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Preclinical phase II study of ifosfamide in human tumour xenografts in vivo*

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Summary. The in vivo effects of the oxazaphosphorine compound ifosfamide (IFO) on human tumour xenografts were assessed in thymus aplastic nude mice. The human origin of the tumours was confirmed by isoenzymatic and immunohistochemical methods. Tumour models were selected from a panel of 180 regularly growing, well-characterized xenografts. The maximum tolerated dose in tumour-bearing nude mice was determined to be 130 mg/kg per day given on days 1–3 and 15–17. After 21 days, lethality was 14% after i.p. and 6% after s.c. administration. A total of 43 human tumours were tested for antineoplastic activity, 15 of which (36%) showed regression: 4/5 breast cancer xenografts, 1/3 colon, 1/1 gastric, 2/7 non-small-cell lung cancers (NSCLC), 3/4 small-cell lung cancers (SCLC), 1/2 sarcomas and 3/3 testicular cancers. Two ovarian, two uterine and six renal cancer xenografts as well as three melanomas and five tumours of various histologies were resistant. In 30 human tumour xenografts, the antineoplastic efficacy of the two oxazaphosphorine derivatives cyclophosphamide and IFO was compared. The maximum tolerated dose of cyclophosphamide was 200 mg/kg per day given i.p. on days 1 and 15; it led to 17% lethality after 21 days. Cyclophosphamide induced tumour regression or remission in 10/30 xenografts (33%) and IFO in 13/30 (43%). In conclusion, the observed efficacy of IFO parallels the clinical situation. Breast, lung and testicular cancer and sarcomas proved to be responsive. The antitumoural activity of IFO shows similarities to that of cyclophosphamide; however, a higher response rate and lower toxicity were noted for the former. Preclinical phase II studies in nude mice seem to offer an effective way of identifying active drugs as well as sensitive tumour types for further clinical development.

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Introduction

The oxazaphosphorine compound ifosfamide (IFO) has been the subject of extensive preclinical and clinical research. Its therapeutic potential has been confirmed in various experimental and human tumours [5, 12] as well as in clinical studies [19]. We report results thus far obtained on the antineoplastic efficacy of IFO in human tumours established as continuous xenograft lines in nude mice. High antitumor activity, with complete and partial remissions, was observed.

Materials and methods

For all experiments, 6- to 8-week-old female, athymic nude mice (NMRI nu/nu strain) were used. The animals were housed in macrolon cages under laminar air-flow conditions and maintained as previously described by Fortmeyer and Bastert [11].

We studied the effect of IFO on 43 human tumours grown s.c. by serial passage in nude mice. The human origin of the tumours was confirmed by isoenzymatic and immunohistochemical methods. Tumour models were selected from a panel of 180 regularly growing xenografts [9, 10]; characterization of these models included histology, growth behaviour, chemosensitivity to 12 standard anticancer drugs in vivo and in vitro (clonogenic assay), isoenzyme phenotype analysis, hormone receptor analysis and DNA histogram as generated by flow cytometry. Characteristics of the selected gynecological tumour xenografts are given in Table 1.

Tumour slices averaging 3 × 3 × 0.5–1 mm were implanted s.c. into both flanks of the animals. The median tumour diameter at the beginning of therapy was 7 mm. Mice were randomly assigned to treatment groups and untreated control groups; each group consisted of 5–6 mice bearing 6–10 evaluable tumours.

Tumour growth was recorded weekly by two-dimensional measurement with calipers; tumour size (TS) was calculated according to the formula length × width ($TS = a \times b$). The antitumour effect of IFO was evaluated following maximal tumour regression (in resistant tumours, after 3–4 weeks).

Data evaluation was carried out using specifically designed software. Relative tumour size (RTS) values were calculated for each single tumour by dividing the tumour size on day X by the tumour size on day 0 at the time of randomisation ($RTS = TS_x \cdot 100/TS_0$). Median RTS values were used for further evaluation. Tumour doubling time (DT) of test and control groups was defined as the period required to reach an RTS of 200%.

Table 1. Characterization of selected gynecological tumour models growing s. c. in nude mice

Tumor designation	Origin	Histology	Established (month/year)	Doubling time (days)[a]
MAXF 401	Breast	Moderately differentiated adenocarcinoma	10/82	9
MAXF 449	Breast	Poorly differentiated adenocarcinoma	2/83	11
MAXF 583	Breast	Moderately differentiated adenocarcinoma	10/84	13
MAXF 857	Breast	Invasive ductal adenocarcinoma	10/86	14
MAXF 1162	Breast	Poorly differentiated comedo carcinoma	7/88	13
OVXF 645	Ovary	Papillary serous adenocarcinoma, well differentiated	6/85	15
OVXF 899	Ovary	Papillary serous adenocarcinoma, moderately differentiated	1/87	7
CEXF 1169	Uterine cervix	Poorly differentiated squamous-cell carcinoma	8/88	13
UXF 1087	Uterine corpus	Moderately differentiated adenocarcinoma	1/88	11

[a] In serial passage

The treatment response was classified as a complete remission (RTS on day 21 or 28, $\leq 10\%$ of initial value), partial remission (11%–50%), minimal regression (51%–75%), no change (76%–124%) or progressive disease ($\geq 125\%$). A tumour was considered to be sensitive if at least a minimal regression was achieved. Additionally, the RTS of treated groups vs controls was compared (T/C value). The specific growth delay (SGD) was calculated with regard to the DT as previously described by Steel et al. [18]:

$$\text{SGD} = \frac{\text{DT treated group} - \text{DT control group}}{\text{DT control group}} .$$

IFO was kindly supplied by Asta Pharma AG (Frankfurt, FRG).

Results

Toxicity

The maximum tolerated dose of IFO in tumor-bearing nude mice was 130 mg/kg per day given on days 1–3 and 15–17. After 21 days, lethality was 14% (12/83) after i.p. and 6% (2/32) after s.c. administration. Other schedules effected higher toxicity. For 100 mg/kg per day given i.p. on days 1–4 and 15–18, 21% lethality (9/42) was observed after 21 days. The i.p. injection of 300 mg/kg per

Table 2. Antineoplastic efficacy of ifosfamide in human tumour xenografts in vivo

Tumour type	Evaluable (*n*)	Efficacy:					CR+PR+MR/ total
		CR	PR	MR	NC	PD	
Breast	5	–	3	1	–	1	4/5
Ovarian	2	–	–	–	1	1	0/2
Uterine cervix	1	–	–	–	1	–	0/1
Uterine corpus	1	–	–	–	–	1	0/1
Gastric	1	–	1	–	–	–	1/1
Colorectal	3	–	1	–	–	2	1/3
Pancreatic	1	–	–	–	–	1	0/1
Lung:							
non-small-cell	7	1	–	1	1	4	2/7
small-cell	4	1	1	1	1	–	3/4
Testicular	3	–	2	1	–	–	3/3
Renal	6	–	–	–	2	4	0/6
Melanoma	3	–	–	–	–	3	0/3
Sarcoma	2	–	1	–	–	1	1/2
Mesothelioma	1	–	–	–	–	1	0/1
Head and neck	1	–	–	–	1	–	0/1
Thymoma	2	–	–	–	–	2	0/2
Total	43	2	9	4	7	21	15/43 (36%)

CR, complete remission; PR, partial remission; MR, minimal regression; NC, no change; PD, progressive disease

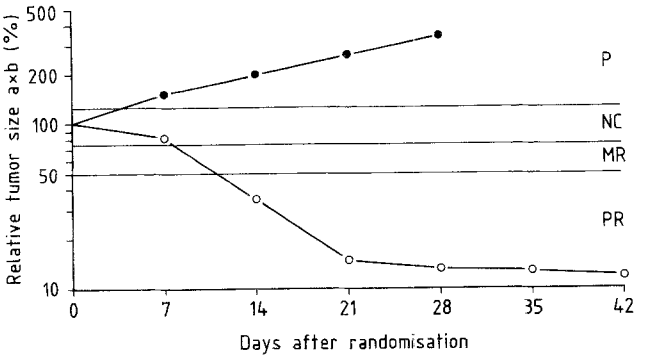

Fig. 1. Effect of ifosfamide in breast cancer MAXF 401. —●—, control; —○—, IFO (i.p.)

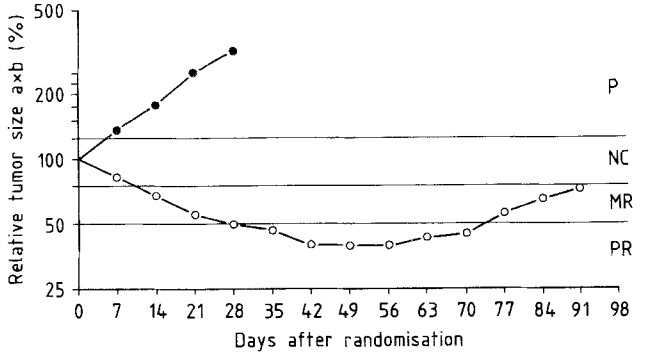

Fig. 2. Effect of ifosfamide in breast cancer MAXF 449.—●—, control; —○—, IFO (s.c.)

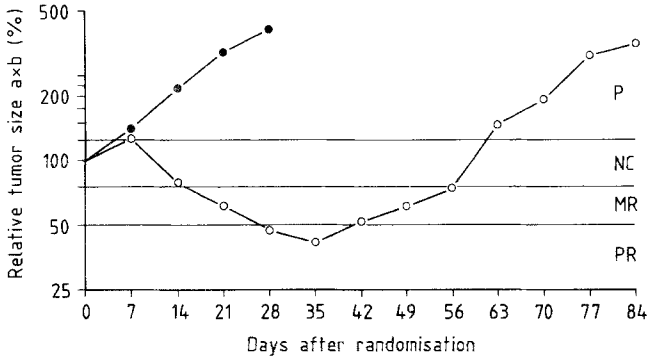

Fig. 3. Effect of ifosfamide in breast cancer MAXF 1162. —●—, control; —○—, IFO (i.p.)

day on days 1 and 15 and of 400 mg/kg per day on days 1–3 resulted in the death of 21% and 44% of the mice, respectively.

Antineoplastic efficacy

The antitumour activity of IFO is summarized in Table 2. In all, 15/43 human tumours (36%) showed a regression and 22/43 (51%) demonstrated a significant inhibition of growth as defined by an SGD of ≥ 2. Complete remissions were observed in two lung cancer xenografts: one small-cell and one large-cell bronchogenic carcinoma. Partial remissions were achieved in nine tumours: three breast

Table 3. Comparison of the antineoplastic activity of cyclophosphamide and ifosfamide in human tumour xenografts growing s.c. in nude mice

Tumour designation	Origin	Efficacy[a]:	
		Cyclophosphamide	Ifosfamide
MAXF 401	Breast	++	++
MAXF 449	Breast	++	++
MAXF 583	Breast	±	+
MAXF 857	Breast	–	–
OVXF 645	Ovary	–	±
OVXF 899	Ovary	–	–
UXF 1087	Uterine corpus	–	–
GXF 97	Stomach	+	++
CXF 158	Colon	–	–
CXF 243	Colon	–	–
CXF 280	Colon	++	++
PAXF 546	Pancreas	–	–
LXFA 629	Lung, adenocarcinoma	±	+
LXFE 247	Lung, squamous-cell	–	–
LXFE 397	Lung, squamous-cell	–	–
LXFE 409	Lung, squamous-cell	±	±
LXFE 638	Lung, squamous-cell	–	–
LXFL 529	Lung, large-cell	+++	+++
LXFS 387	Lung, small-cell	+	+
LXFS 538	Lung, small-cell	++	++
LXFS 605	Lung, small-cell	++	+++
LXFS 650	Lung, small-cell	±	±
TXF 404	Testes	++	++
TXF 593	Testes	±	+
RXF 423	Renal	–	–
RXF 631	Renal	–	–
MEXF 514	Melanoma	–	–
MEXF 520	Melanoma	–	–
SXF 81	Sarcoma	+++	++
PXF 349	Mesothelioma	–	–

[a] Relative tumour size: –, progression; ±, no change; +, minor regression; ++, partial remission; +++, complete remission

cancer xenografts, one gastric, one undifferentiated colorectal, one small-cell lung and two testicular cancer xenografts as well as one sarcoma. In two ovarian, two uterine, one pancreatic, five squamous-cell lung and six renal carcinomas, IFO did not effect a tumour regression.

Activity in gynecological tumours

In all, 9 of 43 xenografts studied were derived from gynecological tumours. In five breast cancer xenografts, three partial remissions (Figs. 1–3) and one minimal regression were observed. An invasive ductal adenocarcinoma did not respond to IFO. Two ovarian carcinoma xenografts were tested; a well-differentiated papillary serous adenocarcinoma showed (transient) no change and a moderately differentiated tumour was resistant. On day 21, a carcinoma of the uterine cervix exhibited no change; however, progressive growth occurred immediately after treatment was stopped. An adenocarcinoma of the uterine corpus was not responsive to IFO and grew progressively.

Cross-resistance and collateral activity

A comparison of the antineoplastic efficacy of the two oxazaphosphorine derivatives cyclophosphamide and IFO was carried out in 30 human tumours (Table 3). Both compounds were tested simultaneously in 11 experiments; in 19 cases, IFO and cyclophosphamide were studied sequentially in the same xenograft model. The maximum tolerated dose for cyclophosphamide was determined to be 200 mg/kg per day given i.p. on days 1 and 15; it led to 17% lethality after 21 days. Cyclophosphamide induced tumour regression or remission in 10/30 xenografts (33%) and IFO in 13/30 (43%). In three other xenografts, IFO effected a higher degree of response than cyclophosphamide.

Discussion

In preclinical animal experiments, IFO has shown a number of advantages over cyclophosphamide, such as a higher therapeutic index and a broader spectrum of activity [12]. In clinical studies, IFO has been shown to be highly active in bronchogenic carcinomas [6, 7], malignant neoplasms of the testes [13, 17], breast [16] and ovarian cancer [20], sarcomas [1, 14] and lymphomas [3].

Our results confirm the broad antineoplastic activity of the alkylating agent IFO in various tumour types. The optimal dose level in nude mice was determined to be 130 mg/kg per day given i.p. or s.c. on days 1–3 and 15–17. This fractionated schedule yielded lower toxicity and a higher response rate, suggesting an important role for the concentration-time product (c x t) at the site of action [2]. These findings are in accordance with previous clinical studies, where toxicity was substantially reduced by continuous infusion of IFO over several days, with the possibility of a 50% increase in the total dose and further improvement of the therapeutic response [15].

With regard to disease regression, IFO was efficacious in 15/43 tumours studied. The observed spectrum of activity parallels the clinical properties of the drug. Lung and testicular cancer xenografts as well as sarcomas were shown to be responsive. Marginal activity as determined by an SGD of $\tilde{x}$ 2 was found in 2/6 renal and 2/5 squamous-cell bronchogenic carcinomas, suggesting a slight effect of IFO on these tumour types. However, regressions were not observed.

For gynecological neoplasms, good activity was seen in breast cancer xenografts, with partial remissions in 3/5 tumours studied and a duration of response in nude mice of 6–10 weeks. In a well-differentiated ovarian carcinoma resistant to cyclophosphamide, IFO produced no change. Another ovarian adenocarcinoma and a carcinoma of the uterine corpus were resistant and grew progressively; one cervical cancer showed no change. These data indicate some effect of IFO on cancers of the ovary and uterine cervix, as demonstrated by previous studies (4, 20]. However, as the present study involved a small number of tumours our results have to be considered as being preliminary.

The comparison of the antineoplastic activity of cyclophosphamide and IFO in 30 selected human tumour models revealed a therapeutic advantage for IFO, with a higher response rate and lower toxicity. In breast cancer MAXF 583 on day 28, cyclophosphamide resulted in no change (RTS, 124%) and IFO led to a minimal regression (RTS, 72%). Similar differences in activity were found in ovarian papillary serous adenocarcinoma OVXF 645, gastric cancer GXF 97, adenocarcinoma of the lung LXFA 629, small-cell lung cancer LXFS 605 and testicular teratocarcinoma TXF 593. Both compounds are structurally related oxazaphosphorine derivatives. In cyclophosphamide, two 2-chloroethyl groups are attached to an exocyclic nitrogen atom, which corresponds to the nitrogen mustard structure. In IFO, one of the 2-chloroethyl groups is transferred onto the cyclic phosphamide nitrogen atom of the oxazaphosphorine ring, resulting in an altered linear distance between the two functional groups. The optimization of the distance for cross-linkage of nucleic acids may be an important cause of the specific chemotherapeutic efficacy of IFO [8].

This study indicates the possibility of a broad preclinical evaluation of interesting new compounds in an in vivo system. In a comparison of tumour response in nude mice and patients, correct predictions for resistance were achieved in 96%, and for tumour response in 90% [9], demonstrating the high predictivity of the human tumour/nude mouse system. Preclinical phase II studies in selected human tumour models growing s.c. in athymic nude mice seem to offer an effective way of identifying active drugs as well as sensitive tumour types for further clinical development.

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