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Urinary excretion of the enantiomers of ifosfamide and its inactive metabolites in children*

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Summary. The precondition for the antineoplastic effect of ifosfamide (ifo) is the oxidation of the oxazaphosphorine ring system, which contains a chiral centre at the phosphorous atom. This "ring oxidation" leads to the formation of alkylating mustard via several steps. A second metabolic pathway produces the cytostatically inactive metabolites 2- and 3-dechloroethyl-ifosfamide (2-d- and 3-d-ifo). The urinary excretion of the optical isomers of unmetabolised ifo and of 2- and 3-d-ifo, which represents the amount of ifo that has not been activated, was investigated by capillary gas chromatography for 18 treatment cycles in 14 children on various therapeutic schedules. The total cumulative excretion in 12 completely sampled cycles ranged from 27% to 50% of the ifo dose. Between 14% and 34% of the dose could be detected as ifo; 9% to 29%, as 3-d-ifo; and 2% to 8%, as 2-d-ifo. At 24 h after the end of therapy, excretion was nearly complete. Without exception, slightly more R-ifo (53%–61%) than S-ifo was excreted. S-2-d-ifo (50%–73%) was the main 2-d-metabolite. S-3-d-ifo (deriving from R-ifo) predominated in 6 of 14 children and R-3-d-ifo, in 8. Enantiomer-specific excretion increased after the end of infusion (up to 73% for R-ifo and 27% for S-ifo). We demonstrated stereospecific metabolism of ifo in children, with two different patterns of side-chain oxidation being observed. There was no evidence of important stereospecific ring oxidation in most children. A benefit should not be expected from the therapeutic application of pure enantiomers.

Introduction

Ifosfamide (ifo), first synthesized in the late 1960s, is an alkylating agent that belongs to the group of oxazaphosphorines and is an isomer of cyclosphosphamide. Ifo has been demonstrated to exhibit significant activity against numerous human malignancies. Currently, ifo is used as a part of first-line therapeutic regimens in approximately half of all children presenting with newly diagnosed cancer in Germany [17]. The current regimens vary widely in dose and scheduling, and both renal [13, 14, 24, 25, 28, 33, 34, 35] and CNS [6, 10, 12, 15, 18, 20, 30–32, 36] toxicity have increasingly been observed.

Ifo is a prodrug and requires biotransformation. The basis of its cytotoxic effect is the hydroxylation of the oxazaphosphorine ring system at the carbon atom in position 4 (see Fig. 1) by the microsomal mixed-function oxidase system of the liver [1, 7, 16]. 4-Hydroxy-ifosfamide (4-oH-ifo) is converted into tautomeric aldoifosfamide by the opening of the oxazaphosphorine ring. Spontaneous elimination of acrolein produces the alkylating compound isophosphoramide mustard [*N*,*N*′-bis(2-chloroethyl)-phosphoric acid diamide]. Further oxidation of 4-OH-ifo results in the inactive metabolites keto- and carboxyifosfamide [7, 16].

In addition to this "ring oxidation", "side-chain oxidation" of the chloroethyl groups at the cyclic (position 3) and exocyclic (position 2) nitrogen has been demonstrated, which leads to the formation of 3-dechloroethyl- (3-d-ifo) and 2-dechloroethyl-ifosfamide (2-d-ifo) and the elimination of chloroacetaldehyde [7, 27]. Dechloroethyl metabolites cannot form the bifunctional alkylating mustard. Drug metabolized by side-chain oxidation does not appreciably contribute to cytotoxicity but may contribute to toxic side effects. In particular, high levels of chloroacetaldehyde have been related to neurotoxicity in children [12, 20]. The chiral center of the enantiomers of ifosfamide is the phosphorous atom in the oxazaphosphorine ring system. All metabolites with intact ring systems exist in an R and an S form [23]. R-ifo is metabolized to R-2- and S-3-d-ifo and metabolism of S-ifo leads to the formation of S-2- and R-3-d-ifo, according to the definition of Cahn, Ingold and Prelog.

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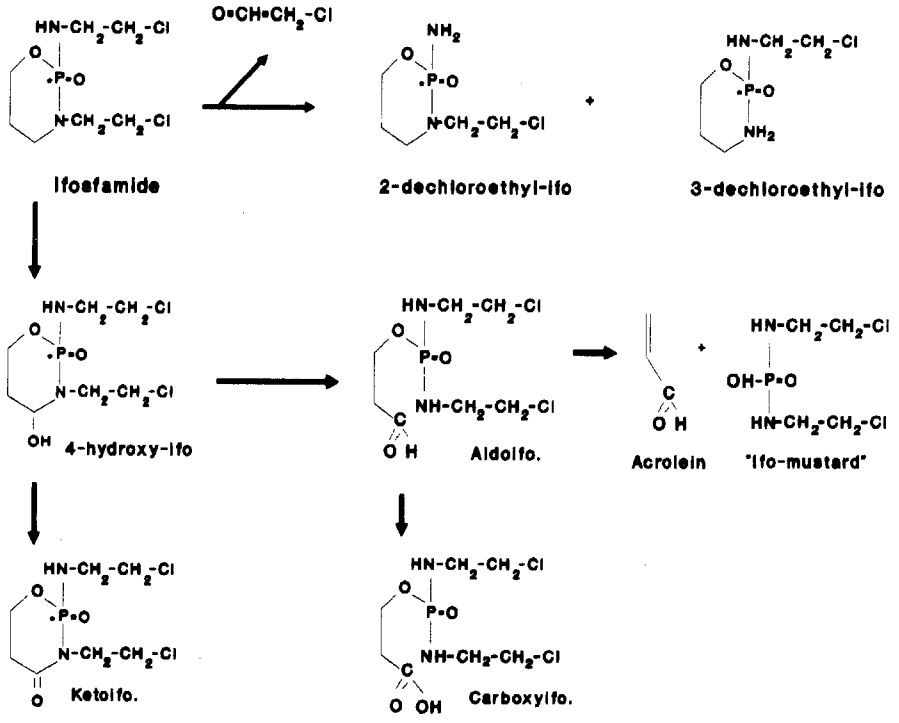

Fig. 1. Metabolism of ifo

Differences in the therapeutic activity of oxazaphosphorine enantiomers were first reported for cyclophosphamide in a mouse tumor model [8].

Likewise, stereodifferentiation of the antitumor effect has been established for the ifosfamide enantiomers. The levorotatory form was significantly more cytotoxic in a mouse tumor model [19]. Metabolic activation of S-ifo is significantly higher in mice, and keto-ifo is highly selectively formed from S-ifo [4]. Nevertheless, Masurel et al. [22] failed to demonstrate significant differences between the efficacy and toxicity of R-ifo, S-ifo and racemic ifo in a mouse-tumor xenograft model [22]. In summary, previous investigations have revealed a considerable variability in enantiomer-specific oxazaphosphorine metabolism and cytotoxicity among different animal species and tumor models ([4, 8, 9, 19, 22, 23]; reviewed in [11]).

In 1983, Misiura et al. [23] first determined the relative metabolism of the enantiomers of ifo in two patients. The urine of both subjects showed a slight enrichment of R-ifo and an excess of S-2-d-ifo. One patient excreted more S-3-d-ifo and the other, more R-3-d-ifo. These data suggest substantial stereoselectivity for ifo metabolism in humans. However, ifo is currently used therapeutically as a racemic mixture.

On this basis, the present study was designed to investigate the following questions:

1. Which quantities of the excreted ifo and side-chain oxidation products represent that part of the dose that has not been activated but is perhaps responsible for undesirable side effects?

2. Is the stereoselectivity of side-chain oxidation or the activating pathway a substantial phenomenon of clinical importance, which might result in a benefit's being gained from selective application of an individual enantiomer?

Patients and methods

Patients. A total of 14 children aged between 5 and 17 years underwent 18 courses of chemotherapy for osteosarcoma (5), soft-tissue sarcoma (3), Ewing's sarcoma (2), acute lymphoblastic leukemia (2), primitive neuroectodermal tumor (PNET, 1), and non-Hodgkins lymphoma (1) and were studied during 1 or 2 treatment cycles. The treatment schedules used are outlined in Table 1. Individual doses ranged from 400 to 3000 mg/m^2. Drugs were given as infusions lasting from 1 h to 5 days (continuous infusion); two protocols prescribed bolus injection. The accompanying cytostatics given on the various schedules included vincristine (VCR), Adriamycin (ADR), daunorubicin (DNR), actinomycin D (AMD), etoposide (VP-16), teniposide (VM26), cisplatin, cytarabine (Ara-C), methotrexate (MTX), dexamethasone (Dexa), thioguanine (TG), and vindesine (VDS).

Table 1. Dosage, scheduling and accompanying cytostatics used in the present study

Patients	Dose (mg/m^2 daily)	Days	Infusion	Other cytostatics
AH, CT, IE, JP, KK	3000	2	Continuous	VCR, ADR, AMD
DF, DR, MB	1000/2000	5	Continuous	VP-16
VG, BBr	2000	5	Continuous	Cisplatin
PB, BB	3000	2	1 h	Cisplatin
DS	800	5	1 h	VM26, Ara-C, MTX, Dexa
MS	400	5	Bolus	Dexa, TG, DNR, VDS, MTX

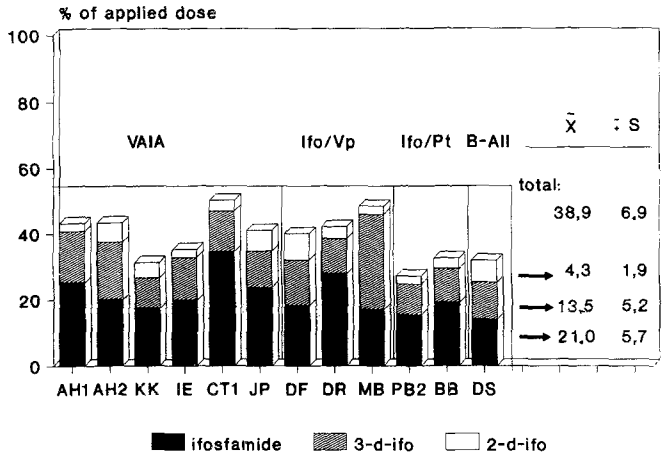

Fig. 2. Cumulative excretion of ifo, 2-d- and 3-d-ifo ($n = 12$)

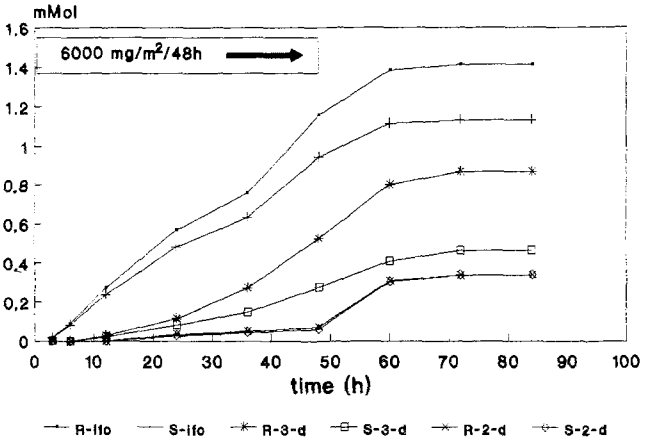

Fig. 3. Cumulative excretion of enantiomers during a single schedule (patient KK; total dose, 3800 mg = 14.6 mmol)

Urinary fractions were sampled at up to 24 h after the end of the therapy, at which time renal excretion was almost complete. Samples were collected at 3- to 12-h intervals and were immediately stored at −20° C. Although urine sampling was incomplete in several courses, 12 courses in 11 children could be examined completely (AH1/2–DS).

Methods. The analysis was performed by capillary gas chromatography (Hewlett-Packard, HP 5830 A) following extraction with dichloromethane. *5,5′*-Dimethylcyclophosphamide was added as on internal standard. Ifo and 2- and 3-d-ifo were separated on a covalently bonded, fused silica phase (OV1701, 5 m × 0.32 mm) and the enantiomers, on a Chirasil-Val fused-silica capillary column (45 m × 0.31 mm) and were detected on a flame ionization detector (FID; for details of this method, see Blaschke and Koch [3]. *5,5′*-Dimethylcyclophosphamide, ifo, and 2- and 3-d-ifo were kindly supplied by ASTA Pharma, (FRG). Standards of the enantiomers were obtained by preparative separation as described elsewhere [5].

Results

A mean of 21% ± 6% of the applied ifo dose (range, 14%–34%) was excreted unmetabolized over all of the 12 completely sampled courses (Fig. 2). Overall, 4% ± 2% of the 2-d-ifo (range, 2%–8%) and 14% ± 5% of the 3-d-ifo (range, 9%–29%) could be recovered. Without exception, 3-d-ifo excretion predominated over 2-d-ifo excretion in the 12 completely sampled courses, with the former reaching levels of up to 11-fold (mean, 3.9; range, 2%–11) those of the latter. One patient (MS) did not excrete any detectable amount of side-chain oxidation products during the first therapy course. In the second course, 9% of the applied dose was recovered as 3-d-ifo and 10%, as 2-d-ifo. However in both therapy courses, sampling was finished at >96 h prior to the fifth dose and therefore remained incomplete.

A total urinary excretion of side-chain-oxidized metabolites ranged from 12% to 31% (mean, 18% ± 5%) and the sum of unmetabolized and side-chain-metabolized ifo was 27%–50% (mean, 39% ± 7%). There was no significant difference between short (PB2–DS) and continuous infusion (AH1–MB) for total renal excretion and for elimination of the individual metabolites. No relationship was found between the excreted amount of ifosfamide or metabolites and age, sex or accompanying therapy.

Figure 3 exemplarily presents the cumulative excretion of the optical isomers of ifo and the side-chain-oxidized metabolites in one child receiving a 48-h continuous infusion of 3 g/m² daily. Excretion of unmetabolized ifo remained nearly constant over the entire infusion period. Excretion of the side-chain-oxidized metabolites began after a 6-h lag time in the 6- to 12-h sampling period and steadily increased up to the end of therapy. At 12 h after the end of the treatment total excretion was nearly complete.

Stereospecific evaluation

An increasing difference was found in the cumulative excretion of the optical antipodes of ifo and side-chain-oxidized products (see Fig. 3). The predominating ifo enan-

Table 2. Percentage of the enantiomers in excreted metabolites

	AH1	AH2	KK	IE	CT1	JP	DF	DR	MB	PB2	BB	DS	MS1	MS2	PB1	BBr	CT2	VG
Ifo-R	57	56	56	57	56	55	57	58	61	54	55	56	53	54	54	52	55	57
Ifo-S	43	44	44	43	44	45	43	42	39	46	45	43	47	46	46	41	45	43
2-d-R	42	34	50	29	33	28	43	32	27	44	41	32		32	49	37	26	36
2-d-S	58	66	50	71	64	72	57	68	73	56	59	65		68	51	63	74	61
3-d-R	60	58	65	36	37	47	60	48	46	67	61	55		33	64	54	37	53
3-d-S	40	42	35	64	62	53	40	52	54	33	39	43		55	30	42	63	45

AH1–DS were completely sampled

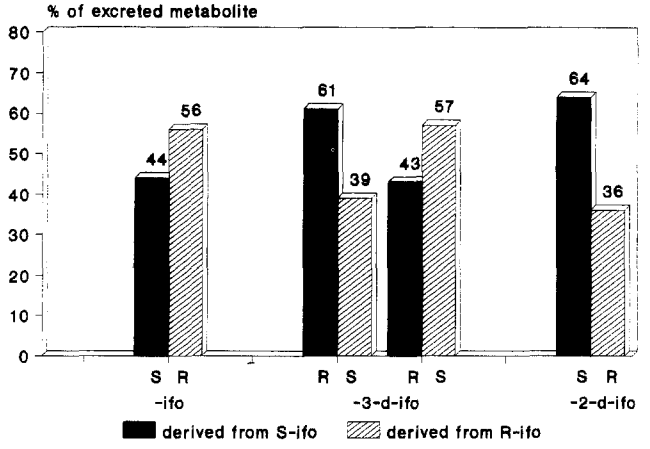

Fig. 4. Mean R/S ratio for excreted ifo, 2-d-ifo and 3-d-ifo, showing two different patterns at 3-d-ifo (12 completely sampled courses) ($n = 12$)

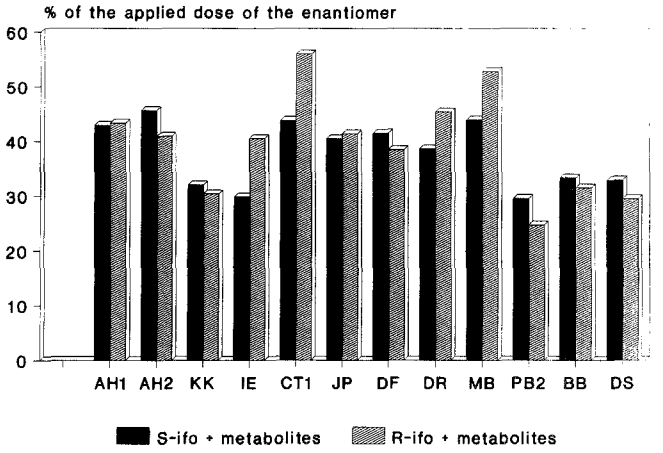

Fig. 5. Sum of R/S-ifo and related metabolites (S-ifo + R-3-d-ifo + S-2-d-ifo and R-ifo + S-3-d-ifo + R-2-d-ifo)

tiomer was R-ifo, and S-ifo was excreted in lower quantities. The main side-chain metabolite was R-3-d-ifo, which is the oxidation product of S-ifo. Without exception, all 14 children undergoing 18 chemotherapy courses metabolized a larger amount of S-ifo and therefore excreted more R-ifo (see Table 2).

The cumulative ifo excretion in all 12 completely sampled courses was 56% ± 2% R-ifo vs 44% S-ifo (see Fig. 4). The ratio of optical antipodes was enhanced in the fractions of urine excreted after the end of therapy. The last fraction sampled showed a mean of 67% R-ifo and 33% S-ifo. The predominating 2-d metabolite was S-2-d-ifo (mean, 64% ± 7%). No child excreted more R-2-d-ifo than S-2-d-ifo, and only one child (see Fig. 3) showed a value of of 50%/50%. In 6 of 14 children, the quantities of S-3-d-ifo, the metabolite of R-ifo, exceeded the amounts of R-3-d-ifo in the urine. These children, however, also displayed a higher excretion of unmetabolized R-ifo. Taking into account only the 12 completely sampled courses, 5/11 children showed this pattern (57% ± 5% S-3-d-ifo); the other 6 excreted more R-3-d-ifo (61% ± 4%; see Fig. 4).

Discussion

Previously published reports on the pharmacokinetics of ifo have shown a wide interindividual variability [21, 26, 29]. Despite the large variation in age, dose, scheduling, and accompanying therapy, we found an overall surprisingly uniform pattern of excretion of unmetabolized ifo and *N*-desalkylated compounds. The recovery of side-chain-oxidized metabolites was in the range previously described by other investigators. For the dechloroethylated forms, Lind et al. [21] reported 3.5% (day 1) to 7.5% (day 5), median recovery, with wide ranges (0.63%–26.63%). Norpoth et al. [27] recovered >50% of a given dose in the 2- and 3-desalkylated form. With a mean of 21%, unmetabolized ifo was the major component recovered in the urine. This amount was clearly higher than the values found by Lind and co-workers (0–6.37%) [21] but was in concordance with those reported by Allen et al. [2], who recovered 20%–50% of the drug in unchanged form. We found no evidence of any relationship between metabolic patterns in urinary excretion and age, sex, or the dose rate of ifo therapy.

There are few data on the stereospecificity of ifo in humans. Misiura et al. [23] published data on two patients and indicated substantial stereoselectivity, especially of side-chain oxidation, for this drug. Without exception, we demonstrated higher cumulative excretion of R-ifo (56%) than of S-ifo (44%). Higher excretion of R-ifo indicates a larger extent of S-ifo metabolism.

The predominating 2-d enantiomer, representing a mean of 64% of the amount of 2-d-ifo recovered, is a metabolite of S-ifo, which was metabolized with preference and excreted to a lesser extent. In Figure 3, the main 3-d enantiomer is R-3-d-ifo, a metabolite of S-ifo, which suggests that S-ifo is excreted in lower amounts because it undergoes stereoselective metabolism to R-3-d-ifo. This metabolic excretion pattern seems conclusive and could be demonstrated in 8 of 14 children; the other 6 children predominantly oxidized the cyclic (position 3) side chain of R-ifo, forming S-3-d-ifo. Nevertheless, they also excreted a higher amount of unmetabolized R-ifo (see Fig. 4). The predominant formation of R- and S-3-d-ifo, respectively, in different individuals is in agreement with the results found in the two patients reported by Misiura and co-workers [23], one of whom showed enhanced R-3-d-ifo excretion and the other, an excess of S-3-d-ifo.

In our patients, the R/S ratio for 3-d-ifo varied between 67%/33% and 36%/64% of the recovered 3-d-ifo. The specific individual pattern, however, was exactly reproduced in children AH, CT, and PB, who were analyzed for more than one therapy course (Table 2). Therefore, the children can be classified into one group that predominantly oxidizes S-ifo to R-3-d-ifo and another that preferentially metabolizes R-ifo to S-3-d-ifo. For each of these groups, the extent of stereospecificity is on the order of that seen in 2-d excretion (see Fig. 4). S-2-d-ifo was the predominant 2-d enantiomer seen in all children. Only the child KK showed equal excretion of R- and S-2-d-ifo (Fig. 3, Table 2). The R/S ratio for 3-d-ifo showed marked interindividual variation. This and the wide range observed in the ratio of 2d/3d formation corroborates that 2-d- and

3-d-ifo are products of different side-chain-oxidizing enzymes.

The sum of either of the enantiomers of the drug and the derived side-chain metabolites, respectively, reflects the amount of the applied optical antipode of ifo that is not activated by hydroxylation of the ring system. If the sum of excreted unmetabolized R-ifo and R-ifo-derived S-3-d- and R-2-d-ifo is compared with the sum of corresponding S formations, a slight difference becomes apparent in the children who predominantly exhibited R-ifo metabolism via side-chain oxidation at position 3. The renal excretion demonstrated an excess of R-ifo and derivatives (Fig. 5; IE, CT, JP, DR, MB). We propose that these children might display a slight preference for S-ifo at the activating cytochrome P_{450} system.

In the other children, the stereospecific side-chain oxidation pathway explains the difference in excretion of R- and S-ifo. Assuming that the unrecovered part of the dose was metabolized by ring oxidation, this is indirect evidence against the stereospecificity of this activating pathway in these children. Given that side-chain oxidation is related to toxic side effects [12, 20], some benefit might theoretically be expected from the application of only that enantiomer that undergoes less side-chain oxidation. However, the main side-chain metabolite was 3-d-ifo, which was preferentially formed from different optical antipodes. Theoretically, an advantage in the form of reduced side-chain metabolism could be expected from the use of the S form of ifo in nearly half of our patients and the R form in the other half (Fig. 4). The maximal theoretical benefit of increasing the activating ring oxidation would be only about 10% (Fig. 5).

In conclusion, we demonstrated moderate stereospecific metabolism of ifosfamide in children exhibiting different patterns of side-chain oxidation. The affinity of the oxidases to the optical isomers, however, is not highly specific at all. Therefore, stereospecific metabolism does not indicate that any clear-cut advantage can be gained from the application of an individual enantiomer.

References

1. Allen LM, Creaven PJ (1972) In vitro activation of isophosphamide (NSC-109724), a new oxazaphosphorine, by rat liver microsomes. Cancer Chemother Rep 56: 603–610
2. Allen LM, Creaven PJ, Nelson RL (1976) Studies on the human pharmacokinetics of ifosfamide (NCI-109724). Cancer Treat Rep 60: 451–458
3. Blaschke G, Koch U (1986) Untersuchungen zum stereoselektiven Metabolismus von Ifosfamid. Arch Pharmacol 319: 1052
4. Blaschke G, Widey W (1989) Metabolismus der Enantiomere des Zytostatikums Ifosfamid. Arzneimittelforschung 39(I): 223–226
5. Blaschke G, Hilgard P, Maibaum J, Niemeyer U, Pohl J (1986) Präparative Trennung der Ifosfamid-Enantiomere und ihre pharmakologisch-toxikologische Untersuchung. Arzneimittelforschung 36 (II): 1493–1495
6. Cerny T, Castiglione M, Brunner K, Küpfer A, Martinelli G, Lind M (1990) Ifosfamide by continuous infusion to prevent encephalopathy. Lancet I: 175
7. Connors TA, Cox PJ, Farmer PB, Foster AB, Jarman M (1973) Some studies of the active intermediates formed in the microsomal metabolism of cyclophosphamide and isophosphamide. Biochem Pharmacol 23: 115–129
8. Cox PJ, Farmer PB, Jarman M, Jones M, Stec WJ, Kinas R (1976) Observations on the differential metabolism and biological activity of the optical isomers of cyclophosphamide. Biochem Pharmacol 25: 993–996
9. Cox PJ, Farmer PB, Jarman M, Kinas RW, Stec WJ (1978) Stereoselectivity in the metabolism of the enantiomers of cyclophosphamide in mice, rats and rabbits. Drug Metab Dispos 6: 617–622
10. Delepine N, Taillard F, Desbois JG, Cornille H, Delepine G, Jasmin C (1986) CNS-side effects induced by ifosfamide-mesna in children with osteosarcomas. Biomed Pharmacother 40: 173–175
11. Farmer PB (1988) Enantiomers of cyclophosphamide and iphosphamide. Biochem Pharmacol 37: 145–148
12. Goren MP, Wright RK, Pratt CB, Pell FE (1986) Dechloroethylation of ifosfamide and neurotoxicity. Lancet II: 1219–1220
13. Goren MP, Wright RK, Horowitz ME, Pratt CB (1987) Ifosfamide induced subclinical tubular nephrotoxicity despite mesna. Cancer Treat Rep 71: 127–130
14. Goren MP, Pratt CB, Viar MJ (1989) Tubular nephrotoxicity during long-term ifosfamide and mesna therapy. Cancer Chemother Pharmacol 25: 70–72
15. Heim ME, Fiene R, Schick E, Wolpert E, Queißer W (1981) Central nervous side effects following ifosfamide monotherapy of advanced renal carcinoma. J Cancer Res Clin Oncol 100: 113–116
16. Hill DL, Russel Laster W, Kirk MC, El Dareer S, Struck RF (1973) Metabolism of iphosphamide [2-(2-chloroethylamino)-3-(2-chloroethyl)tetrahydro-2H-1,3-2-oxazaphosphorine-2-oxide] and production of a toxic iphosphamide metabolite. Cancer Res 33: 1016–1022
17. Jürgens H, Treuner J, Winkler K, Göbel U (1989) Ifosfamide in pediatric malignancies. Semin Oncol 16 [Suppl 3]: 46–50
18. Kellie SJ, Pritchard J, Bowman A, Kraker J de, Lilleyman JS (1987) Ifosfamide neurotoxicity in children. J Clin Oncol 5: 512–514
19. Kusnierczyk H, Radzikowski C, Paprocka M, Budzynski W, Rak J, Kinas R, Misiura K, Stec W (1986) Antitumor activity of optical isomers of cyclophosphamide, ifosfamide and trofosfamide as compared to clinically used racemates. J Immunopharmacol 8: 455–480
20. Lewis LD, Meanwell CA (1990) Ifosfamide pharmacokinetics and neurotoxicity. Lancet I: 175–196
21. Lind J, Roberts HL, Thatcher N, Idle JR (1990) The effect of route of administration and fractionation of dose on the metabolism of ifosfamide. Cancer Chemother Pharmacol 26: 105–111
22. Masurel D, Houghton PJ, Young CL, Wainer I (1990) Efficacy, toxicity, pharmacokinetics, and in vitro metabolism of the enantiomers of ifosfamide in mice. Cancer Res 50: 252–255
23. Misiura K, Okruszek A, Pankiewicz K, Stec WJ, Czownicki Z, Utracka B (1983) Stereospecific synthesis of chiral metabolites of ifosfamide and their determination in the urine. J Med Chem 26: 674–679
24. Moncrieff M, Foot A (1989) Fanconi syndrome after ifosfamide. Cancer Chemother Pharmacol 23: 121–122
25. Newbury-Ecob RA, Noble VW, Barbor PRH (1989) Ifosfamide-induced Fanconi syndrome. Lancet I: 1328
26. Ninane J, Baurain R, Kraker J de, Ferster A, Trouet A, Cornu G (1989) Alkylating activity in serum, urine and CSF following high-dose ifosfamide in children. Cancer Chemother Pharmacol 24 [Suppl]: 82–86
27. Norpoth K, Addicks HW, Witting U, Müller G, Raidt H (1975) Quantitative Bestimmung von Cyclophosphamid, Ifosfamid und Trofosfamid sowie ihrer stabilen Metabolite auf der DC-Platte mit 4-Pyridinaldehyd-2-benzothiazolylhydrazon (PHB). Arzneimittelforschung 25: 1331–1336
28. Patterson WP, Khojaseh A (1989) Ifosfamide-induced renal tubular defects. Cancer 63: 649–651
29. Pearcey R, Calvert R, Mehta A (1988) Disposition of ifosfamide in patients receiving ifosfamide infusion therapy for the treatment of cervical carcinoma. Cancer Chemother Pharmacol 22: 353–355
30. Pinkerton CA, Philip T, Brunat Mentigney M (1985) Ifosfamide, mesna, and encephalopathy. Lancet I: 1399
31. Pratt CB, Green AA, Horowitz ME, Meyer WH, Etcubanas E, Douglass E, Hayes FA, Thompson E, Wilimas J, Igarashi M, Kovnar E (1986) Central nervous system toxicity following the treatment of

pediatric patients with ifosfamide/mesna. J Clin Oncol 4: 1253–1261
32. Salloum E, Flamant F, Goshn M, Taleb N, Akatchereian C (1987) Irreversible encephalopathy with ifosfamide/mesna. J Clin Oncol 5: 1303–1304
33. Skinner R, Pearson ADJ, Price L, Coulthard MG, Craft AW (1989) Nephrotoxicity of ifosfamide in children. Lancet II: 159
34. Skinner R, Pearson ADJ, Price L, Cunningham K, Craft AW (1989) Hypophosphataemic rickets after ifosfamide treatment in children. BMJ 298: 1560–1561
35. Smeitink J, Verreussel M, Schröder C, Lippens R (1988) Nephrotoxicity associated with ifosfamide. Eur J Pediatr 148: 164–166
36. Watkin SW, Husband DJ, Green JA, Warenius HM (1989) Ifosfamide encephalopathy: a reappraisal. Eur J Cancer Clin Oncol 1303–1310