[CANCER RESEARCH 54, 4393–4397, August 15, 1994]

Efficacy and Toxicity of Ifosfamide Stereoisomers in an *in Vivo* Rat Mammary Carcinoma Model[1]

I. W. Wainer, C. P. Granvil, T. Wang, and G. Batist[2]

Experimental Pharmacology Program, Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Quebec H3T-1E2, [T. W., G. B.], and Department of Oncology, Pharmacokinetics Division, McGill University, Montreal, Quebec H3A-2T5 [I. W. W., C. P. G.], Canada

ABSTRACT

Ifosfamide (IFF) is a nitrogen mustard with significant activity against a number of tumors. Since it is a chiral molecule, it has been suggested that enantioselective metabolism could result in different efficacy and toxicity profiles for (*R*)- and (*S*)-ifosfamide. Both experimental animal and clinical data suggest that *N*-dechloroethyl metabolites of (*S*)-IFF are more significantly associated with neurological toxicity, which may limit therapeutic use of IFF. We have used purified ifosfamide enantiomers to examine the pharmacokinetics; spectrum of toxicity including lethality, weight loss, and myelosuppression; and antitumor effects of the mixture compared to each of the purified enantiomers. In the MatB mammary carcinoma grown in female Fischer rats we demonstrated that the antitumor efficacy appears to be the same for (*R*)-IFF and (*S*)-IFF, while the (*R*)-IFF has greater myelotoxicity. Pharmacokinetic analysis of plasma concentration-time confirms that the (*R*)-IFF is metabolized to a greater extent than (*S*)-IFF via the activation pathway. These data suggest that purified (*R*)-IFF may be an effective way to deliver active cytotoxic drug while limiting the generation of neurotoxic metabolites.

INTRODUCTION

IFF[3] is an oxazaphosphorine nitrogen mustard which has shown significant activity against a variety of tumors in the clinic (1–3). IFF is also a chiral molecule which contains an asymmetrically substituted phosphorus atom, and it exists in two enantiomeric forms, (*R*)-IFF and (*S*)-IFF (Fig. 1). In clinical practice, IFF is used as a racemic (50:50) mixture of the two stereoisomers.

IFF must be metabolically transformed into the active cytotoxic agent isophosphoramide mustard (4). The initial metabolic step in the transformation of IFF to isophosphoramide mustard is the oxidation of carbon 4 of the oxazaphosphorine ring by hepatic microsomal enzymes to form 4-hydroxy-IFF (Fig. 1). The hydroxylation can also occur at one of the β-chloroethyl side chains. This pathway produces two inactive dechloroethylated metabolites, 2-dechloroethylifosfamide and 3-dechloroethylifosfamide (Fig. 1). This metabolic route also produces chloroacetaldehyde, a central nervous system toxin (5, 6), and can account for up to 48% of the administered IFF (7).

Since IFF is a chiral molecule, the possibility exists that (*R*)-IFF and (*S*)-IFF will exhibit different pharmacological properties; *e.g.*, enantioselective differences in the metabolism and disposition of IFF isomers have been observed in humans (7–9) and rats (10). Recently we reported an association between clinical neurotoxicity and (*S*)-IFF dechloroethylated metabolites in patients treated with IFF (13). In both of these species, the *N*-dechloroethylation pathway has been shown to be enantioselective for (*S*)-IFF. Enantiomers can also display relatively distinct efficacies and toxicities. For example, (*S*)-IFF was reported to have a higher therapeutic index than (*R*)-IFF in *in vivo* studies in mice using three transplanted tumor models: P388 lymphoid leukemias; Lewis lung carcinoma; and mouse mammary carcinoma 16C MAC (11). (*S*)-IFF was also found to be more toxic (11). However, no difference was found between the antitumor effect of (*R*)-IFF and (*S*)-IFF on L1210 lymphoid leukemias in mice (11) or in acute toxicity and activity against L5222 leukemia in rats (12).

The efficacy and toxicity of IFF enantiomers have also been studied in CBA/CaJ mice (14). The results of the studies indicated that there were no statistically significant differences between the efficacies of (*R*)-IFF, (*S*)-IFF, and (*R,S*)-IFF against childhood rhabdomyosarcoma (HxRh28) grown *in vivo* as a xenograft in immunoincompetent female CBA/CaJ mice. The same was true regarding the acute toxicities of the stereoisomers. No statistically significant differences were found in the plasma pharmacokinetics of (*S*)-IFF and (*R*)-IFF, or in the *in vitro* *N*-dechloroethylation. Since the human pharmacokinetics and metabolism of IFF are enantioselective, the results of this study led the authors to question the validity of mice as a model for human efficacy, toxicity, and pharmacokinetic studies.

In order to help clarify the relative efficacies and toxicities of (*S*)-IFF and (*R*)-IFF, we have investigated the effect of these enantiomers in F344 rats. The *N*-dechloroethylation pathway and pharmacokinetic disposition of IFF have previously been shown to be enantioselective in this species (14). Both non-tumor-bearing animals and those bearing a MatB rat mammary carcinoma were used in this study and the relative lethality, bone marrow suppression, weight loss, antitumor activity, and pharmacokinetics were determined for (*R*)-IFF, (*S*)-IFF and (*R,S*)-IFF. The results of these studies are presented below.

MATERIALS AND METHODS

Chemicals. (*R,S*)-Ifosfamide was supplied from Bristol-Myers Canada (Belleville, Ontario, Canada) and individual enantiomers were prepared by enantioselective high pressure liquid chromatography (15). The purity of each enantiomer was >99.5%. All other reagents and solvents were analytical grade. Stock solutions of IFF and hexobarbital (IS) were prepared in methanol at a concentration of 1 mg/ml and diluted to make calibration standards. These solutions were stored at −20°C until used.

Gas Chromatography-Mass Spectrometry. The analysis of IFF in plasma were performed using gas chromatography-mass spectrometry. The gas chromatograph was a Varian 3400 gas chromatograph equipped with a Finnigan A 200S gas chromatograph autosampler operating in the splitless mode. The mass spectrometer was a Finnigan Mat Model Incos 50 operating in the electron impact and selective ion monitoring mode. The chromatographic separation was carried out on a fused silica capillary column (DB-5; 30 m × 0.25 mm, inside diameter; film thickness, 0.25 μm). The column oven temperature was linearly programmed from 80°C to 250°C. The injector temperature was 250°C in the splitless mode, the helium pressure was 8 psi, and the transfer line was maintained at 260°C. The mass spectrometer was operated in the electron impact ionization mode at an ion source temperature of 180°C and an ionization energy of 70 eV.

Plasma Preparation. To 0.1 ml of plasma sample were added 20 μl IS (hexobarbital, 100 μg/ml in methanol) and 3 ml chloroform. The mixture was vortexed for 1 min and centrifuged at 1000 × *g* for 10 min. The aqueous phase was discarded, and the organic phase was transferred to another tube and evaporated to dryness in a speed-Vac concentrator. The residue was reconsti-

Received 1/25/94; accepted 6/15/94.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants from the Cancer Research Society and Martinex Research, Inc.

[2] To whom requests for reprints should be addressed, at Lady Davis Institute for Medical Research, Jewish General Hospital, 3755 Cote Ste. Catherine, Montreal, Quebec H3T-1E2, Canada.

[3] The abbreviations used are: IFF, ifosfamide; IS, internal standard; AUC, area under plasma concentration-time curve.

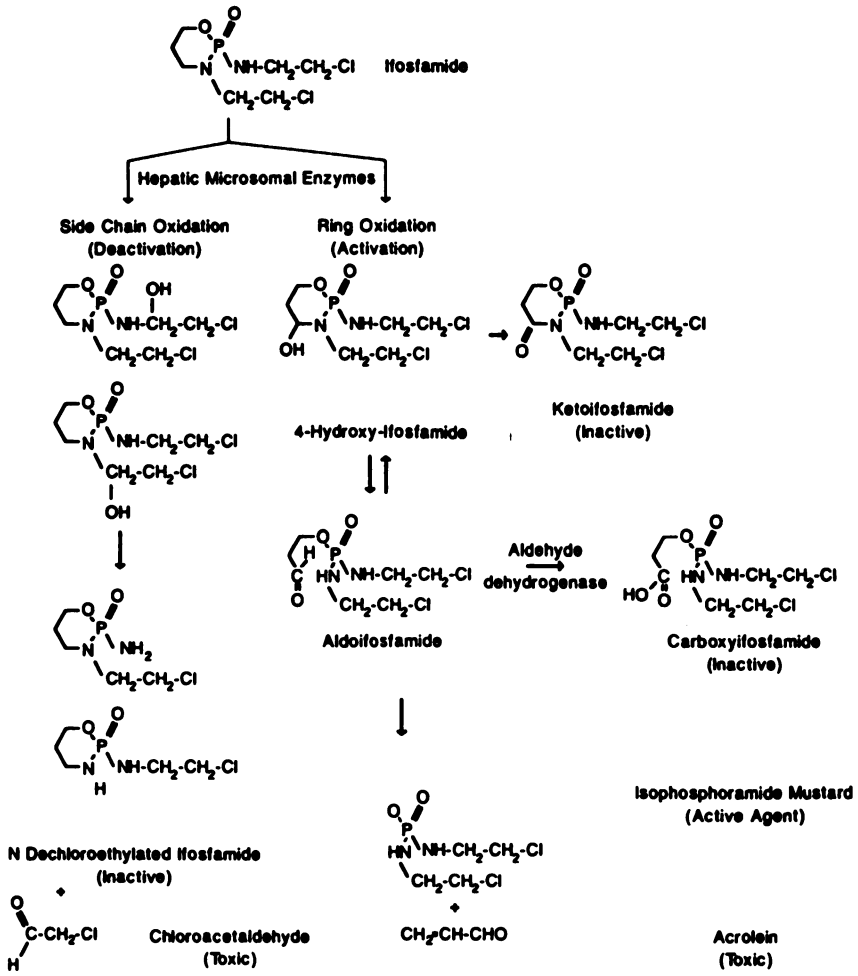

Fig. 1. Pathways of ifosfamide activation and metabolism to potentially toxic products.

tuted in 100 μl methanol and 1 μl was injected onto a Varian 3400 gas chromatograph.

Quantitation. The quantitation of (*R,S*)-IFF, (*R*)-IFF, and (*S*)-IFF was performed in the selected ion monitoring mode at *m/z* 211 [(*R*)-IFF and (*S*)-IFF] and *m/z* 221 (IS). Standard solutions were prepared in drug-free plasma with IFF concentrations of 0.25–120 μg/ml. Two standard curves were used, one between 0.25 and 10 μg/ml and a second one from 10 to 150 μg/ml. For each standard curve, the coefficient correlation was 0.99. The limit of detection was 125 ng/ml. The recovery and precision were good. The interassay precision was 5%. The peak area internal standard ratios of the compounds were used to calculate the concentrations for the standard curves and unknown samples.

Animal Pharmacokinetic Studies. The pharmacokinetics of (*R,S*)-IFF, (*R*)-IFF, and (*S*)-IFF were determined from female Fischer rats (F344; Charles River Canada) weighing 150–180 g. These animals were treated with a single i.v. dose of IFF, (*R*)-IFF, and (*S*)-IFF (125 mg/kg) dissolved in sterile water by the lateral tail vein. The rats were anesthetized with urethan, and the carotid artery was cannulated and fitted with a heparin lock. Blood samples (0.3–0.5 ml) were collected at 0, 5, 15, 30, 60, 90, 120, 180, 240, and 300 min. The collected plasma was centrifuged and stored at −20°C until analysis. Previous studies in the same strain of rats demonstrated that the pharmacokinetics of ifosfamide enantiomers can be adequately described using a 300-min sampling schedule (10, 16).

The AUC was calculated by the trapezoidal method. The values were subjected to an analysis of variance comparison. The extrapolated AUC was estimated as the last measured plasma concentration divided by the terminal elimination rate constant.

Animal Model and Tumor Cells. MatB is a rat mammary carcinoma cell line that grows both *in vitro* and *in vivo*. It has been demonstrated to be sensitive to a variety of bifunctional alkylators when testing in both conditions (17). We have previously demonstrated the potential importance of this model in preclinical studies of breast cancer, since it is a poorly differentiated estrogen receptor negative tumor that grows *in vivo* as a solid mass that develops a vascular supply and metastasizes to regional lymph nodes. It has proved useful in modeling *in vivo* modulation of drug resistance (18). In a recent study we demonstrated that the presence of MatB is associated with increased circulating WBC but no change in platelet count. Furthermore, the presence of the tumor, and moreover of a drug-sensitive *versus* drug-resistant tumor, is shown to affect host factors including response to a variety of drugs (19). Cytokines produced by tumors have been described previously (20). A subline selected *in vitro* for resistance to melphalan, with cross-resistance to other nitrogen mustards, has also been shown to be resistant on *in vivo* examination (17).

MatB cells are maintained *in vitro* in α-minimum essential medium (GIBCO, Grand Island, NY) supplemented with 1.3% sodium pyruvate, 2.6% glutamine, 1.3% nonessential amino acids, 10% fetal calf serum, and 100 units/ml gentamicin. Cells are maintained at 37°C under 5% CO_2. A suspension of 5×10^5 cells is injected s.c. into the flanks of female F344 rats weighing 160–200 g. Within 7–10 days, when there is a palpable nodule, the rats are lightly anesthetized with ether, and a single i.v. dose of ifosfamide is injected via the tail vein. The tumor size and animal weights are monitored every other day. On the basis of the conventional rat:human dose conversion factor of 1:7 and idealized human body weights and surface areas of 70 kg and 1.5 m^2, the dose of 100 mg/kg in the rat represents approximately 600 mg/m^2 in humans, which is in the general range of clinical single doses (1). Statistical analysis was performed using an unpaired Student's *t* test or Pearson's χ^2 analysis.

Determination of Myelotoxicity. For the studies of myelotoxicity, at least three animals/group had blood obtained by cardiac puncture with a 23-gauge needle. The samples were heparinized and both WBC and platelet counts were determined using a Coulter Counter.

RESULTS

There were differences resulting in the presence of a tumor in these animals with regard to the effects of these treatments, and the data are therefore presented for each situation separately.

Toxicity: Lethality

Non-Tumor Bearing. The toxicity was assessed by lethality within 7–10 days after administration of (*R*)-, (*S*)-, or (*R,S*)-ifosfamide in non-tumor-bearing Fischer 344 rats. At 100 and 125 mg/kg there is minimal lethality in the (*R*)- and (*R,S*)-ifosfamide groups (1 of 5 and 1 of 5, respectively) whereas 0 of 5 rats died after the (*S*)-ifosfamide dosing. At 150 mg/kg there was increased toxicity in the (*R*)- and (*R,S*)-IFF-treated groups, where 2 of 4 rats died in each of the groups, while 0 of 4 died in the (*S*)-IFF group. At this dose level the lethality was apparently greater for the (*R*)-IFF-treated animals compared to either of the other groups ($P = 0.045$).

Tumor-bearing Animals. At 100 mg/kg there were 0 of 5 toxic deaths after treatment with (*R,S*)- or (*S*)-IFF, respectively, while (*R*)-IFF resulted in 1 of 5 animals dead. At 125 mg/kg there were 4 of 10 and 5 of 10 toxic deaths after (*R,S*)- and (*R*)-IFF, respectively, and 2 of 10 after (*S*)-IFF treatment. χ^2 analysis shows that there is a significant difference comparing treatment with (*R*)-IFF to (*S*)-IFF ($P = 0.017$); however, there is no difference comparing either (*R*)-IFF or (*S*)-IFF to (*R,S*)-IFF. At 150 mg/kg there were 5 of 5 deaths after either (*R,S*)- or (*R*)-IFF, and 3 of 5 after (*S*)-ifosfamide. This is no significant difference for the (*R*)-IFF *versus* either (*S*)-IFF or (*R,S*)-IFF ($P = 0.067$). More detailed toxicity studies were performed at the dose of 125 mg/kg.

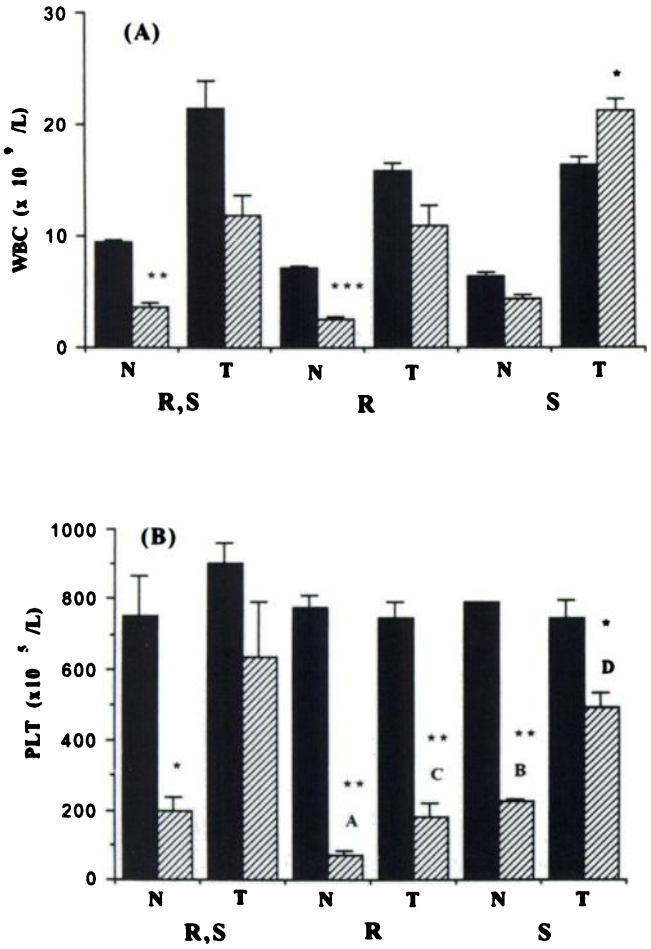

Fig. 2. Peripheral blood counts obtained as described in tumor-bearing rats that received saline or IFF treatment i.v. (*A*) Results of measurement of WBC; (*B*) Effects of treatment on platelets. There were 3–5 animals in each group. *Points*, mean; *bars*, SD; *N*, non-tumor bearing; *T*, tumor bearing. ■, controls; ▨, groups treated with (*R,S*)-IFF, (*R*)-IFF, or (*S*)-IFF. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. *A versus B*, $P < 0.05$; *C versus D*, $P < 0.01$.

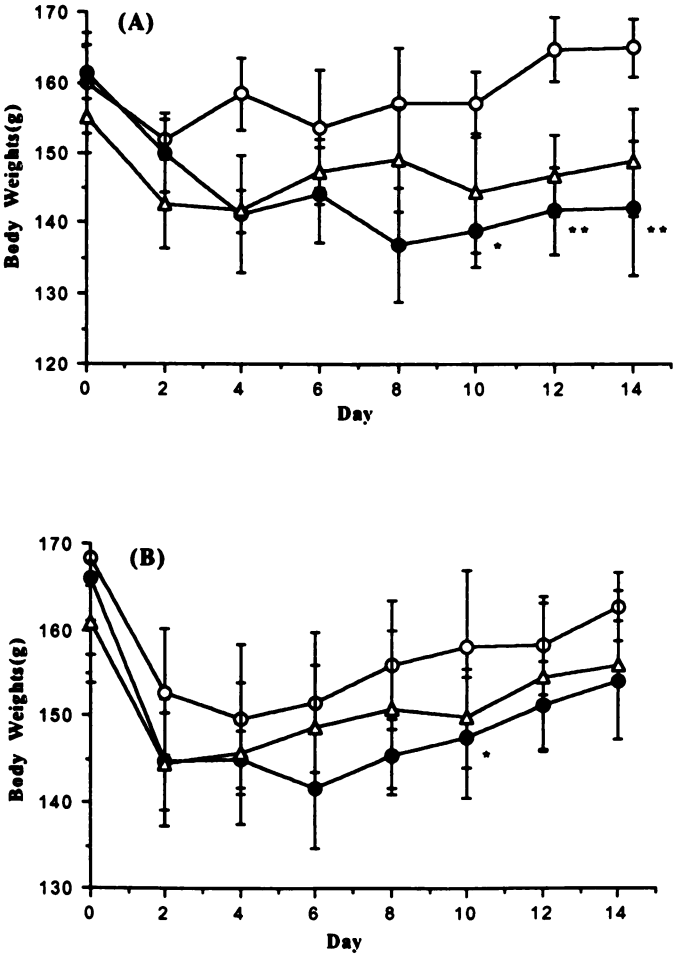

Fig. 3. Weights of rats measured every other day beginning at the time of IFF treatment. (*A*) Tumor-free rats. (*B*) Tumor-bearing rats. There were 3–5 animals/group. *Points*, mean; *bars*, SD ○, IFF; ●, (*R*)-IFF; △, (*S*)-IFF; *, $P < 0.05$; **, $P < 0.01$.

Toxicity: Bone Marrow Suppression

Fig. 2 demonstrates the results of measurement of WBC (Fig. 2*A*) and platelet (Fig. 2*B*) counts in blood obtained by cardiac puncture in non-tumor-bearing and tumor-bearing rats. Measurements were made prior to and 1 week after i.v. injection of 125 mg/kg of (*R,S*)-, (*R*)-, or (*S*)-ifosfamide. In tumor-free rats myelosuppression is observed after all three formulations, although myelosuppression due to (*S*)-IFF is not statistically significant. In the presence of a tumor, the effects of a colony-stimulating factor are evidenced by higher WBC even prior to drug administration. Because the WBC progressively rises over time with exposure to the tumor, the intergroup variation in absolute WBC relates to differences in time from tumor inoculation to baseline WBC measurement and then drug administration. While both (*R,S*)- and (*R*)-IFF treatments result in significant myelosuppression, there is no such effect after (*S*)-IFF where the WBC is higher.

Differences in platelet toxicity are demonstrated in Fig. 2*B*. There is no tumor-induced effect on platelet counts, as is the case with most of the presently identified colony-stimulating factors. There is significant thrombocytopenia 1 week after administration of all 3 drug forms in non-tumor-bearing rats; however, (*R*)-ifosfamide resulted in

greater platelet suppression that was statistically difference from either the (*R,S*)- or the (*S*)-ifosfamide ($P < 0.05$). Platelet suppression in the presence of tumor is significantly less after (*S*)- compared to either (*R*)- or (*R,S*)-ifosfamide.

Toxicity: Weight Loss

Weight loss was determined by every-other-day measurements of groups of at least three rats. Fig. 3 shows the results of these measurements at various days after treatment with 125 mg/kg ifosfamide. In tumor-free rats (Fig. 3*A*) there is significantly greater weight loss in rats treated with (*R*)-ifosfamide compared to the racemic mixture from days 10–14 after treatment, while (*S*)-isosfamide results in an intermediate level of gastrointestinal toxicity. Interestingly, in the presence of the tumor the weight loss profile is different, with all three formulations resulting in weight loss. Although the effect of (*R*)-IFF appears greatest, it is significantly so only at one time point (Fig. 3*B*). The weight loss effects do not correlate with the lethality at this dose, so that while the presence of the (*R*)-IFF does appear to result in greater weight loss at some time points, at this dose it does not translate into greater animal death rate.

Pharmacokinetics

Fig. 4 presents the plasma concentration time curves obtained for the samples at 300 min postinjection. Fig. 5 demonstrates the calculated AUC for IFF as detected in rat plasma after injection of 100 mg/kg i.v. There were statistically significant differences between AUC_{RS} and AUC_S ($P < 0.01$) and AUC_R *versus* AUC_S ($P < 0.01$), while no statistically significant difference was observed between AUC_{RS} and AUC_R. These results are consistent with those obtained for toxicity and efficacy and indicate that (*R*)-IFF is metabolized to a greater degree than (*S*)-IFF via the activation pathway. This is the reverse of the *N*-dechloroethylation pathways where (*S*)-IFF is metabolized to a greater extent than (*R*)-IFF. However, *N*-dechloroethylation takes place at a slower rate than 4-hydroxylation and to a lesser extent.

Antitumor Activity

The wild-type MatB tumors are responsive to ifosfamide, as is shown in Fig. 6*A*. While tumors in saline-treated rats continue to grow, in all treatment groups there was complete disappearance of measurable tumors within 14 days. In order to assess differences among the isomers in antitumor effects at the same dose, we grew a MatB subline selected for resistance to nitrogen mustards in the rats, which are at least 4-fold resistant to another nitrogen mustard, mel-

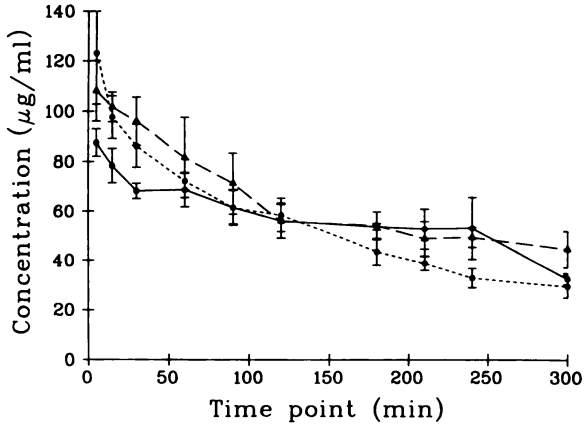

Fig. 4. Plasma concentration time curves for the animals treated with: ○, IFF ($n = 2$); ●, (*R*)-IFF ($n = 5$); △, (*S*)-IFF ($n = 5$).

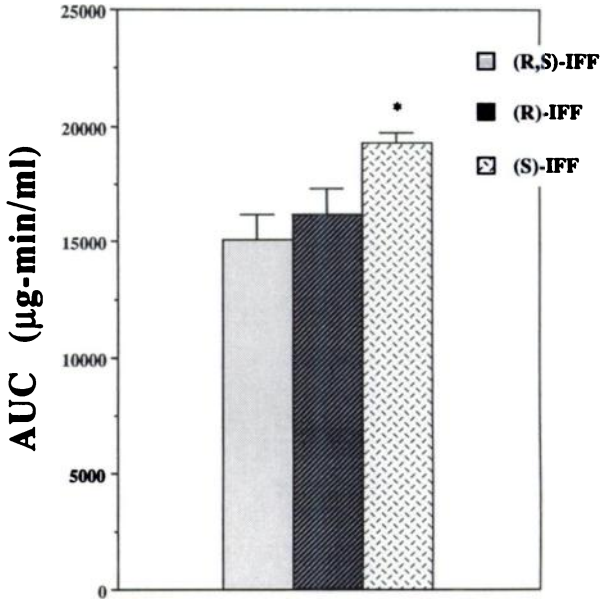

Fig. 5. Systemic exposure expressed as the AUC following i.v. administration of (*R,S*)-, (*R*)-, and (*S*)-IFF. *Columns*, mean; *bars*, SD.

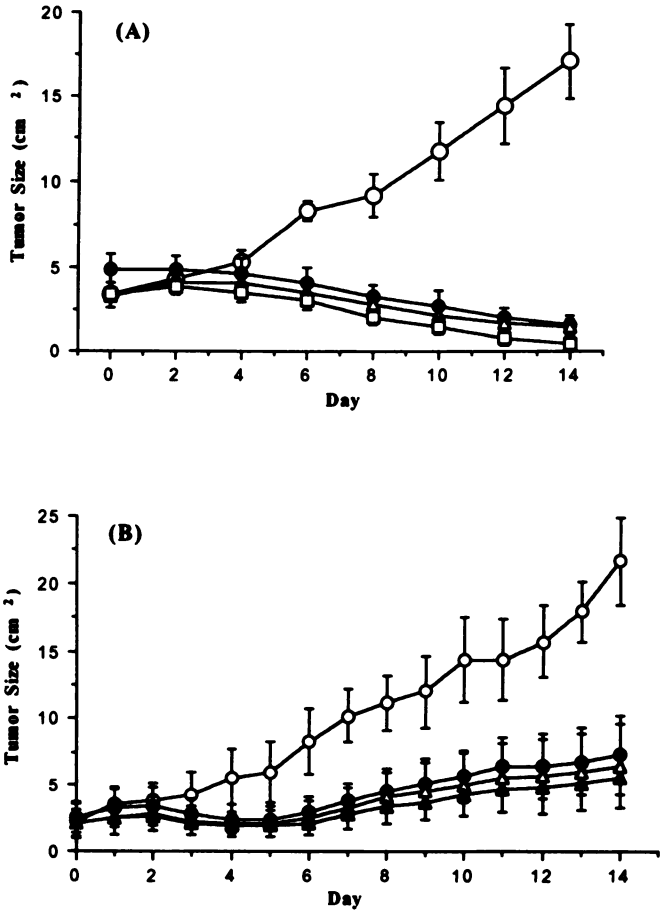

Fig. 6. Tumor size, which is the product of two perpendicular measurements, was measured every other day beginning on the day of IFF administration. *Points*, mean; *bars*, SD in groups of at least 5 animals. ○, saline treated; ●, IFF; □, (*R*)-IFF; in (*A*); ▲, (*R*)-IFF in (*B*); △, (*S*)-IFF. (*A*) Results in animals bearing the wild-type tumors. (*B*) Results for melphalan-resistant tumor-bearing animals.

phalan, *in vivo* (17). Fig. 6*B* shows that all three formulations result in equivalent significant tumor growth delay; there is no difference among the formulations regarding tumor growth duration.

DISCUSSION

The results of this study show no significant difference in the antitumor efficacy of (*R*)-IFF and (*S*)-IFF but a significant increase in lethality and bone marrow suppression of (*R*)-IFF relative to (*S*)-IFF. The pharmacokinetics also indicated that (*R*)-IFF was cleared more quickly than (*S*)-IFF. Since the *N*-dechloroethylation pathway has been shown to be enantioselective for (*S*)-IFF and since the *N*-dechloroethylated metabolites are neither lethal or myelotoxic, it appears that more of the (*R*)-IFF is passing through the 4-hydroxylation pathway leading to the production of the cytotoxic metabolites. The pharmacological and clinical relevance of this possibility is currently under investigation. Elucidation of the specifics of these metabolic pathways could result in the selection of drug and dose on the basis of the pharmacological metabolic phenotype of individual patients.

Another important result of these studies is the finding that rat metabolism of IFF is very likely closer to that of humans than is that of mice at least with regard to enantioselective metabolism. The metabolism of ifosfamide is not enantioselective in the mouse, and since IFF must be activated by microsomal metabolism, no pharmacological difference for (*R*)-IFF *versus* (*S*)-IFF would be expected. However, there is enantioselective metabolism in the rat that is the same as is observed in humans. Further studies in this model should permit detailed exploration of the various metabolic pathways for each of the IFF isomers. The precise cause of death in these animals is not known. At the dose used to examine weight loss, the data suggest that (*R*)-IFF is more toxic at some time points, but this does not result in more deaths than the racemic IFF. While nausea is a common clinical toxicity of IFF, severe and potentially lethal mucositis and diarrhea are not. This will require exploration in the clinical setting. However, the neurotoxicity observed in the clinic was not assessed in these rats, but the data to date suggest that use of the pure (*R*)-IFF enantiomer may substantially reduce the risk of this toxicity.

REFERENCES

1. De Kraker, J. Ifosfamide in paediatric oncology. Anticancer Drugs, *2:* 339–341, 1991.
2. Pratt, C. B., Douglass, E. C., and Etcubanas, E. L. Ifosfamide in paediatric malignant solid tumours. Cancer Chemother. Pharmacol., *24* (Suppl.)*:* 24–27, 1989.
3. Jurgens, H., Treuner, J., Winkler, K., and Gobel, U. Ifosfamide in pediatric malignancies. Semin. Oncol., *3* (Suppl.)*:* 46–50, 1989.
4. Allen, L. M., Creaven, P. J., and Nelson, R. L. Studies on the human pharmacokinetics of ifosfamide (NCI-109724). Cancer Treat. Rep., *60:* 451–458, 1976.
5. Goren, M. P., Wright, R. K., Pratt, C. P., and Pell, F. E. Dechloroethylation of ifosfamide and neurotoxicity. Lancet, *2:* 1219–1220, 1986.
6. Lind, M. J., Roberts, H. L., Thatcher, N., and Idle, J. R. The effect of route of administration and fractionation of dose on the metabolism of ifosfamide. Cancer Chemother. Pharmacol., *26:* 105–111, 1990.
7. Boos, J., Welslau, W., Ritter, J., Blaschke, G., and Schellong, G. Urinary excretion of the enantiomers of ifosfamide and its inactive metabolites in children. Cancer Chemother. Pharmacol., *28:* 455–460, 1991.
8. Misiura, K., Okruszek, A., Pankiewicz, K., Stec, W. J., Czownicki, Z., and Utracka, B. Stereospecific synthesis of chiral metabolites of ifosfamide and their determination in the urine. J. Med. Chem., *26:* 674–679, 1983.
9. Crom, W. R., Kearns, C. M., Wainer, I. W., Meyer, W. H., Pratt, C. B., Heideman, R. L., and Relling, M. V. Urinary excretion of ifosfamide enantiomers in children with cancer. Clin. Pharmacol. Ther., *49:* 195, 1991.
10. Granvil, C. P., Wang, T., Batist, G., and Wainer, I. W. Influence of phenobarbital induction on the enantioselective *N*-dechloroethylation of ifosfamide enantiomers in the rat. Drug Metab. Dispos., *22:* 166–168, 1994.
11. Kusnierczyk, H., Radzikowski, C., Paprocka, M., Budzynski, W., Rak, J., Kinas, R., Misiura, K., and Stec, W. Antitumor activity of optical isomers of cyclophosphamide, ifosfamide and trofosfamide as compared to clinically used racemates. J. Immunopharmacol., *8:* 455–480, 1986.
12. Wainer, I. W., Ducharme, J., Granvil, C. P., Trudeau, M., and Leyland-Jones, B. Ifosfamide stereoselective dechloroethylation and neurotoxicity. Lancet, *343:* 982–983, 1994.
13. Blaschke, G., Hilgard, P., Maibaum, J., Niemeyer, U., and Pohl, J. Präparative Trennung der Ifosfamid-Enantiomere und uhre pharmakologisch-toxikologische Untersuchung. Arzneim. Forsch., *36:* 1493–1495, 1986.
14. Masurel, D., Houghton, P. J., Young, C. L., and Wainer, I. W. Efficacy, toxicity, pharmacokinetics and *in vitro* metabolism of the enantiomers of ifosfamide in mice. Cancer Res., *50:* 252–255, 1990.
15. Masurel, D., and Wainer, I. W. Analytical and preparative HPLC separation of the enantiomers of ifosfamide, cyclophosphamide and trofosfamide and their determination in plasma. J. Chromatogr., *490:* 133–143, 1989.
16. Granvil, C. P., Gehrcke, B., Konig, W. A., and Wainer, I. W. The determination of the enantiomers of ifosfamide and its 2- and 3-*N*-dechloroethylated metabolites in plasma and urine using enantioselective gas chromatography with mass spectrometric detection. J. Chromatogr., *622:* 21–31, 1993.
17. Schecter, R. L., Woo, A., Duong, M., and Batist, G. *In vivo* and *in vitro* mechanisms of drug resistance in a rat mammary carcinoma model. Cancer Res., *51:* 1434–1442, 1991.
18. Alaoui-Jamali, M., Schecter, R. L., Rustum, Y. M., Centurioni, M. G., Lehnert, S., and Batist, G. *In vivo* reversal of doxorubicin resistance by a tiapamil analog Ro11-2933. J. Pharmacol. Exp. Ther., *264:* 93–98, 1993.
19. Batist, G., Mayer, L., Pilkiewicz, F., *et al.* Liposomal doxorubicin in experimental and clinical breast cancer. Proc. Am. Assoc. Cancer Res., *1090:* 183, 1991.
20. Asanoe, S., Urabe, A., Okabe, T., Sato, N., Kondo, Y., Ueyama, Y., Chiba, S., Oshawa, N., and Kosaka, K. Demonstration of granulocytopoietic factor(s) in the plasma of nude mice transplanted with a human lung cancer and in tumor tissue. Blood, *49:* 845–852, 1977.