Peptide Receptor Radionuclide Therapy

Pre-clinical studies

Gerrit Dirk Slooter

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Pre-clinical studies

Peptide Receptor Radionuclide Therapie

Pre klinische studies

proefschrift

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CHAPTER 1

AIMS OF THE THESIS

Radionuclide therapy

Neuroendocrine tumours of the digestive tract include two main types of endocrine tumours: those developed within the pancreatic islets or the duodenal loop and carcinoid tumours. Most neuroendocrine tumours are potentially malignant and have already metastasised at the time of diagnosis. The clinical distress of patients with neuroendocrine tumours is related to the hypersecretion of hormones. Somatostatin analogues, of which octreotide is used most commonly are of considerable help in controlling symptomatology in these of patients. There is also experimental and clinical evidence of tumour growth inhibition in some tumours by somatostatin analogues but the mechanism through which this inhibition is exerted is still to be unraveled. A mechanism mediated through specific somatostatin receptors could be a possibility since these receptors have been identified on most neuroendocrine tumours.

In vivo visualisation of the somatostatin receptors can be performed with somatostatin receptor scintigraphy, which has proven it's clinical value. When used for scintigraphy octreotide is labelled with low dose of Indium-111 (^{111}In), which emits gamma rays. Theoretically high dose radioactivity of ^{111}In could be used for radionuclide therapy since ^{111}In also emits electrons with a short particle range. These electrons should be able to damage the nucleus of somatostatin receptor-positive cells. This peptide receptor radionuclide therapy with radiolabelled somatostatin analogues could be a promising treatment modality for patients with somatostatin receptor-positive tumours.

Tumour growth after partial hepatectomy

Partial hepatectomy is performed clinically as the only curative treatment modality for liver metastases. After partial hepatectomy a well-orchestrated process of liver regeneration takes place which enables the liver to restore it's volume. In the rat this regeneration is completed within 10 days, in human within 30 days. Clinically, after partial hepatectomy for metastases there are a high number of early recurrences. It could be that altered physiological conditions during liver regeneration have a tumour growth stimulating effect on non resected remnant tumour cells. If this process of increased tumour growth after partial hepatectomy can clinically and experimentally be substantiated, there could be a great benefit for systemic adjuvant treatment. However, this adjuvant treatment should not impair the process of liver regeneration.

Aims of the thesis

The aims of this thesis were to investigate the possibilities of antitumour treatment with octreotide and peptide receptor radionuclide therapy with radiolabelled octreotide in the rat. Therefore we used a liver metastases model with somatostatin receptor-positive and somatostatin receptor-negative tumours. We also studied the tumour growth stimulating effect of liver regeneration after partial hepatectomy. Since, if this putative tumour growth stimulation by partial hepatectomy could be demonstrated we wanted to see if treatment with octreotide and radionuclide therapy could be effective also after partial hepatectomy and whether this treatment would subsequently influence liver regeneration. In addition we wanted to investigate the value of somatostatin receptor scintigraphy in patients with liver metastases of neuroendocrine tumours to define patients that could possibly benefit from peptide receptor radionuclide therapy.

CHAPTER 2

SOMATOSTATIN RECEPTOR IMAGING, THERAPY AND NEW STRATEGIES IN PATIENTS WITH NEUROENDOCRINE TUMOURS

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G.D. Slooter, A. Mearadji, W.A.P. Breeman,
M. de Jong, E.P. Krenning and C.H.J. van Eijck

Somatostatin receptor expression

Somatostatin (SS) is a small regulatory peptide; it was isolated in the ovine hypothalamic gland in 1973 as growth hormone (GH) releasing inhibiting factor[1]. Somatostatin is widely distributed in the human body and is found not only in the hypothalamus, but also in various parts of the gastrointestinal tract, indicating that inhibition of GH is not its only function[2]. Apart from its function as neurotransmitter in the central nervous system (CNS), it also has inhibitory effects on the secretion of hormones by pancreatic islands (insulin, glucagon) and on the exocrine pancreatic function. Somatostatin also inhibits normal gastrin production and consequently gastric acid and pepsin production[3]. A number of observations have also suggested an antiproliferative effect of somatostatin and its stable analogues[4-6].

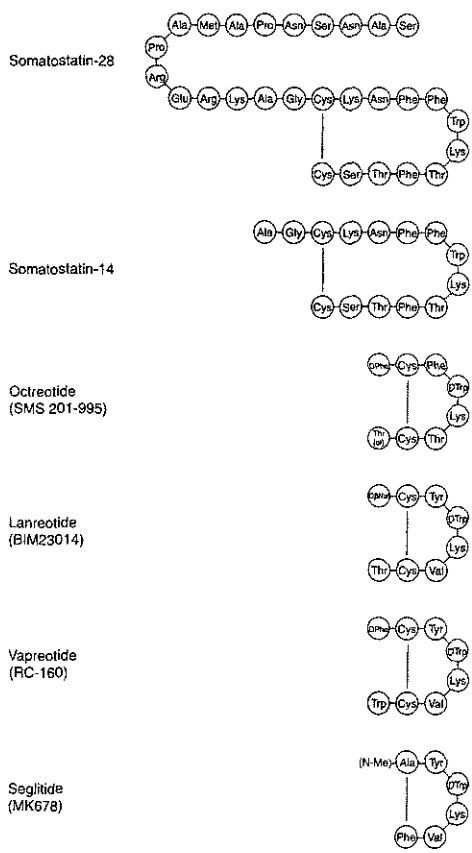

Figure 1 The amino acid sequences of native and synthetic, metabolically stable somatostatin analogues. (Image by T. van Os)

Critical to all these actions is the expression of somatostatin receptors (SS-Rs) on the cell membrane. These SS-Rs subserve two functions: first to recognize the ligand and bind it with high affinity and specificity, and second to generate a transmembrane signal that evokes a biological response. Large number of SS-Rs are found on most tumours with amine precursor uptake and decarboxilation (APUD) characteristics and neuroendocrine properties, such as carcinoids, paragangliomas, pheochromocytomas, medullary thyroid cancers and endocrine pancreatic tumours. In addition large numbers of binding sites with high affinity for somatostatin were also found on breast and brain tumours as well as on various cells of the immune system[7-10]. At least five different human SS-R subtypes have been cloned[11]. All subtypes bind somatostatin with high affinity, while their affinities for the somatostatin analogue SMS 201-995 (Octreotide) differ considerably. Octreotide binds with high affinity to SS-R subtype 2 (sst_2) and sst_5, to a lesser degree to sst_3, while no binding to sst_1 and sst_4 occurs. Other somatostatin analogues that are in clinical use such as BIM 23014 (Lanreotide) and RC-160

(Vapreotide) as well as the hexapeptide MK678 (Seglitide) bind to three to five SS-R subtypes displaying also high affinity for sst_2 and sst_5 and moderate affinity for sst_3[12]. Figure 1 shows the amino acid sequences of native somatostatin and these analogues. Somatostatin receptor messenger RNA subtypes are widely expressed in neuroendocrine tumours, but their distribution does not necessarily correlate with SS-R subtype expression. Furthermore SS-R subtypes show a differential subcellular localisation in human SS-R positive tumours[13]. The majority of human endocrine pancreatic tumours such as gastrinomas, glucoganomas, VIPomas and "non-functioning" islet cells tumours express sst_2. *In vitro* studies have shown that 72 per cent of the insulinomas express SS-Rs, however, these receptors are mainly st_3, which has low affinity for octreotide[14,15]. Although SS-Rs have been demonstrated on exocrine pancreatic cells in experimental animals (mainly on the acinar cells), neither SS-Rs nor neuroendocrine properties could be confirmed on human exocrine pancreatic adenocarcinomas[16]. Carcinoids, paragangliomas, pheochromocytomas and medullary thyroid cancers all have a mixed distribution of the SS-R subtypes but sst_2 is expressed most frequently. The presence of different combinations of SS-R subtypes may explain the variable clinical response to somatostatin analogues and the difference in successful localisation by SS-R scintigraphy (SRS) of these tumours. Metastases of SS-R positive tumours initially express also these receptors, however loss of these receptors has been described after dedifferentiation of tumour cells or after chemotherapy[17]. In addition, SS-R overexpression was identified in peritumoural veins of primary tumours and in veins surrounding lymph node, bone and lung metastases[18].

Somatostatin receptor scintigraphy

The optimal management of patients with neuroendocrine tumours requires accurate imaging and staging. For the visualisation of SS-R positive tumours SRS with [^{111}Indium-diethylenetriaminopenta-acetic acid (^{111}In- DTPA^0)]octreotide (Octreoscan®) has been used for more than 10 years[19,20]. The efficacy of SRS using [^{111}In-DTPA^0]octreotide in patients with histologically or biochemically proven endocrine pancreatic tumours or carcinoids was evaluated in a European multicenter trial[21]. The highest success rates of SRS were observed with glucagonomas (100 per cent), VIPomas (88 per cent), gastrinomas (73 per cent),"non-functioning" islet cells tumours (82 per cent) and carcinoids (87 per cent). Insulinomas were detected in only 46 per cent of cases due to the low incidence of sst_2 on insulinoma cells. The low sensitivity in this study found for some tumours may be related to important difference in scanning procedures such as the amount of radioligand administered, the duration of the acquisition and the use of single photon emission computed tomography (SPECT)[22]. With SPECT, in which a rotating camera is used, image reconstructions can be made similar to spiral computed tomography (CT). This technique gives additional visual information,

especially on tumours in the liver and upper-abdomen[23]. Twenty-five per cent more liver metastases are detected with SPECT than with planar SRS.

In a prospective study comparing the sensitivity of SRS with that of CT, magnetic resonance imaging (MRI), ultrasonography and selective angiography in the detection of primary and metastatic gastrinomas, SRS altered clinical management in 47 per cent of instances and had a superior sensitivity, and specificity[24] (table 1).

Table 1 Imaging methods for the detection of liver metastases and extra-hepatic metastases in patients with Zolinger-Ellison syndrome

	Positive result (%)	
	Extra-hepatic tumour	Liver metastases
Procedure	(n = 80)	(n = 24)
Ultrasonography (US)	9	46
Computed tomography (CT)	31	42
Magnetic resonance imaging (MRI)	30	71
Angiography (Angio)	28	62
US + CT + MRI + Angio (CIM)	48	83
SRS	58	92
SRS + CIM	68	96
SRS Only*	20	12
CIM Only**	10	4

Gibril et al, Ann Intern Med 1996; 125:26-34
Results are expressed as the percentage of the 24 patients with proven liver metastasis and of the 80 with extrahepatic disease. Conventional imaging methods (CIM) included ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI) and angiography.
* *Tumour detected only with somatostatine receptor scintigraphy (SRS)*
** *Tumour detected only with CIM*

Cadiot *et al.* compared the results of SRS with those of conventional imaging techniques including endoscopic ultrasonography, and with surgical findings in 21 consecutive patients with Zollinger-Ellison syndrome[25]. SRS added complementary information to other imaging techniques including echoendoscopy and improved the preoperative detection of extrapancreatic gastrinomas. By combining SRS with echoendoscopy they were able to detect 90 per cent of the tumours in the upper duodenopancreatic area. SRS identified metastatic disease in 20-30 per cent of patients after all other imaging techniques had failed[23]. In another study of 160 patients with biologically and/or histologically proven gastroenteropancreatic tumours, including pancreatic islet cell tumours, SRS changed the surgical therapeutic strategy in 40 patients (table 2) [26].

Table 2 Clinical impact of somatostatin receptor scintigraphy (SRS) in 160 patients with gastroenteropatic tumours.

Initial Classification	Total no.	SRS Classification I	II	III
I No metastasis	90		7	18
II Only liver metastasis	59		46	13
III Extra-hepatic metastasis	11			11

Lebtahi et al., J Nucl Med 1997:38;853-858

Unsuspected liver tumours were discovered only by SRS in seven patients, contralateral liver tumours before hepatectomy in two, and extrahepatic disease in 31 patients.

Finally, difference in SS-R expression between islet cells tumours, especially "non-functioning" tumours and pancreatic duct cancers offers the possibility of differentiating between these tumours preoperatively[27]. This is important, as palliative surgery in patients with islet cell tumours is of value not only in relieving clinical symptoms, but also in decreasing tumour burden, which might enhance the effect of medical treatment and result in improved clinical status and longer survival.

Clinical use of somatostatin analogues

Most endocrine pancreatic tumours, with the exception of insulinomas, have a malignant potential and have already metastasised at the time of diagnosis. These tumours are in general slow growing and most of the clinical distress is related to the hypersecretion of hormones, which often incapacitates the patient and causes long and repeated periods of admission to hospital. The clinical use of the somatostatin analogue octreotide in this type of patients is of considerable help in controlling symptomatology. Debilitating diarrhea, dehydration and hypokalemia (VIPoma) and necrolytic skin lesions (glucagonoma) can be well controlled during chronic treatment with octreotide. There is no doubt that octreotide therapy is of great benefit for most of these patients and improves their quality of life dramatically[28]. In selected patients peptic ulceration and hyperplasia of fundic agyrophil cells (gastrinoma) or life-threatening attacks of hypoglycemia (metastatic insulinoma) octreotide could be of therapeutic benefit[29,30]. Clinical studies in those with hormone producing islet cell tumours have shown a close parallel between the presence of SS-Rs on the tumours and the *in vivo* and *in vitro* suppressive effects of octreotide on hormone release[31]. This indicates that SRS can predict a possible suppressive effect of octreotide on hormonal hypersecretion by endocrine pancreatic tumours. Although octreotide is able to inhibit gastrin release in Zollinger-Ellison syndrome, proton-

pump inhibitors are currently the first choice as over 80 per cent of patients are controlled by omeprazole, lanzoprazole or pantoprazole. A major problem in the treatment of patients with islet-cell tumours and carcinoids with octreotide is that the inhibition of the secretion of tumour related hormones is transient. Most patients become insensitive to octreotide treatment, possibly by downregulation of SS-R expression or outgrowth of SS-R-negative clones[6,32,33]. The beneficial effects on clinical symptomatology in patients with metastatic endocrine pancreatic tumour and carcinoids are highly variable. Increasing the dose of octreotide or intermittent administration reverses these problems in most patients. The median duration of improvement of diarrhoea and flushing attacks by octreotide in patients with metastatic carcinoid disease is over 12 months[5].

Oncologic applications of somatostatin analogues

The observation that somatostatin inhibits the release of various peptide hormones has stimulated interest in its use as an antiproliferative agent. In pre-clinical studies somatostatin analogues have inhibited the growth of a wide variety of SS-R-positive as well as SS-R-negative tumours *in vivo* and *in vitro*[4,6,34-36]. An indirect tumour growth inhibition may be achieved via the inhibition of circulating tumour growth-promoting circulating hormones (GH, insulin-like growth factor 1, insulin) and inhibition of circulating, paracrine- and/or autocrine-secreted stimulatory growth factors. Somatostatin and it's analogues can also modulate the activity of immune cells[37] and potentially influence tumour blood supply; Reubi *et al.* demonstrated a high density of SS-Rs on veins in the peritumoural zone of several types of malignant tumour[38]. A potentiation of the antiproliferative effect of octreotide has also been suggested[39], but no beneficial effect of octreotide combined with tamoxifen was found in patients with metastatic breast cancer[40]. Critical to the direct antiproliferative effects of somatostatin analogues could be the presence of SS-Rs. Both cAMP-dependent and –independent effector mechanisms have been suggested[6,41-44], while stimulation of phosphotyrosine phosphatase activity may play an important role in the inhibition of growth factor-stimulated cell growth[45-47]. The results of clinical trials in patients with GEP tumours, using somatostatin analogues alone or in combination with interferon-α, are rather disappointing with a biochemical response in 77 per cent of patients with a median duration of 15 months but without any reduction of tumour size[48]. Clinically, octreotide has been the most commonly applied somatostatin analogue, yielding biochemical response rates of between 30 and 70 per cent but objective tumour shrinkage in less than 10 – 15 per cent of patients[5,49-55]. Treatment with standard doses of the somatostatin analogue lanreotide does not appear to be any better than with standard doses of octreotide. However, tumour biopsies before and during treatment with high doses of lanreotide have indicated apoptosis in responding patients[56-58]. Side effects of long-term administration of somatostatin analogues are rare. The most relevant side effect is the development of gallstones, which is believed to derive from the inhibition

of gallbladder emptying due to the inhibition of cholecystokinin release[59]. Octreotide might also influence blood sugar levels in patients with diabetes mellitus.

Peptide Receptor Radionuclide Therapy

A fascinating application could be the use of radiolabelled octreotide for Peptide Receptor Radionuclide Therapy (PRRT). Systemic injection of [^{111}In-DTPA^{0}]octreotide for SRS results is a long retention time of radioactivity in the tumours. After binding to the SS-R the ligand-receptor complex is internalised through invagination of the plasma membrane[60]. The radioligand is transported and delivered into the lysosomes[61] and the receptor then recycles back to the plasma membrane. This process is temperature dependent and takes approximately 15 min[60] and a single receptor can deliver numerous ligand molecules to the lysosomes[6,62,63]. The metabolite ^{111}In-DTPA-D-Phe is not capable of passing the lysosomal membrane resulting in a biological half-life of in human tumour tissue of > 700 hours[61,64,65]. ^{111}In not only emits gamma-rays, which can be visualised during scintigraphy, but also internal conversion- and Auger electrons having a medium to short tissue penetration (200-550 μm, 0.02-10 μm, respectively). Therefore, an effect on tumour cell proliferation could be expected, as the radiotoxicity of the radionuclide is very high if the DNA of the cell is within the particle range after internalisation[66,67]. Our hypothesis was that the same agent [^{111}In-DTPA^{0}]octreotide that is used in SRS with a low radioactive dose could be used for radionuclide therapy when given with a high radioactive dose.

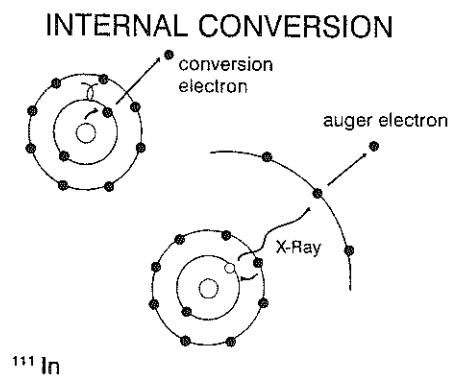

Figure 2 *Schematic representation of gamma-ray emission ("internal conversion") leading to the ejection of conversion-electrons (nuclear photons transfer their energy to orbital electrons, which are then ejected from the atom, theraby leaving an electron vacancy, indicated with the small open circle) and Auger-electrons (the latter by X-ray photon interaction with an loosely bound outer electron).*

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CHAPTER 3

SOMATOSTATIN RECEPTOR DEPENDENT GROWTH INHIBITION OF LIVER METASTASES BY OCTREOTIDE

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Abstract

Rats were administered the somatostatin analogue octreotide 15 μg intraperitoneally twice daily for 4 weeks after intraportal injection of somatostatin receptor-positive pancreatic tumour cells (CA-20948) and somatostatin receptor-negative colonic tumour cells (CC-531). Octreotide significantly inhibited the growth and development of somatostatin receptor-positive tumour cells in the liver. The median number of liver tumours was 286 (range 146 to greater than 500) in the treated animals and more than 500 (range 250 to in 3 excess of 500) in the controls ($p< 0.05$). This significant difference in tumour load was also represented in the mean (s.e.m.) liver weight (14.5±3.7) g in animals given octreotide *versus* 17.9±3.0) g in the controls). No effect of octreotide treatment was found on the growth and development of somatostatin receptor-negative tumour cells in the liver. The median (range) number of tumours was 6.5 (0-425) in the treated animals and 11.0 (0-475) in the controls. Mean (s.e.m.) liver weights were 14.0±5.7) g and 11.8±4.5) g respectively. There was no difference in serum levels of growth hormone, prolactin and insulin-like growth factor between control and octreotide treated rats. The growth inhibition of somatostatin receptor-positive tumour cells was unlikely the result of suppressed secretion of one of these tumour growth factors. Octreotide may be useful for treatment of patients with somatostatin receptor-positive hepatic metastases, which can be demonstrated by somatostatin receptor scintigraphy.

Introduction

Several possible mechanisms of action have been postulated for the tumour growth-inhibitory effect of somatostatin analogues on different tumours[1-11]. Schally et al. in 1987 demonstrated that the reduction of circulating levels of prolactin and growth hormone by somatostatin might contribute to the inhibition of breast and prostate tumour growth[12]. Moreover, somatostatin may inhibit local growth factor activity, as growth hormone stimulates cell differentiation directly and clonal expansion indirectly through local production of insuline-like growth factor[13-16]. Somatostatin can also have a direct antiproliferative effect and, furthermore, by preventing centrosomal separation inhibits the DNA synthesis and cell replication induced by epidermal growth factor (EGF)[17]. In human pancreatic cancer cells, somatostatin activates dephosphorylation of EGF receptors and thereby reverses the stimulatory effect of EGF on cell growth[18,19]. Other possible mechanisms by which somatostatin analogues may inhibit tumour growth are interference with the secretion of autocrine growth factors by tumour cells[17,20,21] and direct inhibition of angiogenesis[22,23]. An increase in natural killer cell activity in humans[24] and stimulation of the activity of the reticuloendothelial system in rats have been reported following treatmnet with somatostatin analogues. These effects indicate that a change in immunological activity may also partly explain the inhibitory effects on tumour growth[9]. Octreotide significantly reduces the growth of tumour in the liver following intraportal injection of Walker cells in rats but has no effect on the growth of tumours after injection of these cells into the thigh. This finding was explained by postulating a stimulatory effect of octreotide on the reticuloendothelial system and/or a reduction in hepatic and tumour blood flow[25]. Another somatostatin analogue (RC-160) also significantly inhibited the incidence and growth of liver metastases of colonic 320 DM and WidR human colonic cancer cells in nude mice[26].

A technique for the *in vivo* visualisation of somatostatin receptor-positive endocrine tumours and their metastases has recently been developed. A parallel between the presence of somatostatin receptors on tumours and the *in vivo* and *in vitro* effects of octreotide on hormonal release from these tumours was found, suggesting that a positive scan predicts a good suppressive effect of somatostatin analogues[27]. The present study therefore investigated whether the growth and development of hepatic metastases could be inhibited by octreotide, whether this was related to the presence of somatostatin receptors on the tumour cells and whether this was the result of inhibition of putative tumour growth factors.

Materials & Methods

Animals

Thirty-two male rats weighing 250-300g of the inbred WAG and Lewis strains were studied. The animals were bred under specific pathogen-free conditions and were 10 to 14 weeks old when used. They were kept under standard laboratory conditions (12-h light-dark cycle) and fed a standard laboratory diet (Hope Farms, Woerden, The Netherlands). The experimental protocols adhered to the rules laid down in the Dutch Animal Experimentation Act and were approved by the Committee on Animal Research of the Erasmus University, Rotterdam.

Tumours

CC-531 is a 1,2-dimethylhydrazine-induced, moderately differentiated colonic adenocarcinoma that is transplantable in syngeneic WAG rats[28]. The tumour was maintained *in vitro* in RPMI 1640 medium supplemented with 5 per cent fetal calf serum (virus and mycoplasma screened), 1 per cent penicillin 5000 units/ml, 1 per cent streptomycin 5000 units/ml and 1 per cent L-glutamine 200mmol/l (all supplements obtained from Gibco, Breda, The Netherlands). Before their use cells were trypsinised (5 min,37°C), centrifuged (5 min at 700g), resuspended in RPMI 1640 and counted before use. Viability was measured with trypan blue exclusion (0,3 per cent in a 0,9 percent sodium chloride solution) and always exceeded 95 per cent. The tumour is immunogenic as determined by the immunisation challenge method[29].

The pancreatic tumour, CA-20948, which was derived from Dr. Klijn[3], was originally induced by azaserine. The tumour is of acinar origin and is maintained in the authors laboratory as a transplantable tumour in Lewis rats. The tumours were removed from the rats, washed in ice-cold RPMI 1640 supplemented with antibiotics, and chopped into small fragments which were passed through a 18-G needle. The resulting slurry was incubated for 10 min in RPMI 1640, centrifuged at 500 g for 5 min and washed twice with cold medium. The pellet was resuspended and adjusted to a suspension containing $200x10^6$ live cells per ml.

Somatostatin receptor binding studies

Reaction conditions were the same as those described by Reubi[30]. The radioligand used in the binding studies was the radiolabelled somatostatin analogue [^{125}I-Tyr^3]octreotide (SMS 204-090; Sandoz, Basel, Switzerland). Briefly, membrane preparations corresponding to 50 µg protein of CC-531 colonic and CA-20948 pancreatic tumour cells were incubated in a total volume of 100 µl RPMI at room temperature for 90 min with 30000-50000 c.p.m. radioligand and increasing concentrations of unlabelled Tyr^3-octreotide in HEPES buffer 10mmol/l HEPES, 5mmol/l $MgCl_2$ and 0,2g/l bacitracin, pH 7,6) containing 0,2 per cent bovine serum albumin. Ice-cold HEPES buffer (1 ml, pH 7,6) was added to the

assay mixture after the incubation, and membrane-bound radioactivity was separated from unbound by centrifugation during 2 min at 14000 r.p.m. in a microcentrifuge[31]. The remaining pellet was washed twice with ice-cold HEPES buffer, and the final pellet was counted in a γ counter. Specific binding was taken to be total binding minus binding in the presence of 10 μmol/l unlabelled Tyr^3-octreotide. Unrelated compounds, such as thyrotropin-releasing hormone, luteinizing hormone-releasing hormone and EGF, added in a 1000-fold excess were not able to displace [^{125}I-Tyr^3]octreotide binding. Somatostatin receptor binding data were analyzed by Scatchard plot[32].

Induction and measurement of liver metastases

Metastatic tumours in the liver were established for both cell lines in 16 rats. A 2.5 cm median abdominal incision was made in each rat after induction of anaesthesia and $1.2x10^6$ viable CA-20948 cells in 0.6 ml Hank's balanced salt solution (HBSS) or $5x10^5$ CC-531 cells in 0.5 ml HBSS were injected slowly into the portal vein. The abdominal wall was closed with one layer of continuous silk suture. The rats were divided into groups after injection of tumour cells: group 1, CC-531 liver metastases treated with octreotide (eight WAG rats); group 2, CC-531 liver metastases used as controls (eight WAG rats); group 3, CA-20948 liver metastases treated with octreotide (eight Lewis rats): and group 4, CA-20948 liver metastases used as controls (eight Lewis rats). All rats were killed by an overdose of ether after 28 days for evaluation of the incidence of liver metastases. The livers were excised and fixed in 10 per cent formalin overnight. The visible tumours on the surface of each liver (up to 500) were counted and scored and, finally, all livers were weighed.

Treatment with the somatostatin analogue octreotide

Octreotide (D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol) (Sandoz) 15 μg was injected intraperitoneally twice daily (approximately 50 μg/kg) starting on the first day after tumour implantation in rats of groups 1 and 3. The dose of octreotide was chosen on the basis of a previous study[33] on the effects of octreotide on growth of 7315a pituitary tumour cells in rats.

Assays

Plasma of 16 rats (eight controls and eight treated with octreotide) was collected 4, 11 and 18 days after starting the administration. Prolactin and growth hormone concentrations were determined by a double antibody radioimmunoasay, as described previously[34,35]. Concentrations of IGF-1 were measured by radioimmunoassay using a commercially available kit from Medgenix Diagnostics (Brussels, Belgium).

Statistical analysis

All data are expressed as means (s.e.m.). Statistical analysis was performed using the Mann-Whitney U-test. $P<0.05$ was considered significant.

Results

Somatostatinreceptor binding studies

Specific binding of the ^{125}I-labelled somatostatin analogue Tyr^3-octreotide to membrane preparations of CA-20948 pancreatic tumour cells was demonstrated, but not to the CC-531 colonic tumour cells (figure 1). Binding [^{125}I-Tyr^3]octreotide to CA-20948 membranes could be displaced in a dose-dependent manner with unlabelled Tyr^3-octreotide. Scatchard analysis of these data revealed a single class of high affinity binding sites with an apparent dissociation constant (K_d) of 0.6 nmol/l and a maximum binding capacity (B_{max}) estimated to be 110 fmol per mg per membrane protein (table 1).

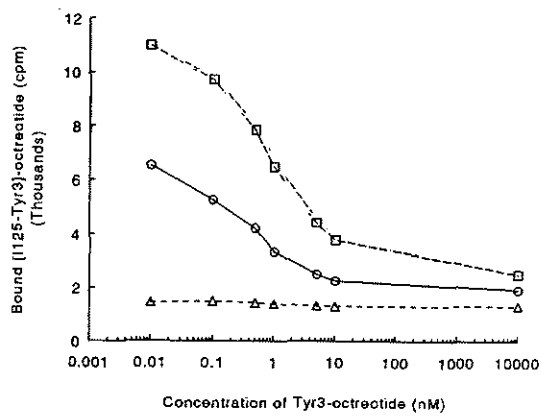

Figure 1 *Dose dependent displacement of [^{125}I-Tyr^3]octreotide binding to membranes of rat brain cortex (□), CA-20948 pancreatic (o) and CC-531 colon (Δ) tumour cells by unlabelled Tyr^3-octreotide.*

Table 1 Dissociation constant (K_d) and maximum binding capacity (B_{max}) of somatostatin receptors.

Cell type	K_d(nmol/l)	B_{max} (fmol per mg membrane protein)
CA-20948	0.6	110
CC-531	n.d.	n.d.
Brain cortex (control)	1.2	243

n.d.:not detectable

Effect of octreotide on the incidence of liver metastases

The number of hepatic metastases of CA-20948 pancreatic tumour cells was significantly reduced after 28 days of treatment with octreotide. The median number of liver tumours was 286 (range 146 to greater than 500) in treated rats and more than 500 (range 250 to in excess of 500) in controls ($P<0.05$). This difference in tumour load also recurred as a difference in mean (s.e.m.) liver weight (14.5 (3.7) g in animals given octreotide and 17.9 (3.0) in controls) (table 2). Regression analysis of liver weight and the number of metastases produced a correlation coefficient of 0.95 ($P<0.01$). Rats injected with colonic tumour cells (CC-531) showed no significant difference in the number of liver tumours (median (range) 6.5 (0 - 425) in the treated animals and 11.0 (0 - 475) in the controls) and the mean (s.e.m.) liver weight (14.0 (5.7) g in the treated animals and 11.8 (4.5) in the controls).

Table 2a Liver weight and incidence of hepatic metastases of CA-20948 and CC-531 cancer cells treated with octreotide.

Treatment	Liver weight (g) Mean (s.e.m.)	Number of tumours Median (range)
CA-20948		
Controls (n=8)	17.9 (3.0)	>500 (250->500)
Octreotide (n=8)	14.5 (3.7)*	286 (146->500)*
CC-531		
Controls (n=8)	11.8 (4.5)	11.0 (0-475)
Octreotide (n=8)	14.0 (5.7)**	6.5 (0-425)**

*$P<0.05$, **not significant (versus controls, Mann-Whitney U test)*

Table 2b Incidence of hepatic metastases of CA-20948 and CC-531 cancer cells treated with octreotide.

	Number of metastases			
Treatment	0	1-100	100-500	>500
CA-20948				
Controls (n=8)	0	0	3	5
Octreotide (n=8)	0	0	7	1
CC-531				
Controls (n=8)	2	4	2	0
Octreotide (n=8)	2	3	3	0

Effects of octreotide on growth hormone (GH), prolactin (PRL) and IGF-1 release

Plasma levels of growth hormone, prolactine and IGF-1 were not affected by chronic administration of octreotide (table 3).

Table 3 Effect of octreotide on plasma hormone levels.

	Prolactine (μg/l)	Growth hormone (μg/l)	IGF-1 (nmol/l)
Day 4			
Controls (n=8)	12.4 (8.6)	10.4 (6.3)	108.0 (11.5)
Octreotide (n=8)	11.4 (8.1)	9.5 (6.7)	99.8 (10.6)
Day 11			
Controls (n=8)	9.6 (6.4)	3.4 (1.7)	108.9 (11.0)
Octreotide (n=8)	11.7 (7.5)	2.8 (2.0)	100.9 (10.7)
Day18			
Controls (n=8)	10.3 (6.9)	5.6 (4.0)	103.8 (11.6)
Octreotide (n=8)	9.7 (7.1)	5.1 (3.1)	92.1 (13.3)

Values are means (s.e.m.)

Discussion

Several studies have demonstrated that somatostatin analogues inhibit tumour growth in experimental tumour models[1-9]. Most tumours used in these experiments contain somatostatin receptors and were studied *in vitro* and *in vivo* in transplantable lesions. Nott *et al.* reported the effects of octreotide pretreatment on hepatic tumour derived from intraportal administration of Walker cells[10,25]. They suggested that the inhibitory effect of octreotide was related to the location of the Walker cells within the liver, as the somatostatin analogue stimulated the activity of the hepatic reticuloendothelial system by 300 per cent. Kupffer cells and large granular lymphocytes form an important natural defence against malignant cells in the liver. Reduced portal venous flow and splanchnic vascular resistance in octreotide-treated animals, both of which decrease the supply of nutrients in the early stages of development of hepatic tumour, were also noted by these authors[25]. The somatostatin analogue RC-160 was also found to inhibit the incidence and growth of liver metastases from two human colonic cancers as demonstrated by a reduced 5-bromo-2'-deoxyuridine labelling index, and DNA and protein content of the tumour, suggesting a cytostatic effect of RC-160 on cellular proliferation[26].

Another possible mechanism of the antitumour action of somatostatin analogues includes the interference with secretion of growth factors such as prolactin, growth hormone and IGF-1. The reduction in prolactin levels produced by the administration of octreotide in animals may contribute to the inhibition of the growth of experimental breast and prostate tumours[12]. The fall in growth hormone levels could also directly or indirectly be of importance for growth inhibition of various tumours. Secretion of IGF-1, which mediates most of the effects of growth hormone at tissue levels and also induces differentiation and proliferation of mesenchyme derived cells, is also indirectly inhibited by somatostatin analogues[13-16]. A decrease in the concentration of IGF-1 or suppression of its activity might therefore reduce or prevent its mitogenic effects shown *in vitro*.

The results of the present study demonstrate that octreotide significantly reduces the growth and development of hepatic tumours induced by intraportal injection of somatostatin receptor-positive CA-20948 pancreatic cells, but not of somatostatin receptor-negative CC-531 colonic cells. As no difference was found in serum levels of prolactin, growth hormone and IGF-1 during treatment with the somatostatin analogue, and no inhibition of growth of somatostatin receptor-negative CC-531 hepatic metastases was observed, the mechanism by which growth of CA-20948 tumour cells is inhibited may be somatostatin receptor dependent and may not be dependent on stimulation of the reticuloendothelial system or a reduction in portal flow. If immunomodulation had taken place, growth inhibition of CC-531 cells should have occured as this tumour is weakly immunogenic[36,37] and susceptible to immunomodulation.

Recently the authors described the use of isotope labelled somatostatin analogues for the visualisation of neuroendocrine tumours and their metastases[27]. *In vitro* detection of somatostatin receptors on these tumours indicated that ligand binding to the tumour *in vivo* represents binding to

specific somatostatin receptors. The identification of such receptors predicted a good suppressive effect of octreotide on hormonal hypersecretion by these tumours. The results of the present study indicate that octreotide might be considered as a useful agent for the treatment of patients with somatostain receptor-positive liver metastases from neuroendocrine tumours, particularly since no side-effects have been observed in patients given octreotide[38].

In conclusion, the somatostatin analogue octreotide inhibits the growth and development of hepatic tumours of somatostatin receptor-positive CA-20948 pancreatic cells. Although other mechanisms can not be ruled out the present findings suggest that this may be mediated via a somatostatin receptor dependent mechanism, as no inhibition of somatostatin receptor-negative CC-531 colonic cells was found and no differences in levels of circulating hormones and growth factors were observed. Additional *in vivo* studies with tumours of different somatostatin receptor expression are needed to confirm this notion.

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CHAPTER 4

THE ANTIPROLIFERATIVE EFFECT OF RADIOLABELLED OCTREOTIDE IN A METASTASES MODEL IN THE RAT

The International Journal of Cancer 1999; 81: 767-71

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Abstract

Most neuroendocrine tumours and several other tumours such as breast carcinoma and malignant lymphoma express somatostatin receptors (SS-R). Lesions expressing these receptors can be visualised by receptor [^{111}Indium-diethylenetriaminopenta-acetic acid (^{111}In- DTPA^{0})]octreotide (Octreoscan®) ([^{111}In-DTPA^{0}]octreotide). This radioligand is internalised and transported to the lysosomes with a long residence time of ^{111}In. The aim of this experimental study in rats was to investigate whether the same agent, given in a high radioactive dose, can be used for therapy of hepatic metastases of different tumour cell lines. The development of hepatic metastases was determined 21 days after direct injection of SS-R-positive or negative tumour cells into the portal vein in rats. On day 1 and/or 8 the animals were treated with 370 MBq (0.5 μg) [^{111}In-DTPA^{0}]octreotide. In one experiment, using SS-R-positive tumour cells, animals were pretreated with a high dose of "cold" octreotide to block the SS-R by saturation. The number of SS-R-positive liver metastases was significantly decreased after treatment with [^{111}In-DTPA^{0}]octreotide. Blocking the SS-R by octreotide substantially decreased the efficacy of treatment with [^{111}In-DTPA^{0}]octreotide, suggesting that the presence of SS-R is mandatory. This was confirmed by the finding that the number of SS-R-negative liver metastases was not affected by treatment with [^{111}In-DTPA^{0}]octreotide. Therefore we conclude that: (1) high radioactive doses with [^{111}In-DTPA^{0}]octreotide for PRRT (peptide receptor radionuclide therapy) can inhibit the growth of SS-R-positive liver metastases in an animal model, (2) PRRT is only effective if SS-R are present on the tumours, (3) the effect of PRRT with [^{111}In-DTPA^{0}]octreotide can be reduced by pretreatment with "cold" octreotide, which indicates that receptor binding is essentail for PRRT. Our data suggest that PRRT with radiolabelled octreotide might be a new promising treatment modality for SS-R-positive tumours.

Introduction

Somatostatin is a small regulatory peptide which inhibits the release of various hormones and may act as neurotransmitter in the central nervous system[1]. A number of observations have also suggested an antiproliferative effect of somatostatin and its analogues[2-4]. Critical to these actions of somatostatin is the presence of somatostatin receptors (SS-Rs). SS-Rs have been demonstrated on a variety of human tumours and their metastases[5]. At least five different human SS-Rs subtypes (sst_{1-5}) have been cloned[6]. All subtypes bind somatostatin with high affinity, while their affinity for the somatostatin analogue octreotide differs considerably. Octreotide binds with high affinity to sst_2 and sst_5 and to a lesser degree sst_3, while no binding to sst_1 and sst_4 occurs. However, the vast majority of human SS-R-positive tumours express sst_2 [7]. After the injection of [^{111}In-DTPA^0]octreotide SS-R-positive tumours show uptake of radioactivity that can be visualised with a gamma camera[8,9]. ^{111}In not only emits gamma rays, which can be visualised, but also internal conversion- and Auger electrons with a medium to short tissue penetration (200-550 μm, 0.02-10 μm, respectively)[10-12]. *In vivo* ^{111}In is internalised and transported into the lysosomes of SS-R-positive cells after the administration of [^{111}In-DTPA^0]octreotide[13] with a long residence time in the tumour (biological half-life > 700 hours)[14]. Therefore, an effect on tumour cell proliferation by these electrons may be expected. Inspired by the ample knowledge of [^{111}In-DTPA^0]octreotide scintigraphy, peptide receptor radionuclide therapy (PRRT) with high doses of radioactivity labelled with this SS analogue has been initiated clinically[14-16]. In addition, three experimental studies demonstrated tumour growth inhibitory effects on solid subcutaneous tumours by radiolabelled somatostatin analogues in animal models[17-19]. This new concept of SS-R mediated, PRRT however, has never been tested for the treatment of SS-R-positive liver metastases with an ^{111}In-labelled somatostatin analogue. In a previous report we demonstrated that growth of SS-R-positive CA-20948 pancreatic tumour cells in the liver could be inhibited by subcutaneous administration of 15 μg "cold" octreotide twice per day, whereas no effect was achieved with SS-R-negative CC-531 colon carcinoma cells[20]. Using the same *in vivo* tumour models, we describe here the marked SS-R dependent efficacy of PRRT with [^{111}In-DTPA^0]octreotide to inhibit the development of SS-R-positive liver metastases.

Materials & Methods

Animals

Male rats of the inbred WAG and Lewis strain, which were 10-14 weeks old and 225-250 g, were obtained from Harlan-CPB (Austerlitz, The Netherlands). Animals were kept under standard laboratory conditions (12 hours light/12 hours dark) and were given standard laboratory diet (Hope Farms, Woerden, The Netherlands) and water *ad libitum*. The experimental protocol adhered to the rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of Erasmus University.

Tumours

The pancreatic tumour, CA-20948, was originally induced by azaserine. The SS-R-positive tumour is of acinar origin and is transplantable in syngeneic Lewis rats. The tumour was transplanted and maintained in the liver after direct injection into the portal vein. To produce artificial liver metastases, tumours were excised from donor livers, cleaned from normal liver tissue and pressed through sieves with decreasing mess size. The resulting suspension was washed twice in RPMI 1640. Viability was measured with trypan-blue exclusion (0.3% in a 0.9% NaCl-solution). A suspension of 2.5x 10^6 living cells/mL was used for direct injection into the portal vein.

Tumour CC-531 is a SS-R-negative, 1,2 dimethylhydrazine-induced, moderately differentiated colon adenocarcinoma, transplantable in syngeneic WAG rats. The tumour is maintained in tissue culture as a monolayer in RPMI 1640 medium (GIBCO, Paisly, UK.) supplemented with 5% fetal calf serum. The cells were harvested from stationary cultures by gentle trypsinisation. A suspension of 2.5x 10^6 living cells/mL was used for direct injection into the portal vein.

The presence/absence of the SS-R on both tumour cell lines was determined by specific binding of [^{125}I-Tyr^3]octreotide. Binding to membrane preparations of CA-20948 pancreatic tumour cells (IC_{50} of 0.6 nM and a B_{max} 110 fmol per mg membrane protein), but not to the membrane preparations of the CC-531 colon tumour cells was demonstrated(Van Eijck *et al.*, 1994).

Radiolabelling and quality control of the radioligand

[DTPA^0]octreotide (Pentetreotide, DRN 4920) and $^{111}InCl_3$ (DRN 4901, 370 MBq/mL in HCl, pH 1.5 - 1.9) were obtained from Mallinckrodt Medical (Petten, The Netherlands). Octreotide was a gift of Novartis, Preclinical Research (Basel, Switzerland). The labelling was performed by diluting the freeze-dried [DTPA^0]octreotide in 1 mL saline and adding this to the $^{111}InCl_3$. Thirty minutes after the start of this procedure quality control was performed by instant thin layer chromatography with silica-gel and 0.1 M sodium-citrate, pH 5 as eluent, as described earlier[21]. Labelling efficiency of [^{111}In-

DTPA^0]octreotide was over 98 %. Each administration of the radioligand consisted of 370 MBq ^{111}In labelled with 0.5 μg [DTPA^0]octreotide, referred as 370 MBq (0.5 μg) [^{111}In-DTPA^0]octreotide.

Experimental procedure

Under ether anesthesia the abdomen was opened through a 2.5 cm midline incision. Then 0.5 x 10^6 viable, SS-R-positive, CA-20948 cells in 0.2 mL RPMI 1640 were injected slowly into the portal vein through a 0.4 X 12-mm needle. The abdominal wall was closed in one layer by a continuous silk suture. The day after the operation the rats were randomised into experimental groups and control groups. Each group consisted of 5 or 6 rats.

In experiment 1, rats in the experimental group were treated with 370 MBq (0.5 μg) [^{111}In-DTPA^0]octreotide iv. on day 1 and 8. Rats in the control group were injected with vehicle, 0.5 μg [DTPA^0]octreotide iv.. In experiment 2 two groups were added in which treatment was given on day 1 or 8 alone. Experiment 3 was designed to investigate the effect of pretreatment with a SS-R blocking concentration of octreotide. The effect of 370 MBq (0.5 μg) [^{111}In-DTPA^0]octreotide iv. on day 1 and 8 was studied with and without SS-R blocking. In the group with receptor blocking rats were treated with subcutaneous (sc.) administration of 1 mg octreotide 30 min. prior to injection of the radioligand, as described earlier (Breeman *et al.*, 1994). Another 6 rats were injected with 1 mg octreotide sc. only, for comparison reasons. In experiment 4 also the importance of the SS-R in PRRT is investigated. In this experiment 0.5x 10^6, SS-R-negative, CC-531 cells in 0.2 mL RPMI were injected into the portal vein. Treatment was given according to the same schedule as used in experiment 1. All rats were sacrificed 21 days after inoculation of tumour cells. Livers were removed, immersed in phosphate-buffered saline. Tumour growth was determined by two investigators counting the number of metastases on the surface of the liver lobes (up to 100), while blinded for treatment modality.

Statistical analysis

Statistical analysis was performed using Mann-Whitney U-test on categorised outcomes and Fisher's exact test on proportions. Statistical significance was defined as $p<0.05$.

Results

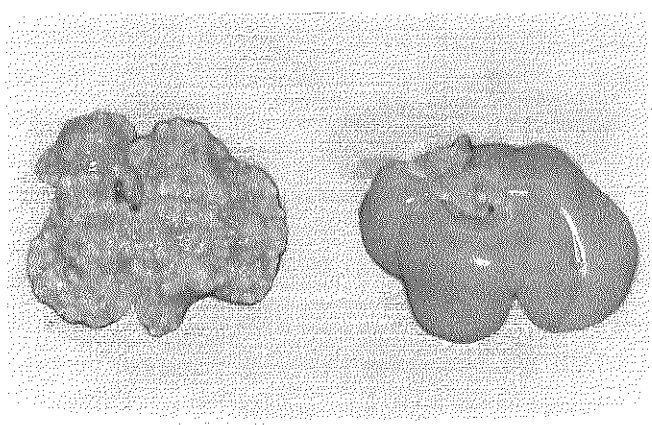
In experiment 1, PRRT with administration of 370 MBq (0.5 μg) [^{111}In-DTPA^0]octreotide on day 1 and day 8 induced a significant ($p<0.01$) decrease in the number of hepatic metastases 21 days after direct injection of CA-20948 pancreatic SS-R-positive tumour cells into the portal vein. In the control group all animals showed tumour colonies in the liver. After PRRT, tumour colonies were found in only 2 of 6 animals (table 1). Examples of livers from both experimental and control groups are shown in Figure 1.

Table 1 The effect of PRRT on SS-R-positive liver metastases.

	Number of metastases			
Treatment	0	1 -20	21 – 100	>100
Controls	-	2	2	2
PRRT	4	2	-	-

Number of animals with given range of metastases, 21 days after direct injection of SS-R-positive CA-20948 tumour cells into the portal vein. The effect of PRRT on days 1 and 8 with 370 MBq (0.5 μg) [^{111}In-DTPA^0]octreotide is significantly different ($p < 0.01$) from the effect of treatment with 0.5 μg "cold" [DTPA^0]octreotide (controls).

Figure 1 The effect of PRRT on SS-R-positive liver metastases.



Livers from the control and experimental groups (left and right, respectively); data presented in table I. Left: liver with >100 metastases (control). Right: Liver with no visible metastases after PRRT on days 1 and 8 with 370 MBq (0.5 μg) [^{111}In-DTPA^0]octreotide.

In table 2 the results are shown of experiment 2 in which two groups were added in which treatment was given on day 1 or on day 8 only. Treatment on day 1 and 8 alone also decreased tumour load significantly ($p<0.01$) while no differences could be found between treatment on either day. Treatment on both days however resulted in lesser liver colonies than when a single treatment was given ($p<0.05$).

Table 2 The effect of PRRT on SS-R-positive liver metastases, given at different times.

Treatment	Number of metastases			
	0	1 -20	21 – 100	>100
Controls	-	-	-	6
PRRT day 1	-	4	1	-
PRRT day 8	-	3	2	-
PRRT days 1 & 8	3	2	-	-

Number of animals with given range of metastases (5 or 6 animals per group), 21 days after direct injection of SS-R-positive CA-20948 tumour cells into the portal vein. PRRT with 370 MBq (0.5 μg) [^{111}In-DTPA^0]octreotide was given on day 1 or 8 or on both days 1 and 8. The effect of all treatment schedules was significantly different ($p< 0.01$) from the effect of 0.5 μg "cold" [DTPA^0]octreotide on days 1 and 8 (Controls). Treatment on days 1 and 8 was significantly different from treatment on day 8 alone ($p<0.05$). No significant difference was found between the effect of treatment on day 8 or day 1.

In the third experiment (table 3) PRRT again induced a significant decrease in the number of tumour colonies ($p <0.01$). Pretreatment with 1 mg octreotide prior to PRRT resulted in a significantly higher number of liver tumour colonies compared to PRRT without receptor blocking ($p <0.01$). In a third group that was only treated with octreotide without PRRT all rat livers contained over 100 colonies.

Table 3 The effect of pre-blocking of the SS-R with octreotide on PRRT of SS-R-positive liver metastases.

Treatment	Number of metastases			
	0	1 - 20	21 – 100	>100
PRRT	3	3	-	-
PRRT + block	-	-	4	1
Octreotide	-	-	-	6

Number of animals with given range of metastases, 21 days after direct injection of SS-R-positive CA-20948 tumour cells into the portal vein. Effect of PRRT on days 1 and 8 with 370 MBq (0.5 μg) [^{111}In-DTPA^0]octreotide without or with 1 mg octreotide subcutaneously to block the SS-R. A third group of rats were used as control to investigate the effect of 1 mg octreotide sc..
Group 1 vs. group 3: $p < 0.01$; group 1 vs. group 2: $p < 0.01$; group 2 vs. group 3: $p < 0.05$.

The results of PRRT on the formation of liver metastases after direct injection of SS-R-negative CC-531 tumour cells into the portal vein are shown in table 4. No difference in the number of liver tumour colonies was found between the experimental group and the control group.

Table 4 The effect of PRRT on SS-R-negative liver metastases.

	Number of metastases			
Treatment	0	1 -20	21 – 100	>100
Controls	-	-	3	3
PRRT	-	-	2	4

Number of animals with given range of metastases, 21 days after direct injection of SS-R-negative CC-531 tumour cells into the portal vein. The effect of PRRT on days 1 and 8 with 370 MBq (0.5 μg) [^{111}In-DTPA^{0}]octreotide is not significantly different from the effect of treatment with 0.5 μg "cold" [DTPA^{0}]octreotide (controls).

Discussion

Several studies have demonstrated that non-radioactive somatostatin analogues inhibit tumour growth in experimental tumour models[4,22,23]. Most tumours used in these experiments contain SS-Rs and were studied *in vitro* and *in vivo* as a transplantable tumour. Possible mechanisms of the antiproliferative effects of these non-radioactive analogues include stimulation of the hepatic reticuloendothelial system activity, reduction of portal venous flow and interference with secretion of growth factors such as prolactin, growth hormone and insulin-like growth factor-1[2]. Results of our previous studies however indicated that tumour growth inhibition by octreotide was mediated via SS-R, since no inhibition of tumour growth could be found when SS-R-negative tumour cells were used[20]. To demonstrate the presence of SS-Rs on tumour cells, *in vivo* peptide receptor scintigraphy with radiolabelled somatostatin analogues, such as [^{111}In-DTPA^0]octreotide has been proven to be a sensitive and specific technique. Using this technique, primary tumours and their, often unrecognised, metastases of a wide variety of human SS-R-positive tumours can be localised[9].

When PRRT with radiolabelled octreotide is considered it is important to know whether octreotide has a high affinity for SS-Rs expressed on the tumour cells and whether the radioligand is actively internalised by the same cells. Five different SS-R subtypes (sst_{1-5}) have been cloned and characterised on tumour cells, which show a distinct pharmacological binding profile to octreotide. Octreotide was found to bind with high affinity to sst_2 and sst_5 only and showed a low or no affinity to sst_3, sst_1 and sst_4, respectively. SS-R subtype expression in different types of human cancers has now been studied using in situ hybridisation, RNAse protection assays and RT-PCR[6,24,25]. These results indicate that most tumours express multiple SS-R subtypes at the same time. In the vast majority of human SS-R-positive tumours however sst_2 is predominantly expressed. Since several *in vivo* and *in vitro* studies demonstrated that [^{111}In-DTPA^0]octreotide retained high affinity to sst_2 this compound might be suitable for PRRT. The internalisation by tumour cells of the radioligand is another important aspect of PRRT with radiolabelled octreotide. Internalisation by receptor mediated endocytosis may bring the radionuclide closer to it is target, the DNA of the tumour cell. Hofland *et al.* showed that the radioiodinated [Tyr^3]octreotide is rapidly internalised by mouse AtT20 pituitary tumour cells and by the primary cultures of human GH-secreting pituitary tumour cells[25]. Duncan *et al.* reported internalisation of [^{111}In-DTPA^0]octreotide *in vivo* into the lysosomes[13]. In agreement with these studies Andersson *et al.* recently demonstrated internalisation of ^{111}In into human carcinoid and gastrinoma cells after incubation with [^{111}In-DTPA^0]octreotide[26]. PRRT with high doses of [^{111}In-DTPA^0]octreotide might therefore be effective because of the emission of Auger- and internal conversion electrons by ^{111}In[11,12]. Due to internalisation of [^{111}In-DTPA^0]octreotide the radiotoxicity of these electrons is high since the DNA of the cell is within the micrometer range from the internalised radionuclide.

We investigated the antiproliferative effect of [^{111}In-DTPA^0]octreotide on SS-R-positive and -negative tumour cells in a rat liver metastases model. The major finding from these experiments is that PRRT with [^{111}In-DTPA^0]octreotide leads to a marked inhibition of intrahepatic growth of SS-R-positive tumour cell colonies. Furthermore, in repeated experiments, most treated animals showed no SS-R-positive tumour colonies, an outcome that was not observed in earlier experiments using non-radioactive octreotide[20]. Possibly the reduced number of tumour colonies could be due to interference with either adherence or growth of the tumour cells when therapy was given on day 1, but treatment given eight days after tumour cell inoculation also led to a significant tumour growth inhibitory effect.

The results of the present experiments suggest that tumour growth inhibition was predominantly due to the specific binding of [^{111}In-DTPA^0]octreotide to SS-Rs and not to a systemic or a secondary mechanism, since PRRT had no effect on SS-R-negative tumour cells. Furthermore, pretreatment with 1 mg octreotide, resulting in a saturation of the SS-Rs, led to a diminished effect of the PRRT. The finding that blocking the SS-R did not completely abolish the therapeutic effect of PRRT may be due to the inability to completely block all the receptors by pretreatment, since there is a competitive equilibrium[22,27]. The presence or abcence of the SS-R is not the only difference between the models; however, no data are available on differences in radiosensitivity.

^{111}In, however, is not the optimal radionuclide for PRRT, since it lacks the higher energies of α- and ß-particles: the ß-particle emitter ^{90}Y with a maximum ß-energy of 2.3 MeV and a half-life of 64 hr may be more suitable. Since ^{90}Y-DTPA is not stable *in vivo*, octreotide has been derivatized with the DOTA (tetraazacyclododecanetetraacetic acid) chelator for stable radiolabelling with ^{90}Y. This resulted in the radioligand: [^{90}Y-DOTA^0,D-Phe^1,Tyr^3]octreotide, a single intraperitoneal injection of 500μCi [^{90}Y-DOTA^0,D-Phe^1,Tyr^3]octreotide led to a significant decrease (25%) in the size of SS-R-positive AR42J pancreatic tumours in nude mice[18]. Stolz *et al.* also reported the curative potential of [^{90}Y-DOTA^0,D-Phe^1,Tyr^3]octreotide for the SS-R-positive tumour CA-20948 inoculated at the hindlegs in Lewis rats[19]. The presence of sst_2 and sst_5 was reported for this model in this study.

Clinical phase I studies with this radioligand in patients with neuroendocrine tumours have already started. A third experimental study similarly demonstrated an effect on tumour growth by radiolabelled analogues in animal models. Multiple intratumour injections of ^{188}Re-labelled RC-160, another somatostatin analogue, resulted in a reduction of tumour size and prolonged survival in nude mice bearing positive PC-3 human prostatic adenocarcinomas [17]. PRRT of SS-R-positive tumours with [^{111}In-DTPA^0]octreotide has also been carried out in several patients with inoperable, metastasised neuroendocrine tumours. After multiple high doses of radioactivity of [^{111}In-DTPA^0]octreotide (up to 53 GBq) impressive effects on hormone production and a decrease in tumour volume have been observed[14,15]. Typical radiation doses to tissues with administered doses of 6000-7000 MBq [^{111}In-DTPA^0]octreotide are: kidneys 300-1400 cGy (depending on the relative biological effectiveness (RBE, 1-20) for Auger electrons), spleen 20 cGy, liver 50 cGy, bone marrow 13 cGy, thyroid gland 25 cGy, and pituitary 70

cGy[28]. In addition, within these administrated doses of 6000-7000 MBq the estimated/calculated tumour radiation dose are for a 10-g tumour (assumptions: 1% uptake; effective half-life equal to the physical half-life) 1700 and 6700 cGy (RBE for Auger electrons 1 and 20, respectively) and for a 100-g tumour (1 % uptake) 250 and 750 cGy, respectively[29]. We are currently also performing toxicology studies after 370 MBq (0.5 μg) [^{111}In-DTPA^{0}]octreotide in control rats not bearing tumours. Since we do realise that tumour load in our experiments was relatively small and the doses used relatively high, more PRRT experiments, in more advanced stages of tumour development and with different doses of [^{111}In-DTPA^{0}]octreotide, are necessary. However, our present results suggest that PRRT may be a promising new treatment modality for patients with inoperable, locally advanced or disseminated SS-R-positive tumours. The results of these experimental methods suggest that the antiproliferative effect is due to selective binding to the SS-R and not to a systemic or secondary mechanism.

Therefore we conclude that: (1) high radioactive doses with [^{111}In-DTPA^{0}]octreotide for PRRT can inhibit the growth of SS-R-positive liver metastases in an animal model, (2) PRRT is only effective if SS-R are present on the tumours, and is therefore receptor mediated and (3) the effect of PRRT with [^{111}In-DTPA^{0}]octreotide can be reduced by pre-treatment with "cold" octreotide, which indicates that receptor binding is essentail for PRRT.

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CHAPTER 5

PEPTIDE RECEPTOR RADIONUCLIDE THERAPY WITH RADIOLABELLED OCTREOTIDE; TIMING AND DOSAGE

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Abstract

Most neuroendocrine tumours and several other tumours such as breast carcinoma and malignant lymphoma express somatostatin receptors (SS-Rs). Lesions expressing these receptors can be visualised *in vivo* by receptor scintigraphy using low radioactive doses of the radiolabelled somatostatin analogue [^{111}In-DTPA^{0}]octreotide. The same agent given in a high radioactive dose can be used for Peptide Receptor Radionuclide Therapy (PRRT). The aim of this study was to investigate the effect of different radioactive doses and different treatment schedules for PRRT with [^{111}In-DTPA^{0}]octreotide.

SS-R-positive CA-20948 tumour cells were injected into the portal vein on day 0 to form tumour colonies in the liver. Tumour growth was evaluated 21 days later. In the first experiment PRRT was performed with 3.7, 37 or 370 MBq [^{111}In-DTPA^{0}]octreotide on day 1. In further experiments PRRT was performed with 370 MBq on day 1 and/or 8, day 6 or 12 and on day 7 or 14 respectively.

PRRT with 370 MBq [^{111}In-DTPA^{0}]octreotide significantly reduced tumour growth compared to controls ($p<0.01$) and was superior to doses of 3.7 and 37 MBq ($p<0.05$). Other experiments were continued with 370 MBq which was an effective dose on days 1 through 12. PRRT given twice on day 1 and 8 was more effective than single dose on day 1 or 8. There was no evidence of severe toxicity.

PRRT with high doses of [^{111}In-DTPA^{0}]octreotide is effective in SS-R-positive tumours. Also established tumours in the liver could effectively be treated and there was no severe toxicity. Therefore PRRT could be a promising treatment modality for patients with inoperable SS-R-positive tumours.

Introduction

Somatostatin is a small regulatory peptide which inhibits the release of various hormones and may act as neurotransmitter in the central nervous system[1,2]. The tetradecapeptide somatostatin itself is unsuitable for treatment, because of its very short half-life of approximately 3 minutes in man after intravenous injection. The somatostatin analogue octreotide has a half-life of 30 – 60 minutes and a more potent action than somatostatin. Octreotide has clinically been used widely in controlling symptomatology of excess hormone production of gastroenteropancreatic (GEP) tumours like carcinoid tumours, VIPoma, glucagonoma, insulinoma, and gastrinoma[3-5]. Several experimental and clinical studies also suggest an antiproliferative effect of somatostatin and its analogues[6-12]. Critical to these actions is the presence of somatostatin receptors (SS-R), which like other membrane receptors subserve two functions: (1) to recognise the ligand and bind it with high affinity and specificity, and (2) to generate a transmembrane signal that evokes a biological response. At least five different human SS-R subtypes (sst_{1-5}) have been cloned[13]. All subtypes bind somatostatin with high affinity, while their affinity for the somatostatin analogue, octreotide, differs considerably. Octreotide binds with high affinity to sst_2 and sst_5, to a lesser degree to sst_3, while no binding to sst_1 and sst_4 occurs. SS-R's have been demonstrated on a variety of human tumours and their metastases[14]. The vast majority of human SS-R-positive tumours express sst_2[15].

For the visualisation of SS-R-positive tumours *in vivo* somatostatin receptor scintigraphy with the radioactive somatostatin analogue [^{111}Indium-diethylenetriaminopenta-acetic acid (^{111}In-DTPA^0)]octreotide (Octreoscan®) ([^{111}In-DTPA^0]octreotide) is used and this technique has become an important diagnostic tool in the management of patients with SS-R-positive tumours[16-19]. ^{111}In emits not only gamma rays, which can be visualised, but also internal conversion- and Auger electrons with a medium to short tissue penetration [20-22]. With the active process of internalisation the radionuclide is brought close to its possible target, the DNA of the tumour cell. Therefore, an effect on tumour cell proliferation is to be expected. We previously reported the antiproliferative effect of Peptide Receptor Radionuclide Therapy (PRRT) with 370 MBq [^{111}In-DTPA^0]octreotide on the growth of SS-R-positive CA-20948 pancreatic tumour cells in the rat liver. We demonstrated that this effect of PRRT is SS-R dependent since pre-blocking the receptors with a high dose of non-radioactive octreotide almost completely cancelled out the effect. Moreover, no effect was achieved with SS-R-negative CC531 colon carcinoma cells[23].

In the present study we investigated the effect of different treatment schedules of PRRT with [^{111}In-DTPA^0]octreotide in a liver metastases model in the rat. Therapy was given with different doses ranging from 3.7 MBq to 370 MBq to investigate the optimal treatment dose and with different time intervals up to 14 days after tumour cell injection into the portal vein to determine whether PRRT with [^{111}In-DTPA^0]octreotide is also effective on more established tumours. To investigate short term toxicity kidney and liver tissue were histologically examined and biochemical parameters were monitored.

Materials & Methods

Animals

Male rats of the inbred Lewis strain, which were 10-14 weeks old and 225-250 g, were obtained from Harlan-CPB (Austerlitz, The Netherlands). The animals were kept under standard laboratory conditions (12 hours light/12 hours dark) and were given standard laboratory diet (Hope Farms, Woerden, The Netherlands) and water *ad libitum*. The experimental protocol adhered to the rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee of the Erasmus Medical Centre.

Tumour

The pancreatic tumour, CA-20948, was originally induced by azaserine[24]. The SS-R-positive tumour is of acinar origin and is transplantable in syngeneic Lewis rats. The tumour was transplanted and maintained in the liver after direct injection into the portal vein. To produce artificial liver metastases, tumours were excised from donor livers, cleaned from normal liver tissue and pressed through sieves with decreasing mess size. The resulting suspension was washed twice in RPMI 1640. Viability was measured with trypan-blue exclusion (0.3% in a 0.9% NaCl-solution). A suspension of 2.5x 10^6 cells/mL was used for direct injection into the portal vein.

The presence of the SS-R was demonstrated by specific binding of [^{125}I-Tyr^3]octreotide to membrane preparations of CA-20948 pancreatic tumour cells: IC_{50} of 0.6 nM and a B_{max} 110 fmol per mg membrane protein[12].

Labelled peptides

[DTPA^0]octreotide (Pentetreotide, DRN 4920) and $^{111}InCl_3$ (DRN 4901, 370 MBq/mL in HCl, pH 1.5 - 1.9) were obtained from Mallinckrodt Medical (Petten, The Netherlands). [DTPA^0]octreotide was labelled with $^{111}InCl_3$ as described previously[25 26].

Experimental procedure

On day 0 in each experiment, the abdomen was opened through a 2.5 cm midline incision under ether anaesthesia. Then 0.5 x 10^6 viable, SS-R-positive, CA-20948 cells in 0.2 mL RPMI 1640 were injected slowly into the portal vein through a 0.4 X 12-mm needle. The abdominal wall was closed in one layer by a continuous silk suture. The day after the operation the rats were randomised into experimental groups and control groups. Each group consisted of 5 to 8 rats.

The first experiment was designed to determine the minimal effective dose of PRRT. Groups of 8 animals were injected with 3.7 or 37 or 370 MBq (0.5µg) [^{111}In-DTPA^0]octreotide intravenously on day 1. In further experiments PRRT was given with 370 MBq on day 1 and/or 8, 6 and 12 and 7 and 14 respectively. All rats were sacrificed 21 days after inoculation of tumour cells. The livers were removed,

immersed in phosphate-buffered saline and then dried and weighed. Tumour growth was determined by two investigators counting and ranking the number of metastases on the surface of the liver lobes, while blinded for treatment modality. The kidneys were removed for histological examination.

Statistical analysis

Statistical analysis was performed using Mann-Whitney U-test on categorised outcomes and Fisher's exact test on proportions. Statistical significance was defined as $p< 0.05$.

Results

Dose-effect relation

The results of PRRT with different radiation doses of [^{111}In-DTPA^0]octreotide on the growth of tumours in the liver are given in table 1. PRRT with 370 MBq dose reduced the number of tumour colonies and wet liver weight compared to control animals ($p<0.05$). PRRT with 370 MBq dosage had more effect on the tumour score and wet liver weight than the 37 MBq and 3.7 MBq doses.

Table 1 Dose effect relation of PRRT with [^{111}In-DTPA^0]octreotide on the tumour score of SS-R-positive liver colonies in the rat.

Radiation dose	Liver weight (g) Mean (s.e.m.)	Tumour score 0	1+	2+	3+	4+	5+
3.7 MBq (n=8)	16.4 (3.7)	-	-	-	2	5	1
37 MBq (n=8)	17.9 (5.0)	-	-	-	-	4	4
370 MBq (n=8)	14.1 (5.8)*	-	-	4	4	-	-
Controls (n=4)	17.4 (6.0)	-	-	-	-	-	4

** $p<0.05$ versus control, 3.7 MBq and 37 MBq based on liver weight and tumour score*
Liver weight and number of animals with given range of tumour score, 21 days after direct injection of SS-R-positive CA-20948 tumour cells into the portal vein. PRRT with 3.7 MBq, 37 MBq or 370 MBq [^{111}In-DTPA^0]octreotide was given on day 1. PRRT with 370 MBq induced a significant reduction of tumour growth compared to control and other radiation doses.

Effects of PRRT with [^{111}In-DTPA^0]octreotide

Since only a significant decrease in tumour growth was found with PRRT with a dose of 370 MBq we continued our experiments with this dose. In table 2 the results of treatment on day 1 or 8 and on days 1 and 8 are expressed compared to controls. PRRT on day 1 or 8 decreased the number of

tumour colonies and the wet liver weight ($p<0.05$) compared to controls, while no difference could be found between treatment on either day.

Table 2 The effect of PRRT with 370 MBq [^{111}In-DTPA^{0}]octreotide in different treatment schedules on the tumour score of SS-R-positive liver colonies in the rat.

Treatment	Liver weight (g) Mean (s.e.m.)	Tumour score 0	1+	2+	3+	4+	5+
PRRT day 1 (n=5)	13.0 (5.6)	-	2	1	2	-	-
PRRT day 8 (n=5)	10.8 (1.1)	-	-	3	2	-	-
PRRT day 1 & 8 (n=5)	12.3 (3.3)**	3	2	-	-	-	-
Controls (n=6)	21.6 (1.7)*	-	-	-	-	-	6

** $p<0.05$ versus all PRRT groups; based on tumour score and liver weight*
*** $p<0.05$ versus control, PRRT day 1, PRRT day 8; based on tumour score*
Liver weight and number of animals with given range of tumour score, 21 days after direct injection of SS-R-positive CA-20948 tumour cells into the portal vein. PRRT with 370 MBq (0.5μg) [^{111}In-DTPA^{0}]octreotide was given on day 1 or 8 or on both days. PRRT on day 1 and 8 induced a significant reduction of tumour growth compared to control treatment. Treatment on both days was superior to treatment on either day.

Treatment on both days, however, resulted in a further decrease compared to single treatment ($p<0.05$). The results of the experiment with PRRT on days 6 or 12 are summarised in table 3. PRRT on day 6 or 12 decreased both the number of tumour colonies and wet liver weight compared to controls. In the experiment with PRRT on day 7 and 14 the antiproliferative effect was significant ($p<0.05$) based on liver weight and tumour score when PRRT was given on day 7 but not significant when given on day 14 (data not shown).

Table 3 The effect of PRRT with 370 MBq [^{111}In-DTPA^{0}]octreotide in different treatment schedules on the tumour score of SS-R-positive liver colonies in the rat.

Treatment	Liver weight (g) Mean (s.e.m.)	Tumour score 0	1+	2+	3+	4+	5+
PRRT day 6 (n=8)	11.5 (1.3)	3	-	5	-	-	-
PRRT day 12 (n=8)	9.5 (1.7)	1	-	3	4	-	-
Controls (n=8)	16.8 (3.3)*	-	-	-	-	-	8

** $p<0.05$ versus PRRT on day 6 and day 12; based on tumour score and liver weight*
Liver weight and number of animals with given range of tumour score, 21 days after direct injection of SS-R-positive CA-20948 tumour cells into the portal vein. PRRT with 370 MBq (0.5μg) [^{111}In-DTPA^{0}]octreotide was given in on day 6 or 12. PRRT on day 6 and 12 induced a significant reduction of tumour growth compared to controls that were injected with saline.

Discussion

Most carcinoid tumours and endocrine pancreatic tumours, with the exception of insulinomas, have a malignant potential and have already metastasised at the time of diagnosis[18]. These tumours are generally slow growing and most of the clinical distress is related to the hypersecretion of hormones, which often incapacitates the patient and causes long and repeated hospital stay. Since the majority of the patients have both intrahepatic and extrahepatic dissemination curative resection of the liver is only possible for a selective group though also cytoreductive hepatic surgery leads to sustained palliation of symptoms, reduces the need for additional treatment and prolongs survival in selected groups of patients[27-31]. Because a limited number of patients can be cured by surgery there is a need for systemic therapy. The clinical use of the somatostatin analogue octreotide in this type of patients is of considerable help in controlling symptomatology. Debilitating diarrhoea, dehydration and hypokalemia (VIPoma), peptic ulceration (gastrinoma), life-threatening attacks of hypoglycemia (insulinoma) and necrolytic skin lesions (glucagonoma) can be well controlled during chronic treatment with octreotide[3-5]. The observation that somatostatin inhibits the release of various peptide hormones has stimulated the interest in its use as an antiproliferative agent. In pre-clinical studies somatostatin analogues inhibit the growth of a wide variety of SS-R-positive as well as SS-R-negative tumours *in vivo* and *in vitro*[7,9,32]. We demonstrated earlier in the same tumour model as in the present paper that tumour growth of SS-R-positive tumour cells was decreased by non-radioactive octreotide while no effect was found for SS-R-negative tumour cells suggesting that the antiproliferative effect of octreotide is SS-R dependent[12].

To demonstrate the presence of SS-R on tumour cells *in vivo* somatostatin receptor scintigraphy (SRS) with [^{111}In-DTPA^{0}]octreotide has been proven to be a sensitive and specific technique. Using this technique, primary tumours and their, often unrecognised, metastases of a wide variety of human SS-R-positive tumours can be localised[16-19]. After systemic injection of [^{111}In-DTPA^{0}]octreotide the radionuclide is internalised and transported into the lysosomes by SS-R specific and temperature dependent process starting with endocytosis[33,34]. The metabolite ^{111}In-DTPA-D-Phe is not capable of passing the lysosomal membrane resulting in a biological half-life of in human tumour tissue of > 700 hours[17,35]. ^{111}In not only emits gamma-rays, which can be visualised during scintigraphy, but also internal conversion- and Auger electrons having a medium to short tissue penetration (200-550 μm, 0.02-10 μm, respectively). Therefore, an effect on tumour cell proliferation could be expected, as the radiotoxicity of the radionuclide is very high if the DNA of the cell is within the particle range[20,21]. [^{111}In-DTPA^{0}]octreotide has an appropriate distribution profile in humans to perform SRS with low doses of radioactivity and PRRT with high doses of radioactivity[36,37]. In previous studies in our model with liver metastases we found that PRRT with 370 MBq [^{111}In-DTPA^{0}]octreotide decreased the growth of SS-R-positive CA-20948 tumours. The necessity of the SS-R was proven since blocking the SS-Rs by pre-treatment with octreotide annihilated the effect of PRRT. Moreover no effect was found on SS-R-negative cells.

In the present study we demonstrated a dose-effect relation for PRRT with [^{111}In-DTPA^{0}]octreotide. The 370 MBq dose had more effect on tumour growth than 37 MBq or 3.7 MBq. The dose of 37 MBq in our rat experiments equals the dose used in humans in dose/kg which is 110-160 MBq/kg. Since PRRT with 370MBq was demonstrated to be superior to lower doses we continued our experiments with 370 MBq. In the experiments with different treatment schedules of PRRT with [^{111}In-DTPA^{0}]octreotide we confirmed that tumour growth can be inhibited with PRRT, even with a single dose 12 days after inoculation of the tumour cells. These findings suggest that even on established tumours reduction of tumour volume can be obtained and thereby support the results of case reports that describe reduction of tumour volume[36].

For clinical radiotherapeutic use the toxicity of PRRT on normal tissues should be considered. Side effects of PRRT are to be expected in organs consisting of tissues that express SS-Rs, such as adrenals and pituitary gland. Also kidney function could be influenced by PRRT since [^{111}In-DTPA^{0}]octreotide is cleared mostly excreted through the urinary system and binds selectively to the excreting tubuli which is demonstrated clinically by high uptake in normal kidney tissue . Histological examination of kidneys in our experiments with [^{111}In-DTPA^{0}]octreotide showed no alterations compared to kidneys of untreated animals. Glomerular filtration rates of creatinine and urea were not influenced by PRRT with 370 MBq (data not shown). To investigate the possible side effects our group has performed toxicity studies with PRRT with 370 MBq in non tumour-bearing rats. Preliminary results from this study with a follow up to 6 months post PRRT were that no alterations were found in hematological - and endocrinological factors; including white - and red blood cell count, platelet count and the serum level of thyroid stimulating hormone, follicle stimulating hormone, glyco-haemoglobin, alanine transaminase and aspartate transaminase. Also renal excretion profiles were not influenced (manuscript in preparation).

It has been demonstrated that the distrubution of SS-Rs is not homogeneous throughout established solid tumours. It is to be expected that heterogeneity of SS-R expression in large tumours will cause incomplete responses during PRRT with the radionuclide ^{111}In because of the short particle range of its Auger- and conversion-electrons. For radiotherapeutic applications, also other radionuclides like Yttrium-90 (^{90}Y) have been proposed for coupling to somatostatin analogues. ^{90}Y, with a half-life time of 2.7 days, is a pure β-emitter, with a tissue range up to 1 cm. The electrons have a maximum energy of 2.3 Mev. ^{90}Y shows dissociation from DTPA-conjugated peptides in serum, resulting in hematopoietic toxicity *in vivo*, therefore, Tyr^{3} –octreotide, which has a higher binding affinity for sst_2 than octreotide itself, has been derivatised with the chelator DOTA (tetracyclododecanetetraacetic acid) enabling stable radiolabelling with both ^{90}Y and ^{111}In. Preclinical studies and clinical studies with [DOTA^{0},Tyr^{3}]octreotide showed favourable biodistribution and tumour uptake characteristics[25,38]. In our model we also performed an experiment with PRRT with [^{90}Y-DOTA^{0},Tyr^{3}]octreotide. PRRT with 93 MBq [^{90}Y-DOTA^{0},Tyr^{3}]octreotide on day 1 resulted in a decrease ($p<0.05$) in tumour growth

compared to animals injected with saline (controls) and compared to animals injected with 2.5 µg non radioactive [DOTA^0,Tyr^3]octreotide (vehicle). There was no difference between control and vehicle groups. The non-radioactive vehicle [DOTA^0,Tyr^3]octreotide did not influence tumour growth compared to controls, indicating that the tumour growth inhibition of PRRT is due to the radionuclide ^{90}Y. Theoretically the β-particles of ^{90}Y might have additional beneficial characteristics, due to the effect of "cross-fire". Tumour cells lacking the SS-R might be hit by an electron coming from a neighbouring SS-R-positive cell, which has internalised the radioligand. This may lead to a high and more homogeneous radiation dose in larger parts of the tumour. Further experiments with PRRT with ^{111}In and ^{90}Y labelled somatostatin analogues on more established tumours should reveal these beneficial effects.

Three other experimental studies with radiolabelled somatostatin analogues demonstrated the antiproliferative potential of PRRT on solid subcutaneously transplanted tumours, using [^{64}Cu-TETA^0]octreotide, [^{90}Y-DOTA^0,Tyr^3]octreotide and ^{188}Re-RC-160, respectively[39-41]. In these experiments therapy was given by intratumour or intraperitoneal injection, resulting in a significant decrease in tumour size. We also demonstrated tumour growth inhibition of subcutaneous SS-R-positive tumours as has been published previously[42]. In these experiments PRRT was given intravenously. In addition, PRRT with high radioactive doses of [^{111}In-DTPA^0]octreotide or [^{90}Y-DOTA^0,Tyr^3]octreotide has been initiated clinically [37,43-46].

New developments in molecular biology have made it possible to transfect SS-R-negative tumour cells with a SS-R-gene. The group of Susini has developed an approach using sst_2-gene transfer for the treatment of pancreatic cancer[47]. By inducing the SS-R on SS-R-negative tumours, treatment with PRRT might be possible. Moreover, transfection of SS-R-positive tumours with a SS-R-gene can increase the homogeneity of distribution of tumour cells expressing the SS-R and thereby increase the efficacy of PRRT. We are currently investigating this strategy of treatment of cancer with SS-R-negative colon adenocarcinoma cells CC531 in our model.

Peptide receptor radionuclide therapy (PRRT) with intravenously administered radiolabelled somatostatin analogues is effective in our liver metastases model in the rat. In our experimental model there were no signs of acute toxicity. Further investigations will focus on treatment of SS-R-negative tumours and treatment with other radionuclides. Our preclinical studies will help to optimise PRRT clinically.

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CHAPTER 6

LIVER REGENERATION AND TUMOUR GROWTH: AN INTRODUCTION

Introduction

Surgical excision of liver tumours represents the only curative treatment for primary and metastatic liver malignancies. This operation in which the tumours in the liver are excised together with surrounding normal liver tissue is called partial hepatectomy (PHx). For colorectal tumours the results of PHx compare favourably with the natural outcome for selected groups of patients[1]. However, despite the curative potential of PHx, recurrence has been reported in 65-80 % of the patients[2]. The predominant site of recurrence is within the liver remnant and the majority of recurrence is diagnosed within the first year after the operation[1,3-6]. The high number of early recurrences suggests that PHx might act as a 'double-edged sword'. On one hand survival after PHx is increased but on the other hand resection might provoke enhanced tumour growth.

Several groups have found experimental evidence for stimulation of tumour growth by PHx in various animal models (table 1); both intrahepatic and extrahepatic lesions were found to grow more quickly after the operation[7-27]. Clinical support for this theory was published recently. Elias *et al.*[28] demonstrated that during liver regeneration following right portal embolisation the growth rate of liver metastases in the left liver lobes was increased and even more rapid than the growth of liver parenchyma. These findings are in agreement with studies in rats which demonstrate accelerated tumour growth in regenerating liver lobes and inhibited tumour growth in atrophied liver segments after selective portal embolisation[29]. Various explanations for this phenomenon have been described. Growth factors, like hepatocyte growth factor (HGF), transforming growth factor-alpha (TGFα), epidermal growth factor (EGF) and other growth regulators, produced by the regenerating liver, that orchestrate the process of liver regeneration, have also been implicated as tumour growth promoting agents [30]. Other possible mechanisms include: the detrimental effect of surgical manipulation[31], the generalised immunosuppression provoked by PHx[13,32], and impaired Kupffer cell function[33-35].

If this phenomenon of increased tumour growth after PHx can clinically be substantiated early systemic chemotherapy after PHx could be of great benefit [36-38]. However, this adjuvant treatment should not interfere with liver regeneration[39]since it is the unique capacity of the liver to regenerate that enables the patient to recover from extensive liver loss.

Liver regeneration

The ancient Greeks recognised liver regeneration in the myth of Prometheus. Having stolen the secret of fire from the gods of Olympus, Prometheus was condemned to having a portion of his liver eaten daily by an eagle. His liver regenerated overnight, thus providing the eagle with eternal food and Prometheus with eternal torture[40]. In those days they must have known that the mammalian liver has the unique ability to restore its volume after tissue loss. In present times we are able to

quantify liver regeneration since there are techniques available, like computerised tomography and magnitic resonance imaging. Restoration of liver volume in human is complete within one month. In a group of patients that underwent right hepatectomy in a living donor liver transplantation program the mean residual liver mass was 600 g. The average liver volume was doubled in 7 days and the remnant liver reached its maximum volume after 30 days[41]. However, in adults, after PHx the liver does not always regain its pre-operative size[42].

If, in the rat, two thirds of the liver volume is removed the residual liver tissue induces compensatory hyperplasia to regain the original volume within 10 days. The magnitude of this regeneration process is proportional to the volume removed or damaged[43]. The core reference for experimental studies on liver regeneration in the rat is the work of Higgins and Anderson, published in 1931[44]. They described a simple technique to resect 70% of the liver volume by ligating and dissecting the left lateral and median liver lobes at their vascular pedicle (70% PHx). Regeneration after PHx is a precise, well orchestrated process involving a complex scheme of stimulatory and inhibitory effects on cell growth. The role of polypeptide growth factors has been investigated most. The numerous growth regulators that are involved in hepatic regeneration are listed in table 1 [45]. Liver regeneration after PHx is carried out by proliferation of all the existing mature cellular populations composing the intact organ. These include hepatocytes, biliary epthelial cells, fenestrated endothelial cells, Kupffer cells and hepatic stellate cells. Hepatocytes, are the first to proliferate and most of the hepatocytes participate; 95% in young - and 75% in very old rats[46]. 24 hours after PHx there is a peak in DNA synthesis followed by a smaller peak between 36 and 48 hours. The first peak of DNA synthesis of the other cells of the liver is after 48 hours[40]. The tissue and serum levels of most growth regulating substances show a peak within a few hours after PHx and have become normal within 48 hours. Since the first 48 hours of liver regeneration are so outspoken the putative effect of regeneration on tumour growth is expected to occur most within these 48 hours.

Table 1 Hepatic growth regulators.

Polypeptide Growth Factors:	Hepatocyte growth factor
	Epidermal growth factor
	Transforming growth factor α
	Insulin-like growth factors and growth hormone
	Tumour necrosis factor α
	Augmenter of liver regeneration
	Heparin-binding growth factors
	Fibroblast growth factors
	Hepatic stimulatory substance
	Hepatopoietins
	Keratinocyte growth factor
	Miscellaneous hepatotrophic factors
Hormones and Nutritients:	Adrenal cortical hormones
	Catecholamines
	Estrogens and androgens
	Insulin and glucagon
	Nutrients
	Parathyroid hormone, calcium, vitamin D
	Prolactin
	Prostaglandins
	Thyroid hormones
	Vasopressin
Growth Inhibitory Factors:	Transforming growth factor β
	Hepatocyte proliferation inhibitor
	Interleukin 1β
	Other growth inhibitors

Source: Kren et al. 1997, Ann N Y Acad Sci

An interesting phenomenon is the existence of stem cells. Ductular oval cells in the liver have the possibility of differentiation into hepatocytes under appropriate experimental conditions[47]. Also it has been shown in animal models that hepatocytes and ductal cells can derive from bone marrow cells[48]. In patients that had undergone bone marrow transplantations, populations of hepatocytes of

donor origin were found in the livers at autopsy[49]. In non-regenerative conditions liver repopulation occurs when transplanted cells have a growth advantage over damaged recipient liver cells[50]. Significant contribution of the stem cells to the regeneration process only occurs under circumstances in which the residual differentiated cells are functionally compromised and/or cannot proliferate[51].

Origin of recurrent liver metastastases

There are different theories about the origin of new metastases in the liver that give rise to recurrences. Tumour cells can spread from a remnant of the primary tumour or from extra hepatic secondaries to form *de novo* metastases in the liver. Also tumour cells that are spread during PHx can settle again in the liver[5253]. But most likely (micro)metastases in the liver that had not been detected by pre-operative imaging and remained undetected at laparotomy (occult metastases) grow out to form detectable metastases[54,55]. It has been suggested that most of the occult metastases are non-proliferating single tumour cells that are spread throughout the liver[16]. These "dormant" tumour cells could be triggered to start proliferating by changes in extracellular host factors. Clinical studies of tumour recurrence in melanoma show that single tumour cells or small groups of tumour cells can undergo a period of dormancy followed by rapid growth during relapse[56]. This could be explained by a balance between proliferation and apoptosis of tumour cells[57] or by the lack of vascularisation of the tumour which is necessary to enlarge to a size beyond a few mm[58]. Liver regeneration after partial resection could provide such a trigger for metastatic growth and act as an alarm clock, awaking the dormant tumours.

Mechanisms of increased tumourgrowth after partial hepatecomy

Hepatocyte growth factor

Hepatocyte Growth Factor (HGF) also known as Scatter Factor has been identified in 1984 by Nakamura et al.[59] as a specific factor to trigger liver regeneration. HGF is produced in many cell types in the liver including Kupffer cells, endothelial cells, fibroblasts and fat storing cells[60]. Following PHx there is a marked increase in the level of circulating HGF and increased expression of HGF mRNA in liver cells but also in cells of other organs, e.g. the kidney[61,62]. Infusion of HGF is known to accelerate liver regeneration. HGF may be important in the growth and development of cancer metastases by several mechanisms. Over-expression of HGF receptors on the tumour cells might increase motility and alter the invasive nature of malignant cells[60]. Recombinant human HGF ,injected directly into the tumours, increased proliferation of the established tumours of three hepatoblastoma cell lines[63]. There is also evidence of paracrine and autocrine regulation by HGF[60,64,65]. HGF may also stimulate primary and secondary tumour growth by stimulation of angiogenesis[66,67].

Transforming Growth Factor and Epitelial Growth Factor

De Jong and Lont *et al.* describe extensively the putative role of transforming growth factor (TGF-α) and epithelial growth factor (EGF)[18]. Both the level of TGF-α and EGF are increased during liver regeneration[68,69].These growth factors stimulate hepatocyte growth *in vivo* and *in vitro*. Expression of EGF receptor and production of TGF-α that can result in autocrine and paracrine stimulation was found in various colorectal cancers[18]. The role of TGF-β is more complex. It appears to be the main negative growth regulator peptide of the regenerating process[70]. Picardo et al. found that TGF-α and TGF-β levels within the regenerating liver in the rat are increased[23]. *In vitro* experiments with Morris hepatoma cells showed a growth stimulation with TGF-α but a growth inhibition of TGF-β. TGF-β, however, in other experiments has shown to be a promotor of tumour growth[71,72]. TGF-β might therefore be a bifunctional regulator of cellular growth[73,74] of which its impact on tumour growth in conditions of regeneretion is yet to be elucidated.

Other growth factors

Insulin-like growth factor-1 and -2, (IGF) production by the liver during regeneration is not increased. There is evidence to suggest, however that IGF may play a role in regulation of liver growth as autocrine factors since IGF-1 receptors are expressed in regenerating rat liver and not in non-proliferating cells from rat and human livers[45]. Basic fibroblast growth factor (bFGF) plays a role in angiogenesis and influences tumour growth by promoting vascularisation. Also a direct stimulating effect on various colon carcinoma cell lines has been demonstrated[75].

Surgical trauma

The relationship between tumour growth and wound or tissue healing is an area that has particular relevance to all surgeons with an interest in cancer management. In a series of experiments with over 800 rats Fisher and Fischer in 1959 found that repeated laparotomy increased the incidence of metastases to the liver from 24% to 47 %. If manipualation of the liver was added to the repeated laparotomy the incidence was as high as 84%[31]. The impact of surgical trauma might be limited in the experiments listed in table 2, which all use the procedure described by Higgins and Anderson in 1931. In this procedure there is only limited manipulation of the remnant liver. The liver consists of 7 lobes, each with separate vascularisation and biliary ducts. During PHx the entire left-lateral and median lobes are removed by ligating their vascular pedicles. The lobes of the remnant liver are left intact and do not have wound surfaces. Furthermore, the experiments on liver regeneration and tumour growth were carried out with sham operated control animals with equivalent manipulation to the liver.

Table 2 Effects of partial hepatectomy on the growth of tumour within the liver or at extra hepatic locations in rats and mice.

Reference	Method	Tumour growth within liver	Extra hepatic tumour growth
Paschkis[7] 1955	70% PHx	-	↑ subcutaneous
Fisher[8] 1959	70% PHx	↑	-
Gershbein[9] 1963	70% PHx	↑	= subcutaneous
Ichihashi[10] 1984	70% PHx	↑ solid	-
Ramantanis[11] 1985	sera or spleen cells of 70% PHx rats iv.	-	↑ subcutaneous
Van Dale[12] 1988	70% PHx	↑ i.po.	↑ bulbus vestibuli
Panis[13] 1990	70% PHx	= i.po.	-
Loizidou[14] 1991	70% PHx	↑ i.po.	-
Asaga[15] 1991	70% + 35% PHx	-	↑
Panis[16] 1992	70% PHx	↑ i.po.	-
Mizutani[17] 1992	70% PHx	↑ i.po.	-
De Jong[18] 1995	70% PHx	↑ solid	= retroperetoneal = renal subcapsular
Karpoff [19] 1996	70% PHx	↑	-
Loizidou[20] 1996	Fibroblasts of 70% PHx rats	-	↑ subcutaneous
Schindel[21] 1997	70% PHx	↑ solid	↑ subcutaneous
Ikeda[22] 1997	70% PHx	-	↑ lung
Picardo[23] 1998	70% + 30% PHx	↑ i.po.	= lung
Ono[24] 1986	70% Phx	-	↓ subcutaneous
Leith[25] 1992	70% PHx	-	↑ subcutaneous
Morimoto[26] 1992	40% PHx	↑	↑ lung
Gutman[27] 1994	70% PHx	-	↑ subcutaneous

(↑) increased tumour growth, (↓) inhibition of tumour growth,
(=) no change in tumour growth, (-) not investigated
(solid) outgrowth of solid tumour implanted in the remnant liver
(i.po.) tumour growth within the liver after injection of tumour into the portal vein

Immunosuppression

Panis *et al.* performed several studies with induction of tumour growth in the liver in which immunosuppression was induced by Cylosporin A[13,16,32]. In one experiment they used a group of rats that had no detectable liver metastases 8 weeks after portal injection of colon carcinoma cells. They found a similar increase in development of liver tumours after PHx or after Cyclosporin A treatment within the same experiment[16]. However, a study by Ono *et al.* in mice disagrees with these findings since they found that PHx did not lead to stimulation of tumour growth but rather to growth inhibition. They transplanted 3 different tumour cell lines subcutaneously 3 to 10 days after PHx and found complete regression of a hepatoma cell line whereas no effect on tumour growth was seen on two other cell lines. Activation of killer cells and natural killer cells were *in vitro* induced by PHx, suggesting an immunostimulatory effect[24]. At least three other studies in mice oppose the findings of Ono *et al.* since they show an increased growth of subcutaneously implanted tumours in similar experiments with subcutaneous tumours[25-27]. A recent study of Jarnagain *et al.*[76] demonstrated an effect of immunomodulation on tumour growth after PHx. They found that neoadjuvant interleukin-12 immunogene therapy protects against cancer recurrence. In experiments with colon adenocarcinoma CC531 immunosuppression could be an explanation for the observed effect of PHx since this tumour is weakly immunogenic[77]. Our research group demonstrated earlier that CC531 exhibits enhanced growth when recipients are treated with Cyclosporine A[78].

Kupffer cell function

Kupffer cells are the representatives in the liver of the reticulo endothelial system (RES). Via the formation of glycoprotein, lymfokines and growth factors, Kupffer cells can direct the production of proteins by hepatocytes and their ability to proliferate. Kupffer cells are important for hepatocyte regeneration after PHx. In experiments with Kupffer cell-depleted rats the production of growth-stimulating cytokines is imbalanced and liver regeneration impaired[79]. Kupffer cells are suggested to play a relevant role in arresting circulating tumour cells. They also seem to play a major role in clearing neoplastic cells from the liver parenchyma, in controlling tumour growth in the very early stages of metastatic development and in modulating the host immune response to cancer cells[80]. Heuff et al.[35] found, in a liver metastases model with CC531 tumour cells, that selective elimination of Kupffer cells resulted in a strongly enhanced growth of liver metastases. Karpoff et al[19] found that Kupffer cell stimulation by interferon γ (IFN-γ) attenuated the tumour growth enhancement of PHx *in vivo* which, *in vitro*, is associated with a significant enhancement of Kupffer cell mediated tumour activity. Although not based on direct evidence it is tempting to speculate that PHx leads to a depression of the natural cytotoxicity normally exhibited by Kupffer cells, resulting in accelerated tumour growth.

Systemic treatment after partial hepatectomy

The initial postoperative period after PHx is not only critical for the formation of metastatic lesions, but is also important for the regeneration of the resected liver. Applied antitumour strategies during rat liver regeneration are listed in table 3[10,17,19,21,81,82].

Table 3 Effects on tumour growth of adjuvant treatment during liver regeneration after 70% partial hepatectomy in rats.

Reference	Treatment	Dose	Schedule		Result
Ichihashi[10] 1984	Mitomycin C	0.4 mg/kg	3x iv.	Day 0 – 2	↓ tumour in liver
Mizutani[17] 1992	Mitomycin C	0.2 mg/kg	3x iv.	Day 0 – 2	↓ tumour in liver
	5 fluorouracil	20 mg/kg	3x iv.	Day 0 – 2	↓ tumour in liver
Karpoff[19] 1996	IFN γ	$5x10^4$ U	4x i.p.	Day -3 – 0; or Day 0 – 3	↓ tumour in liver
Schindel[21] 1997	Octreotide	50 µg/kg	2 dd, i.p.	Day 0 – 15	↓ tumour in liver
					↓ tumour sucutaneously
Davies[81] 1997	Octreotide	2 µg	2 dd, s.c.	Day 0 – 21	↓ tumour in liver
Ikeda[82] 1998	Cilostazol	50 mg/kg	5x orally	Day 0 – 8	↑ survival

(↑) increased tumour growth, (↓) inhibition of tumour growth

The initial period after PHx could theoretically be favourable for chemotherapy since the rapidly dividing tumour cells are more susceptible to several cytostatic agents and the tumour burden of occult metastases, after resection of the larger lesion, is low[37,38]. However, adjuvant treatment aiming on reduction of tumour growth stimulation caused by PHx will also impair regeneration and is therefore not recommended clinically [83,84]. Currently routine practice is to wait 4 weeks after resection before initiating adjuvant treatment for fear of detrimentally altering the process of altering DNA synthesis[39]. Several reports on experimental studies with adjuvant chemotherapy during liver regeneration describe notable adverse side effects[85,86]. 5-fluorouracil (5-FU) was found to reduce the tumour growth stimulation by 70 per cent PHx but also resulted in a lower wet weight of the remaining liver[17]. Mitomycin C (MMC) is found to be effective with less impact on regeneration. Therefore the effect of MMC may not be due to suppression of hepatic regeneration but to a direct cytocidal effect against rapidly dividing tumour cells[10,17]. Doxorubicin administered, by a single intra hepatic artery injection one day after 70 per cent PHx, did not increase survival. In this experiment

tumour cells were injected into the portal vein directly after PHx. In the same experiment, cilostazol, a cyclic AMP phosphodiesterase inhibitor and a suppressor of platelet aggregation was found to increase survival when given 24 hours before PHx and tumour cell injection. This effect might be due to a decrease in tumour cell lodging which plays an important role in the early stages of development of metastases[82].

IFN-γ treatment 3 days prior to PHx attenuated the tumour growth enhancement. Growth factor release and liver regeneration were not affected. The effect of IFN-γ on tumour growth is associated with significant enhancement of Kupffer cell mediated tumouricidal activity[19].

The proinflamatory cytokine tumour necrosis factor α (TNF-α) could be of benefit after PHx since TNF is known to stimulate rather than impair liver regeneration[87-89]. After PHx TNF-α is one of the earliest cytokines to be induced. It stimulates the production of other cytokines, including Il-6, which is required for hepatocyte proliferation[87,90]. TNF-α has shown antitumour activity on colon cancer in rats[91]. Therefore treatment with TNF-α could be an interesting option after Phx. However, in the clinical situation the use of TNF is limited to isolated perfusion because of severe toxicity after systemic administration[92,93].

New treatment options

Somatostatin analogues

Octreotide, a long acting somatostatin analogue, was found to reduce the enhanced tumour growth by PHx in different models with intrahepatic - and subcutaneous tumours[21,81]. Octreotide is suggested to influence tumour growth in the regenerating liver through various pathways. Liver regeneration is reduced by octreotide treatment started before PHx, possibly by an indirect effect via suppression of insulin levels[94] or inhibition of the release and end organ effect of many growth factors and cytokines[95]. Octreotide increases hepatic RES activity probably by stimulation of Kupffer cells. Furthermore, portal pressure after 70 per cent PHx is reduced by continuous infusion of octreotide[81]. The tumour growth inhibiting effect of somatostatin analogues could also be caused by a direct effect on the tumour cells mediated through binding of the analogue to specific somatostatin receptors as was demonstrated by the present authors[96]. Somatostatin receptors on tumours are *in vivo* demonstrated by somatostatin receptor scintigraphy in which a low dose of radiolabelled octreotide is used. This binding to the somatostatin receptor is highly selective. Therefore high doses of radiolabelled octreotide could theoretically have a local irradiation effect on the tumours as was demonstrated earlier[97]. The tumour growth inhibiting effect of octreotide and radiolabelled octreotide could be of benefit in patients with SS-R-positive tumours of which neuroendocrine tumours are the most common.

In patients with liver metastases of neuroendocrine tumours liver resections are performed with both curative and palliative intent[98]. While most tumour tissue in the liver is removed surgically adjuvant treatment can exert it's antitumour effect on residual intrahepatic tumour tissue and extrahepatic secondaries. However, if treatment with octreotide or radiolabelled octreotide after PHx demonstrates an antitumour effect it is important to investigate the effects on liver regeneration since octreotide is reported to suppress liver regeneration which could impair functional recovery clinically[81,99].

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CHAPTER 7

TUMOUR GROWTH STIMULATION AFTER PARTIAL HEPATECTOMY CAN BE REDUCED BY TREATMENT WITH TUMOUR NECROSIS FACTOR

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Abstract

This study investigated whether partial hepatectomy enhances the growth of experimental liver metastases of colonic carcinoma in rats and whether treatment with recombinant human tumour necrosis factor (TNF-α) can reduce this increased growth. Resection of 35 or 70 per cent of the liver was performed in inbred WAG rats with sham operated controls. Immediately after surgery 5 x 10^5 CC531 colonic tumour cells were injected into the portal vein. After 28 days the animals were killed and the number of liver metastases counted. A 35 per cent hepatectomy induced a significant increase in the number of liver colonies (median 28 colonies, *versus* 3 in controls), whereas a 70 per cent resection provoked excessive growth, consistently leading to more than 100 liver metastases and a significantly increased wet liver weight in all animals. TNF-α was given intravenously to rats following PHx 70% or sham operation in a dose of 160 μg/kg, three times per week until sacrifice. Treatment with TNF-α was only marginally effective on tumour development in sham operated rats, but was very effective following 70 per cent hepatectomy or sham operation in a dose of 160μg/kg three times per week. This had only a marginal effect on tumour development in sham operated rats but was very effective following partial hepatectomy (median 45 liver metastases). These observations confirm previous findings that surgical metastasectomy may act as a 'double-edged sword' by provoking outgrowth of dormant tumour cells and suggest that adjuvant treatment with TNF-α may be of benefit in patients undergoing resection of metastases.

Introduction

Surgical excision by partial hepatectomy is the only available method to prolong survival for patients with hepatic metastases from colorectal carcinoma. The 5-year survival rate of patients that are eligible for surgical resection (i.e. those with a solitary metastasis or small tumour burden confined to the liver) is 25-40 per cent[1-4]. These results compare favourably with the natural outcome for this selective group of patients, who have a 5-year survival rate of less than 1 per cent[1,4]. However, despite the curative potential of hepatic resection, recurrence has been reported in 65-80 per cent of patients[2-4], the majority being diagnosed within the first year after operation[2,3]. This suggests that the first period after resection may be of crucial importance for prognosis.

It is obvious that the favourable survival rates achieved by surgery validate a continued policy of resection of metastatic liver disease. On the other hand, the high number of early recurrences suggests that resection might provoke enhanced tumour growth of occult metastases. Experimental research may provide clues for a modified surgical strategy or appropriately timed adjuvant therapy. Several groups have found experimental evidence for tumour growth stimulation by partial hepatectomy in various animal models; both intrahepatic lesions and subcutaneously transplanted tumours were found to grow more quickly after this operation[5-9]. There are various explanations for this phenomenon. Growth factors produced by the regenerating liver, such as hepatocyte growth factor, transforming growth factor-ß and epidermal growth factor, have been implicated as tumour growth promoting agents[10-12]. Other possible mechanisms include the detrimental effect of surgical manipulation[13], the generalised immunosuppression provoked by partial resection[14], and impaired Kupffer cell function[15].

The aim of the present study was to investigate whether evidence could be found for enhanced growth of fresh liver metastases after partial resection in a rat model of colonic adenocarcinoma. Furthermore, it was investigated whether the putative growth stimulation could be inhibited or reversed by adjuvant treatment TNF-α, an agent with proven efficacy against the colonic tumour under investigation[16].

Materials & Methods

Animals and Tumour

Male inbred WAG rats, 10-14 weeks old, weighing between 250 - 275 g, and bred under specific pathogen-free conditions were used. The rats were given standard rat food and water *ad libitum*. The experimental protocol adhered to the rules laid down by The Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of Erasmus University. Tumour CC531 is a moderately differentiated, weakly immunogenic colonic adenocarcinoma, induced in a WAG rat by 1,2-dimethylhydrazine[17]. The tumour is maintained in tissue culture as a monolayer in RPMI 1640 medium (Gibco, Paisley, U.K.), supplemented with 5 per cent fetal calf serum. A total of 5 x 10^5 cultured tumour cells were injected into the portal vein. The cells were harvested from stationary cultures by gentle trypsinisation, providing cell suspensions with a viability greater than 95 per cent. Tumour CC531 is relatively insensitive to chemotherapy, but sensitive to the effects of biological response modifiers like the interferon-inducer Bropirimine, Interferon γ and TNF-α[16].

Experimental procedure

Under ether anaesthesia the abdomen was opened through a midline incision. The left lateral and median liver lobes, representing 70 per cent of the liver volume, were freed of fibrous attachments and exteriorised. The circulation in these lobes was temporary interrupted by ligation of the hilar vessels. CC531 tumour cells in 0.2 ml. RPMI 1640 were injected into the portal vein through a 0.4 x 12 mm. needle. Over 2 minutes the tumour cells were directed through the remaining 30 per cent of the liver that was not ligated. The rats were then randomised into a partial hepatectomy group and a non-hepatectomy (sham) group. In the 70 per cent hepatectomy group the temporary ligation was replaced by a permanent 2/0 silk tie and the ligated lobes were resected. In the 35 per cent hepatectomy group only the left lateral lobe was resected. In the sham group the temporary ligation was removed to re-establish the circulation. The liver was then returned to the peritoneal cavity and the laparotomy wound closed in one layer.

Rats were killed after 28 days by an overdose of ether. The liver was removed, immersed in phosphate buffered saline (PBS), and tumour growth was determined by counting the number of colonies at the surface of the liver lobes. The livers were put on blotting paper for 30 seconds to remove excess of PBS and were than weighed immediately. Experimental groups contained five to eight animals.

Tumour Necrosis Factor α

TNF-α was provided in lyophilized form by Knoll (Ludwigshafen, Germany). The product was 99 per cent pure, contained less than 10 pg. of endotoxin per mg protein and had a specific

activity of 6.63 X 10^6 Units/mg. It was reconstituted in PBS prior to injection and was given intravenously in a dose of 160 μg/kg in 0.2 ml PBS, three times per week, starting on the day of operation. Control treatment consisted of 0.2 ml PBS according to the same schedule. On completion of sham operation or 70 per cent hepatectomy the rats were randomised and assigned to control or TNF-α treatment.

Statistical analysis

The number of tumour colonies at the surface of the liver lobes was counted. When more than 100 metastases were present, the number was scored as >100. Data were analysed with the Mann-Whitney U-test for comparison of non-parametric data and expressed as median (range). The mean liver weight was compared using Student's unpaired *t* test. Significance was accepted at $p < 0.05$.

Results

Effect of partial hepatectomy on tumour growth

The number of metastases 28 days after 35 or 70 per cent hepatectomy are shown in table 1. A 35 per cent resection induced a significant increase in the number of metastases ($p=0.044$) but not in wet liver weight. The number of colonies in the control group ranged from 0 to 20 (median 3), and from 10 to 57 (median 28) in the 35 per cent hepatectomy group. A 70 per cent resection induced an even greater increase in tumour development. In all rats receiving this operation the number of tumour colonies exceeded 100, whereas that in controls ranged from 0 to 31 (median 7).

Table 1 Liver metastases and wet liver weight 28 days after tumour cell injection.

Operation	Incidence	No. of metastastases Median (range)	Wet liver weight (g) Mean (S.D.)
30 % PHx	6 of 6	> 100	22.5 (8.9)‡
Sham*	5 of 6	7 (0 – 31)	10.6 (0.9)
70 % PHx	5 of 5	28 (10 – 57)†	10.5 (1.3)
Sham*	5 of 6	3 (0 – 20)	10.2 (0.3)

** Remaining lobes ligated during injection of tumour cells, but not removed.*
† $P < 0.05$ versus sham (Mann – Whitney U test)
‡ $P < 0.05$ versus sham (Student's t test)

Following 70 per cent hepatectomy there also was a significant increase in wet liver weight ($p= 0.006$). The experiment involving 70 per cent resection was repeated four times showing similar, significant results. Figure 1 shows the explosive tumour growth in a 70 per cent resected liver and can

be compared with the appearance of a control liver in figure 2. In sham operated rats no tumour was ever found in the lobes ligated during the tumour cell injection.

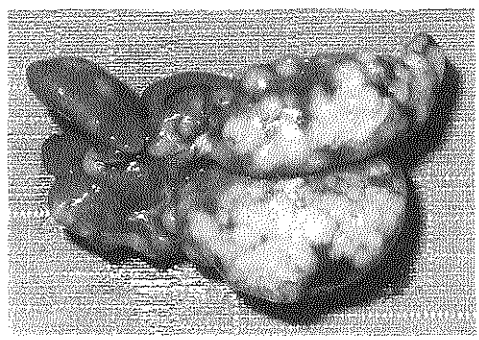

Figure 1

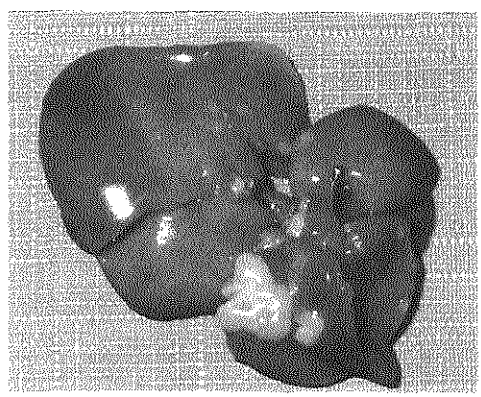

Figure 2

Antitumour effect of TNF-α

The results of treatment of sham operated and 70 per cent hepatectomised rats with TNF-α are shown in table 2. In the sham operated group treatment with TNF-α had no significant effect on tumour growth compared with that in non-treated sham operated controls (0-7 *versus* 0->100 colonies respectively). Following 70 per cent resection, treatment with TNF-α was very effective and resulted in a significant inhibition of tumour growth: range 5->100 (median 45) tumours in the treated group *versus* > 100 colonies in all non-treated animals. The decrease in tumour load following TNF-α

treatment was confirmed by a significantly decreased mean(S.D.) liver weight (12.4±6.5 *versus* 22.4±8.4 g, respectively). Clinical signs of toxicity due to TNF-α treatment were not observed.

Table 2 Effect of tumour necrosis factor α on growth of liver metastases 28 days after 70 per cent hepatectomy or sham operation.

Treatment/Operation	Incidence	No. of metastases Median (range)	Wet liver weight (g) Mean (S.D.)
TNF-α treatment			
70 % PHx	6 of 6	45 (5 - >100)†	12.4 (6.4)
Sham*	2 of 5	0 (0 – 7)	9.0 (0.6)
Control treatment			
70 % PHx	5 of 5	> 100‡	22.3 (8.4) §
Sham*	5 of 8	3.5 (0 – > 100)	8.7 (1.2)

TNF-α: tumour necrosis factor-α
** Remaining lobes ligated during injection of tumour cells, but not removed.*
† P < 0.05 versus sham and 70 per cent hepatectomy control treatment
‡ P <0.05 versus sham (Mann-Whitney U-test)
§ P < 0.05 versus sham (Student's t test)

Discussion

The first finding in these experiments is that partial hepatectomy provokes increased tumour growth in the liver remnant. In repeated experiments an increase was found in wet liver weight and in the number of tumours after partial hepatectomy. To investigate whether the degree of growth promotion was proportional to the size of the resection, the effects of 70 per cent and 35 per cent hepatectomy were compared, and it was found that this was indeed the case. Although both procedures significantly increased tumour growth, the effect of a 70 per cent resection was far greater. These findings confirm previous results obtained with various tumour models in rats[5-9]. Using the same CC531 tumour model as described in the present study de Jong *et al.*(personal communication) recently found that partial hepatectomy also enhanced the growth of established liver metastases. It thus appears that, in this model, partial resection affects not only the outgrowth of fresh artificial metastases but also the proliferation of established tumours, thereby mirroring the clinical situation to an even greater degree. However, results of studies performed predominantly in mice disagreed with these findings, and showed that partial resection did not lead to stimulation of tumour growth but

rather to growth inhibition[18-20]. The inhibition of growth observed in the latter models has been ascribed to the immunostimulatory effects of partial resection, whereas immunosuppression and the release of growth factors have been held responsible for the enhanced tumour growth in rats. In the present rat model a likely explanation for the observed effect of partial resection is immunosuppression. A previous study showed that tumour CC531, which is weakly immunogenic, exhibits enhanced growth when recipients are treated with the immunosuppressive drug cyclosporine[21]. In addition Heuff *et al*[22], using exactly the same model as the present authors, found that selective elimination of Kupffer cells resulted in a strongly enhanced growth of liver metastases, similar to the explosive growth following partial resection in the present study. Although there is no direct evidence, it is tempting to speculate that partial resection leads to a depression of the natural cytotoxicity normally exhibited by Kupffer cells, resulting in accelerated tumour growth.

There are different theories regarding the origin of new metastases in the liver that give rise to detectable recurrence. Tumour cells can spread from primary tumour cells in the liver remnant or from extrahepatic secondaries to form *de novo* metastases in the liver. Also, tumour cells that are spread during partial hepatectomy can settle again in the liver[1]. However, it is most likely that micrometastases not detected by preoperative or intraoperative screening grow out to form detectable metastases[23,24]. It has been suggested that most of the occult metastases are single tumour cells that are spread throughout the liver[8]. These dormant tumour cells could be triggered to start proliferating by changes in extracellular host factors. Liver regeneration after partial resection could provide such a trigger for metastatic growth. It is conceivable that, in this phase of tumour development adjuvant therapy would be particularly effective.

Treatment with TNF-α was found to reduce the number of metastases in the liver remnant. The antitumour effect was more prominent in the regenerating than in sham operated liver, suggesting that the process of regeneration provides optimal circumstances for TNF-α to be effective. However tumour growth in the sham operated livers was low, so firm conclusions on the relative inadequacy of TNF-α in non-hepatectomised livers can not be drawn.

Earlier studies on the efficacy of adjuvant therapy following partial hepatectomy in the rat provided evidence that 5-fluorouracil (5-FU) and mitomycin C (MMC) both were effective in reducing the enhanced tumour growth[9]. However, whereas the effect of mitomycin was found to be due to direct antitumour cytotoxicity, that of 5-FU was indirect and could be ascribed to inhibition of liver regeneration. In the present study the inhibition of tumour growth by TNF-α was not likely to be due to inhibition of liver regeneration. A pilot study has shown that treatment with TNF-α in doses ranging from 10 to 160 μg/kg on days 0 and 3 did not affect liver regeneration as assessed by wet liver weighing on day 6 after 70 per cent hepatectomy (unpublished results). In addition, repeated injections of TNF-α to hepatectomised and intact rats stimulates DNA synthesis in hepatocytes resulting in an increased liver cell mass[25,26]. If confirmed, these latter findings imply that, depending on dose and

timing of administration, TNF-α may show two opposing effects: inhibition of tumour growth on the one hand and a counterproductive stimulation of liver regeneration on the other. In the clinical situation this undesired effect might be annihilated by combining TNF-α with chemotherapy.

The clinical results so far obtained with systemic administration of TNF-α have not met the expectations raised by numerous animal studies[27]. The few studies performed with TNF-α in patients with colorectal cancer have provided no evidence of antitumour efficacy[28,29]. However, interest in using TNF-α clinically has recently been rekindled by the remarkable efficacy of locoregional treatment with TNF-α (combined with Interferon-gamma and chemotherapy), in patients with melanoma and sarcomas[30,31]. In agreement with this new approach are the positive results obtained with locoregional treatment of liver metastases from colorectal cancer. Van der Schelling *et al*[32] found stable disease after injection of TNF-α directly into liver metastases, whereas Mavligit and colleagues[33] observed a 14 per cent partial tumour response following TNF-α infusion via the hepatic artery. These studies indicate that TNF-α has definite antitumour activity in colorectal cancer metastatic to the liver. This observation, combined with the results obtained in the present study, supports the notion that a combination of metastasectomy and locoregional adjuvant therapy with TNF-α, preferably combined with chemotherapy may be of benefit for patients with liver metastases from colorectal cancer.

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CHAPTER 8

THE INHIBITORY EFFECT OF RADIOLABELLED OCTREOTIDE ON INTRAHEPATIC TUMOUR GROWTH AFTER PARTIAL HEPATECTOMY

Submitted for publication

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Abstract

Most neuroendocrine tumours and several other tumours such as breast carcinoma and malignant lymphoma express somatostatin receptors. Lesions expressing these receptors can *in vivo* be visualised by receptor scintigraphy using low radioactive doses of the somatostatin analogue [^{111}In-DTPA^{0}]octreotide. The same agent given in a high radioactive dose can be used for peptide receptor radionuclide therapy (PRRT). The aim of this study was to evaluate whether PRRT with [^{111}In-DTPA^{0}]octreotide is able to reduce tumour growth even under tumour growth stimulating conditions induced by partial hepatectomy.

Rats underwent 70% partial hepatectomy (PHx) or sham operation. The development of hepatic metastases was determined 21 days after direct injection of somatostatin receptor-positive (SS-R-positive) or SS-R-negative tumour cells into the portal vein. Groups of 8 or 9 animals that underwent PHx or sham operation were treated with octreotide 50µg/kg sc. twice daily or with 370 MBq [^{111}In-DTPA^{0}]octreotide iv. on day 1 and 8. Both treatments were compared to control treatment. Forty non tumour-bearing rats were used to determine the influence of [^{111}In-DTPA^{0}]octreotide therapy on liver regeneration after PHx.

PHx induced an increase in tumour growth in all experiments ($p<0.01$). Octreotide treatment did not influence tumour growth after PHx or sham operation. [^{111}In-DTPA^{0}]octreotide could effectively reduce tumour growth in the liver of SS-R-positive tumours also under conditions of increased tumour growth as generated by PHx ($p<0.01$). [^{111}In-DTPA^{0}]octreotide was also effective on SS-R-negative tumours after PHx ($p=0.01$) but not after sham operation. Furthermore, [^{111}In-DTPA^{0}]octreotide therapy did not influence liver regeneration or liver function after PHx.

PRRT with [^{111}In-DTPA^{0}]octreotide is effective in SS-R-positive tumours. During liver regeneration also the growth of SS-R-negative tumours is reduced. This effect is not induced by impairment of liver regeneration or liver function. Radionuclide therapy could therefore be a promising treatment modality for patients with symptomatic liver metastases of neuroendocrine tumours in combination with liver resection.

Introduction

Somatostatin (SS) is a small regulatory peptide, produced by degradation of a precursor protein, which inhibits the release of various hormones and may act as neurotransmitter in the central nervous system[1]. Several experimental and clinical studies also suggest an antiproliferative effect of somatostatinand its analogues[2-8]. Critical to these actions is the presence of somatostatin receptors (SS-R), which like other membrane receptors subserve two functions: (1) to recognise the ligand and bind it with high affinity and specificity, and (2) to generate a transmembrane signal that evokes a biological response. At least five different human SS-R subtypes (sst_{1-5}) have been cloned[9]. All subtypes bind somatostatinwith high affinity, while their affinity for the somatostatin analogue, octreotide, differs considerably. Octreotide binds with high affinity to sst_2 and sst_5, to a lesser degree to sst_3, while no binding to sst_1 and sst_4 occurs. SS-Rs have been demonstrated on a variety of human tumours and their metastases[10]. The vast majority of human SS-R-positive tumours express sst_2[11]. For the visualisation of SS-R-positive tumours *in vivo* somatostatin receptor scintigraphy with [^{111}Indium-diethylenetriaminopenta-acetic acid (^{111}In-DTPA^0)]octreotide (Octreoscan®) is used and this technique has become an important diagnostic tool in the management of patients with SS-R-positive tumours[12-15]. ^{111}In emits not only gamma rays, which can be visualised with a gamma camera, but also internal conversion- and Auger electrons with a medium to short tissue penetration (200-550 μm, 0.02-10 μm, respectively)[16-18]. *In vivo*, [^{111}In-DTPA^0]octreotide binds to the SS-R and the ligand, including ^{111}In, is internalised and transported into the lysosomes with a long residence time of ^{111}In in the tumour cells (biological half life > 700 hrs)[19,20]. This internalisation by tumour cells of the radioligand *in vivo* is an important aspect for peptide receptor radionuclide therapy (PRRT). We previously reported the antiproliferative effect of PRRT with [^{111}In-DTPA^0]octreotide on the growth of SS-R-positive CA-20948 pancreatic tumour cells in the liver. We demonstrated that this effect of PRRT is SS-R dependent since blocking the receptors with a high dose of non-radioactive octreotide reduced this growth inhibitory effect almost completely. Moreover, no effect was achieved with SS-R-negative CC531 colon carcinoma cells[21].

Resections of hepatic metastases of gastroenteropancreatic (GEP) tumours, which are predominantly SS-R-positive, are mostly performed with palliative intent since in most cases, a diffuse pattern of metastases is present at laparotomy. As we demonstrated earlier, PHx stimulates tumour growth within the regenerating liver dramatically[22]. Therefore we wanted to see whether the effect of PRRT on the growth of intrahepatic tumours in a model with accelerated intrahepatic tumour growth was also effective. In addition, we studied whether this effect of PRRT on tumour proliferation after PHx could probably be due to inhibition of liver regeneration. The putative tumour growth inhibiting effect of PRRT after PHx could have implications for further clinical trials in patients with SS-R-positive tumours and liver metastases.

Materials & methods

Animals

Male rats of the inbred WAG and Lewis strain, which were 10-14 weeks old and 225-250 g (Harlan-CPB,Austerlitz, The Netherlands) were kept under standard laboratory conditions (12 hours light/12 hours dark) and were given standard laboratory diet (Hope Farms, Woerden, The Netherlands) and water *ad libitum*. The experimental protocol adhered to the rules laid down by the Dutch Animal Experimentation Act and was approved by the Committee on Animal Research of the Erasmus Medical Centre Rotterdam.

Tumours

The pancreatic tumour, CA-20948, was originally induced by azaserine[23]. The SS-R-positive tumour is of acinar origin and is transplantable in syngeneic Lewis rats. The tumour was transplanted and maintained in the liver by direct injection into the portal vein. To produce artificial liver metastases, tumours were excised from donor livers, cleaned from normal liver tissue and pressed through sieves with decreasing mess size. The resulting suspension was washed twice in RPMI 1640 (Gibco, Paisly, UK.). Viability was measured with trypan-blue exclusion (0.3% in a 0.9% NaCl-solution). A suspension of 2.5x 10^6 living cells/mL was used for direct injection into the portal vein.

Tumour CC531 is a SS-R-negative, 1,2 dimethylhydrazine-induced, moderately differentiated colon adenocarcinoma, transplantable in syngeneic WAG rats[24]. The tumour is maintained in tissue culture as a monolayer in RPMI 1640 medium supplemented with 5% fetal calf serum. The cells were harvested from stationary cultures by gentle trypsinisation. A suspension of 2.5x 10^6 living cells/mL was used for direct injection into the portal vein. The presence/absence of the SS-R on both tumour cell lines was determined by specific binding of [^{125}I-Tyr^3]octreotide. This binding was demonstrated to membrane preparations of CA-20948 pancreatic tumour cells (IC_{50} of 0.6 nM and a B_{max} 110 fmol per mg membrane protein), while no binding was found to the membrane preparations of the CC531 colon tumour cells [8].

Radiolabelling and quality control of the radioligand

[DTPA^0]octreotide (Pentetreotide, DRN 4920) and $^{111}InCl_3$ (DRN 4901, 370 MBq/mL in HCl, pH 1.5 - 1.9) were obtained from Mallinckrodt Medical (Petten, The Netherlands). Octreotide was a gift of Novartis, Preclinical Research (Basle, Switzerland). The labelling was performed by diluting the freeze-dried [DTPA^0]octreotide in 1 mL saline and adding this to the $^{111}InCl_3$. Thirty minutes after the start of this procedure quality control was performed by instant thin layer chromatography with silica-gel and 0.1 M sodium-citrate, pH 5 as eluent, as described earlier [25]. Labelling efficiency of [^{111}In-DTPA^0]octreotide was over 98 %. Each administration of the radioligand into the dorsal penis vein

consisted of 370 MBq ^{111}In labelled with 0.5 µg [DTPA^0]octreotide, referred as 370 MBq [^{111}In-DTPA^0]octreotide.

Experimental procedure in tumour-bearing rats

Under ether anaesthesia the abdomen was opened through a midline incision. The left lateral and median liver lobes, representing 70% of the liver volume, were freed of fibrous attachments. The circulation in these lobes was temporary interrupted by ligation of the hilar vessels. Then 0.5 x 10^6 viable, SS-R-positive CA-20948 cells, suspended in 0.2 mL RPMI 1640 were injected slowly into the portal vein through a 0.4 x 12-mm needle. During 2 minutes the tumour cells were directed through the remaining 30 per cent of the liver that was not ligated. The rats were then randomised into a 70 % partial hepatectomy group (PHx) and a non-hepatectomy group (sham). In the PHx group the temporary ligation was replaced by a permanent 2-0 silk tie and the ligated lobes were resected. In the sham group the temporary ligation was removed 2 minutes after the injection to re-establish the circulation. At the end of both procedures the liver was returned to the peritoneal cavity and the laparotomy wound was closed in one layer. On day 1 after the operation rats from both PHx and sham groups were randomised into experimental and control groups. All rats were sacrificed 21 days after inoculation of tumour cells. The livers were removed, immersed in phosphate-buffered saline, dried and weighed. Tumour growth was determined by two investigators counting the number of metastases on the surface of the liver lobes, while blinded for treatment modality, as described earlier[21]. In the parallel experiment the same procedure was performed when 0.5 x 10^6 viable, SS-R-negative CC531 cells were injected. Experimental groups contained 8 or 9 animals.

Treatment with octreotide

Sixteen rats were injected with SS-R-positive CA-20948 tumour cells into the portal vein. Eight rats were treated with octreotide (Sandostatin®) 50 µg/kg in 0.2 mL RPMI, subcutaneously in the neck. Treatment was given twice daily starting on the first day after the operation. Control treatment consisted of 0.2 mL RPMI injections according to the same schedule.

Treatment with [^{111}In-DTPA^0]octreotide

Sixteen rats were injected with SS-R-positive CA-20948 tumour cells into the portal vein and, in a parallel experiment, 16 rats were injected with SS-R-negative CC531 tumour cells. Rats in the experimental groups were treated with 370 MBq [^{111}In-DTPA^0]octreotide into the tail vein on days 1 and 8. Rats in the control groups were injected with vehicle, 0.5 µg [DTPA^0]octreotide.

Effects of [^{111}In-DTPA^{0}]octreotide on liver regeneration

Fourty non tumour-bearing WAG rats underwent PHx according to the procedure described above and randomised into PRRT- and control groups. Animals in the PRRT group were injected with 370 MBq [^{111}In-DTPA^{0}]octreotide iv., 24 hours after PHx. Rats in the control group were injected with 0.5 µg [DTPA^{0}]octreotide iv. Of each group 4 rats were sacrificed at 2, 4, 8, 16, and 32 days after PHx. Functional recovery of the liver was determined with Bromosulfalein (BSP) ($C_{20}H_8Br_4Na_2O_{10}S_2$) clearance during 45 minutes[26]. One hour before sacrifice the animals were injected into the dorsal penis vein with 8 mg BSP per 100 g body weight. Blood samples were drawn from the tail at 1 and 45 minutes after the injection of BSP. BSP concentrations in these samples were determined by colorometry at 586 nm wave length. Clearance of BSP in 45 minutes is expressed as (1-(BSP level after 45 minutes/BSP level after 1 minute)) x100.

Liver function analysis was performed by determining the serum levels of alanine transaminase, aspartate transaminase, γ-glutamyl transferase, alkaline phosphatase, bilirubin, protein, and albumin. All livers were weighed and DNA synthesis was measured by the 5-bromo-5-iododeoxyuridine (BrdU) labeling index. In short, 90 minutes before sacrifice, 50mg BrdU per kg body weight were administered intraperitoneally. Samples of the remnant liver were fixed and embedded in parafin. The labeling index of BrdU was expressed as the rate of 100 hepatocyte nuclear positivity in five fields at high power (x400).

Statistical analysis

Statistical analysis was performed using Mann-Whitney U-test on categorised outcomes. Statistical significance was defined at $p<0.05$.

Results

Effects of treatment with octreotide

The results of octreotide treatment on the growth of SS-R-positive CA-20948 tumours in the liver are given in table 1. In sham operated animals there was no difference in tumour growth between octreotide treatment and controls, 21 days after tumour cell injection. As found in earlier experiments PHx resulted in a significant increase in tumour growth, compared to sham operation, in both octreotide treated animals and controls ($P< 0.01$)[22]. Octreotide treatment in rats that underwent PHx did not decrease tumour growth.

Table 1 The effect of octeotide on SS-R-positive liver metastases after sham operation or 70% partial hepatectomy.

Treatment	Liver weight (g) Mean (s.e.m.)	Tumour score 0	1+	2+	3+	4+	5+
Sham operation							
Controls (n=8)	10.4 (0.9)	-	1	3	3	1	-
Octreotide (n=8)	10.3 (1.0)	-	1	4	3	-	-
70% PHx							
Controls (n=8)†	16.1 (4.3)	-	-	-	-	2	6
Octreotide (n=8)‡	14.8 (2.6)	-	-	-	1	1	6

†‡ *($p < 0.01$) vs. Sham operation*

Liver weight and number of animals with given range of hepatic metastases (8 animals per group), 21 days after sham operation or 70% partial hepatectomy and direct injection of SS-R-positive CA-20948 tumour cells into the portal vein. Octreotide treatment was given subcutaneously, twice daily 50 µg/kg. No significant effect was found of octreotide treatment in sham operated animals or in 70% partial hepatectomised animals.

Effects of PRRT with [^{111}In-DTPA^0]octreotide

The results of PRRT with 370 MBq [^{111}In-DTPA^0]octreotide on the growth of SS-R-positive CA-20948 tumours in the liver are summarised in table 2 . PRRT on days 1 and 8 induced a significant decrease in tumour growth in sham operated animals ($p < 0.01$). PHx again showed an increased tumour growth compared to sham operation ($p < 0.01$). Under these conditions of increased tumour growth, PRRT also induced a decrease in tumour growth ($p < 0.01$).

In the parallel experiment with SS-R-negative CC531 tumour cells (table 3). PHx increased tumour growth both in PRRT and control groups ($p < 0.01$) . PRRT did not induce a difference in tumour growth in sham operated animals. However, there was a significant decrease of tumour growth by PRRT after PHx also for these SS-R-negative tumours ($P = 0.01$). In this experiment 1 rat died by an overdose of ether. Another animal died due to incomplete ligation of the hilar vessels.

Table 2 The effect of PRRT with [^{111}In-DTPA^{0}]octreotide on SS-R-positive liver metastases after sham operation or 70% partial hepatectomy.

Treatment	Liver weight (g) Mean (s.e.m.)	Tumour score 0	1+	2+	3+	4+	5+
Sham operation							
Controls (n=8)	17.3 (3.1)	-	1	-	-	2	5
PRRT (n=8)	10.1 (0.4)	1	7	-	-	-	-
70% PHx							
Controls †(n=8)	23.0 (2.7)	-	-	-	-	-	8
PRRT (n=8)‡	9.3 (1.1)	-	7	1	-	-	-

† *($p < 0.01$) vs. Sham operation*
‡ *($p < 0.01$) vs. Controls*
Liver weight and number of animals with given range of hepatic metastases (8 animals per group), 21 days after sham operation or 70% partial hepatectomy and direct injection of SS-R-positive CA-20948 tumour cells into the portal vein. PRRT with 370 MBq [^{111}In-DTPA^{0}]octreotide, given on days 1 and 8, induced a significant reduction of tumour growth both in sham operated animals and in 70% partial hepatectomised animals.

Table 3 The effect of PRRT with [^{111}In-DTPA^{0}]octreotide on SS-R-negative liver metastases after sham operation or 70% partial hepatectomy.

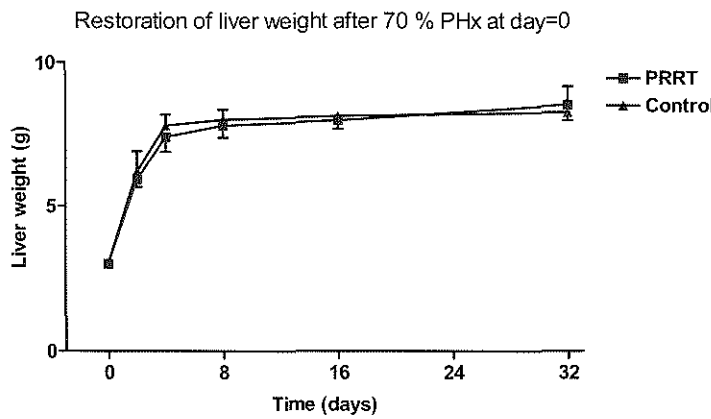
Treatment	Liver weight (g) Mean (s.e.m.)	Tumour score 0	1+	2+	3+	4+	5+
Sham operation							
Controls (n=9)	14.1 (5.3)	1	2	5	1	-	-
PRRT (n=9)	18.5 (8.0)	1	1	5	2		
70% PHx							
Controls †(n=9)	43.4 (8.0)	-	-	-	-	2	7
PRRT (n=7)‡	30.8 (5.6)	-	7	-	5	2	

† *($p < 0.01$) vs. Sham operation*
‡ *($p = 0.01$) vs. Controls*
Liver weight and number of animals with given range of hepatic metastases (7 or 9 animals per group), 21 days after sham operation or 70% partial hepatectomy and direct injection of SS-R-negative CC531 tumour-cells into the portal vein. PRRT with 370 MBq [^{111}In-DTPA^{0}]octreotide on days 1 and 8 induced a significant reduction of tumour growth after partial hepatectomy. No significant effect of PRRT was found in sham operated rats.

Effects of PRRT on liver regeneration

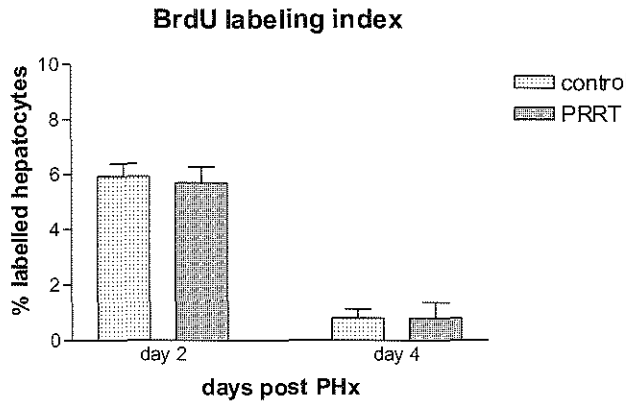
The influence of PRRT on liver regeneration after PHx was investigated in non tumour-bearing animals. During the first 4 days there was a rapid increase in liver weight after which restoraton of liver weight was almost completed. No difference in wet liver weight was found after 2, 4, 8, 16, and 32 days between rats that underwent PHx with or without PRRT (figure 1). There was also no difference in BrdU labeling index between these 2 groups at days 2 and 4 after PHx (figure 2). At later intervals in both groups labelled hepatocytes were only identified sporadically again without difference. Basic liver function tests after PHx showed no alteration by PRRT (data not shown). In adition, BSP clearance was not influenced by PRRT (figure 3).

Figure 1 Peptide receptor radionuclide therapy with [^{111}In-DTPA^{0}]octreotide and liver regeneration after 70 % partial hepatectomy.



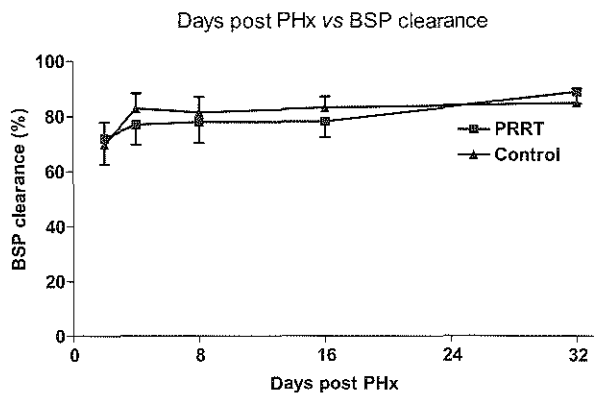
Liver weights of 40 rats that underwent 70% partial hepatectomy. The influence was studied of PRRT with 370 MBq [^{111}In-DTPA^{0}]octreotide intravenously on day 1 compared to control treatment with saline. Groups of animals (n=4) were sacrificed on days 2, 4, 8, 16, or 32 after 70 % partial hepatectomy.

Figure 2 Peptide receptor radionuclide therapy with [^{111}In-DTPA^{0}]octreotide and liver regeneration after 70 % partial hepatectomy.



Labelling index of hepatocytes in rats that underwent 70% partial hepatectomy. The influence was studied of PRRT with 370 MBq [^{111}In-DTPA^{0}]octreotide intravenously on day 1 compared to control treatment with saline. Animals were injected 90 minutes before sacrifice with 50 mg/kg BrdU. Groups of animals (n=4) were sacrificed on days 2, 4, 8, 16, or 32 after 70 % partial hepatectomy. After 4 days labelled hepatocytes were only identified sporadically.

Figure 3 Peptide receptor radionuclide therapy with [^{111}In-DTPA^{0}]octreotide and liver function after 70 % partial hepatectomy.



Clearance of Bromosulfalein (BSP) in 40 rats that underwent 70% partial hepatectomy (PHx). The influence was studied of PRRT with 370 MBq [^{111}In-DTPA^{0}]octreotide intra venously on day 1 compared to control treatment with saline. The clearance of 8 mg/kg BSP in 45 minutes was expressed as (1 – (BSP level after 45 minutes / BSP level after 1 minute) x 100. BSP clearance was measured 2, 4, 8, 16, and 32 days after 70 % PHx in groups consisting of 4 animals per measure point.

Discussion

Surgical excision of liver tumours represents the only curative treatment for primary and metastatic liver malignancies. For colorectal tumours the results of surgical resection compare favourably with the natural outcome for these selected groups of patients[27]. However, despite the curative potential of hepatic resection, recurrence has been reported in 65-80 % of the patients. The predominant site of recurrence is within the remnant liver and the majority is diagnosed within the first year after the operation[28,29]. The high number of early recurrences suggests that resection might act as a 'double-edged sword'. On one hand survival after PHx is increased but on the other hand resection might provoke enhanced tumour growth. Clinical support for increased tumour growth after PHx was published recently. Elias *et al.*[30] demonstrated that during liver regeneration following right portal embolisation the growth rate of liver metastases in the left liver lobes is increased and even more rapid than the growth of liver parenchyma. These findings are in agreement with studies in rats which demonstrate accelerated tumour growth in regenerating liver lobes and inhibited tumour growth in atrophied liver segments after selective portal embolisation[31].

Several groups have found experimental evidence for stimulation of tumour growth by PHx in various animal models; both intrahepatic - and extrahepatic tumours were found to grow more rapidly after PHx[32-37]. We reported earlier, using the same model as in the present study, that the growth of SS-R-negative CC531 colonadenocarcinoma tumours in the remnant liver after resection of 70% of the liver rats was significantly increased compared to sham operated animals[22]. We then reported that tumour necrosis Factor α (TNF-α), administered intravenously, reduced this tumour growth stimulating effect of PHx. TNF-α could be of benefit after PHx since TNF-α is known to stimulate rather than impair liver regeneration[38,39]. However, in the clinical situation the use of TNF-α is limited to isolated perfusion because of severe toxicity after systemic administration[40,41].

If tumour growth is stimulated after PHx the number of replicating tumour cells will be increased. Theoretically this phase of tumour growth stimulation could be favourable for adjuvant chemotherapy, applied as adjuvant treatment, since the rapidly dividing tumour cells might be more susceptible to cytostatic agents. However, adjuvant treatment aiming at reduction of tumour growth stimulation caused by PHx might also impair liver regeneration and must therefore clinically be applied with great care for it is the regenerating capacity of the liver that makes patients able to recover from large resections [42,43]. Several reports on studies with adjuvant chemotherapy, including doxyrubicin and 5-fluorouracil, describe notable adverse effects caused by impairment of liver regeneration [34,44,45]. An effective adjuvant treatment that does not impair regenereration could therefore be of value for patients with hepatic metastases.

For neuroendocrine liver metastases, that are mostly SS-R-positive, clinical considerations are different since surgery is not only undertaken with curative intent but also for palliation. Cytoreductive hepatic surgery leads to sustained palliation of symptoms, reduces the need for additional treatment and prolongs survival in selected groups of patients[46-50]. Adjuvant strategies after liver resection could be of great value for patients with hepatic neuroendocrine tumours since (occult) residual tumour burden within the liver will be low after the resection. Of these patients with liver metastases of neuroendocrine tumours 65 – 75 % have extrahepatic disease, as can be detected by scintigraphy[14,51]. Development of effective adjuvant treatment after cytoreductive hepatic resection might make more patients eligible for surgery.

The first finding from this study is that the growth of SS-R-positive pancreas carcinoma CA-20948 tumours is increased by PHx to the same extent as we found earlier for tumour CC531[22,52]. Therefore our model with PHx seemed appropriate to investigate the effect of octreotide and PRRT on SS-R-positive and SS-R-negative tumours during accelerated growth. We did not find a reduction of tumour growth after PHx in our experiments using octreotide alone. However, we were able to demonstrate that PRRT with 370 MBq [^{111}In-DTPA^{0}]octreotide on day 1 and 8 significantly inhibits the growth of SS-R-positive CA-20948 tumours in the liver. After PRRT, both in sham operated and PHx animals there were only few tumour colonies present while control animals all had a large tumour burden in their livers after 21 days. In previous studies, in animals without PHx, we found that PRRT significantly decreased the growth of SS-R-positive tumours in the liver even when PRRT was performed 12 days after inoculation of the tumour[21,52]. The nessecity of the SS-R for this tumour growth-inhibiting effect was proven since blocking the SS-R's by pre-treatment with octreotide reduced the effect of PRRT almost completely. Moreover no effect was found on SS-R-negative CC531 tumours as also was found in the current study for sham operated animals. Three other experimental studies with radiolabelled somatostatin analogues demonstrated the antiproliferative potential of PRRT on solid subcutaneously transplanted tumours, using [^{64}Cu-TETA^{0}]octreotide, [^{90}Y-DOTA,Tyr^{3}]octreotide and ^{188}Re-RC-160, respectively[53-55]. However, the effect of PRRT was never demonstrated for liver tumours. Recently the first results of PRRT with [^{111}In-DTPA^{0}]octreotide in patients with neuroendocrine tumours were documented using a cumulative dose of 74 GBq. There were no major clinical side effects and there were promising beneficial effects on clinical symptomes, hormone production and tumour proliferation. Of the 21 patients who received a cumulative dose of more than 20 GBq eight patients showed stabilisation of disease and six other patients showed a reduction in tumour size[56].

In this study we reconfirmed that PRRT has no significant effect on SS-R-negative CC531, therefore, no effect was expected after PHx. However, tumour growth was significantly reduced by PRRT after PHx also for this SS-R-negative tumour, albeit far less than observed for SS-R-positive tumours. It could be that liver regeneration is impaired by PRRT as has been described for octreotide[57,58]. This putative inhibition of regeneration could also lead to less tumour growth stimulation. Therefore, we

investigated the effect of PRRT on liver regeneration in non-tumour-bearing animals. PRRT was given one day after PHx since liver regeneration is most profound during the first 48 hours after PHx[59]. The restoration of liver weight was not found to be influenced. In both groups there was a fast increase in liver weight during the first days after PHx. At different time intervals up to 32 days, when complete restoration of liver volume is to be expected, no difference was found in wet liver weight. Also no difference in BrdU labeling index was found indicating that there was no difference in the number of regenerating hepatocytes. Functional recovery of the liver, studied by BSP clearance and basic liver function tests, was not different. Therefore we conclude that the decrease in tumour growth by PRRT after PHx is not caused by an inhibitory effect on liver regeneration. An explanation for the effect of PRRT on SS-R-negative tumours could be that the neovasculature of regenerating livers express a high density of SS-R's as has been demonstrated in peritumoural veins in primary tumours and their metastases[60,61]. The effect of PRRT on these SS-R-negative tumours could then be ascribed to accumulation of the radionuclide close to the tumour cells and the effect on angioneogenesis.

This study demonstrates that PRRT can reduce increased tumour growth after PHx. This effect was very strong for SS-R-positive tumours, probably mediated through the SS-R, however also for SS-R-negative tumours PRRT could reduce the increase of tumour growth. Liver regeneration is not impaired by PRRT nor the function of the remaining liver. This could imply that PRRT might be an effective option after PHx clinically.

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CHAPTER 9

SOMATOSTATIN RECEPTOR SCINTIGRAPHY IN PATIENTS WITH LIVER METASTASES OF NEUROENDOCRINE TUMOURS

Submitted for publication

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Abstract

Somatostatin receptors are present on most neuroendocrine tumours. Somatostatin receptor scintigraphy (SRS) with [^{111}In-DTPA^{0}]octreotide (Octreoscan®) is a technique which has shown to localise the primary as well as intra- and extrahepatic metastatic disease of neuroendocrine tumours with a high sensitivity. For the optimal management of patients with liver metastasis of neuroendocrine tumours accurate imaging and staging is essential. The aim of the present study was to compare the value of conventional imaging methods (CIM) and SRS in the diagnosis and staging of patients with proven liver metastases of neuroendocrine tumours.

Forty-eight patients with liver metastases of neuroendocrine tumours detected by CIM were referred to our hospital. Liver metastases of carcinoid tumours were found in 39 patients, in 5 of pancreatic islet cell tumours and in 4 of "non-functioning" endocrine tumours. The results of CIM and SRS in the detection of both intrahepatic- and extrahepatic tumours were compared. In 26 patients in which single photon emission computed tomography (SPECT) was performed together with planar SRS the additional value of SPECT imaging in the detection of intrahepatic metastases was determined.

With CIM 50 extrahepatic tumours were detected in 22 patients, while with SRS 114 extrahepatic tumours were detected in 37 patients. With planar SRS liver metastases were visualised in 35 of the 48 patients (73%). In all 26 patients (100%) in whom both planar SRS and SPECT were performed liver metastases were demonstrated.

Planar SRS is superior in the detection of extrahepatic metastases of neuroendocrine tumours compared to the combination of conventional imaging methods. With SPECT imaging additional to planar imaging with SRS liver metastases are demonstrated with high sensitivity. Therefore SRS with SPECT should be recommended as the first imaging method for the staging of patients with neuroendocrine tumours.

Introduction

The optimal management of patients with neuroendocrine tumours (NETs) requires accurate imaging and staging. Proof of extrahepatic metastases will eliminate patients for curative liver resection or liver transplantation. Treatment of liver burden will then only be possible with palliative intent. In addition accurate localisation of intra- and extrahepatic tumours will make operative strategies more efficient. In the past therapeutic strategy was based on the results of conventional imaging methods (CIM) such as computed tomography (CT), ultrasound (US) and recently of magnetic resonance imaging (MRI) in combination with bone scanning. For the visualisation of somatostatin receptor (SS-R) positive tumours like neuroendocrine tumours, somatostatin receptor scintigraphy (SRS) with [^{111}Indium-diethylenetriaminopenta-acetic acid (^{111}In- DTPA^{0})]octreotide (Octreoscan®) has been used for more than 10 years[1,2]. There are two complementary imaging methods in SRS: planar SRS imaging which yields two-dimensional images with which extrahepatic localisations of primary and metastatic tumours can be identified and SRS-SPECT, in which a rotating camera is used, to make image reconstructions similar to computed tomography. This technique renders additional visual information, especially on tumours in the liver and upper-abdomen[3-5].The efficacy of SRS using [^{111}In-DTPA^{0}]octreotide in patients with histologically or biochemically proven endocrine pancreatic tumours or carcinoids was evaluated in a European multicenter trial[6]. The highest success rates of SRS for the visualisation of primary tumours were observed with glucagonomas (100%), vipomas (88%), gastrinomas (73%), "non-functioning"islet cell tumours (82%) and carcinoids (87%).

Three clinical studies have demonstrated the additional value of SRS to CIM, consisting of CT, US magnetic resonance imaging (MRI), selected angiography and endoscopic ultrasonography in patients with neuroendocrine tumours[7-9]. SRS in these studies altered clinical management in 21% to 47% of the patients and had superior sensitivity and specificity compared to CIM. In these studies SRS was performed to identify primary tumours and intra- and extrahepatic metastases. The aim of the present study however, was to assess the value of planar SPECT and SRS for the localisation of intrahepatic and extrahepatic tumour lesions in patients with histologically proven liver metastases of NETs.

Patients & methods

A retrospective study of all patients with hepatic metastases of NETs analysed at our hospital between 1989 and 1998 was performed. Forty-eight patients with liver metastases detected by either abdominal ultrasound or spiral CT underwent SRS. Evidence of neuroendocrine origin of these metastases was rendered by histological examination of liver biopsies or resection specimen.

Conventional imaging methods (CIM) consisted of abdominal trifasic spinal CT scanning, at 5 cm thickness and with oral contrast, and US of upper abdomen in all patients. Chest CT was added in 7 patients, bone scintigraphy in 3 and colonoscopy in 2 because clinical symptoms were suspect for metastatic disease. Before 1992 SRS was performed only planar in 22 patients; thereafter planar SRS was combined with SPECT imaging of the abdomen in 26 patients. Of all 48 patients the reports of CIM were compared with the reports of SRS. The time between the investigations was less than 30 days. Extrahepatic lesions were scored in total numbers of tumours and number of regions of the body affected. The body was therefore divided into 6 localisation segments: head and neck, thorax, upper abdomen (above lower kidney poles), lower abdomen (beneath lower kidney poles), spine and extremities. If multiple tumours were visualised at one location this was scored only once. The sensitivity for the detection of intrahepatic lesions was determined in patients that had only planar SRS performed and in patients that had both planar SRS and SRS SPECT performed. For the 26 patients that had both planar SRS and SRS SPECT performed, the images were reviewed to score the additional value of SPECT imaging to planar SRS for the detection of intrahepatic metastases.

Scanning protocol

Planar images were obtained with a double head or large field of view gamma camera, equipped with medium-energy parallel-hole collimators, 24 h after injection of 220 MBq [^{111}In-DTPA^{0}]octreotide (Mallinckrodt, Petten, the Netherlands). Windows were centred over both ^{111}In photon peaks (172keV and 245 keV) (window width 20%). The acquisition parameters for planar spot view images were 3000,000 pre-set counts or 15 min per view for the head and neck, and 500,000 counts or 15 min for the remainder of the body. Repeat scintigraphy after 48 h was performed for the abdomen to differentiate radioactive bowel content from presumed pathology. For SPECT images with a triple-head camera the acquisition parameters were: 40 steps of 3 degrees each, and 30 sec per step. SPECT analysis was performed with a Metz filter on original data.

Results

Origin of liver metastases

Forty-eight patients were evaluated of whom 39 (81%) had liver metastases of carcinoid tumours, 5 of pancreatic islet cell tumours, and in 4 patients liver metastases were present of "non-functioning" neuroendocrine tumours. All patients had intrahepatic tumours demonstrated by CIM. In 24 patients the primary tumour had been surgically removed previously.

Detection of extrahepatic tumours

With SRS extrahepatic tumours were detected in 37 patients while with CIM extrahepatic tumours were detected in 22 patients. In 15 patients SRS was the only imaging method to detect extrahepatic tumours while SRS detected extrahepatic tumours in all patients that had tumours detected by CIM (table 1). This means that SRS detected extrahepatic tumours in 58 % of the patients in which extrahepatic disease could not be demonstrated by CIM.

SRS showed 114 extrahepatic tumours at 86 different localisations in 37 patients whereas CIM showed 50 extrahepatic lesions at 33 localisations in 22 patients. The results, listed in table 2, demonstrate that SRS detected significantly more tumours at different localisations in 68 % more patients than CIM.

Table 1 Detection of extrahepatic metastases CIM *vs.* SRS.

	Number of patients		
	CIM Positive	CIM Negative	Total
SRS Positive	22	15	37
SRS Negative	--	11	11
			48

The localisations at which extrahepatic tumours were found with SRS or CIM alone or both CIM and SRS are listed in table 3. The total number of localisations was 91 of which SRS was the only imaging method in 60. In 24 patients the primary tumour had been removed surgically. In 13 patients the localisation of the primary tumour was not demonstrated with SRS. In 11 patients the primary tumour was known and identified with SRS.

Detection of intrahepatic tumours

In table 4 the detection of intrahepatic tumours is listed. In 22 patients only planar SRS was performed. In 17 of these 22 patients the presence of intrahepatic tumours was found. In 26 patients both planar SRS and SRS SPECT were performed. Re-examination of planar SRS and SRS SPECT in this group of 26 patients demonstrated that planar SRS demonstrated intrahepatic tumours in 18 patients. So with planar SRS in the group of 48 patients liver metastases were found in 35 (73%). With the addition of SRS SPECT intrahepatic tumours were demonstrated in all 26 patients (100%).

Table 2 Detection of extrahepatic metastases CIM vs. SRS.

Imaging method	No. of patients	No. of tumours	No. of localisations
CIM	22	50	33
SRS	37 (+68%)	114 (+128%)	86 (+161%)

Comparison between conventional imaging methods (CIM) and somatostatin receptor scintigraphy (SRS) in the detection of extrahepatic metastases in 48 patients with liver metastases of histologically proven neuroendocrine tumours. Table shows the number of patients in whom extrahepatic metastases were demonstrated, the number of tumours detected and the number of localisations at which tumours were found. The number of additional patients, tumours and localisations detected by SRS are expressed in percentages.

Table 3 Localisations of extrahepatic metastases CIM vs. SRS.

	CIM only	CIM + SRS	SRS only	
Head and neck	-	3	5	
Thorax	-	6	14	
Upper abdomen*	3	8	14	
Lower abdomen	-	5	14	
Spine	1	2	5	
Extremities	1	2	7	
Total	5	26	60	91

*Comparison between conventional imaging methods (CIM) and somatostatin receptor scintigraphy (SRS) in the detection of extrahepatic metastases in 48 patients with liver metastases of histologically proven neuroendocrine tumours. Table shows the number of patients in whom extrahepatic metastases were demonstrated at the 6 localisations listed. The total number of 91 localisations was found. SRS was the only imaging method to identify these localisations in 60 (66%) whereas SRS did not demonstrate lesions suspected to be metastases by CIM at 5 localisations 5 (5%). * The abdomen is divided into upper abdomen and lower abdomen by the imaginary line connecting the lower poles of the kidneys.*

Table 4 Detection of intrahepatic metastases somatostatin receptor scintigraphy.

	Planar only	Planar only	Planar + SPECT
Yes	17	18	26
No	5	8	-
Total	22	26	

Detection of intrahepatic metastasis with somatostatin receptor scintigrapy (SRS) in 48 patiens who had intrahepatic tumours demonstrated by conventional imaging methods(CIM). 22 patiens had SRS-planar performed only and 26 patients had both SRS-planar and SRS-SPECT performed. In the patients that had both SRS-planar and SRS-SPECT performed intrahepatic tumours were found in all patients. Review of the images in the group that had both SRS-planar and SPECT performed demonstrated that in 8 of the cases the intrahepatic tumours were not detected SRS-planar only.

Table 5 Imaging methods for the detection of liver metastases and extra-hepatic metastases in patients with Zolinger-Ellison syndrome.

	Positive result (%)	
Procedure	Extra-hepatic tumour ($n = 80$)	Liver metastases ($n = 24$)
Ultrasonography (US)	9	46
Computed tomography (CT)	31	42
Magnetic resonance imaging (MRI)	30	71
Angiography (Angio)	28	62
US + CT + MRI + Angio (CIM)	48	83
SRS	58	92
SRS + CIM	68	96
SRS Only*	20	12
CIM Only**	10	4

Gibril et al, Ann Intern Med 1996; 125:26-34

Results of tumour localisation for the identification of liver metastasis and an extra-hepatic tumour in patients with Zollinger-Ellison syndrome. Results are expressed as the percentage of the 24 patients with proven liver metastasis and of the 80 with extrahepatic disease.
Conventional imaging methods (CIM) included ultrasonography, computed tomography(CT), magnetic resonance imaging (MRI) and angiography.
* *Tumour detected only with somatostatine receptor scintigraphy (SRS)*
** *Tumour detected only with CIM*

Discussion

Several research groups have demonstrated the additional value of SRS in the diagnosis of NETs. Their studies included patients with and without liver metastases. In a prospective study from the National Institutes of Health, Bethesda, comparing the sensitivity of SRS with that of CT-scanning, MRI, US and selective angiography in the detection of primary and metastatic gastrinomas, SRS altered clinical management in 47% and had a superior sensitivity, and specificity (table 5)[7,10]. At the Hopital Bichat – Claude Bernard, Paris the results of SRS were compared with the results of CIM including endoscopic US, and with surgical findings in 21 consecutive patients with Zollinger-Ellison syndrome[11]. SRS added complementary information to other imaging techniques including endoscopic US and improved the preoperative detection of extrapancreatic gastrinomas. By combining SRS with endoscopic US they were able to detect 90% of the tumours in the upper duodenopancreatic area. SRS identified metastatic disease in 20-30% of patients after all other imaging techniques had failed[3]. In their prospective study concerning 160 patients with neuroendocrine gastroenteropancreatic (GEP) tumours including carcinoid and pancreatic islet cell tumours, Lebtahi et al. described that SRS changed the surgical therapeutic strategy in 40 (25%) patients. Unsuspected liver tumours were discovered only by SRS in 7 patients, contralateral liver tumours before hepatecomy in 2 and extrahepatic disease in 31 patients.[9] A study from the Institutio Nazionale Tumori, Milan, demonstrated a modification of scheduled treatment by SRS in 21 % and a detection of previously unknown tumours in 28 % of the patients[8].

The aim of our study was to stage patients with proven hepatic metastases of NETs more accurately in order to improve clinical management. First of all we demonstrated that SRS is superior in the detection of extrahepatic tumours compared to the combination of CIM which consisted of abdominal CT and US in all patients and additional chest CT, bone scintigraphy and colonoscopy in some patients. In 15(31%) of the 48 patients investigated, SRS was the only imaging modality to detect extrahepatic metastases. Our results are in agreement with two other studies in which SRS demonstrated extrahepatic metastases in 22% and 54% of the patients that had no evidence of extrahepatic tumour with other imaging techniques[9,12]. For these patients treatment by means of liver resection only would not be curative and the detection of extrahepatic disease furthermore excludes these patients for liver transplantation. The great advantage of whole body planar scintigraphy is that with one single method 'whole body' imaging is obtained (i.e. from head to pelvis), and that this includes information on possible metastatic sites that are not usually investigated by CT, US, and MRI. Also, the nature of SRS, which is based on the presence of SS-Rs on the tumour cells, instead of the criterion of tissue invasion or lymphnode enlargement, which is identified in CIM, may yield additional information. This explains that with SRS in our group of 48 patients 60 additional tumour localisations were visualised which had not been detected with CIM. Interestingly bone metastases in

extremities and spine were demonstrated in 16 (33 %) patients with liver metastases. Gibril et al. detected bone metastases in 31 % of the patients with liver metastases from gastrinomas. Lebtahi et al. found similar accuracy of SRS and bone scintigraphy for the detection of bone metastases in patients with NETs[13]. Because bone metastases can occur initially outside the axial skeleton, SRS is recommended the initial localization method of choice to screen for bone metastases in patients with liver metastases of NETs. Patients with pancreatic endocrine tumours with liver metastases should undergo SRS every 6 months to 1 year to detect bone metastases[14].

Because there is a relatively high uptake in liver and kidney, the interpretation of planar SRS in the upper abdomen and especially within the liver is difficult. With the addition of SPECT imaging, the upper abdomen can be analysed with higher sensitivity[4]. In the group of 26 patients with proven liver metastases in our study in all patients intrahepatic tumours were detected by SPECT and in only 18 patients (72%) with only planar SRS which demonstrates the importance of systematic SPECT imaging in the management of patients with NETs.

Since 37 patients in our series (77%) were found to have extrahepatic disease and another 7 (16%) multiple bilateral liver metastases, most patients were no candidates for curative liver resection or transplantation and should be treated systemically. Clinical studies in patients with hormone producing islet cell tumours showed a close parallel between the presence of SS-Rs on the tumours and the *in vivo* and *in vitro* suppressive effects of octreotide on hormone release[15]. This indicates that SRS can predict a possible suppressive effect of octreotide on hormonal hypersecretion by endocrine pancreatic tumours. The observation that somatostatin inhibits the release of various peptide hormones has stimulated the interest in its use as an antiproliferative agent. In preclinical studies somatostatin analogues inhibit the growth of a wide variety of SS-R positive as well as SS-R negative tumours *in vivo* and *in vitro*[16-18]. The results of clinical trials in patients with NETs, using somatostatin analogues alone or in combination with interferon-α are rather disappointing with a biochemical response in 77% of patients with a median duration of 15 months but without reduction in tumour size[19-21]. Octreotide has been clinically the most commonly applied somatostatin analogue, yielding biochemical response rates between 30 – 70 % but objective tumour shrinkage in less than 2%-7% of the patients[22-26].

A new application is the use of radiolabelled octreotide for peptide receptor radionuclide therapy (PRRT) in patients with disseminated neuroendocrine tumours . After systemic injection of [^{111}In-DTPA^{0}]octreotide the radionuclide is specifically internalised and transported into the lysosomes[27,28]. ^{111}In not only emits gamma-rays, which can be visualised during scintigraphy, but also internal conversion- and Auger electrons having a medium to short tissue penetration (200-550 μm, 0.02-10 μm, respectively). Therefore, an effect on tumour cell proliferation could be expected, as the radiotoxicity of the radionuclide is very high if the DNA of the cell is within the particle range[29,30]. Experimental studies demonstrated tumour growth-inhibition of solid subcutaneous tumours by radiolabelled somatostatin analogues in animal models[31-33]. We demonstrated in a liver model that the

antiproliferative effect of [^{111}In-DTPA^{0}]octreotide is dependent on the presence or abundance of the SS-R which can be clinically demonstrated by SRS[2].

A phase 1 study on the side effects and antiproliferative effect of high, multiple radiotherapeutic doses of [^{111}In-DTPA^{0}]octreotide started in 1995[35,36]. Twenty-one patients received a total cumulative dose of at least 20 GBq [^{111}In-DTPA^{0}]octreotide with a maximum of 75 GBq. With a maximum follow up of 26 months no major clinical side effects were observed except for a transient decline in platelets count and the number of white blood cells. Kidney function was only influenced slightly and without clinical relevance. Impressive effects on the clinical condition of the patient and hormone or tumour marker production were observed after the administration of high doses of [^{111}In-DTPA^{0}]octreotide. Prior to the start of treatment all 21 patients had progressive disease. In 8 patients treatment resulted in stable disease and in 6 patients in actual tumour shrinkage, monitored with CT or MRI. In these patients beneficial effects on hormone production and clinical symptoms were found. SRS in these patients predicted the clinical result since a better result of PRRT was observed in patients whose tumours has a high accumulation of the radioligand on their staging SRS scintigram. Our findings are in agreement with the results of other reports on radionuclide therapy with [^{111}In-DTPA^{0}]octreotide that have described smaller groups of patients with end stage NETs[37-41].

We conclude that SRS plays a crucial role in the staging and managemant of patients with NETs since we found that SRS detected extrahepatic tumours in 58% of patients in which extrahepatic disease could not be demonstrated by CIM. In addition SPECT imaging of the upper abdomen renders a high sensitivity for intrahepatic tumour detection. Therefore, planar SRS with SPECT imaging should be recommended as the first imaging method in patients with neuroendocrine tumours. With proof of extrahepatic tumours patients with liver metastases will be excluded for curative liver surgery. Systemic PRRT with radiolabelled somatostatin analogues for these patients could become a promising treatment modality in the future.

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CHAPTER 10

SUMMARY AND CONCLUSIONS

SAMENVATTING EN CONCLUSIES

Summary

The aims of this thesis were to investigate the possibilities of antitumour therapy with octreotide and radiolabelled octreotide in the rat. We also studied a tumour growth stimulating effect of liver regeneration after partial hepatectomy. Since, if this putative tumour growth stimulation by partial hepatectomy could be demonstrated we wanted to see whether treatment with octreotide and radionuclide therapy could be effective after hepatectomy and whether this treatment would influence liver regeneration. In addition we wanted to investigate the value of somatostatin receptor scintigraphy in patients with liver metastases of neuroendocrine tumours to define patients that could possibly benefit from peptide receptor radionuclide therapy.

Chapter 2 gives a review on somatostatin receptor expression and the use of somatostatin receptor scintigraphy in patients with neuroendocrine tumours. The clinical use of somatostatin analogues in the treatment of neuroendocrine diseases and the oncological applications of somatostatin analogues are described. An introduction is given on new treatment options for patients with neuroendocrine tumours with peptide receptor radionuclide therapy (PRRT) with the somatostatin analogue octreotide. The hypothesis was that radiolabelled octreotide, that is used in scintigraphy with a low radioactive dose, could be used for PRRT when given with a high radioactive dose, which was tested in a rat model (chapter 4).

In **chapter 3** the effect of the somatostatin analogue octreotide on the growth of tumours in the rat liver is described. Octreotide, administered intravenously, inhibits the growth of tumours in the liver after direct injection of somatostatin receptor-positive tumour cells into the portal vein. The growth of tumours in the liver after administration of somatostatin receptor-negative tumour cells was not inhibited. Since we also demonstrated that the serum levels of circulating hormones and growth factors, which could be mediators for tumour growth, was not influenced by treatment with octreotide, a direct mechanism for the antitumour effect was suggested. Since no effect of octreotide treatment was found for somatostatin receptor-negative tumours this direct effect might be mediated via a somatostatin receptor-dependent mechanism.

Chapter 4 describes the antiproliferative effect of PRRT with radiolabelled octreotide in our rat liver metastases model. In a similar model as described in chapter 3 tumour growth was inhibited by PRRT when somatostatin receptor-positive tumour cells were used. The effects of PRRT are impressive compared to the effect of non-radioactive octreotide. We were able to demonstrate the necessity of the somatostatin receptor for this effect since pre-blocking with non-radioactive octreotide, leading to saturation of the receptors annihilated this effect. Moreover, no effect was found

when somatostatin receptor-negative tumour cells were used. Our data suggest that PRRT with radiolabelled octreotide could be a new promising treatment modality for patients with neuroendocrine tumours and other somatostatin receptor-positive tumours.

In **chapter 5** the results are described of different treatment schedules of PRRT with radiolabelled octreotide. The radioactive dose of 370 MBq as was also used in the experiments in chapter 4 appeared to be most effective. With this dose no severe toxicity was noted. PRRT was also effective when tumours were allowed 12 days to settle in the liver. Multiple dose treatment was more effective than single dose treatment.

Chapter 6 gives an overview on the putative tumour growth stimulating effect of partial hepatectomy (PHx). PHx is the only curative treatment for patients with primary or secondary tumours in the liver. However the high number of early recurrences suggests that PHx might act as a "double-edged sword". On one hand survival after PHx is increased but on the other hand PHx might enhance tumour growth. Several explanations for this phenomenon are described but most likely growth factors that orchestrate the process of liver regeneration after PHx are responsible for tumour growth stimulation. Options for adjuvant treatment after PHx are limited since systemic chemotherapy might also influence liver regeneration. TNF-α could be a treatment option since TNF-α is known to stimulate rather than inhibit liver regeneration. Treatment with octreotide and radiolabelled octreotide could provide a therapeutic option if liver regeneration is not affected.

Chapter 7 describes the effects of PHx on tumour growth in our liver metastases model. We designed a rat model in which tumour cells are injected into the portal vein during vascular occlusion of one or more liver lobes. Resection of 35 per cent of the liver induced a significant increase in tumour growth of somatostatin receptor-negative tumours whereas resection of 70 per cent provoked excessive tumour growth leading to more than 100 liver metastases and a significantly increased wet liver weight in all animals. This means that tumour growth stimulation is proportional to the extent of the resection. TNF-α, given intravenously, only had a marginal effect in rats that did not undergo PHx but was very effective after PHx. However the clinical use of TNF-α is limited to isolated perfusion because of severe systemic toxicity.

In **chapter 8** the effect of octreotide and PRRT with radiolabelled octreotide on tumour growth after partial hepatectomy is described. The first finding in these experiments is that also the growth of somatostatin receptor-positive tumours is stimulated by PHx. Treatment of somatostatin receptor-positive tumours with non-radiolabelled octreotide after PHx did not decrease this tumour growth stimulation. After PRRT with radiolabelled octreotide, however, both in sham operated and

PHx animals there were only few somatostatin receptor-positive tumour colonies present while control animals all had a large tumour burden in their livers. PRRT was also effective on somatostatin receptor-negative tumours after PHx but not after sham operation. This effect on somatostatin receptor-negative tumours after partial hepatectomy was not expected. It was hypothesised that this effect of PRRT could have been caused by influence on liver regeneration. Therefore we investigated the effect of PRRT on liver regeneration. Liver regeneration and liver function after PHx was not influenced by PRRT. This could imply that PRRT might be a safe treatment option after PHx clinically.

For the optimal management of patients with neuroendocrine tumours accurate imaging and staging is essential. Somatostatin receptor scintigraphy (SRS) is a visualisation technique which has shown to localise neuroendocrine tumours with high sensitivity. The aim of the study described in **chapter 9** was to compare the value of conventional imaging methods and SRS in the detection of both intra- and extrahepatic metastases in 48 patients with liver metastases of neuroendocrine tumours. Conventional imaging consisted of CT of chest and abdomen and ultrasound examination of upper abdomen in all patients and additional bone scintigraphy and colonoscopy in a few patients. SRS consisted of planar scintigraphy which yields whole body, one dimensional imaging in all patients and in 26 patients additional SPECT imaging in which a rotating camera is used to make reconstructions similar to CT. All conventional imaging methods together detected extrahepatic metastases in 22 of the 48 patients. SRS detected extrahepatic metastases in all of these 22 patients but also in 15 additional patients. SRS detected 128 % more extrahepatic tumours in these patients that conventional imaging methods. With the addition of SPECT imaging to planar SRS liver metastases were detected in all patients. Therefore were conclude that SRS with the addition of SPECT imaging should be recommended as the first imaging method for the staging of patients with neuroendocrine tumours. Our paper demonstrates that nearly all patients with liver metastases of neuroendocrine tumours have extrahepatic metastases and are therefore excluded for curative liver surgery. Systemic PRRT with radiolabelled somatostatin analogues for these patients could be a promising treatment modality.

Conclusions

1. Octreotide inhibits the growth of somatostatin receptor-positive tumours and not of somatostatin receptor-negative tumours in a liver model in the rat.

2. PRRT with radiolabelled octreotide has an impressive inhibiting effect on the growth of somatostatin receptor-positive tumours in the rat.

3. The effect of PRRT is mediated through the somatostatin receptor since no effect is found after blocking of the somatostatin receptors. Moreover, no effect is found with PRRT on somatostatin receptor-negative tumours.

4. PRRT can be effective on established tumours since also tumour growth was inhibited when treatment was given 12 days after tumour cell inoculation.

5. Resection of 35 per cent or 70 per cent of the liver volume induces tumour growth stimulation in the regenerating remnant liver in the rat.

6. The extent of tumour growth stimulation after partial hepatectomy is proportional to the volume of the liver that is resected.

7. Tumour growth stimulation after 70 per cent hepatectomy in the rat is reduced by systemic treatment with TNF- α.

8. PRRT inhibits the growth of tumours in the liver even under tumour growth stimulating conditions caused by 70 per cent hepatectomy. This effect is at least partially not receptor-mediated since also tumour growth inhibition was found for somatostatin receptor-negative tumours after partial hepatectomy.

9. PRRT with radiolabelled octreotide does not influence liver regeneration after 70 per cent hepatectomy in the rat.

10. Somatostatin receptor scintigraphy in superior to all conventional imaging methods together in the imaging and staging of patients with neuroendocrine tumours.

11. Of the patients with liver metastases 78 per cent also have extrahepatic metastases and are therefore excluded from curative liver resection. These patients could possibly benefit from PRRT.

Samenvatting

Doel van dit proefschrift was om een nieuwe behandeling van tumoren te onwikkelen waarbij gebruik gemaakt wordt van radioactief octreotide. Met behulp van een rattenmodel wilden wij tevens aantonen dat tumorgroei wordt gestimuleerd door leverregeneratie die optreedt na resectie van een deel van de lever. Indien deze tumorgroei-stimulatie tijdens leverregeneratie kon worden aangetoond wilden wij bestuderen of behandeling met octreotide en radioactief octreotide deze tumorgroeistimulatie kan beïnvloeden.

Het tweede doel van dit proefschrift was om de aanvullende waarde van somatostatinereceptor scintigrafie aan te tonen bij de diagnostiek van patiënten met levermetastasen van neuro-endocrien tumoren. Somatostatinereceptor-scintigrafie is een techniek die, met behulp van een lage dosis radioactief octreotide, tumoren kan afbeelden die specifieke somatostatinereceptoren tot expressie brengen. De belangrijkste groep tumoren die deze receptoren dragen zijn neuro-endocrien tumoren. Met behulp van somatostatinereceptor-scintigrafie kan men patiënten selecteren die in aanmerking komen voor behandeling met octreotide en radioactief octreotide.

Hoofdstuk 2 geeft een overzicht van het voorkomen van somatostatinereceptoren bij neuro-endocrien tumoren en het gebruik van somatostatinereceptor-scintigrafie bij patiënten met neuro-endocriene aandoeningen. Beschreven wordt hoe somatostatine-analoga zoals octreotide klinisch worden gebruikt in de behandeling van neuro-endocrien ziekten en het effect dat deze somatostatine-analoga hebben op de groei van tumoren. Het principe van peptide receptor radionuclide therapy (PRRT) met radioactief octreotide wordt beschreven. Het is onze hypothese dat radioactief octreotide, dat in een laag radioactieve dosis wordt gebruikt voor scintigrafie, in een hoog radioactieve dosis kan worden gebruikt voor PRRT.

In **hoofdstuk 3** wordt het effect beschreven van het somatostatine-analogon octreotide op de groei van tumoren in de lever bij de rat. Octreotide remt de groei van tumoren die somatostatinereceptoren tot expressie brengen. Bij tumoren die deze receptoren niet hebben remt octreotide de groei niet. Wij konden aantonen dat door behandeling met octreotide de waarde in het serum van enkele groeiregulerende hormonen niet veranderde waarmee een direct effect op de tumorcellen van octreotide aannemelijk werd gemaakt. Omdat er geen effect werd gevonden bij receptor-negatieve tumoren menen wij dat directe effect van octreotide afhankelijk is van de aanwezigheid van specifieke somatostatinereceptoren.

Hoofdstuk 4 beschrijft het tumorgroei remmend effect van PRRT met radioactief octreotide in ons levermetastase model. PRRT was zeer effectief bij somatostatinereceptor-positieve tumoren. De resultaten waren indrukwekkend beter dan in de experimenten met niet-radioactief octreotide zoals

beschreven in hoofdstuk 3. Wij konden aantonen dat de aanwezigheid van somatostinereceptoren noodzakelijk is voor dit effect doordat blokkeren van de receptoren het effect van radioactief octreotide vrijwel volledig te niet deed. Tevens werd geen effect gezien van behandeling met radioactief octreotide bij somatostatinereceptor-negatieve tumoren. Gesteund door onze bevindingen zou PRRT met radioactief octreotide een veelbelovende behandelingsmogelijkheid kunnen zijn voor patiënten met neuro-endocrien tumoren en andere receptor-positieve tumoren.

In **hoofdstuk 5** worden de resultaten besproken van experimenten waarin PRRT met radioactief octreotide wordt toegepast in verschillende doseringen van radioactiviteit en volgens verschillende behandel schemata. PRRT met indium gelabeld octreotide met een dosering van 370MBq, zoals gebruikt in de experimenten in hoofdstuk vier bleek, het meest effectief. Met deze dosering werden geen bijwerkingen gevonden van PRRT. Herhaalde behandelingen waren effectiever dan een eenmalige behandeling.

Hoofdstuk 6 geeft een overzicht van literatuur over en inzichten in het groeistimulerend effect dat op kan treden tijdens leverregeneratie na resectie van een deel van de lever. Deze operatie, waarbij tumoren in de lever worden verwijderd met mede nemen van gezond lever weefsel, wordt partiële hepatectomie (PHx) genoemd. PHx is de enige behandelingsmogelijkheid met kans op genezing bij patiënten met primaire levertumoren of levermetastasen van andere maligne aandoeningen. De overleving van patiënten wordt door de operatie duidelijk verhoogd maar indien zich recidief tumorgroei ontwikkelt wordt dit bij het merendeel van de patiënten al gediagnostiseerd binnen één jaar na de operatie. De meeste recidieven worden gevonden in de lever zelf. PHx zou dus kunnen werken als een 'mes dat aan twee kanten snijdt'. Aan de ene kant verbetert de overleving door de operatie maar aan de andere kant lijkt het dat tumorgroei wordt gestimuleerd in de regenererende lever. Er zijn verschillende verklaringen voor versterkte tumorgroei na PHx. De meeste waarde wordt gehecht aan de verklaring dat groeifactoren die betrokken zijn bij het regeneratieproces van de lever tevens een tumorgroei-stimulerende werking hebben. Mogelijkheden voor adjuvante behandeling direct na PHx worden beperkt doordat chemotherapie de regeneratie kan ontregelen. Behandeling met tumor necrosis factor α (TNF-α) zou een optie kunnen zijn direct na PHx omdat TNF-α de leverregeneratie zou stimuleren. Ook behandeling met octreotide of PRRT met radioactief octreotide zouden effectief kunnen zijn als adjuvante behandeling na PHx. Indien er tumorgroei-remming gevonden wordt dient tevens het effect van deze stoffen op het proces van leverregeneratie onderzocht moeten worden.

Hoofdstuk 7 beschrijft het effect van PHx op de groei van tumoren in ons lever model. Resectie van 35 procent van de lever veroorzaakte een significante toename in het aantal somatostatinereceptor-negatieve tumoren. Resectie van zeventig procent veroorzaakte een explosieve

tumorgroei waarbij in alle dieren meer dan 100 tumoren werden gevonden in de lever. Tevens werd een significante toename gevonden van het gewicht van de levers. Hieruit concluderen wij dat de omvang van het tumorgroei-stimulerend-effect gerelateerd is aan de grootte van de leverresectie. Behandeling met TNF-α bleek het effect van tumorgroei stimulatie door PHx te kunnen remmen. Klinisch worden de mogelijkheden van systemische toediening van TNF-α beperkt door ernstige toxiciteit. Wel wordt TNF-α gebruikt bij geïsoleerde perfusie van extremiteiten en solide organen.

In **hoofdstuk 8** wordt het effect beschreven van behandeling met octreotide en PRRT met radioactief octreotide op tumorgroei na PHx. Aangetoond werd dat ook somatostatine-receptor-positieve tumoren tot versnelde groei komen tijdens leverregeneratie. Behandeling met niet-radioactief octreotide had geen invloed op de tumorgroei stimulatie van PHx. PRRT met radioactief octreotide resulteerde in een significant verminderde tumorgroei na PHx en controle operatie zoals beschreven in hoofdstuk 4 en 5. Tegen de verwachting in werd ook de groei geremd van receptor-negatieve tumoren na PHx. Wij hadden de theorie dat de verminderde tumorgroei stimulatie van PHx veroorzaakt zou kunnen worden door remming van de leverregeneratie. In onze experimenten werd geen invloed worden aangetoond van PRRT op de leverregeneratie. Het feit dat de regeneratie niet wordt geremd door PRRT kan betekenen dat PRRT veilig kan worden toegepast direct na leverresectie.

Om patiënten met neuro-endocriene tumoren effectief te kunnen behandelen is het noodzakelijk om over goede diagnostiek te beschikken om te komen tot de juiste stagering. Somatostatinereceptor-scintigrafie (SRS) is een afbeeldingtechniek waarmee neuro-endocrien tumoren, met hoge sensitiviteit, kunnen worden afgebeeld. Het doel van de studie in **hoofdstuk 9** was om de waarde van de SRS te vergelijken met conventionele diagnostiek in de detectie van metastasen binnen en buiten de lever in 48 patiënten met levermetastasen van histologisch bewezen neuro-endocrien tumoren. Conventionele diagnostiek bestond uit CT en echo van de buik, eventueel aangevuld met een botscan of colonoscopie. Bij alle patiënten werd SRS als ééndimensionaal onderzoek verricht. Bij 26 patiënten werd dit onderzoek uitgebreid met SPECT afbeelding waarbij met scintigrafie-beelden reconstructies worden gemaakt zoals bij spiraal-CT afbeeldingen. Met conventionele diagnostiek werden in 22 van de 48 patiënten metastasen gevonden buiten de lever. SRS bevestigde deze metastasen buiten de lever bij alle 22 patiënten maar detecteerde deze bij nog eens 15 andere patiënten. Met SRS werden 128 procent meer metastasen gevonden buiten de lever. Met de toevoeging van SPECT werden alle patiënten de aanwezigheid van levermetastasen bevestigd. Wij concluderen daarom dat bij alle patiënten met neuro-endocrien tumoren eerst SRS moet worden verricht en dan pas conventionele diagnostiek op indicatie. Wij vonden dat bijna alle patiënten die levermetastasen hebben van neuro-endocrien tumoren ook metastasen hebben buiten de lever. Hierdoor komen deze patiënten niet in aanmerking voor lever resectie met kans op genezing. PRRT

met radioactieve somatostatine-analoga zou voor deze patiënten een veelbelovende behandelingsmogelijkheid kunnen zijn.

Conclusies

1. Behandeling met octreotide remt de groei van somatostatinereceptor-positieve tumoren in de rat en niet van receptor-negatieve tumoren.

2. PRRT met radioactief octreotide resulteert in een indrukwekkende groeidaling van receptor-positieve tumoren in de rat.

3. Voor het effect van PRRT is de aanwezigheid van specifieke somatostatine receptoren noodzakelijk omdat blokkering van deze receptoren het effect van PRRT te niet doet. Daarnaast wordt geen effect gevonden bij receptor-negatieve tumoren.

4. PRRT is effectief in de vroege fase van tumorgroei in de lever, ook bij solide tumoren die zich gedurende 12 dagen hebben ontwikkeld.

5. Resectie van 35 of 70 procent van het levervolume resulteert in versterkte tumorgroei in de regenererende restlever.

6. De intensiteit van tumorgroei-stimulatie is afhankelijk van de uitgebreidheid van de resectie.

7. Tumorgroei-stimulatie na 70 procent partiele hepatectomie kan worden geremd met systemisch toegediend TNF-α.

8. PRRT met radioactief octreotide remt de groei van tumoren in de lever zelfs onder condities van tumorgroei-stimulatie door 70 procent partiele hepatectomie. Dit effect is ten dele niet receptor afhankelijk omdat na partiele resectie ook tumorgroei-remming werd gezien bij receptor-negatieve tumoren.

9. PRRT met radioactief octreotide heeft geen invloed op leverregeneratie bij de rat.

10. Somatostatine-receptor-scintigrafie heeft een grotere sensitiviteit dan conventionele diagnostiek bij de detectie en stagering van patiënten met neuro-endocriene tumoren.

11. Zevenentachtig procent van de patiënten met levermetastasen van neuro-endocrien tumoren heeft ook metastasen buiten de lever en komt daarvoor niet in aanmerking voor curatieve leverresectie. Deze patiënten zouden mogelijk baat hebben bij een behandeling met PRRT.

CHAPTER 11

FUTURE ASPECTS OF PEPTIDE RECEPTOR RADIONUCLIDE THERAPY

Introduction

Based on the concept of somatostatin receptor scintigraphy with radiolabelled peptides our experiments on peptide receptor radionuclide therapy (PRRT) were initialised. This thesis has demonstrated the possibilities for PRRT with the radiolabelled somatostatin analogue [^{111}In-DTPA^0]octreotide. Our experimental results and also clinical findings indicate that PRRT with radiolabelled somatostatin analogues could be a promising new treatment modality for patients with somatostatin receptor-positive tumours. PRRT, however, is still in its infancy and further developments are necessary to optimise therapy.

New radionuclides

For radiotherapeutic applications, besides ^{111}In other radionuclides such as ^{90}Y (Yttrium) and ^{177}Lu (Lutetium) have also been proposed for coupling to somatostatin analogues. ^{90}Y , with a half-life of 2.7 days, is a pure high-energy β emitter with a tissue range up to 12 mm, and ^{177}Lu, with a half-life of 6.7 days emits γ radiation (suitable for visualisation), and intermediate-energy β particles with approximately 2 mm tissue penetration. For tumours with a heterogeneous distribution of somatostatin receptors, ^{90}Y- and ^{177}Lu-labelled somatostatin analogues might have extra beneficial properties because of an effect known as "cross-fire". A tumour cell lacking somatostatin receptors might be hit by an electron coming from a neighbouring cell that has internalised the radioligand. This mechanism may lead to a higher and more homogeneous radiation dose in larger parts of the tumour.

Promising results with regard to growth inhibition were shown in pre-clinical studies with eradication of CA-20948 tumours[1,2] and in patient studies using [^{90}Y-DOTA^0,Tyr^3]octreotide (OctreoTher®). Preliminary clinical reports indicate that relatively high activities of [^{90}Y-DOTA^0,Tyr^3]octreotide can be administered with low risk of myelotoxicity, although the radiation doses to the kidneys require careful consideration. Tumor doses can be high enough to obtain objective therapeutic responses[3-5].

High radiotherapeutic efficacy of PRRT with [^{177}Lu-DOTA^0,Tyr^3]octreotate was found in experimental studies[6]. [^{177}Lu-DOTA^0,Tyr^3]octreotate shows the highest tumour uptake of all tested octreotide analogues so far, with excellent tumour to kidney ratios. PRRT with this analogue started only very recently in our centre[7].

New somatostatin analogues

Currently the most promising somatostatin analogue is [Tyr^3]octreotate, where the alcohol Thr(ol) at the C-terminus is replaced with the natural amino acid Thr. This analogue has a very high affinity for the somatostatin receptor subtype2 (sst_2) and showed the highest CA-20948 tumour uptake in a biodistribution study in rats comparing different ^{111}In-labelled somatostatin analogues[8].

Reduction of toxicity

A major problem in PRRT is caused by the uptake of radioactivity in the kidneys: small peptides in the blood plasma are filtered through theglomerular capillaries in the kidneys and subsequently reabsorbed by and retained in the proximal tubular cells. Renal uptake of radioactivity can be reduced by 40 per cent by intravenous administration of a combination of L-lysine and L-arginine. Clinnically PRRT with somatostain analogues should be carried out with co-infusion of these aminoacids[7]. Other methods to reduce toxicity are currently under investigation. Another critical organ is bone marrow. Based on dosimetry (for PRRT with ^{111}In or ^{177}Lu with conventional gamma cameras and for PRRT with ^{90}Y with position emission tomography (PET) using ^{86}Y) the cumulative radiation dose has to be kept below 2-3 Gy. Higher doses may result in myelodysplasic syndrome and/or leukemia.

Coupling to chemotherapeutic agents

Another approach in treating tumours expressing somatostin receptors is to substitute the radionuclide with a chemotherapeutic agent. Schally's group has developed a cytotoxic somatostatin analogue containing 2 pyrrolino-doxorubicin (AN-201), which is 500 to 1000 times more potent *in vitro* than its parent compound[9,10]. By linking AN-201 to RC-121, a somatostatin analogue, a new cytotoxic somatostatin analogue, (AN-238), was obtained. Various *in vitro* experiments with AN-238 on human and rat somatostatin receptor-positive tumours have shown effective inhibition of tumour growth[11,12].

Transfection of somatostatin receptors

New developments in molecular biology have made it possible to transfect somatostatin receptor-negative tumour cells with a somatostatin receptor gene. Susini's group has developed an approach using somatostatin receptor subtype 2 (sst_2) -gene transfer for the treatment of pancreatic cancer[13]. By inducing the somatostatin receptor on these tumour cells, antitumour effects were obtained which might be attributed to several mechanisms. First, an autocrine negative feedback loop in which transfected tumour cells start to produce somatostatin, which binds in an autocrine manner to the induced somatostatin receptor, may provide an inhibitory effect on tumour cell growth. Second, binding of somatostatin to sst_2 upregulates p27, a tumour suppressor gene, which leads to cell cycle arrest in the G0-G1 phase, and subsequently causes apoptosis. Local and distant bystander effects have also been noted. The local bystander effect might be attributed in part to apoptosis. When sst_2-positive cells undergo apoptosis, these cells release apoptotic vesicles and enzymes, which in turn might kill neighbouring cells. The distant bystander effect may be explained on a paracrine effect. Somatostatin can upregulate the expression of sst_1 on parental tumour cells, thereby rendering them sensitive to the antiproliferative effect of somatostatin. All the above mentioned mechanisms may contribute to successful treatment of certain types of cancers with gene therapy.

Another reason why transfection of tumour cells with a somatostatin receptor gene may be beneficial involves peptide receptor radionuclide therapy (PRRT). By inducing the somatostatin receptor on somatostatin receptor-negative tumours, treatment with radionuclides might become an option. Moreover, transfection of somatostatin receptor-positive tumours with a somatostatin receptor gene can increase the homogeneity of distribution of tumour cells expressing the somatostatin receptor and so increase the efficacy of PRRT.

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CURRICULUM VITAE

Gerrit Dirk Slooter werd op 13 oktober 1965 geboren in Rotterdam. Vanaf 1977 tot 2001 volgde hij onderwijs op het Dr. Molewaterplein te Rotterdam. Na het beëindigen van de middelbare school in 1984 (Gymnasium Erasmianum) studeerde hij gedurende één jaar geschiedenis en biomedische wetenschappen aan de S.R. University of Pennsylvania met een studiebeurs van the Netherlands America Committee of Educational Excange. In 1985 startte hij met de studie geneeskunde aan de Erasmus Universiteit Rotterdam en behaalde het doctoraalexamen in 1990. De studie werd voor een jaar onderbroken om te werken als wetenschappelijk medewerker op de afdeling radiodiagnostiek van de Erasmus Universiteit. Hij studeerde af als arts in 1992 en werd aangesteld als wetenschappelijk medewerker op het Laboratorium voor Chirurgie. Een jaar later werd onderzoek gecombineerd met klinisch werk op de afdeling Heelkunde. Hij volgde de opleiding tot chirurg vanaf 1995 in het Zuiderziekenhuis (opleider Dr. K.J. Brouwer) en vanaf 1998 in het Academisch Ziekenhuis Rotterdam- Dijkzigt (opleiders Prof. dr. H.A. Bruining en Prof. dr. H.J. Bonjer, hoofd Prof. dr. J. Jeekel). In zijn laatste opleidingsjaar volgde hij de officiële differentiatie in de traumatologische chirurgie bij Prof. dr. A.B. van Vugt. Op 1 januari 2001 werd hij geregistreerd als algemeen chirurg. Gedurende de eerste drie maanden van 2001 volbracht hij een AO-stipendium Unfallchirurgie in München (opleider Prof. dr. B. Claudi). In mei 2001 zal hij toetreden tot de maatschap Algemene Chirurgie van het St. Joseph Ziekenhuis te Veldhoven (in associatie met Dr. F.A.A.M. Croiset van Uchelen, M.H.M. Bender, J.A. Charbon, Dr. R.M.H. Roumen, Dr. M.R.M. Scheltinga en met M.G. Luiting). Op 7 april 1995 is hij getrouwd met Christiana Wilhelmina Perquin. Zij hebben thans twee dochters: Floor en Lotte.

LIST OF ABBREVIATIONS

AMP	Adenosine monophosphate
APUD	Amine precursor uptake and decarboxylation
Bmax	Maximum binding capacity
BIM 23014	Lanreotide
CIM	Conventional imaging methods
CNS	Central nervous system
CT	Computed tomography
DNA	Deoxyribonucleic acid
DOTA	tetracyclododecanetetraacetic acid
DTPA	Diethylenetriamino pentaacetic acid
EGF	Epidermal growth factor
FCS	Fetal calf serum
FGF	Fibroblast growth factor
FU	Fluorouracil
GBq	Giga Becquerel
GEP	Gastro-entero pancreatic tumours
GH	Growth hormone
Gy	Gray
HGF	Hepatocyte growth factor
IC50	Concentration, needed to achieve 50% inhibition of binding
IFN	interferon
IGF-1	Insulin-like growth factor-1
K_d	Dissociation constant
MBq	Mega Becquerel
MMC	Mitomycin C
MRI	Magnetic resonance imaging
NET	Neuroendocrine tumours
PBS	Phosphate buffered saline
PET	Position emission tomography
PHx	Partial hepatectomy
PRRT	Peptide receptor radionuclide therapy
RC 160	Vaptreotide
RES	Reticulo endothelial system
RNA	Ribonucleic acid
RT-PCR	Transcriptase polymerase chain reaction
SPECT	Single photon emission computed tomography
SRS	Somatostatin receptor scintigraphy
SS	Somatostatin
SS-R	Somatostatin receptor
sst	Somatostatin receptor subtype
TGF	Transforming growth factor
TNF	Tumour necrosis factor
US	Ultra sound
WAG	White Agouti