infusion device in 24 h. In contrast to the observed loss of bioactivity of EuroCetus rIL-2, no loss of bioactivity was noted for Hoffmann–LaRoche, Glaxo and two Amgen rIL-2 muteins.

DISCUSSION

We found that the dramatic loss of bioavailability of EuroCetus rIL-2, when dissolved in 10 ml saline and infused slowly through a plastic infusion device, resulted from a concentration-dependent precipitation of rIL-2 in saline and adherence of the protein to the tubing material. Accordingly, in the package insert (June 1989) EuroCetus states that the product has to be dissolved in 5% dextrose with or without 2% albumin.

Other rIL-2 muteins tested [Hoffmann-LaRoche, Glaxo, Amgen (two different muteins)] could easily be dissolved in normal saline at a concentration of 33.3 μg/ml, and had no evident tendency to adhere to the tubing material. The various rIL-2 muteins differ only a little in amino acid sequence. In EuroCetus rIL-2 and in one of the Amgen rIL-2 muteins, cys^{125} has been replaced by serine and alanine, respectively. EuroCetus rIL-2 has the alanine at position one deleted, while Hoffmann–LaRoche, Glaxo and both Amgen rIL-2 muteins have an additional methionine at the N-terminal end. These changes may dramatically influence the biophysical characteristics. Phase separation studies demonstrated that EuroCetus rIL-2 is strongly hydrophobic and that the ala^{125} Amgen mutein is mildly hydrophobic, while muteins with an additional methionine but without replacement at cys^{125} are as hydrophilic as the non-glycosylated natural IL-2 [9].

In our experiments no difference in behaviour was observed between Amgen (ala^{125}) and the rIL-2 muteins without cys^{125} replacement. The aberrant behaviour of EuroCetus rIL-2 may, therefore, not only be determined by the cys^{125}→ser^{125} mutation but also by the alteration at the N-terminal end of the molecule.

The rIL-2 muteins with replaced cys^{125} have a strong tendency to form aggregates likely to adhere to solid material [9–11]. Our data indicate that the addition of albumin prevents adherence to the tubing material of EuroCetus rIL-2 dissolved in water. Other groups have also demonstrated the importance of adding albumin to EuroCetus rIL-2 dissolved in 5% glucose [2, 4, 5].

Based on this study, we strongly advocate testing the influence of the mode of administration on the bioavailability of biological agents before giving them to patients.

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Expression of the *mdr*1 Gene in Bone and Soft Tissue Sarcomas of Adult Patients

Ulrike Stein, Volker Wunderlich, Wolfgang Haensch and Peter Schmidt-Peter

The expression of the *mdr*1 gene was evaluated at the RNA level by northern and slot blot analysis, and at the protein level by immunohistochemistry, in a total of 29 bone and 32 soft tissue sarcomas. All patients, mainly adults, had not received previous chemotherapy. Of the tumours investigated, 69% were *mdr*1-positive. An intermediate *mdr*1 expression was observed most frequently, with the exception of osteosarcomas (high) and malignant fibrous histiocytomas (low). Detection of P-glycoprotein in selected tumours revealed consistent results. However, no conclusion can be drawn as yet regarding correlation of *mdr*1 expression and drug resistance in patients.

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INTRODUCTION

BONE AND soft tissue tumours constitute a major histogenetic class of neoplasms with rather high malignant potential. By using multi-agent, now well established chemotherapy, the past decade has seen major changes in treatment strategies for most of these tumours [1, 2].

Multidrug resistance (MDR), either intrinsic or acquired, often limits successful chemotherapy. It is mediated in humans by the *mdr*1 gene product P-glycoprotein [3]. Increased expression of the *mdr*1 gene is frequently observed in a variety of human tumours [4]. A few reports [5–7] cover *mdr*1 expression in bone and soft tissue sarcomas, mostly childhood cancers.

Table 1. mdr1 *mRNA expression in bone and soft tissue sarcomas*

Tumours		Age (years)		*mdr*1 positive	
Type	No.	Median	Range	No.	%
Bone tumours					
Osteosarcomas	11	22	10–56	10	90
MFH bone	4	32	12–74	1	25
Chondrosarcomas	7	55	17–79	5	71
Ewing's sarcomas	3	25	24–26	2	66
Others	4	30	17–41	3	75
Total	29			21	72
Soft tissue sarcomas					
MFH soft tissue	9	63	49–78	3	30
Rhabdomyosarcomas	4	47	36–69	2	50
Leiomyosarcomas	2	72	64–79	2	100
Synovial sarcomas	3	35	26–51	2	66
Neurofibrosarcomas	8	42	24–54	7	88
Others	6	47	26–66	5	83
Total	32			21	66
Overall	61			42	69

For each tumour slot blot and northern analysis were done. MFH, malignant fibrous histiocytomas.

Patients admitted to the Robert-Rössle-Clinic are mainly adults nd treatment regimes include drugs of the MDR family. All investigations reported here were conducted prior to chemotherapy, so that only the intrinsic *mdr*1 expression was detected.

MATERIALS AND METHODS

Tumour material

Tumours were obtained mainly from adults; some bone tumour patients were in the age range from 10-years-old upwards. The male/female ratio was 1:1. Patients were assigned to two groups: the first included 29 malignant bone tumours and the second included 32 soft tissue sarcomas (Table 1). Sarcoma types with few representatives were classified as "others" for each group. Non-chemosensitive tumours (e.g. chondrosarcomas in group 1) were also included. The grading was from 2B to 3B as defined by WHO criteria. Lung tumours served as controls (courtesy of Dr Engelmann, Berlin-Buch, Germany).

Northern analysis

Total cellular RNA from frozen tumours was isolated by the LiCl/urea method [8]. For northern and slot blot analysis 10 μg of each tumour RNA were used according to standard protocols. Hybridisations (68°C; 15% formamide) were carried out by using the ^{32}P-labelled *mdr*1-specific RNA probe (1×10^9 cpm/μg DNA) pHDR 5.A (9; ATCC-No. 61360). The same RNAs were hybridised with a glyceraldehyde phosphate dehydrogenase (GAPDH)-specific ^{32}P-labelled DNA probe [10] as an internal standard. The *mdr*1 expression in the ovarian cancer cell line A 2780 [11], sensitive or resistant to doxorubicin (ADR), served as an external control.

Immunohistochemistry

For the immunohistochemical detection of P-glycoprotein in cryostat sections (5 μm), the monoclonal antibody MRK 16 ([11]; Behring-Werke, Marburg), which recognises an extracellular epitope, was used at a dilution of 1:50 APAAP (alkaline phosphatase anti-alkaline phosphatase) detection system [12] including inhibition of endogenous alkaline phosphatase and amplification of immunoenzymatic signals. Tumour sections were counter-stained with haematoxylin. Sections treated without antibody served as controls.

Statistical analysis

The probability of survival in *mdr*1-positive versus *mdr*1-negative patients was estimated by the Kaplan–Meier method. Comparison was made using SPSS-X [13] modified Wilcoxon analysis.

RESULTS

Northern analysis

The *mdr*1-specific mRNA was detected in 42 of 61 (69%) tumours investigated (Table 1). These intrinsic expressions were observed to be rather uniform in bone (72%) as well as in soft tissue sarcomas (66%) and seemed to be mostly independent of tumour type and location. Besides northern analysis, slot blot hybridisations were carried out with the same RNAs and the same probes to estimate not only the frequency but also the stringency of *mdr*1-specific signals (Table 1). Of 14 lung carcinomas, eight showed no *mdr*1 expression and six showed a low *mdr*1 expression (cf. 4 below). Hybridisations using RNAs of A 2780 and A 2780/ADR detected *mdr*1-specific mRNA in the resistant, but not in the sensitive line.

For the sarcomas investigated a classification described for other tumours [4] was used:

(1) High *mdr*1 expression levels at high frequency; osteosarcomas.
(2) Intermediate *mdr*1 expression levels at high or intermediate frequency; chondrosarcomas, Ewing's sarcomas, rhabdomyosarcomas, leiomyosarcomas, synovial sarcomas, neurofibrosarcomas.
(3) Intermediate *mdr*1 expression levels at low frequency; malignant fibrous histiocytomas of bone and soft tissue.
(4) Almost always undetectable or low *mdr*1 expression levels; lung carcinomas (controls).

Immunohistochemistry

The P-glycoprotein was detected by MRK 16. Tumour sections with membrane-stained areas and with diffuse staining, as occasionally seen in tumours of intermediate or low *mdr*1 mRNA expression, were classified as positive. No staining was observed in control sections. In 11 out of 19 sarcomas analysed (58%), P-glycoprotein was detectable. Within the group of osteosarcomas specific membrane staining was observed in most cases in accordance with the results of *mdr*1 mRNA expression. Two cases showed a particularly intense staining: an osteoblastic osteosarcoma of high malignancy and a chondroblastic osteosarcoma, mainly in the chondroblastic compartment (Fig. 1). It was noticed that in 14 out of 19 sarcomas (74%) consistent results were obtained at both expression levels.

Correspondence to U. Stein.
U. Stein and V. Wunderlich are at the Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Straße 10, D - 0 - 1115 Berlin-Buch, Germany; W. Haensch is at the Department of Pathology and P. Schmidt-Peter is at the Department of Orthopedic Surgery, Free University of Berlin, Robert-Rössle-Clinic, Lindenberger Weg 80, D - 0 - 1115 Berlin-Buch, Germany.
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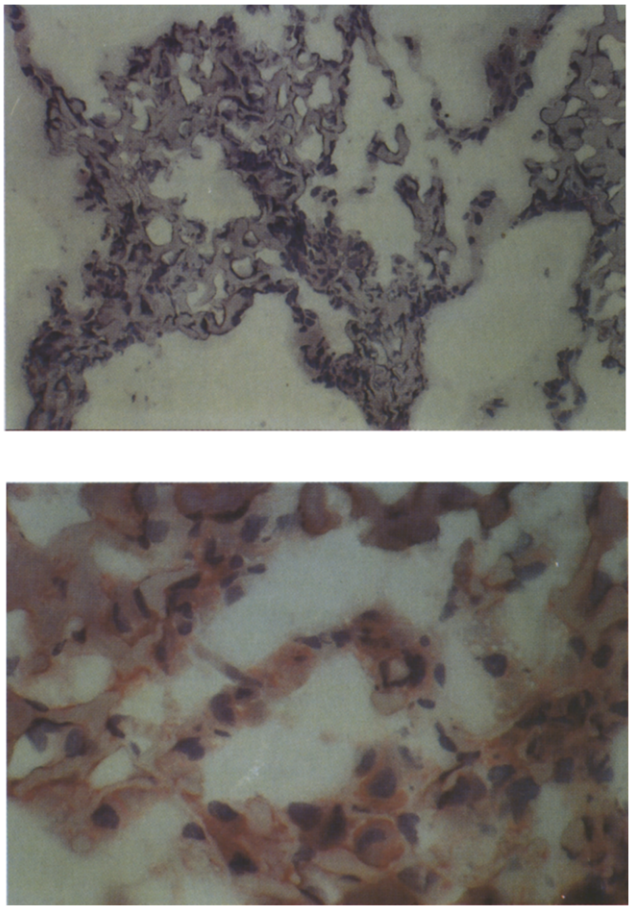

Fig. 1. P-Glycoprotein in osteosarcomas. (Upper) Osteosarcoma negative for P-glycoprotein. (Lower) Osteosarcoma positive for P-glycoprotein.

Clinical parameters

There appears to be no relationship between the level of *mdr*1 expression and clinical data which included histopathological tumour grade, tendency to metastasise, rate of tumour necrosis following later chemotherapy and, in bone sarcomas, level of serum alkaline phosphatase before and after chemotherapy. Evaluation of the final outcome requires more data.

Statistical analysis

Statistical analysis of the data revealed no significant differences ($P > 0.05$) between survival time in *mdr*1-positive and -negative patients either in the whole group or in any of the subgroups.

DISCUSSION

This study describes rather high expression of the *mdr*1 gene in tumours without previous exposure to cytotoxic drugs. Given the considerable improvement due to adjuvant chemotherapy [1, 2], the results, pointing to high intrinsic resistance of these tumours to drugs of the MDR family, are rather unexpected. Among bone sarcomas, the unresponsiveness of most chondrosarcomas to any type of chemotherapy as compared with the good success of chemotherapy in the cases of osteosarcomas and Ewing's sarcomas obviously does not depend solely on the expression of the *mdr*1 gene. Likewise, the intermediate *mdr*1 expression in most soft tissue sarcomas may not be linked directly to the limited response rates of chemotherapy [2], as osteosarcomas have even higher levels at the time of diagnosis.

A number of recent publications have addressed the issue of whether increased *mdr*1 expression is correlated with an unfavourable prognosis. Chan *et al.* reported retrospective studies of paediatric soft tissue sarcomas [6] showing a strong correlation between P-glycoprotein expression and lack of response to chemotherapy. However, these findings were not confirmed by other studies [14]. The results of our study do not favour the suggestion that the pretreatment *mdr*1 expression predicts the outcome of therapy.

The possibility that other tumour resistance mechanisms involving other proteins may influence the multiagent therapy of individual tumours should also be considered [15].

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