Multidrug resistance (mdr) genes in human cancer

K. Nooter1 & H. Herweijer2

1Department of Pharmacology and Experimental Chemotherapy, Institute of Applied Radiobiology and Immunology TNO, PO Box 5815, 2280 HV Rijswijk; and 2Department of Medical Oncology, Rotterdam Cancer Center, Rotterdam, The Netherlands.

Results of treatment with anticancer agents have steadily improved over the years following the introduction of more effective drugs and the establishment of better designed chemotherapy strategies. Still, chemotherapy failure due to cellular drug resistance remains a major problem in most cancer patients. Using cell lines made resistant to anticancer agents, several types of drug resistance have been characterised, among which are alterations in target proteins (Cabral et al., 1980; Flintoff & Essani, 1988), carrier mediated drug uptake (Redwood & Colvin, 1980; Sirotnak et al., 1981), cellular drug metabolism (Aronow et al., 1984) and cellular repair mechanisms (Bedford & Fox, 1982). A very intriguing development in drug resistance research is the discovery of the phenomenon of multidrug resistance (MDR) (Bradley et al., 1988; van der Bliek & Borst, 1989).

In MDR cells, selection for resistance to ‘naturally occurring’ drugs, e.g. anthracyclines, vinca alkaloids, podophyllotoxins, and colchicine, results in the development of cross-resistance to other members of the MDR drug family (Bech-Hansen et al., 1976; Dane, 1972; Inaba & Johnson, 1977; Skovsgaard, 1978). The MDR related drugs are structurally dissimilar and have different intracellular targets. What these drugs have in common is that they are lipophilic compounds derived from various natural products. In general, MDR cells are not cross-resistant to alkylating agents (e.g. chlorambucil and cyclophosphamide), antimetabolites (e.g. cytarabine, methotrexate, and 5-fluorouracil), or cisplatin.

A striking feature of the classical MDR phenotype is its reduced ability to accumulate drugs, as compared to the parent cell lines. This reduced drug accumulation is most likely the main cause of multidrug resistance (Dane, 1973; Kessel & Bosmans, 1970; Riehn & Biedler, 1972; Sirotnak et al., 1986, among other references). It is assumed that the reduced drug accumulation is due to activity of an energy dependent unidirectional drug efflux pump with broad substrate specificity. This drug pump is composed of a transmembrane glycoprotein (P-glycoprotein) with a molecular weight of 170 kDa (Chen et al., 1986; Gerlach et al., 1986; Gros et al., 1986). It uses energy in the form of ATP to transport drugs through a channel formed by the transmembrane segments (Hamada & Tsuibo, 1988; Horio et al., 1988).

Different P-glycoprotein isoforms have been identified, and these are referred to as pgp genes in hamsters and mdr genes in humans and mice (Ng et al., 1989). In humans, two P-glycoprotein isoforms (mdr1 and mdr2) with 80% amino acid homology have been identified (Roninson et al., 1986; van der Bliek et al., 1987). By cross-hybridisation of human genomic DNA, Roninson and co-workers isolated two mdr specific genomic clones designated as mdr1 and mdr2 (Roninson et al., 1986). Independently, Borst and co-workers isolated mdr clones from cDNA libraries prepared from human liver tissue and the human liver cell line HepG2 (van der Bliek et al., 1987). Sequences for two mdr genes were isolated. One corresponded to the previously reported human mdr1 sequence (Chen et al., 1986); the other appeared to be the human homologue of the hamster ppg3 gene and was therefore called mdr3 (van der Bliek et al., 1987; van der Bliek et al., 1988a). It is now known that the mdr3 gene of Borst et al. is identical in sequence to the mdr2 gene of Roninson et al. (Chin et al., 1989). Both the human mdr1 and mdr3 genes were found to be localised on the long arm of chromosome 7 (Callen et al., 1987) and to be linked within 330 kilobases (Chin et al., 1987). Direct proof for the role of mdr1 in MDR was obtained by transfection experiments. Expression of a full length cDNA clone of the human mdr1 gene in a drug-sensitive cell conferred a complete MDR phenotype (Ueda et al., 1987). However, the human mdr3 gene does not seem to be involved in drug resistance and no function of the gene product has yet been identified (van der Bliek et al., 1988a).

Expression of the mdr1 gene in normal tissues

Using slot blot analysis Fojo et al. (1987b) reported substantial expression of the human mdr1 gene in normal adrenal, kidney, jejunal, rectal, liver and lung tissues. Other organs and tissues (skin, subcutaneous tissue, skeletal muscle, heart, spleen, bone marrow, lymphocytes, oesophagus, stomach, ovary, kidney cortex and spinal cord) had low or undetectable mdr1 levels.

Expression was further studied at the cellular level by in situ hybridisation and immunohistochemical techniques (Cordon-Cardo et al., 1990; Thiebaut et al., 1987; van der Valk et al., 1990). P-glycoprotein was mainly found in specialised epithelial cells with secretory or excretory functions. Thiebaut et al. (1987) used the monoclonal antibody MRK16, which is directed against an external epitope of the human P-glycoprotein (Hamada & Tsuibo, 1986). In the liver, P-glycoprotein was found on the biliary surface of hepatocytes and small biliary ductules, in the pancreas on the luminal surface of the epithelial cells of small ductules and, in the kidney, on the brush border of the proximal tubules. The colon and jejenum both showed high levels of P-glycoprotein on the luminal surfaces of the mucosa. Cordon-Cardo et al. (1990) and van der Valk et al. (1990) reported P-glycoprotein expression in other specialised epithelial cells such as the sweat glands in the skin, cells lining the trachea and major bronchi in the lung, glandular epithelial cells of the prostate, breast endometrium and thyroid, acinar cells of the pancreas, and trophoblasts in the placenta. P-glycoprotein expression was also detected in capillary endothelial cells in the human brain, suggesting a role of P-glycoprotein in the blood-brain barrier (Cordon-Cardo et al., 1990). Although the natural substrate for the mdr1 gene encoded P-glycoprotein is not yet known, these expression data suggest that the P-glycoprotein drug efflux pump plays a role in the normal physiology of the organism and in the process of detoxification of xenobiotic substances.
Expression of the mdr1 gene in tumours

It is an attractive hypothesis that the clinical observation of resistance to multidrug based chemotherapy is due to enhanced mdr1 expression in the resistant tumour. Using monoclonal antibodies or nucleic acid probes, many investigators have screened tumour biopsies for mdr1 expression. Expression of mdr1 has been detected in virtually all tumour types, carcinomas, sarcomas, leukemias, and lymphomas (Tables I and II). Yet, the relevance of this phenomenon to clinical drug-resistance is not understood. Here, we present an overview of the literature on mdr1 expression in human tumour materials and discuss some aspects that in our opinion are essential for a full appreciation of the role of mdr in human cancer treatment.

Most of the studies on the expression of the mdr1 gene in human tumours have employed bulk techniques (Northern-, Western- or dot blotting, and RNAase protection) for the detection and quantification of P-glycoprotein or its mRNA. The disadvantage of such techniques is that the frequently observed contamination with nontumour cells in the biopsy as well as the heterogeneity within the tumour cell population with regard to the level of P-glycoprotein expression are ignored (Epstein et al., 1989; Ma et al.; 1987; Rothenberg et al., 1989; Tsuruo et al., 1987; Weinstein et al., 1990). But there are also studies that searched for expression of the gene in individual cells, by using either immunohistochemistry with specific antibodies or in situ hybridisation with specific RNA probes. Although these in situ methods are more subjective in interpretation than are bulk methods, they provide specific information on, e.g. the percentage of mdr positive cells, the expression levels in individual cells, the morphology of the mdr expressing cells and the localisation of the mdr expressing cells in tumours. A tumour with a low percentage of cells expressing high levels of mdr1 might give a low level of expression on average. Yet, such a small clone of high mdr1 expressing cells may be sufficient to prevent effective chemotherapy in the patient.

The literature data on the detection of mdr1 expression in solid tumours are summarised in Table I. Bulk techniques were used in all studies, except for those of Schneider et al. (1989), Salmon et al. (1989) and Chan et al. (1990), in which immunohistochemistry was used. In most studies, the tumour samples were obtained from patients who had not received prior chemotherapy. In some, treatment status was not indicated (Fojo et al., 1987a; Gerlach et al., 1987; Lai et al., 1989; Moscow et al., 1989) and only a few compared both treated and untreated tumour samples (Bourhis et al., 1989a, b, Chan et al., 1990; Gerlach et al., 1987; Goldstein et al., 1989; Schneider et al., 1989). We have arbitrarily divided the solid tumours into three expression groups. For tumours in all three groups, controversial reports on mdr1 expression levels have been published, which to a great extent can be attributed to methodological differences, among others, the sensitivity of the applied assays. Therefore, we have placed the tumours in a specific group based on a general judgement and the selection of literature references in Tables I and II is provided to support this classification.

Group I represents the tumours that developed from tissues normally expressing intermediate to high mdr1 levels, e.g. colon, liver, kidney, adrenal and pancreas. Clinically, these tumours are all intrinsically drug resistant, i.e. have a very low response rate to chemotherapy. In these, high mdr1 expression levels are frequently found, although, even in this group, incidental tumour biopsies with undetectable levels of mdr1 have been encountered.

Group II includes the tumours that occasionally have high, yet mostly intermediate mdr1 expression levels, but also quite often lack expression. This group contains the neuroblastomas, soft tissue sarcomas, breast carcinomas, and, in our opinion, the haematological malignancies (which we have placed in a separate table (Table II) and which will be discussed below). In general, group II tumours respond better to chemotherapy than those of group I and even complete responses can be achieved. Unfortunately, a high percentage of patients relapse and become resistant to chemotherapy.

In tumours belonging to the last group (III), mostly undetectable or incidental low mdr1 expression levels are

| Table I | Expression of mdr1 in human solid tumours |
|-----------------|------------------------------------------|
| Group I High mdr1 expression levels at a high frequency |
| Renal cell cancer | Fojo et al., 1987a* |
| Colon cancer | Kakehi et al., 1988 |
| | Goldstein et al., 1989 |
| | Kanamaru et al., 1989 |
| Hepatocellular carcinoma | Goldstein et al., 1989 |
| Adrenocortical cancer | Goldstein et al., 1989 |
| Phaeochromocytoma | Goldstein et al., 1989 |
| Pancreatic cancer | Goldstein et al., 1989 |
| Group II Intermediate mdr1 expression levels at a lower frequency |
| Neuroblastoma | Goldstein et al., 1989; 1990** |
| Soft tissue sarcomas | Bourhis et al., 1989a |
| | Gerlach et al., 1987** |
| | Chan et al., 1990** |
| Breast cancer | Goldstein et al., 1989 |
| | Moscow et al., 1989* |
| | Schneider et al., 1989** |
| | Salmon et al., 1989 |
| | Keith et al., 1990 |
| Group III Almost always undetectable or low mdr1 expression levels |
| Ovarian cancer | Gerlach et al., 1987* |
| | Goldstein et al., 1989 |
| | Moscow et al., 1989* |
| | Bourhis et al., 1989** |
| | Goldstein et al., 1989 |
| | Moscow et al., 1989 |
| Head and neck cancer | Kakehi et al., 1988 |
| Wilms' tumour | Goldstein et al., 1989 |
| Oesophageal cancer | Goldstein et al., 1989 |
| Bladder cancer | Moscow et al., 1989 |
| Lung cancer (small cell and non small cell) | Goldstein et al., 1989 |
| | Moscow et al., 1989 |
| | Lai et al., 1989* |

* Not indicated whether the patients had received prior chemotherapy. ** Studies that permit a comparison between untreated and treated patients.

| Table II | Expression of mdr1 in human haematological malignancies |
|-----------------|------------------------------------------|
| Tumour type | Untreated* | Treated* | Reference(s) |
| AML* | 7/38 | 20/27 | Ma et al., 1987 |
| | | | Goldstein et al., 1989 |
| | | | Holmes et al., 1989 |
| | | | Nooter et al., 1990 |
| ALL* | 9/39 | 7/24 | Fojo et al., 1978b |
| | | | Goldstein et al., 1989 |
| | | | Rothenberg et al., 1989 |
| | | | Nooter et al., 1990 |
| CML chronic | 0/3 | 10/10 | Goldstein et al., 1989 |
| blast | 7/7 | 9/19 | Tsuruo et al., 1987 |
| | | | Pirket et al., 1981 |
| | | | Nooter et al., 1990 |
| CLL* | 11/14 | 23/36 | Fojo et al., 1989 |
| Multiple myeloma | 5/10 | 15/21 | Dalton et al., 1989a, 1989b |
| Non-Hodgkin's lymphoma | 8/31 | 9/19 | Goldstein et al., 1989 |
| | | | Dalton et al., 1989b |
| | | | Moscow et al., 1989 |
| | | | Salmon et al., 1989 |

* Number of patients with mdr1 expression/total number of patients investigated. *Acute myelocytic leukaemia. *Acute lymphocytic leukaemia. Chronic myelocytic leukaemia, chronic phase or blast crisis. Chronic lymphocytic leukaemia.
MDR GENES IN CANCER

observed. Remarkable are the results obtained with ovarian tumours, which were placed in this group. The first report on P-glycoprotein expression in human tumour materials involved ovarian cancer (Bell et al., 1985). Two of five drug resistant ovarian tumours showed relatively high P-glycoprotein levels as quantified by Western blotting using the C219 monoclonal antibody directed against an internal epitope of the P-glycoprotein (Kramer et al., 1985). However, later studies showed only low expression in three of a total of 88 ovarian tumours (Bourhis et al., 1989b; Gerlach et al., 1987; Goldstein et al., 1989; Moscow et al., 1989). Nevertheless, the observation of Bell et al. has encouraged large scale screening for expression of the mdr1 gene in human cancers. For group III tumours, chemotherapy can be effective, but, again, acquired chemo-resistance is the rule rather than the exception.

MDR related cytotoxic drugs represent a substantial part of the chemotherapeutic arsenal for the treatment of haematological malignancies. Furthermore, in these cancers, initial periods of effective cytoreduction are often followed by a state of acquired drug resistance, making them particularly interesting to study with regard to MDR. The great advantage of studying leukaemias is the ease of obtaining tumour samples both before and after treatment. Therefore, in contrast to the solid tumours, many data are available on mdr1 expression in recurrent, chemotherapy treated haematological malignancies. Expression of mdr1 in such treated and untreated malignancies is summarised in Table II.

In normal haematopoietic cells (total bone marrow, spleen, purified peripheral blood lymphocytes), only low to very low mdr1 expression levels are found (Fojo et al., 1987b; Holmes et al., 1990). However, in almost all types of leukaemias, multiple myelomas and non-Hodgkin's lymphomas, either untreated or treated, elevated mdr1 levels are reported. The mdr1 expression levels can range from low to high and even in untreated tumours relatively high levels are sometimes observed (Goldstein et al., 1989; Herweijer et al., 1990; Nooter et al., 1990a). We can only speculate on the cause of the elevated expression sometimes observed in the untreated tumours. It is possible that the tumours developed by out-growth of rare mdr1 expressing cells present in the originating tissues or that the elevated mdr1 expression developed as a consequence of the malignant transformation (i.e., genetic instability) which took place in the tumour cells.

Expression of the mdr3 gene

Using Northern and dot blotting assays, expression of the human mdr3 gene has been detected only in the liver (van der Bieie et al., 1988a). However, Roninson et al. used a very sensitive and specific assay for human mdr1 and mdr3 expression based on enzymatic amplification of mRNA sequences by polymerase chain reaction (Chin et al., 1989). In human MDR cell lines, increased expression of mdr1 mRNA was paralleled by a smaller increase in levels of mdr3 mRNA, suggesting that mdr1 and mdr3 gene expression in these cells may be regulated by a common mechanism. Using the same technique, mdr1 and mdr3 expression was analysed in normal human tissues. In the colon, lung, stomach, oesophagus, breast, muscles, and bladder, only mdr1 expression was detected (Chin et al., 1989). In the liver, kidneys, adrenals and spleen, both mdr1 and mdr3 expression was observed. This distribution suggests that mdr1 and mdr3 gene products may be involved in some of the same processes or that coexpression of these mRNAs may reflect a common regulatory pathway. Due to the high degree of homology between the mdr1 and mdr3 gene products, it was initially speculated that the mdr3 gene also encodes for an efflux pump with broad specificity (van der Bieie et al., 1988a). However, there is no experimental evidence that the human mdr3 gene and the homologous mouse mdr2 gene are involved in MDR; that transfection and expression of full length cDNA copies of these genes inserted into mammalian expression vectors have so far failed to induce resistance to drugs (Gros et al., 1988; van der Bieie et al., 1988a).

We have recently found that, besides the mdr1 gene, also the mdr3 gene is expressed at relatively high levels in certain types of human leukaemias (acute and chronic lymphocytic leukaemia) (Herweijer et al., 1990). The available data suggest that the mdr3 gene is selectively expressed in malignant cells of the B-cell lineage, specifically in B-cell acute and chronic lymphocytic leukaemia, B-cell prolymphocytic leukaemia (PLL) and hairy cell leukaemia (HCL). Un-treated patients appeared to express the mdr3 gene without detectable levels of mdr1 (Nooter et al., 1990a). In vitro drug uptake studies showed that daunorubicin accumulation in PLL cells was increased by cyclosporin A (Herweijer et al., 1990; Nooter et al., 1990a). Since cyclosporins are inhibitors of the mdr1 encoded P-glycoprotein drug pump, the suggestion is that mdr3 also can encode for a drug efflux pump in PLL cells. These data are in contradiction with the earlier mentioned transfection experiments and we cannot exclude the possibility that the presence of mdr3 mRNA and cyclosporin sensitive drug accumulation in PLL cells is merely coincidental. Further studies are needed to answer the question of whether mdr3 contributes to the primary resistance of (B-cell) leukaemias.

Intrinsic and acquired MDR phenotype

In tumours developed from tissues that normally have a substantial mdr1 expression such as those of colon and kidneys, the mdr1 expression is an inherent characteristic of the tumour cells. There are several observations that suggest that the MDR phenotype also can be acquired by tumours as a consequence of chemotherapy. For some tumour types, high mdr1 expression levels are more frequently observed in treated tumours than in untreated ones. This has been found for acute myeloid leukaemias (Goldstein et al., 1989; Herweijer et al., 1990; Holmes et al., 1989; Nooter et al., 1990b), neuroblastomas (Bourhis et al., 1989a; Goldstein et al., 1990) and breast cancer (Schneider et al., 1989). It is very likely that the acquisition of mdr1 expression by the tumour occurs in the patient by selection of pre-existing mdr1 expressing cells. However, there is increasing evidence that the mdr1 promoter can be activated by chemical stress-inducing agents (Chin et al., 1990; Kohno et al., 1989). Recently, it was found that exposure of a human renal adenocarcinoma cell line to sodium arsenite or cadmium chloride led to a 7- and 8-fold increase in mdr1 mRNA and P-glycoprotein levels. This increase in P-glycoprotein correlated with a transient increase in resistance to vincristine (Chin et al., 1990). In another study, using an in vitro transient expression assay, it was found that the mdr1 promoter could be activated directly by the addition of anticancer agents, including vincristine, daunorubicin and doxorubicin (Kohno et al., 1989). These data suggest that chemotherapeutic agents might themselves directly cause the activation of the mdr1 gene at the transcription level.

Can mdr1 expression account for clinical drug resistance?

Does the presence of mdr1 expressing tumour cells limit successful chemotherapy? This of course, is the ultimate question. One of the strongest pieces of evidence that mdr1 expression in vivo can induce acquired drug resistance, is provided by a transgenic mouse model (Galski et al., 1989). Transgenic mice expressing mdr1 in the haematopoietic tissues, appeared to be resistant to leukaemia induced by the anticancer agent daunomycin. Expression levels of mdr1 in human tumours can be as high as those of in vitro generated MDR cell lines (Dalton et al., 1988b; Fojo et al., 1987a; Goldstein et al., 1990; Herweijer et al., 1990; Kanamaru et al., 1989). However, we do not know whether such levels of resistance (3 to 10-fold) can enable a tumour to survive the currently used chemotherapeutic treatment. Chemosensitivity studies with tumour biop-
sies from breast cancer, myeloma and renal cell cancer have established inverse correlations between mdr1 expression and in vivo sensitivity to MDR related drugs (Kakehi et al., 1988; Keith et al., 1990; Salmon et al., 1989). However, for most drugs, we do not know the in vivo concentrations to which the tumour cells are exposed.

On the surface, there seems to be a fine correlation between the clinical manifestations of drug resistance and mdr1 expression in these particular tumour type. Notorious inherent drug resistant tumours such as colon and renal cell cancers have the highest mdr1 expression levels and tumours with low or undetectable mdr1 levels such as Wilms' tumours respond much better to chemotherapy. However, this correlation is misleading and does not prove a contribution of mdr1 in clinical drug resistance. Many intrinsic drug resistant tumours with high levels of mdr1 expression also do not respond to other, non MDR unrelated drugs. The MDR phenotype is most likely one of the many detoxification systems in these tumour cells (Moscow & Cowan, 1990). For these tumours, the relative contribution of MDR to clinical drug resistance might be very small. However, recent evidence suggests that in some specific malignancies mdr1 expression in the untreated tumour can indeed affect the outcome of subsequent chemotherapy. For neuroblastomas (Bourhis et al., 1989a), acute myelocytic leukaemia (AML) (Sato et al., 1990) and soft tissue sarcomas (Chan et al., 1990), high levels mdr1 expression appeared to be associated with poor prognosis.

Bourhis et al. (1989a) determined the clinical response of primary neuroblastomas to first-line chemotherapy including vincristine, doxorubicin and VP16. Two groups could be distinguished. In the first, all 15 patients with undetectable or low mdr1 mRNA levels showed significant reduction in measurable disease. In the second group of 11 patients with high levels of mdr1 expression, six showed significant reduction in measurable disease, two showed no response and in three disease progression occurred during the course of treatment. In AML, mdr1 levels appeared to be most frequent in patients with the poorest response to chemotherapy (Sato et al., 1990). Four of five patients in whom mdr1 expression was minimal or absent showed complete remission, which lasted for relatively long periods of time. In contrast, seven of 10 patients whose leukaemic cells contained significant mdr1 levels failed to show complete remission. In the other three patients, a complete remission was achieved only after prolonged chemotherapy.

A very impressive longitudinal study was published by Chan et al. who observed a highly significant correlation between P-glycoprotein expression and the clinical outcome of drug treatment in soft tissue sarcomas in childhood (Chan et al., 1990). Chan et al. (1990) markedly improved an immunohistochemical technique for P-glycoprotein detection that can even be applied to formalin fixed, paraffin embedded tissue sections. In nine of 29 soft tissue sarcomas, small patches of P-glycoprotein positive cells were detected. These would probably have been missed in using bulk techniques and they appeared to be of crucial importance in the development of drug resistance. All nine patients with P-glycoprotein positive tumours relapsed after MDR related chemotherapy, as compared with only one in 20 with P-glycoprotein negative tumours. Even low levels of P-glycoprotein expression comparable with 8-fold relative resistance to vincristine in vitro were finally associated with clinically significant drug resistance. As the disease progressed, the number of P-glycoprotein positive cells and the expression levels in individual cells increased.

Circumvention of MDR by resistance modifying agents

Tsuruo et al. made the exiting observation that noncytotoxic doses of the calcium channel blocker verapamil could restore the sensitivity of MDR cells (Tsuruo et al., 1981). As of now, a large number of such so-called resistance modifying agents (RMAs) has been found including: other calcium antagonists, e.g. diltiazem, nicardipine, niludipine (Tsuruo et al., 1985); phenothiazines (Ford et al., 1989); indole alkaloids, e.g. reserpine (Beck et al., 1988) and reserpin analogs (Pearce et al., 1989) as well as other alkaloids and amines (Zamora et al., 1988); analogs of triparanol, e.g. tamoxifen (Ramu et al., 1984), dipyridamole (Ramu & Ramu, 1989), and dihydropyridine (Nogae et al., 1989) and cyclosporins (Nooter et al., 1989; Slater et al., 1986; Twentyman, 1986). For a number of these compounds, activity relationship studies have indicated physical and chemical features necessary to modulate MDR (Beck et al., 1988; Ford et al., 1989; Pearce et al., 1989; Ramu & Ramu, 1989). In most cases, the reversal of resistance by RMAs is accompanied by increased accumulation of cytotoxic agents by the resistant cells as determined by radiolabelled drugs, fluorescence microscopy or laser flow cytometry (Hofsl & Nisho-Meyer, 1990; Kapsimalis et al., 1985; Krishnatry et al., 1986; Nooter et al., 1989; Tsuruo et al., 1984; Tsuruo et al., 1982; Willingham et al., 1986; Yalowich & Ross, 1985). The current hypothesis on the mode of action of RMAs is that they correct the defective cytotoxic drug accumulation by competing for outward transport directly through an interaction, i.e., binding with P-glycoprotein (Akiyama et al., 1988; Cornwell et al., 1987; Foxwell et al., 1989; Naito & Tsuruo, 1989; Safa, 1988).

Clinical trials with resistance modifying agents

The finding that elevated mdr1 expression can occur in tumours and that specific agents can circumvent MDR in model systems has stimulated the development of clinical protocols in which RMAs are used in conjunction with cytotoxic drugs. Pilot studies and phase I/II trials using different RMAs and MDR related cytotoxic drugs in cancer patients have been reported. Verapamil was used with doxorubicin in ovarian cancer (Ozols et al., 1987) and with vinblastine and VP-16 in pediatric drug resistant tumours (Cairo et al., 1989). Verapamil was also used in combination with tamoxifen and doxorubicin, vincristine plus etoposide as the initial chemotherapy in small cell lung cancer (Figueroed et al., 1990). The combination of trifluoperazine and doxorubicin was given for a variety of refractory malignancies (Miller et al., 1988). In colon and renal cancer, cyclosporin A was combined with epidoxorubicin and vinblastine, respectively (Verweij et al., 1990). Epidoxorubicin was also given in combination with quindine as first line chemotherapy in advanced breast cancer (Jones et al., 1990). These investigations, which were primarily intended as feasibility studies, have shown that there is no dramatic increased toxicity for normal tissues such as renal, hepatic, or intestinal epithelia with high levels of P-glycoprotein. There was also no evidence that RMAs potentiated the acute toxicities of the cytotoxic drugs. The clinical efficacy of the experimental protocols was assessed by the occurrence of otherwise unexpected tumour responses and the results overall are disappointing. A shortcoming of the above mentioned studies is a lack of data on P-glycoprotein expression in the tumours, making an evaluation difficult. Most of these studies would seem to be the haematological malignancies, because of the possibility of repeated tumour sampling. Promising results have been obtained in two studies, one in multiple myeloma (Dalton et al., 1989a) and another in acute myelocytic leukaemia (Sonneveld & Nooter, 1990). Verapamil was added to the standard regimen of vincristine, doxorubicin and dexamethasone (VAD) in patients with refractory multiple myeloma (7) or non-Hodgkin's lymphoma (1) (Dalton et al., 1989a). Objective clinical responses were observed in three of eight patients who previously had been refractory to vincristine and doxorubicin. Six of these eight patients had evidence of P-glycoprotein expression in their tumour cells; of these, two showed a partial response and one gave a complete response for 6 months. However, three of six P-glycoprotein positive patients did not show objective response with the combined VAD+verapamil treatment.
From a therapeutic point of view, important features of MDR cells are their reduced drug accumulation and the resulting reduced drug sensitivity, which can both be restored by RMAs. We have shown that, in leukemic cells expressing \textit{mdr1}, the steady-state accumulation of daunorubicin could be significantly increased by cyclosporin A or verapamil (Herweijer et al., 1990; Nooter et al., 1990b). Since these RMAs inhibit the \textit{mdr1} encoded drug pump, our data suggest that this pump is functional in leukemias expressing the \textit{mdr1} gene. We recently reported treatment of a refractory AML patient with daunorubicin and cytarabine combined with cyclosporin A (Sonneveld & Nooter, 1990). In that case, the emergence of the MDR phenotype was monitored during clinical progression of the disease. At relapse, a decrease in daunorubicin accumulation by AML blasts was associated with elevated \textit{mdr1} expression and a decreased \textit{in vitro} sensitivity to daunorubicin. Intracellular daunorubicin accumulation and \textit{in vitro} sensitivity could be completely restored by adding cyclosporin A to the cells. During progressive relapse, the patient was treated with reinduction therapy to which cyclosporin A was added and this result in elimination of the \textit{mdr1} positive AML clone. After 12 weeks, the resistant \textit{mdr1} expressing clone reappeared in the blood and bone marrow.

In our opinion, future studies along this line in haematological malignancies should preferably include the following: (a) RMAs are added to the cytotoxic protocols as early as possible in the development of clinical drug resistance; and, (b) the efficacy of the currently used protocols, and those to which RMAs are added, in killing \textit{mdr1} expressing tumour cells in relationship to the level of \textit{mdr1} expression are monitored by \textit{in situ} methods. Since \textit{mdr1} is also frequently expressed in untreated haematological malignancies, combination therapy should also be considered in previously untreated patients.

Another point of consideration is that the pharmacokinetics and, as a consequence of that the toxicity and efficacy of cytotoxic drugs, might be influenced by the simultaneous use of RMAs (Bright & Buss, 1990; Fedeli et al., 1989; Kert et al., 1986; Nooter et al., 1987). Therefore, we strongly recommend animal studies in which pharmacokinetics, optimal schedules and toxicity of combined drugs can be determined.

Supported by a grant from the Dutch Cancer Society. Dr J. Verweij is thanked for helpful discussions and a critical reading of the manuscript.

References

AKIYAMA, S., CORNWELL, M.M., KUWANO, M., PASTAN, I. & GOTTESMAN, M.M. (1988). Most drugs that reverse multidrug resistance also inhibit photoaffinity labeling of P-glycoprotein by a vinblastine analog. Biochem. Biophys. Res. Commun., 153, 959.

BEDFORD, P. & FOX, B.W. (1982). Repair of DNA interstrand crosslinks after busulphan. A possible mode of resistance. Cancer Chemother. Pharmacol., 8, 8.

BELL, D.R., GERLACH, J.H., KARTNER, N., BUICK, R.N. & LING, V. (1985). Detection of P-glycoprotein in ovarian cancer: a molecular marker associated with multidrug resistance. J. Clin. Oncol., 3, 311.

BOURJIS, J., BÉRARD, J., HARTMANN, O., BOCON-GIBOD, L., LEMERLE, J. & RIOT, G. (1986a). Correlation of MDR1 gene expression with chemotherapy in neuroblastoma. J. Natl Cancer Inst., 81, 1401.

BOURJIS, J., GOLDSTEIN, L.J., RIOT, G., PASTAN, I., GOTTESMAN, M.M. & BÉRARD, J. (1986b). Expression of a human multidrug resistance gene in ovarian carcinomas. Cancer Res., 49, 5062.

BRADLEY, G., JURANKA, P.F. & LING, V. (1988). Mechanism of multidrug resistance. Biochem. Biophys. Acta, 948, 87.

BRIGHT, J.M. & BUSS, D.D. (1990). Effects of verapamil on chronic doxorubicin-induced cardiotoxicity in dogs. J. Natl Cancer Inst., 82, 963.

CABRAL, F., SOBEL, M.E. & GOTTESMAN, M.M. (1980). CHO mutants resistant to colchicine, colcemid or griseofulvin have an altered betatubulin. Cell, 20, 29.

CAIRO, M.S., SIEGEL, S., ANAS, N. & SENDER, L. (1989). Clinical trial of continuous infusion verapamil, bolus vinblastine, and continuous infusion VP-16 in drug-resistant pediatric tumors. Cancer Res., 49, 1063.

CALLENI, D.F., BAKER, E., SIMMERS, R.N., SESHADRI, R. & RONINSON, I.B. (1987). Localization of the human multiple drug resistance gene, \textit{MDR1} to \textit{7q21.1}. Hum. Genet., 77, 142.

CHAN, H.S.L., THORNER, P.S., HADDA, G. & LING, V. (1990). Immunochemical detection of P-glycoprotein: prognostic correlation in soft tissue sarcoma of childhood. J. Clin. Oncol., 8, 689.

CHEN, C., CHIN, J.E., UEDA, K. & others (1986). Internal duplication and homology with bacterial transport proteins in the \textit{mdr} 1 (P-glycoprotein) gene from multidrug-resistant human cells. Cell, 47, 381.

CHIN, J.E., SOFFIR, R., NOONAN, K.E., CHOL, K. & RONINSON, I.B. (1989). Structure and expression of the human \textit{MDR} (P-glycoprotein) gene family. Mol. Cell. Biol., 9, 3808.

CHIN, K.Y., TANAKA, S., DARLINGTON, G., PASTAN, I. & GOTTESMAN, M.M. (1990). Heat shock and arsenite increase expression of the multidrug resistance (\textit{MDR1}) gene in human renal carcinoma cells. J. Biol. Chem., 265, 221.

CORDERO-CARDO, C., O’BRIEN, J.P., BOCCA, J., CASALS, D., BERTINO, J.R. & MELAMED, M.R. (1990). Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. J. Histochim. Cytochem., 38, 1277.

CORNWELL, M.M., PASTAN, I. & GOTTESMAN, M.M. (1987). Certain calcium channel blockers bind specifically to multidrug-resistant human KB carcinoma membrane vesicles and inhibit drug binding to P-glycoprotein. J. Biol. Chem., 262, 1666.

DALTON, W.S., GROGAN, T.M., MELTZER, P.S. & 5 others (1989a). Drug-resistance in multiple myeloma and non-Hodgkin’s lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. J. Clin. Oncol., 7, 415.

DALTON, W.S., GROGAN, T.M., RYBSKI, J.A. & 6 others (1989b). Immunochemical detection and quantitation of P-glycoprotein in multiple drug-resistant human myeloma cells: association with level of drug resistance and drug accumulation. Blood, 73, 747.

DANO, K. (1972). Cross-resistance between Vinca alkaloids and anthracyclines in Ehrlich ascites tumor in vivo. Cancer Chemother. Rep., 56, 701.

DANO, K. (1973). Active outward transport of daunomycin in resistant Ehrlich ascites tumor cells. Biochem. Biophys. Acta., 323, 466.

EPESTEIN, J., XIAO, H. & OBA, I.K. (1989). P-glycoprotein expression in plasma-cell myeloma is associated with resistance to VAD. Blood, 74, 913.

FEDELI, L., COLOZZA, M., BOSCHETTI, E. & 8 others (1989). Pharmacokinetics of vincristine in cancer patients treated with mifedipine. Cancer, 64, 1805.

FIGUEREDO, A., ARNOLD, A., GOODYEAR, M. & 4 others (1990). Addition of verapamil and tamoxifen to the initial chemotherapy of small cell lung cancer. A phase II/II study. Cancer, 65, 1895.

FLINTOFF, W.F. & ESSANI, K. (1990). Methotrexate resistance Chinese hamster ovary cells contain a dihydrofolate reductase with an altered affinity for methotrexate. Biochemistry, 19, 4321.

FOJO, A.T., SHEN, D., MICKLEY, L.A., PASTAN, I. & GOTTESMAN, M.M. (1987a). Intrinsic drug resistance in human kidney cancer is associated with expression of a human multidrug-resistance gene. J. Clin. Oncol., 5, 1922.

FOJO, A.T., UEDA, K., SLAMON, D.J., POPPLACK, D.G., GOTTESMAN, M.M. & PASTAN, I. (1987b). Expression of a multidrug-resistance gene in human tumors and tissues. Proc. Natl Acad. Sci. USA, 84, 265.
KOHNO, K., SATO, S., TAKANO, H., MATSUO, K. & KUWANO, M. (1989). The direct activation of human multidrug resistance gene (mdr1) by anticancer agents. Biochem. Biophys. Res. Commun., 165, 1415.

KRISHAN, A., SAUERTEIG, A., GORDON, K. & SWINKIN, C. (1986). Flow cytometric monitoring of cellular anthracycline accumulation in murine leukemia cells. Cancer Res., 46, 1768.

LAI, S., GOLDSTEIN, L.J., GOTTESMAN, M.M. & others (1989). MDR1 gene expression in lung cancer. J. Natl Cancer Inst., 81, 1144.

MA, D.F., DAVEY, R.A., HARMAN, D.H. & others (1987). Detection of a multidrug resistant phenotype in acute non-lymphoblastic leukemia. Lancet, 1, 135.

MILLER, R.L., BUKOWSKI, R.M., BUDD, G.T. & others (1988). Clinical modulation of doxorubicin resistance by the calmodulin-inhibitor, trifluoperazine: a phase I/II trial. J. Clin. Oncol., 5, 1452.

MOSCOW, J.A. & COWAN, K.H. (1990). Multidrug resistance. In Cancer Chemotherapy and Biological Response Modifiers Annual 11, Pinedo, H.M., Chabner, B.A. & Longo, D.L. (eds) p 97. Elsevier Science Publishers: Amsterdam.

MOSCO, J.A., FAIRCHILD, C.R., MADDEN, M.J. & others (1989). Expression of anionic glutathione-S-transferase and P-glycoprotein genes in human tissues and tumors. Cancer Res., 49, 1422.

NAITO, M. & TSURUO, T. (1989). Competitive inhibition by verapamil of ATP-dependent high affinity vincristine binding to the plasma membrane of multidrug-resistant K562 cells without calcium ion involvement. Cancer Res., 49, 1452.

NG, W.F., SARANGI, F., ZASTAWNY, R.L., VEINOTT-DREBOT, L. & LING, V. (1989). Identification of members of the P-glycoprotein resistance family. Mol. Cell. Biol., 9, 641.

NOGAE, T., KOHNO, K., KIKUCHI, J. & others (1989). Analysis of structural features of dihydropyridine analogs needed to reverse multidrug resistance and to inhibit photoaffinity labeling of P-glycoprotein. Biochem. Pharmacol., 38, 519.

NOOTER, K., OOSTRUM, P. & DEURloo, J. (1987). Effects of verapamil on the pharmacokinetics of daunomycin in the rat. Cancer Chemother. Pharmacol., 20, 176.

NOOTER, K., OOSTRUM, R. & DORNKEN, H., STOKDIJK, W. & VAN DEN ENGH, G. (1989). Effect of cyclosporin A on daunorubicin accumulation in multidrug-resistant P388 leukemia cells measured by real-time flow cytometry. Cancer Chemother. Pharmacol., 23, 296.

NOOTER, K., SONNEVELD, P., JANSEN, A. & others (1990a). Expression of the mdr3 gene in prolymphocytic leukemia: association with cyclosporin-A-induced increase in drug-accumulation. Int. J. Cancer, 45, 626.

NOOTER, K., SONNEVELD, P., OOSTRUM, R., HERWEIJER, H., HAGENBEEK, A. & VALERIO, D. (1990b). Outer expression of the mdr1 gene in blast cells from patients with acute myelocytic leukemia is associated with decreased multidrug resistance in patients that are restored by cyclosporin-A. Int. J. Cancer, 45, 263.

OZOLS, R.F., CUNNION, R.E., KLECKER, R.W. & others (1987). Verapamil and adriamycin in the treatment of drug-resistant ovarian cancer patients. J. Clin. Oncol., 5, 492.

PEARCE, H.L., SAFA, A.R., BACH, N.J., WINTER, M.A., CURTAIN, M.C. & BECK, W.T. (1989). Essential features of the P-glycoprotein pharmacophore as defined by a series of reserpine analogs that modulate multidrug resistance. Proc Natl Acad Sci USA, 86, 5128.

PKRIBER, R., GOLDSTEIN, L.J., LUDWIG, H. & others (1989). Expression of a multidrug resistance gene in blast crisis of chronic myelogenous leukemia. Cancer Commun., 1, 141.

RAMU, A., GLAUBIGER, D. & FUKS, Z. (1984). Reversal of acquired resistance to doxorubicin in P388 murine leukemia cells by tamoxifen and other triparanol analogues. Cancer Res., 44, 4392.

RAMU, N. & RAMU, A. (1989). Circumvention of adriamycin resistance by dipyriddyamine analogues: a structure-activity relationship study. Int. J. Cancer, 43, 487.

REDWOOD, W.R. & COURT, M. (1980). Transport of melphalan by sensitive and resistant L1210 cells. Cancer Res., 40, 1144.

RIEM, H. & BIEDLER, J.L. (1972). Potentiation of drug effect by TWEEN 80 in Chinese hamster cells resistant to actinomycin D and daunomycin. Cancer Res., 32, 1195.

RODOMONSON, I.B., CHIN, J.E. & others (1986). Isolation of human mdr DNA sequences amplified in multidrug-resistant KB carcinomas cells. Proc Natl Acad Sci USA, 83, 4538.

ROTHENBERG, M.L., MICKLEY, L.A., COLE, D.E. & others (1989). Expression of the mdr1-P-170 gene in patients with acute lymphoblastic leukemia. Blood, 74, 1388.

SAFA, A.R. (1988). Photoaffinity labeling of the multidrug-resistance-related P-glycoprotein with photoactive analogs of verapamil. Proc Natl Acad Sci USA, 85, 7187.
SALMON, S.E., GROGAN, T.M., MILLER, T., SCHEPER, R. & DALTON, W.S. (1989). Prediction of doxorubicin resistance in vitro in myeloma, lymphoma, and breast cancer by P-glycoprotein staining. J. Natl Cancer Inst., 81, 96.

SATO, H., GOTTESMAN, M.M., GOLDSTEIN, L.J. & 4 others (1990). Expression of the multidrug resistance gene in myeloid leukemias. Leukemia Res., 14, 11.

SCHNEIDER, J., BAK, M., EFFERTH, T., KAUFMANN, M., MATERN, J. & VOLM, M. (1989). P-glycoprotein expression in treated and untreated human breast cancer. Br. J. Cancer, 60, 815.

SIROTNAK, F.M., MOCCIO, D.M., KELLEHER, L.E. & GOUTAS, L.J. (1981). Relative frequency and kinetic properties of transport-defective phenotypes among methotrexate-resistant L1210 clonal cell lines derived in vivo. Cancer Res., 41, 4447.

SIROTNAK, F.M., YANG, C., MINES, L.S., ORIBÉ, E. & BIEDLER, J.L. (1986). Markedly altered membrane transport and intracellular binding of vincristine in multidrug-resistant Chinese hamster cells selected in resistance to vinca alkaloids. J. Cell. Physiol., 126, 266.

SKOVSGAARD, T. (1978). Mechanism of cross-resistance between vincristine and daunorubicin in Ehrlich ascites tumor cells. Cancer Res., 38, 4722.

SLATER, L.M., SWEET, P., STUPECKY, M. & GUPTA, S. (1986). Cyclosporin A reverses vincristine and daunorubicin resistance in acute lymphatic leukemia in vitro. J. Clin. Invest., 77, 1405.

SONNEVEILD, P. & NOOTER, K. (1990). Reversal of drug-resistance by cyclosporin-A in a patient with acute myelocytic leukaemia. Br. J. Haematol., 75, 208.

THIEBAUT, F., TSURUO, T., HAMADA, H., GOTTESMAN, M.M., PASTAN, I. & WILLINGHAM, M.C. (1987). Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc. Natl Acad. Sci. USA, 84, 7735.

TSURUO, T., IDA, H., KITATANI, Y., YOKOTA, K., TSUKAGOSHI, S. & SAKURAI, Y. (1984). Effects of quinidine and related compounds on cytotoxicity and cellular accumulation of vincristine and adriamycin in drug-resistant tumor cells. Cancer Res., 44, 4303.

TSURUO, T., IDA, H., TSUKAGOSHI, S. & SAKURAI, Y. (1981). Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res., 41, 1967.

TSURUO, T., IDA, H., TSUKAGOSHI, S. & SAKURAI, Y. (1982). Increased accumulation of vincristine and adriamycin in drug-resistant P388 tumor cells following incubation with calcium antagonists and calmodulin inhibitors. Cancer Res., 42, 4730.

TSURUO, T., KAWABATA, H., NAGUMO, N. & 4 others (1985). Potentiation of antitumour agents by calcium channel blockers with special references to cross-resistance patterns. Cancer Chemoher. Pharmacol., 15, 16.

TSURUO, T., SUGIMOTO, Y., HAMADA, H. & 5 others (1987). Detection of multidrug resistance markers, P-glycoprotein and mdr1 mRNA, in human leukemia cells. Jpn. J. Cancer Res. (Gann), 78, 1415.

TWENTYMAN, P.R. (1988). Modification of cytoxic drug resistance by non-immunosuppressive cyclosporins. Br. J. Cancer, 57, 254.

UEDA, K., CARDARELLI, C., GOTTESMAN, M.M. & PASTAN, I. (1987). Expression of a full-length cDNA for the human 'MDR' gene confers resistance to colchicine, doxorubicin, and vinblastine. Proc. Natl Acad. Sci. USA, 84, 3004.

VANDER BLIEK, A.M., BAAS, F., TEN HOUTE-DELANGE, T., KOOMAN, P.M., VAN DER VELDE-KOERTS, T. & BORST, P. (1987). The human mdr3 gene encodes a novel P-glycoprotein homologue and gives rise to alternatively spliced mRNAs in liver. EMBO J., 6, 3325.

VANDER BLIEK, A.M., BAAS, F., VAN DER VELDE-KOERTS, T. & 6 others (1988). Genes amplified and overexpressed in human multidrug-resistant cell lines. Cancer Res., 48, 5927.

VANDER BLIEK, A.M. & BORST, P. (1989). Multidrug-Resistance. In Advances in Cancer Research 52, van de Woude, G.F. & Klein, G. (eds) p. 165. Academic Press: New York.

VAN DER BLIEK, A.M., KOOIMAN, P.M., SCHNEIDER, C. & BORST, P. (1988a). Sequence of mdr3 cDNA encoding a human P-glycoprotein. Gene, 71, 401.

VAN DER VALK, P., VAN KALKEN, C.K., KETELAARS, H. & 8 others (1990). Distribution of multidrug resistance-associated P-glycoprotein in normal and neoplastic human tissues. Am. Oncol., 1, 56.

VERWEIJ, J., HERWEIJER, H., PLANTING, A.S.T. & 4 others (1990). In vitro and in vivo studies on the effect of cyclosporin-A (Cy-A) in the circumvention of clinical multidrug resistance (MDR). Proc. Am. Soc. Clin. Oncol., 9, 74.

WEINSTEIN, R.S., KUZAK, J.R., KLUJSKENS, L.F. & COON, J.S. (1990). P-glycoproteins in pathology – the multidrug resistance gene family in humans. Hum. Pathol., 21, 34.

WILLINGHAM, M.C., CORNEWELL, M.M., CARDARELLI, C.O., GOTTESMAN, M.M. & PASTAN, I. (1986). Single cell analysis of daunomycin uptake and efflux in multidrug-resistant and -sensitive KB cells: effect of verapamil and other drugs. Cancer Res., 46, 5941.

YALOWICH, J.C. & ROSS, W.E. (1985). Verapamil-induced augmentation of etoposide accumulation in L1210 cells in vitro. Cancer Res., 45, 1651.

ZAMORA, J.M., PEARCE, H.L. & BECK, W.T. (1988). Physical-chemical properties shared by compounds that modulate multidrug resistance in human leukemic cells. Mol. Pharmacol., 33, 454.