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Chemoresistance of renal cell carcinoma: 1986–1994

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Summary. Multidrug resistance (MDR) in a variety of human tumors such as renal cell carcinoma (RCC) is thought to be caused by expression of the *mdr1* gene and may be reversed by applying chemosensitizers such as Dexverapamil that inhibit the *mdr1* gene product P-glycoprotein. On the basis of our preclinical analysis, we inititated a clinical (GCP) study with vinblastine (VBL), the most effective – if at all – chemotherapeutic agent; dexverapamil; and dexamethasone in patients with RCC. All patients had histologically proven RCC that was metastatic and progressive at study entry. The statistical design featured a preliminary study of two cycles of VBL alone followed by tumor evaluation. If no response was documented, with all patients thus serving as their own control, dexverapamil and dexamethasone were added for a minimum of three cycles of combination therapy. Having obtained institutional permission by the ethical review committee (MEC 124, 106–1993/12), we enrolled 24 patients on this protocol starting on May 3, 1993. In the preliminary study, 1 complete response (CR) was achieved with VBL alone, and myelotoxicity led to an adequate dose reduction from 2 mg/m^2 VBL per day given as a 5-day continuous infusion (days 1–5) in 6/10 yet evaluable patients to 1.4 mg/m^2 per day. In 8/11 yet evaluable patients, dexverapamil doses reached ≥ 3000 mg/day by 7-day oral uptake (days 0–6, supported by 20 mg dexamethasone given twice daily), which is significantly higher than those previously reported. The combination of VBL given at 1.4 mg/m^2 per day plus, dexverapamil given at 3000 mg per day was felt to be safe and well tolerated. Nine patients were yet evaluable for response. One partial response and three minor responses were noted in this heavily pretreated study population. It appears that this innovative approach may have some activity in RCC and may eventually lead to a rational treatment modality. Careful evaluation in ongoing studies is warranted.

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Renal cell carcinoma (RCC) is the third most common urologic malignancy and accounts for approximately 3% of all adult tumors. The incidence of RCC in the Netherlands is about 1000 new cases every year, roughly 75% of those presenting originally with organ-confined malignancy. The treatment of choice for nondisseminated disease relies on surgery spanning from organ-sparing tumor resection to radical nephrectomy. The 5-year survival for all stages was 40% 40 years ago, reached 50% 20 years ago, and amounted to 60% in the recent literature. It is widely accepted that in addition to refinements in operative strategies and general hospital care, paramount use of ultrasound in medicine has led to earlier tumor detection, thus enabling surgical resection. However, further improvement in the prognosis of RCC is most likely to depend on the development of an eventually effective systemic treatment for the persistantly high number of patients with metastatic disease (reviewed in [10]).

Therapeutic options for advanced stages, including hormonal, immuno-, and chemotherapy, have no proven efficacy, and there is an abundance of recent investigations using innovative forms of immunotherapy [30], gene therapy [29], and chemosensitization-enhanced chemotherapy [11]. The development of the latter is reviewed in this paper.

Chemotherapy of renal cell carcinoma

Until relatively recently, treatment of cancer was the exclusive province of surgeons and radiotherapists. However, only close to half of the patients with newly diagnosed malignancy present with disease localized at the original site and will be cured by such an ablative therapy. The remaining malignancies include systemic cancers such as leukemia and lymphoma and unifocal tumors that have spread by metastasis. The only hope for cure of these neoplasms resides in systemic treatments such as immunotherapy and chemotherapy.

Chemotherapy is a relatively new discipline. It arose in the 1940s and is largely based on two principles. The first

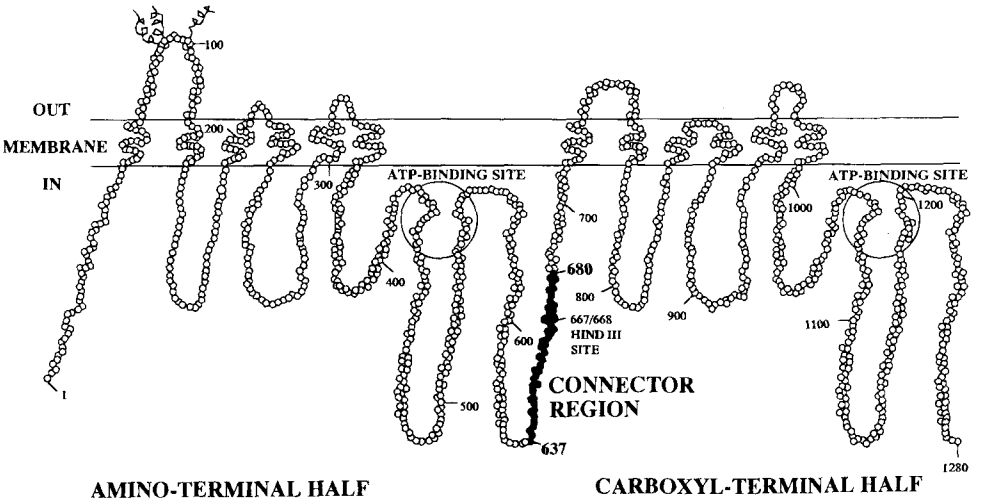

Fig. 1. Two-dimensional model of Pgp (modified after Gottesman and Pastan [5] and Gottesman et al. [6]). Pgp is a 1280-amino acid protein that contains 12 transmembranous regions and belongs to the adenosine triphosphate *(ATP)*-binding cassette superfamily of transport proteins. It functions as an energy-dependent multidrug transporter that evacuates natural-product chemotherapeutic agents from the cytoplasm or from the lipid bilayer of the membrane

is that certain types of cancers originate from differentiated cell types that have special properties and can be targeted if these characteristics persist in the cancer (e.g., via immunotoxins). The second is that cancer cells have metabolic differences that distinguish them from normal cells and these can be exploited. In urologic oncology, chemotherapy is the most widely used systemic cancer treatment. However, only in testicular cancer are cure rates beyond the 90% level, and in metastatic bladder cancer, initial response rates may be up to 70%, but long-term survival usually does not exceed 10%–15%.

A strong interest in chemotherapy of RCC has been noted in the past. By 1967, 30 drugs had been given to 247 patients; by 1977, 42 drugs had been given to 1703 patients; and by 1983, 53 drugs had been given to 2416 patients and introduced into controlled clinical trials. The most recent analysis, from 1983 to 1989, included 39 new drugs that had been given to 2120 patients. The results were very disappointing, however, with less than 7% of the patients achieving objective remissions. As a consequence, the discussion on chemotherapy of RCC once more became laboratory-based and experimental. Current investigations mainly focus on the detection of well-defined drug-resistance mechanisms in RCC and on strategies to reverse them [9].

Multidrug resistance

Cancers such as RCC are often resistant to drugs of more than one type with varying structures and different mechanisms of action. This phenomenon is termed multidrug resistance (MDR). A search for the cause or causes of MDR has occupied the attention of cancer researchers for more than four decades, and it is widely believed that if the biochemical and molecular basis of drug resistance is fully elucidated, it should become possible to devise new strategies for the circumvention of this resistance, hence increasing the number of cancers that can be cured.

The high frequency of MDR, seen both in the clinical course of the disease and in tissue-culture models, suggests that renal cancer cells can express genes that confer simultaneous resistance to different kinds of anticancer drugs. Three such mechanisms have been biochemically and genetically investigated in RCC and comprise *P*-glycoprotein, the product of the *mdr1* gene [3, 7, 8, 13]; glutathione metabolism [14]; and topoisomerase enzymes [31]. Others, such as MDR-associated protein (MRP), are the subject of ongoing studies (G. Mickisch et al., manuscript in preparation).

For the last 8 years, we and other investigators have embarked on studies of drug-resistance mechanisms in urologic cancers in the hope that this molecular analysis will eventually support our clinical research by defining pathways by which chemoresistance can be surmounted or reversed. It now appears that RCCs are unique in differentially expressing a minimum of three distinct factors associated with MDR, thus making RCC a tumor model of importance far beyond urologic oncology. A multitude of clinical studies has emerged since then, all with the same aim of intensifying chemotherapy by interfering with one or several resistance mediators. In RCC, thus far only attempts to inhibit *P*-glycoprotein have led to clinical investigations, and I therefore confine my personal annotations to this particular mechanism.

Expression of *mdr1* and its functional relevance

The human MDR gene *mdr1* (reviewed in [5, 27]), which encodes a 170,000-Da plasma membrane protein named P-glycoprotein (Pgp), (Fig. 1), is widely expressed in normal human tissues. It is found on the surfaces of epithelia of the kidney, intestine, liver, and pancreas; in the adrenal cortex; in the placenta; and in capillary endothelial cells in the testis and brain. Pgp functions as a multidrug-transport protein that extrudes hydrophobic compounds from cells (Fig. 2). When transfected with the *mdr1* gene, sensitive cells become highly resistant to many natural-product chemotherapeutic drugs that are recognized and expelled by the multidrug transporter. This accumulated evidence has led to the suggestion that in normal cells the multidrug transporter has an important role in removing from the body toxic agents ingested in food or inhaled in air, in trans-

porting steroids in the adrenal, and in protecting vulnerable tissues such as the fetus, the brain, or the testis.

When tumors were examined for expression of *mdr1*, it was found to be present in many cancers derived from normal tissue in which it is constitutively detectable. In kidneys, the highest levels of *mdr1* expression were determined in the cells of the proximal tubules, and RCCs arise from these cells. Gene expression could be quantitated by bulk methods such as Northern blotting, slot blotting, or RNAse protection assay [3, 7, 8, 22, 33], and tissue specificity was established by in situ hybridization followed by autoradiography (G. H. Mickisch et al., manuscript in preparation). Despite the possible presence of the closely related *mdr2* transcript, with appropriate care these assays can be made quite specific and relatively quantitative [5, 6].

Expression of Pgp has also been measured by antibodies, which for obvious reasons appeared to be an attractive methodology in a clinical setting. The initial analysis was a negative one, since among many other tissues, two RCCs were also assessed and reported to be Pgp-negative [32]. In our first study, 6 of 20 surgical RCC specimens were found to express Pgp [1], and with further refinement of the techniques, we were capable of detecting Pgp in 19 of 35 RCCs [14]. In general, it is currently accepted that with the help of a panel of monoclonal antibodies, Pgp can be routinely traced in more than 90% of RCCs [34].

These current methods involve obtaining quick-frozen tumor samples in large enough quantities to prepare RNA or to apply a panel of antibodies on frozen sections/archival materials. This appears easy for many primary cancers but is hardly feasible for core biopsies from recurrent or metastatic lesions. The latter difficulty in sample size can be surmounted using new technology based on reverse transcription followed by polymerization chain reaction, which amplifies the RNA signal in a small number of renal tumor cells (G. H. Mickisch et al., submitted for publication).

In well-differentiated parts of RCC with a tendency to tubule formation, apical staining, mimicking the staining pattern in normal proximal tubules, was observed [13, 14], whereas the clear cell variant showed faint heterogeneous positivity [34] less pronounced than that of tubular differentiated tumor cells. This finding is in agreement with the higher *mdr1* mRNA levels described in (well) differentiated (grade 1–2) tumors as compared with poorly differentiated (grade 3-4) RCC [8]. Since grading contributes to some extent to the prognosis of patients with RCC [10] and since patients with rapidly progressive disease tend to suffer from poorly differentiated or sarcomatoid subtypes, Pgp expression appeared not to be a marker for an unfavorable prognosis for these tumor entities [13]. On the basis of these retrospective studies [8, 13, 34], we recently started a prospective trial measuring, among many other molecular parameters, *mdr1* expression (mRNA + protein levels) in 59 consecutive tumor nephrectomy specimens and monitoring the clinical course of disease [24]. Time and a multivariate analysis will tell whether Pgp might eventually emerge as an independent prognostic factor in RCC.

Finally, a possible relationship between Pgp expression and in vitro chemoresistance has been addressed. Pgp has been discovered in 6 of 8 doxorubicin-resistant [3] and in 12 of 14 vinblastine-resistant [7] RCC cultures. In addition, a significant increase in in vitro vinblastine or doxorubicin cytotoxicity with dexverapamil or trifluoperazine, related to the expression of Pgp, has been found in cells obtained from patients with RCC [13, 35].

Chemosensitization in vitro

As noted above, the presence of the multidrug transporter in RCC has been conclusively demonstrated and an association with intrinsic chemoresistance has been established. One goal of current cancer research is to explore ways to reverse, circumvent, or overcome MDR due to *mdr1* expression; this strategy is termed chemosensitization. Clinically, there is profound skepticism with regard to the use of classic chemotherapy in RCC, but since there is no reliable therapy for metastatic disease, innovative approaches including chemosensitization seem warranted.

In general, reversal of MDR has been accomplished by exposing drug-resistant cells to a variety of alternate substrates of Pgp (see Fig. 3), which in themselves are only slightly cytotoxic, if at all. There seems to be no connection among these chemosensitizing agents on a mechanistic level, but they all share a common feature of being membrane-active.

In RCC, this modulation of MDR has initially been achieved in vitro by applying calcium antagonists such as verapamil [3, 13] or the antiarrhythmic drug quinidine [3, 8]. The anti-tumor effects of doxorubicin or vinblastine have been strongly enhanced as deteced by soft-agar clonogenic assay, [^{3}H]-thymidine incorporation, or colorimetric conversion of tetrazolium dye (MTT), respectively. Unfortunately, these prototype low-molecular-weight MDR-reversing agents are potent pharmacologic compounds that produce undesirable side effects when given at the levels required, to surmount MDR substantially. In the case of verapamil, the first chemosensitizing effects were documented at concentrations of 1–2 μM, with optimal resistance modulation occurring at approximately 5–7 μM, far beyond a clinically useful range. Thus, a multitude of new agents with an improved therapeutic index have been and remain under development.

We therefore systematically investigated different classes of calcium antagonists [13] in RCC. Papaverine derivatives such as verapamil and its analogues seemed to bear the most adequate properties for further refinement. Two different strategies appeared feasilbe for reversing MDR in a clinical setting. One involved the evaluation of new chemosensitizers derived from verapamil by selection for higher potency in reversing MDR without inflicting excessive toxicity. Therefore, lower doses should be needed to achieve reversal of MDR, and some of these drugs have been classified as being attractive candidates for future clinical trials [16]. The other concept was to explore resistance modifiers such as dexverapamil, the R(+)-stereoisomer of verapamil, which exhibits strongly reduced cardiovascular activity as compared with racemic verapamil, while maintaining its ability to reverse MDR [13]. Because much higher doses of dexverapamil can be given, clinical trials have been initiated [11].

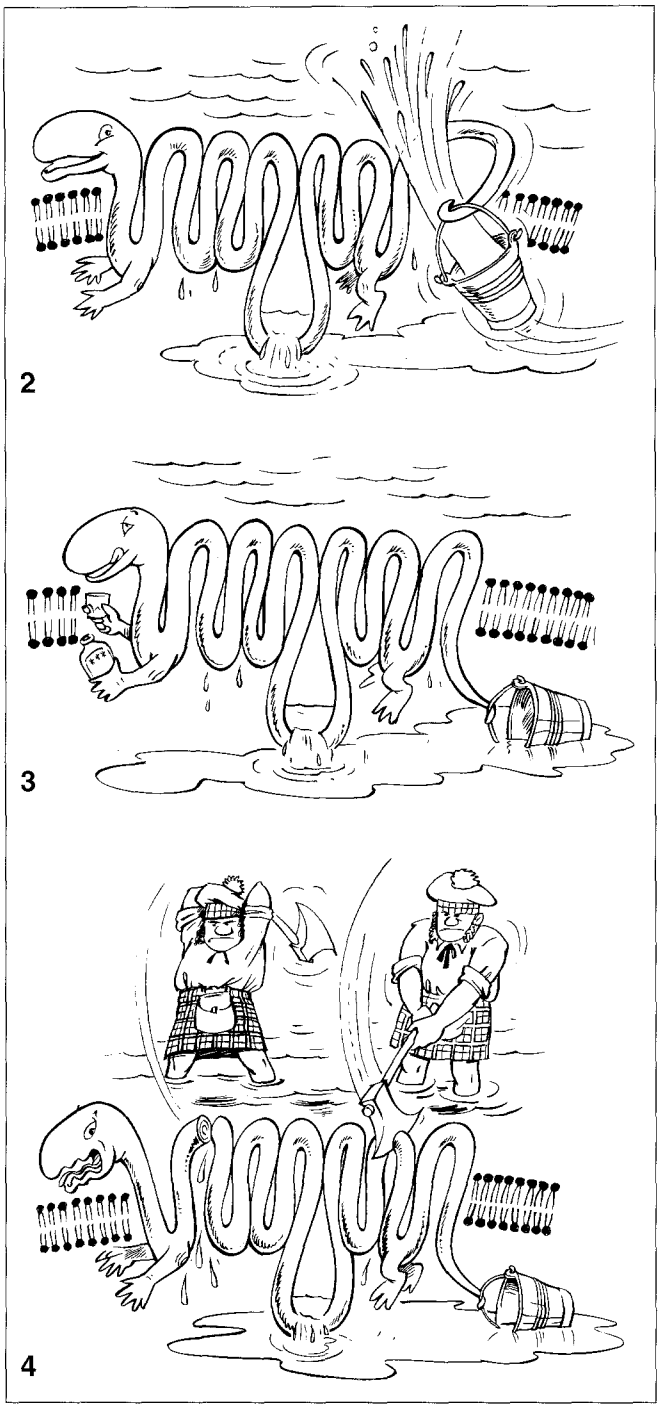

Fig. 2. Schematic illustration of the pump mechanism of the *mdr1* gene encoding for Pgp, the "multidrug transporter," also known as the "Loch Ness monster of drug resistance." Chemotherapeutic agents enter the cell by passive diffusion. Pgp rapidly and effectively expels them by an energy-dependent process; the cell becomes "resistant"

Fig. 3. Illustration of the current concept of chemosensitization used for clinical studies. The most commonly applied method to reverse MDR mediated by Pgp consists of distracting the pump by the use of alternate substrates, which in themselves are only slightly cytotoxic, if at all

Fig. 4. Illustration of how MDR due to Pgp can be cirumvented. By disrupting the phospholipid structure of membranes (e.g., via liposomes), functional or steric alterations of Pgp may be introduced, thus reducing its efficacy

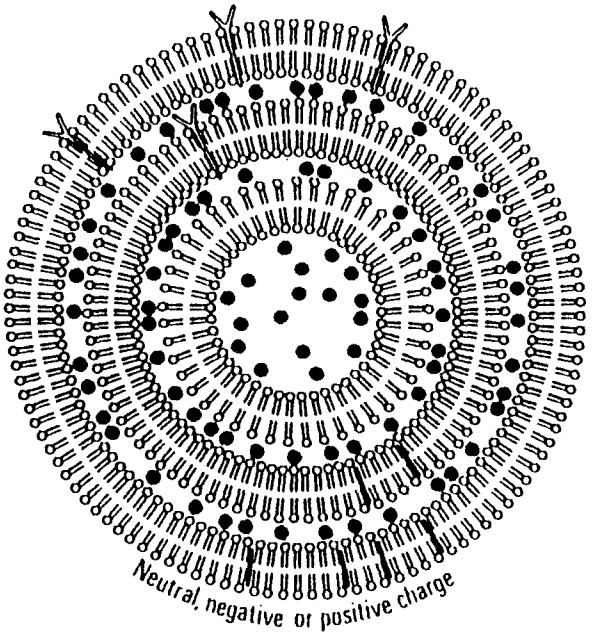

Fig. 5. Model of a chemotherapeutic drug encapsulated in a multilamellar liposome (adapted from Gregoriadis [6a]. ● Water-soluble molecules; ▮ lipid – soluble molecules; ⚚ water-soluble molecules with hydrophobic moiety penetrating lipid phase

An alternate method of chemosensitization relies on circumventing (Fig. 4) rather than distracting the activity of Pgp via false substrates (cf. Fig. 3). Liposomal encapsulation of drug (Fig. 5) may serve this purpose, since (a) it may reduce the binding affinity to Pgp, (b) liposomes themselves may inhibit the pump function of Pgp directly or indirectly, (c) liposomes may modify the phospholipid membrane strucuture and subsequently introduce functional and/or steric alterations to Pgp, and (d) delivery of drugs via liposomes may bypass the plasma membrane, directly discharging the drug into the cytoplasm, from which MDR purging of the drug may be less efficient [20]. In RCCs, our feasibility study indicated that liposomes composed of cardiolipin, phosphatidyl choline, cholesterol, and doxorubicin (approximately 12% by weight) enhanced the therapeutic ratio by a factor of 2–4 as compared with doxorubicin alone [23]. However, our liposomal formulation was developed empirically, and rational liposome design needs to be applied directly to the problem, as this will ultimately lead to the best therapeutic index (G. Mickisch et al., submitted for publication).

Another approach to eliminate the activity of Pgp is based on overcoming MDR by selectively killing cells that express Pgp on their surfaces (Fig. 6). This goal can be achieved experimentally using protein toxins such as *Pseudomonas* exotoxin (PE), that are chemically linked or recombinantly attached to certain anti-Pgp antibodies such as MRK16. For targeted drug delivery to be successful, it is necessary that the cytotoxic agent be extremely active, since the internalization efficiency of conjugates tends to be low. These toxins are catalysts with high turnover numbers, and very few molecules need to reach the cytoplasm of the cell to kill the target cell. For the construction of active immunotoxins (Fig. 7), the toxin must

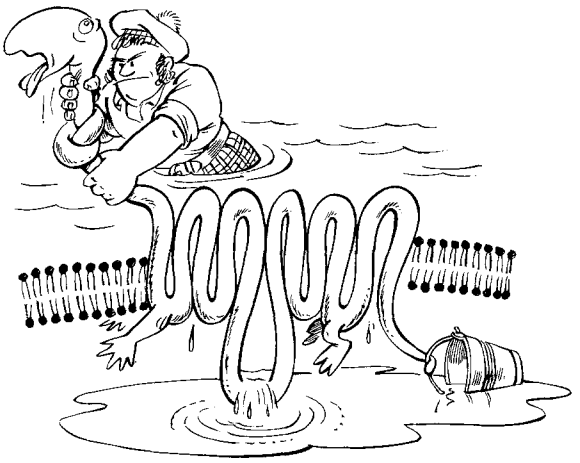

Fig. 6. Illustration of how MDR due to Pgp can be overcome. Another attempt to eliminate the activity of the multidrug transporter is based on overcoming MDR by selectively killing cells that express Pgp on their surfaces. This can be accomplished by molecular targeting via antisense oligonucleotides or via bacterial toxins chemically linked or recombinantly attached to certain anti-Pgp antibodies

be modified such that its interactions with cellular receptors are diminished or abolished, thus preventing unspecific toxicity. As a consequence, toxin entry is mediated by antibody binding. With PE, this can be accomplished by coupling MRK16 to domain I of PE, which strongly reduces the binding of domain I to the ubiquitous PE receptor β-2 microglobulin. In fact, our investigations with MRK16-PE documented effective and specific killing of Pgp-expressing renal carcinoma cells [21, 22]. Alternatively, RCCs were exposed to selected Pgp antisense oligonucleotides, resulting in significant tumor growth delay in the presence of cytotoxic agents (G.H.Mickisch et al., manuscript in preparation).

Animal studies

The development of novel pharmacologic agents for the treatment of human and animal diseases requires considerable time and expense. Typically, a new agent is either synthesized from existing materials or extracted from natural sources and then tested extensively in vitro for desired pharmacologic properties. Once potent agents are obtained, they must be tested in animals to determine whether they maintain their biological activity in the face

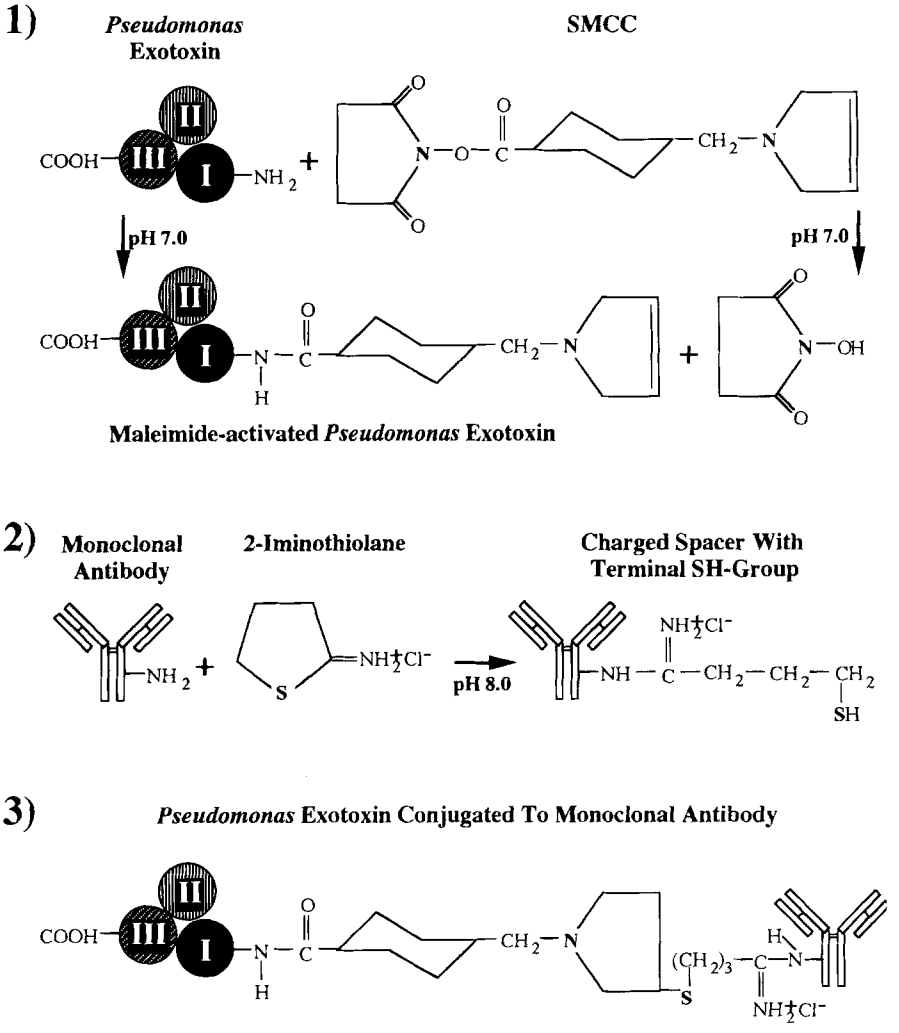

Fig. 7. Construction of an immunoconjugate directed against an external epitope of Pgp (modified after Mickisch et al. [21, 22]). This immunoconjugate (MRK16-PE) was used to eliminate Pgp-expressing renal carcinoma cells effectively and specifically

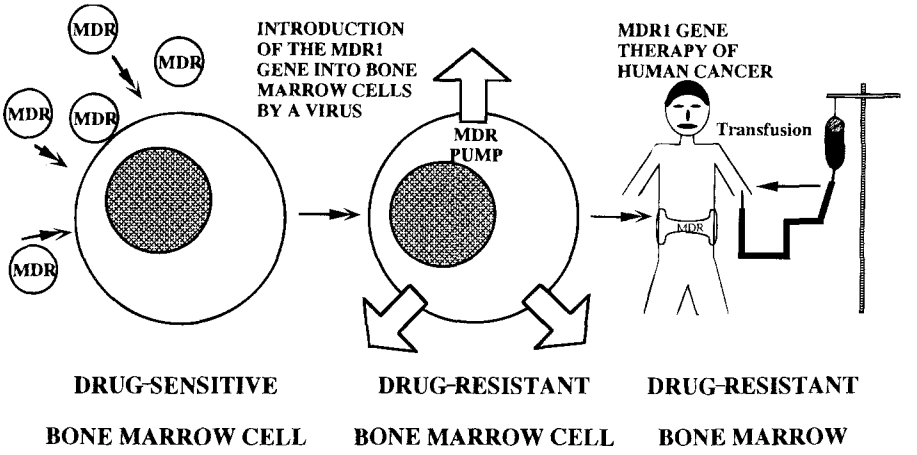

Fig. 8. Potential clinical scenario of engaging the *mdr1* gene in a gene therapeutic setting to intensify classic chemotherapy (adapted from Mickisch and Schroeder [12])

of mechanisms that excrete or chemically modify them and whether they have unexpected toxic properties unrelated to their pharmacologic effect. Animal testing of this kind is expensive, requires many months, consumes large numbers of animals, and often delays the introduction of effective new drugs for medical and veterinary use [17].

Similarly, when new drugs appear to be useful in overcoming MDR on the basis of tissue-culture data (see above), it is necessary to show appropriate bioactivity in an animal system before clinical trials can be justified. Using a traditional approach, the efficacy and toxicity of reversing agents can be evaluated in xenograft-bearing-mouse models of human tumors such as RCC. Multidrug-resistant tumors are implanted in mice and the activity of a cytotoxic drug in the presence and absence of a chemosensitizer is assessed. These types of assays are slow, require large numbers of animals, and are not highly reproducible because of variability in the growth rate of the tumors.

In the past few years it has become apparent that the use of recombinant DNA technology to engineer animals for the specific testing of new classes of pharmacologic agents can speed the development of new drugs. The general principle is that proteins with which drugs interact can be introduced into transgenic mice and used to predict the activity of the drugs in certain disease states.

Our goal was to obtain expression of the human *mdr1* gene in a drug-sensitive tissue in mice that did not express mouse-endogenous *mdr1* genes. We began with two different constructions: the first contained a truncated chicken β-actin promoter sequence joined to a full-length *mdr1* cDNA, and the second used a zinc-inducible human metallothionein promoter controlling the expression of the *mdr1* cDNA. Both promoters should allow high-level expression of *mdr1* RNA in a variety of mouse tissues. As is frequently the case in the construction of transgenic mice, the results we obtained were unexpected but extremely informative [17].

Lines of transgenic mice carrying the *mdr1* cDNA under the control of either the β-actin promoter or the inducible metallothionein promoter were established. The latter expressed Pgp in several tissues, including skeletal muscle, the central nervous system, and the lung, after induction with zinc, which made these animals resistant to the lethal effects of colchicine. The biochemical basis of this resistance is presently being characterized.

The chicken β-actin promoter linked to the *mdr1* cDNA resulted in transgenic mice whose phenotype was much easier to interpret. These mice have made it possible to study the function of human Pgp in a living animal and have formed the basis for strategies aimed at developing new anticancer drugs [15].

Several lines of mice in which the β-actin-*mdr1* transgene was stably integrated into the germ line were obtained. One of these lines (MDR39) has been studied in great detail; *mdr1* RNA levels were determined in many different organs of MDR39 mice, and transcripts of the *mdr1* gene were detected at the highest levels in bone marrow cells and, to a lesser extent, in the spleen. Very small amounts of *mdr1* RNA were also found in the ovary and muscle. The level of *mdr1* mRNA expression observed in bone marrow cells was comparable with that seen in an in-vitro-selected cell line (KB-8-5) that exhibits 3- to 18-fold drug resistance, depending on the drug. This amount of resistance should be clinically significant and, in fact, corresponds to the levels detected in many drug-resistant human tumors such as RCC.

Since expression of Pgp was discovered in the bone marrow of MDR-transgenic mice, it appeared to be of particular importance to determine whether the transgene could protect bone marrow cells against chemotherapy. Bone marrow cells are normally very susceptible to anticancer drugs and, thus, toxicity to bone marrow is often dose-limiting for many kinds of chemotherapy. Differential WBCs of normal and MDR-transgenic mice demonstrated that all major peripheral blood cells of the MDR-transgenic mice had a selective advantage over normal blood cells due to the protection afforded by the *mdr1* gene. This protective advantage of the *mdr1* gene could be transferred to recipient animals by bone marrow transplantation, indicating that protection of marrow was intrinsic to the marrow itself [19]. These data provide clear evidence that expression of the human *mdr1* gene confers MDR on an intact animal at levels of expression seen frequently in human cancers. Transfection of the *mdr1* gene into CD34+ bone marrow cells to intensify classic chemotherapy of human cancer by virtue of *mdr1* anti-myelosuppressive protection is

Table 1. Summary of chemosensitization studies performed in the *mdr 1*-transgenic mouse model

Chemosensitizer	MTD (mg/kg)	WBC at MTD (%)	WBC_{50} (mg/kg)	Therapeutic window (MTD/ WBC_{50})
Verapamil	40	29	~ 1.1	36.4
Quinine	150	28	~ 12.0	12.5
Quinidine	50	33	~ 11.0	4.5
Cyclosporin A	150	22	~ 4.0	37.5
R-verapamil	150	30	~ 1.5	100.0
Amiodarone	100	38	~ 12.0	8.3
Progesterone[a]	> 150 +	46	~ 52.0	–[a]
LU 48895 (Knoll)	25	34	~ 1.5	16.7
LU 49940 (Knoll)	50	32	~ 1.0	50.0
LU 49667 (Knoll)	75	24	~ 0.4	187.5
LU 51903 (Knoll)	60	27	~ 0.5	120.0
PAK 104 (Nissan Chemical)	150	29	~ 14.0	10.7
PAK 200 (Nissan Chemical)	150	24	~ 0.9	166.7
RS 12103-190 (Syntex)	3	72	–	–
RS 36186-193 (Syntex)	60	90	–	–
D-Tetrandine (NSC 77037)	50	93	–	–
12288B-124 (Lederle)	150	93	–	–
B8509-035 (Byk Gulden)	20	28	~ 0.9	22.2
mAb MRK16 (Hoechst Japan)[a]	> 2 +	40	~ 1.5	–[a]
mAb MRK16F(ab')2 (Hoechst)[a]	> 1 +	55	–	–
MRK16-PE[b]	4[b]	33	~ 1.5[b]	–[b]

Data were compiled as outlined by Mickisch et al. [15, 17]. WBC_{50} = Dose of chemosensitizer that reduces the WBC by 50% within 5 days of chemosensitization. Data were generated in conjunction with the administration of 10 mg/kg daunomycin as a single i.p. bolus injection and were expressed as a percentage of the remaining WBC on day 5 as compared with the pretreatment value. *MTD*, Maximum tolerated dose as defined by Mickisch et al. [18]

[a] Not the MTD, but the highest dose tested
[b] Values expressed in μg

Table 2. Efficacy of combinations of chemosensitizers in reversing daunomycin resistance in MDR-transgenic mice

	Single dose	Verapamil (0.5 mg/kg)	Quinine (5 mg/kg)	Quindine (5 mg/kg)	Cyclosporin A (3 mg/kg)	PAK 200 (0.5 mg/kg)
Verapamil (0.5 mg/kg)	62%	48%	28%	25%	22%	45%
Quinine (5 mg/kg)	64%	28%	55%	57%	52%	42%
Quindine (5 mg/kg)	59%	25%	57%	53%	49%	40%
Cyclosporin A (3 mg/kg)	60%	22%	52%	49%	55%	35%
PAK 200 (0.5 mg/kg)	59%	45%	42%	40%	35%	50%

For a description of the methods used, see the footnote to Table 1. Column 2, Single dose of one chemosensitizer; columns 3–7, combinations of different sensitizers; underlined values, combinations of the same sensitizer (i.e., doubling of the dose)

currently being pursued in a clinical gene therapeutic setting [12] (Fig. 8).

To measure the potency of the inserted transgene, we performed dose-escalation studies in the MDR-transgenic mice using taxol. Taxol is a novel investigational antimicrotubule agent currently under study as an anticancer drug, which seemed suitable for our purposes due to its known bone marrow specificity. In fact, it became clear that expression of the *mdr1* transgene resulted in a 10-fold resistance to the cytotoxic activity of taxol in vivo [18].

The development of a preclinical model for the rapid testing of agents that circumvent MDR in cancer is a high priority of research on drug resistance. The clear-cut difference between MDR-protected and normal bone marrow made it possible to evaluate the effect of chemosensitizers [15, 16, 21]. In addition, these transgenic mice served as a simple and reliable test system to determine whether drugs that appeared on the basis of tissue-culture samples to be useful in overcoming MDR would act in a similar manner in an animal. Examples of our studies are depicted in Table 1. They show the efficacy of chemosensi-

tization (WBC_{50}), measured as a fall in the peripheral WBC of MDR-transgenic mice, the toxicity encountered with this approach (MTD), and the calculated therapeutic window (MTD/WBC_{50}), clearly indicating differences among the various reversing agents.

Moreover, we systematically investigated whether this unique system would be adequate and suitable for the assessment of combinations of prototype and readily available chemosensitizers in the hope of avoiding the toxic complications inherent to the use of higher concentrations of these drugs. Pilot studies had revealed that small amounts of drugs such as verapamil and quinine [15] or verapamil and cyclosporin A [21], which produced only partial sensitization of the MDR-transgenic bone marrow cells in vivo, were fully sensitizing when used in combination. Table 2 summarizes data obtained with only one chemosensitizer (column 2), with one chemosensitizer after doubling of the dose (underlined numbers), and with combinations of different chemosensitizers (columns 3–7). There is no doubt that certain combinations give a much greater effect than does either drug alone, whereas other combinations do not lead to substantial enhancement of efficacy.

Suffice it to say that alternative pathways such as circumvention of MDR via liposomes (Fig. 4) or overcoming of MDR via immunotoxins (Fig. 6) have been tested in these intact animals and shown to be feasible [20, 22]. However, given the considerable time required until a new drug has been approved to enter the market, we suggest second-generation chemosensitizers such as dexverapamil to be the most appropriate candidates for the initiation of rational clinical investigation adopting this principle in RCC [11].

Clinical investigations

We are now faced with the emergence of an array of clinical investigations that all have the same aim of attempting to intensify classic chemotherapy by inhibiting the underlying resistance mechanism, e.g. *mdr1* expression. Thus far, hematologic neoplasms and lymphomas have been considered appropriate diseases for the initiation of clinical evaluation. As these are tumors in which many active chemotherapeutic agents are handled and subsequently expelled by Pgp, an alteration in drug efflux via chemosensitization should indeed have an impact on response.

Recent/ongoing clinical studies in RCC are conducted under phase I/II conditions. Hence, the need for well-controlled, randomized trials to evaluate these chemomodulators will remain imperative for the near future. To date, three such investigations in RCC have been reported. As is frequently the case with innovative approaches, the initial results were not extremely rewarding. There was one attempt to circumvent MDR in 15 patients with RCC by combining bolus vinblastine with infusional cyclosporin A [28]. Despite the ambitious title of their report, these authors did not strive to determine *mdr1* expression or to document resistance against vinblastine. Median cyclosporin plasma levels reached a modest 5668 ng/ml. Thus, the toxicity was described as being minimal, as was the efficacy.

Lately, dexniguldipine, a dihydropyridine derivative, was given orally in conjunction with i.v. doxorubicin, a cytotoxic agent that has no activitiy in RCC when given as monotherapy. A total of 30 patients were recruited for this study, to render only 20 eligible cases [4]. Gastrointestinal toxicity was considerable, preventing a reasonable dose escalation, which might have contributed to a more favorable therapeutic outcome.

More recently, in a pilot study, continuous i.v. vinblastine and oral dexverapamil, the R(+/+)-stereoisomer of racemic verapamil, were given concomitantly to 12 patients [26] with continuation of patient accrual. Response rates have not yet been reported, but the study design, featuring four equal daily doses of dexverapamil, led to plasma levels associated with cardiovascular side effects such that most patients did not tolerate more than a daily dose of 1500 mg dexverapamil.

On the basis of our preclinical analysis (see above), we started a clinical (GCP) study in patients with RCC [25]. Vinblastine, the most effective – if at all – chemotherapeutic agent in RCC, was combined with dexverapamil as a chemosensitizer. Since our intensive animal testing had indicated that dexamethasone significantly increased dexverapamil tolerance, the former drug was also included in our protocol. The treatment schedule included 2 mg/m^2 (patients 1–10; optional dose reduction) vinblastine and 1.4 mg/m^2 (patient 11 through the study end; optional dose intensification) vinblastine given as a 5-day continuous infusion. Dexverapamil was given orally six times daily starting at 250 mg/dose until dose escalation reached the individual maximum tolerated dose (days 0–6), and 20 mg dexamethasone was given twice daily as a short-term infusion (days 0–6). The cycle duration was 3 weeks. All patients had histologically proven RCC (in which an *mdr1*-expression analysis will be carried out) that was metastatic and progressive at study entry. The statistical design featured a preliminary study of two cycles of vinblastine alone followed by tumor evaluation. If no response was documented, with all patients thus serving as their own control, dexverapamil and dexamethasone were added for a minimum of three cycles of combination therapy (Fig. 9). To meet the objectives of this study, i.e., to determine the toxicity and efficacy of this particular regimen in RCC, a minimum of 15 and a maximum of 25 evaluable patients were required. Having obtained institutional permission by the ethical review committee (MEC 124, 106-1993/12), we enrolled 24 patients on this protocol from May 1993 until February 1994.

In the preliminary study, 1 complete response (CR) was achieved with vinblastine alone, and myelotoxicity led to an adequate dose reduction from 2 mg/m^2 vinblastine per day given as a 5-day continuous infusion (days 1–5) in 6/10 yet evaluable patients to 1.4 mg/m^2 per day. In 8/11 yet evaluable patients, dexverapamil doses reached $\geq$ 3000 mg per day by 7-day oral uptake (days 0–6, supported by 20 mg dexamethasone given twice daily), which is significantly higher than those previously reported. The combination of vinblastine given at 1.4 mg/m^2 per day plus dexverapamil given at 3000 mg per day was felt to be safe and well tolerated (Fig. 9B). Nine patients were yet evaluable for response. One partial re-

Fig. 9. a Schematic illustration of a contemporary clinical study to reverse MDR in patients with metastatic, progressive, and vinblastine-resistant RCC (protocol MEC 124,106-1993/12). **b** This study design (protocol MEC 124,106-1993/12) was felt to be safe and well tolerated

sponse (PR; WHO criteria, > 50% reduction in tumor volume) and three minimal responses (MR; WHO criteria, > 25% and < 50% reduction in tumor volume) were noted in this heavily pretreated study population.

It appears that this innovative approach may have some activity in RCC and may eventually lead to a rational treatment modality. Careful evaluation in ongoing studies is warranted.

Conclusions

Within a period of 8 years, MDR in RCC has evolved from a laboratory curiosity to a clinical reality that has been introduced into standard textbooks such as *Campbell's Urology* [2]. The rational approach starting with expression analysis and determination of functional significance, followed by chemosensitization in vitro and in animal experiments, and ultimately ending in the initiation of clinical studies has helped to speed this process. Thus, MDR in RCC may serve as a model system for the integration of recent developments in basic science into clinical practice, clearly prompting the use of modern biotechnology to support clinical urologic research.

There is no doubt that the present therapeutic situation in metastatic RCC warrants novel approaches including experimental chemotherapeutic strategies. Moreover, since preclinical data on RCC as well as initial clinical reports on the management of hematologic malignancies using chemosensitization-enhanced chemotherapy seem to be encouraging, clinical trials on RCC patients in a controlled setting are easily justified. However, it must be stated that in spite of the successful present breakthrough in the innovative chemotherapy of RCC, there is nonetheless a long way to go into the future.

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