies by our group and in world literature concerning these topics are addressed in a general discussion.

CONCLUSIONS

Overall we have demonstrated that balloon catheter technology does not provide adequate leakage control in any of these clinical perfusion settings, and is therefore associated with considerable toxicity. It is associated with poor response rates and can not be considered in any setting as a standard of care for any type of tumour. Therefore these procedures should not be conducted outside the setting of rigorous controlled clinical trials. Because of the failure to achieve adequate leakage control we question the clinical potential of these methods.

The augmentation of anti-tumour effects by TNF, can also be achieved in other perfusion settings than ILP. This effect is tumour type dependent, as tumour characteristics like micro-vasculature density determine if this effect can come about. Hypervascular tumours respond very well to the combination of TNF and melphalan, in contrast to hypovascular tumours like colorectal metastases. Addition of TNF to these perfusion procedures in their present form is not feasible, due to the significant systemic leakage, which would result in severe systemic toxicity.

SAMENVATTING

Regionale perfusie is theoretisch een aantrekkelijke behandelingsoptie bij patiënten met niet-resectabele maligniteiten. Dit concept resulteert in een verhoogde expositie van tumorweefsel aan anti-tumormiddelen, terwijl de systemische blootstelling aan deze vaak zeer toxische middelen beperkt blijft. Perfusieprocedures van lever, abdomen en bekken vereisen complexe en tijdvergende chirurgische ingrepen, waardoor klinische toepasbaarheid hiervan op grote schaal niet mogelijk is. Daarbij hebben patiënten met een niet-resectabele tumor vrijwel altijd een slechte prognose en een kleine kans op curatie. Grote, ingrijpende procedures zijn niet gewenst bij de, meestal in opzet palliatieve, behandeling van deze patiëntengroep. Regionale perfusie heeft dan ook slechts een klinische toekomst, indien minder invasieve perfusiemethoden ter beschikking komen. Met balloncathetertechnologie zou het vaatbed van een orgaan of lichaamsregio relatief of volledig kunnen worden geïsoleerd. Daar balloncatheterperfusie-procedures minder tijd vergen dan de conventionele chirurgische procedures, kunnen deze perfusies onder locale hypoxie kunnen worden uitgevoerd, waardoor de noodzaak van een kostbare hart-longmachine vervalt.

Tumor-necrosisfactor-alpha (TNF) is een cytokine met sterke anti-tumoreigenschappen. De ernstige systemische toxiciteit van dit eiwit, maakt intraveneuze toediening echter onmogelijk. Geïsoleerde extremiteitsperfusie (GEP) resulteert in effectieve concentraties TNF, zonder dat zich ernstige systemische bijwerkingen voordoen. GEP met TNF en melphalan ter behandeling van patiënten met weke- delen-sarcomen en melanoom-in-transit-metastasen resulteert in tumorresponses tot 90%. Mogelijk kan de toepasbaarheid van deze middelen worden uitgebreid naar andere regionale perfusieprocedures, zoals die van lever, abdomen en bekken.

Hoofdstuk één belicht de hierboven kort uiteengezette achtergrond van deze studie. De toepasbaarheid van anti-tumormiddelen met hoge systemische toxiciteit, wordt mogelijk mede bepaald door het beschikbaar komen van minder invasieve en relatief lekkage-vrije regionale perfusietechnieken. Het doel van de in dit proefschrift beschreven studies was te onderzoeken of regionale of geïsoleerde perfusie van lever, abdomen of bekken mogelijk is met minder invasieve balloncathetermethoden. Binnen dit kader werd onderzocht of TNF in de toekomst zou kunnen worden toegevoegd aan klinische balloncatheterperfusies. Tevens werd onderzocht of de TNF-gemedieerde anti-tumoreffecten bij GEP, zich ook voordoen bij andere regionale perfusiebehandelingen en werd onderzocht of de TNF-gemedieerde anti-tumoreffecten afhankelijk zijn van tumortype en bepaalde tumoreigenschappen.

In **hoofdstuk twee** werd onderzocht of de synergistische anti-tumoreffecten tussen melphalan en TNF bij GEP, zich ook voordoen bij geïsoleerde leverperfusie (GLP) met deze middelen. Hiertoe ondergingen ratten met experimentele BN-175-liposarcoma tumoren geïmplanteerd in de lever, een GLP met diverse combinaties van deze

middelen. De regionale en systemische TNF-plasmaconcentraties gedurende de procedures toonden een lekkagevrije leverperfusie aan. GLP met alleen perfusiemedium had een geringere groeivertraging ten gevolg, vergeleken met tumoren in niet-geperfundeerde ratten. GLP met alleen melphalan resulteerde in minimale anti-tumoreffecten. Perfusie met alleen TNF had een geringe groeistimulatie van de levertumoren ten gevolg. Wanneer werd geperfundeerd met een combinatie van TNF en melphalan was een sterk anti-tumoreffect het gevolg, vergelijkbaar met de observaties in eerdere GEP-studies in ratten met experimentele BN-175-sarcomen van de extremiteit. Opvallend was dat de dosering van TNF, waarbij synergie optrad, veel lager was dan de effectieve dosering bij GEP in ratten.

Met deze studie werd aangetoond dat lekkagevrije GLP mogelijk is in dit rattenmodel en dat TNF-gemedieerde anti-tumoreffecten zoals deze zich voordoen bij GEP, zich ook voor doen bij GLP.

In **hoofdstuk drie** werd onderzocht of deze TNF gemedieerde anti-tumoreffecten afhankelijk zijn van de vascularisatiegraad van de tumor. Hiertoe werden levertumoren met verschillende vascularisatiegraad behandeld met GLP. Ratten met CC531-coloncarcinoom, ROS-1-osteosarcoom en BN-175-liposarcoom levertumoren, ondergingen GLP met melphalan met en zonder toevoeging van TNF. GLP met alleen melphalan resulteerde in tumorgroeireductie in alle drie tumoren. Toevoeging van TNF resulteerde alleen in de ratten met BN-175-tumoren in een versterkt anti-tumoreffect. Bij de meerderheid van deze ratten deed zich een complete tumorrespons voor. In *in vitro*-studies werd geen (CC531, BN175) of slechts een geringe (ROS-1) gevoeligheid van de tumorcellen voor TNF aangetoond, waardoor een direct anti-tumoreffect werd uitgesloten. Het versterkte anti-tumoreffect in ratten met BN-175-liposarcoom levertumoren ging gepaard met een 5 maal verhoogde weefselconcentratie melphalan. Immuno-histochemisch onderzoek toonde aan dat de vascularisatiegraad van de BN-175-tumor significant hoger lag dan die van CC531- en ROS-1-tumoren.

De resultaten van deze studie suggereren een direct verband tussen microvascularisatiegraad van de tumor en TNF-gemedieerde anti-tumoreffecten.

Hoofdstuk vier beschrijft een dierexperimentele studie waarin de methodologie van geïsoleerde hypoxische leverperfusie (GHLP) met balloncathetertechnieken werd gevalideerd en de toepasbaarheid van TNF bij deze procedure werd onderzocht. Varkens ondergingen GHLP met TNF, melphalan en mitomycine C (MMC). Een controle groep kreeg equivalente doseringen van deze middelen systemisch toegediend. Na perfusie werd 'gespoeld' met perfusiemedium, waarna de vasculaire isolatie werd opgeheven. Gedurende GHLP lagen de loco-regionale plasmaconcentraties van de geperfundeerde middelen 20 - 40 maal hoger dan in de systemische circulatie. Vergeleken met de varkens uit de controlegroep, waren de loco-regionale plasmaconcentraties 10 maal hoger. Gedurende perfusie was er geen systemische lekkage. De regionale TNF-concentraties namen slechts gering af, ten

gevolge van minimale lever-uptake. De regionale MMC- en melphalanconcentraties namen echter wel snel af, waarschijnlijk ten gevolge van opname van deze middelen door het leverweefsel. Na opheffen van de vasculaire isolatie was er slechts een zeer geringe verhoging van de systemische TNF-concentraties, waarmee de effectiviteit van de 'spoelprocedure' werd aangetoond.

Op grond van deze bevindingen concludeerden wij dat een lekkagevrije perfusie van de lever mogelijk is met deze minder invasieve balloncathetermethodologie, waarop werd gestart met een klinische fase I-II studie naar de toepasbaarheid van GLP met balloncatheters.

Hoofdstuk vijf beschrijft de resultaten van een experimentele studie naar de toepasbaarheid van hypoxische abdominale perfusie (HAP) en hypoxische bekkenperfusie (HBP) met balloncathetertechnologie en de mogelijkheid TNF aan deze procedures toe te voegen. Ten gevolge van de anatomie is volledig lekkagevrije perfusie van bekken en abdomen onmogelijk, wat het gebruik van dit middel bij deze procedures mogelijk in de weg staat. Varkens ondergingen HAP en HBP met TNF, melphalan en MMC. We toonden aan dat HAP en HBP gedurende perfusie resulteren in tijdelijk verhoogde loco-regionale plasmaconcentraties van de toegediende middelen. De regionale concentraties van TNF bij deze procedures waren echter te laag om TNF-gemedieerde effecten mogelijk te maken. Tevens was er een vrijwel volledige lekkage van TNF naar de systemische circulatie.

We concludeerden dat toekomstige klinische toepassing van TNF bij deze procedure onwaarschijnlijk is. De aangetoonde regionale concentratie voordelen van MMC en melphalan rechtvaardigden klinische studies naar toepasbaarheid van HAP en HBP met deze middelen.

Hoofdstuk zes beschrijft ons fase I-II onderzoek naar de toepasbaarheid van geïsoleerde hypoxische leverperfusie (GHLP) met balloncatheters bij patiënten met niet-resectabele levermetastasen. 18 opeenvolgende patiënten met niet-resectabele colorectale of oculair melanoom metastasen werden geïncludeerd. Twee perfusiemethoden werden gehanteerd, resulterend in orthograde of retrograde flow. Bij beide technieken vond infusie plaats via de arteria hepatica met melphalan. Bij de eerste 8 patiënten werd de vena porta geoccludeerd en geschiedde de afvloed via de venae hepaticae naar een dubbel-ballonscatheter geplaatst in de vena cava. Deze orthograde leverperfusie was geassocieerd met 56% lekkage. Bij de volgende 10 patiënten werd een retrograde leverperfusie verricht. Hierbij werd de leverafvloed in de vena cava geblokkeerd door een drie-ballonscatheter. Veneuze afvloed was nu via de vena porta. Deze methode resulteerde nog steeds in een forse gemiddelde lekkage van 35%, resulterend in matig tot ernstige toxiciteit. 'Partial responses' werden bij 12% van de patiënten gezien en 'stable disease' bij 81%. Mediane tijd tot locale ziekteprogressie was 4,8 maanden.

Deze resultaten hebben ertoe geleid dat de occlusie balloncathetertechniek voor GHLP werd verlaten, daar met de huidige methodologie geen controle over systemische lekkage kan worden verkregen.

In **hoofstuk zeven** worden de resultaten beschreven van een fase I-II-studie naar de toepasbaarheid van HAP met balloncathetertechnieken bij de behandeling van patiënten met locaal uitgebreid pancreascarcinoom. Bij 21 patiënten werd HAP verricht met MMC en melphalan, 6 weken later gevolgd door truncus coeliacus infusie (TCI) met dezelfde middelen. HAP resulteerde in verhoogde regionale concentraties MMC en melphalan. Een patiënt overleed na TCI tengevolge van darm-ischaemie. In de fase I-studie overleed een patiënt mogelijk ten gevolge van toxiciteit. HAP en TCI resulteerden bij toediening van de maximaal toedienbare dosering (MTD) in een aanzienlijke hematologische toxiciteit. Geen van de behandelingen resulteerden in een alsnog resectabele tumor. Mediane overleving was 6 maanden (1 - 29 maanden). Een analgetisch effect was er slechts bij 5/18 patiënten en in alle gevallen was dit van korte duur.

HAP en TCI met MMC en melphalan heeft geen voordeel bij de behandeling van locaal uitgebreid pancreascarcinoom, voor wat betreft tumorrespons, mediane overleving en pijnreductie, ten opzichte van minder invasieve behandelingsopties. Daar de behandeling gepaard ging met aanzienlijke toxiciteit, morbiditeit en zelfs procedure-gerelateerde toxiciteit, concluderen wij dat deze behandelingsmodaliteit geen plaats heeft bij de behandeling van patiënten met pancreaskanker.

In **hoofdstuk acht** worden de resultaten beschreven van een fase I-II-studie naar de toepasbaarheid van HBP met balloncathetertechnieken bij de behandeling van patiënten met locaal uitgebreide bekkentumoren. Zestien patiënten ondergingen in principe 2 HBP-procedures met melphalan en MMC, gevolgd door radiotherapie. HBP resulteerde in verhoogde regionale concentraties met relatief lage systemische concentraties. Desalniettemin deed zich, evenals bij HAP, aanzienlijke systemische toxiciteit voor. Een patiënt overleed ten gevolge van een procedure gerelateerde complicatie. Analgetische effecten waren wederom slechts van korte duur. Bij 10 patiënten was er radiologisch 'No Change', bij 2 patiënten 'Progressive Disease' en bij 1 patiënt een 'Partial Response'. 11 patiënten kwamen in aanmerking voor chirurgische resectie, wat microscopisch radicaal bleek in 6 gevallen. Mediane overleving was 26,8 maanden (1 - 86 maanden) met nog drie patiënten in leven ten tijde van afronden van de studie. Bij 6 patiënten was er een bekend locaal recidief bij overlijden. Vergeleken met alleen pre-operatieve radiotherapie, lijkt er geen voordeel van toevoeging van HBP aan de neo-adjuvante behandeling, als men kijkt naar overleving, lokaal recidief en pijnreductie.

We concludeerden dat er, mede gezien het invasieve karakter van, de opnameduur, de behandeling gerelateerde morbiditeit en zelfs mortaliteit, geen plaats lijkt voor HBP met MMC en melphalan bij de behandeling van patiënten met locaal uitgebreide bekkenmaligniteiten.

Hoofdstuk negen vormt de discussie van dit proefschrift. Hierin worden de resultaten van de in dit proefschrift beschreven studies, evenals die van gerelateerde, eerdere studies van onze groep besproken en vergeleken met de literatuur

CONCLUSIES

De in dit proefschrift beschreven studies tonen aan dat met de huidige balloncathetertechnologieën geen lekkagevrije perfusie kan worden bewerkstelligd van lever, abdomen, of kleine bekken, waardoor deze procedures resulteren in een aanzienlijke systemische toxiciteit. Daarbij zijn deze minder invasieve perfusiebehandelingen geassocieerd met procedure-gerelateerde morbiditeit en mortaliteit. De beschreven regionale perfusiemethodologieën resulteren slechts in een geringe tumorrespons, en dienen dan ook niet als standaard behandeling te worden beschouwd, maar slechts toegepast te worden binnen het kader van gecontroleerde trials. Daar lekkagevrije perfusie niet kan worden bewerkstelligd, stellen we het klinisch toepassingsperspectief van deze modaliteiten ter discussie.

Het toegenomen anti-tumoreffect door toevoeging van TNF aan GLP met melphalan, doet zich ook voor bij toevoeging van dit cytokine aan andere regionale perfusieprocedures met melphalan. De TNF-gemedieerde effecten zijn echter tumorsoort afhankelijk, daar tumorkarakteristieken, zoals de mate van micro-vascularisatie, bepalen of een TNF-effect optreedt. Bij de pefusiebehandeling van hypervasculaire tumoren blijkt toevoeging van TNF zeer effectief in tegenstelling tot bij de behandeling van minder gevasculariseerde tumoren zoals colorectale metastasen. Toevoeging van TNF aan balloncatheterperfusie-procedures van bekken, abdomen en lever heeft geen toekomstperspectief, daar de lekkage hiervan naar de systemische circulatie zou leiden tot ernstige toxiciteit.

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CURRICULUM VITAE

De auteur van dit proefschrift werd op 6 oktober 1967 geboren te Delft. In 1987 behaalde hij zijn VWO-diploma op het Christelijk Lyceum te Delft. Vervolgens vervulde hij zijn dienstplicht bij de Geneeskundige Troepen van de Koninklijke Landmacht. In 1989 begon hij met zijn studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. De doctoraalfase werd afgerond met een afstudeeronderzoek op het laboratorium voor experimentele chirurgie onder begeleiding van dr. R.L. Marquet. Alvorens de co-schappen aan te vangen deed hij een onderzoeksstage bij het Shriner Burns Institute en Massachusetts General Hospital, Harvard Medical School, Boston, USA, onder supervisie van prof. R.G. Tompkins, MD, PhD. Na het afronden van de co-schappen, die gedeeltelijk werden doorlopen in het St. Elisabeths Hospitaal, Willemstad, Curaçao, behaalde hij in 1996 zijn artsexamen. Vervolgens was hij als arts-onderzoeker verbonden aan de afdeling Chirurgische Oncologie van de Dr. Daniel den Hoed Kliniek te Rotterdam (afdelingshoofd prof. dr. Th. Wiggers). Onder leiding van prof. dr A.M.M. Eggermont werd in deze periode de basis gelegd voor dit proefschrift. De opleiding tot algemeen chirurg werd in 1998 begonnen in het huidige Erasmus Medisch Centrum te Rotterdam (opleiders prof. dr. H.A. Bruining en prof. dr. H.J. Bonjer) en werd vanaf 2001 voortgezet in het Ikazia ziekenhuis te Rotterdam (opleider dr. W.F. Weidema). Sinds april 2004 werkt hij met veel plezier als algemeen chirurg in het Van Weel-Bethesda Ziekenhuis op Goeree-Overflakkee.

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