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ORIGINAL ARTICLE

Penetration of ifosfamide and its active metabolite 4-OH-ifosfamide into cerebrospinal fluid of patients with CNS malignancies

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Abstract
Purpose The aim of this study was to examine the penetration of ifosfamide (IFO) and 4-hydroxy-ifosfamide (4-OH-IFO) into the CSF of human adults and to evaluate the influence of blood–CSF barrier (BCB) function.
Methods In 12 adult patients with a malignant CNS disease treated with IFO 1,300–2,000 mg/m^2/d as a 3-hour intravenous infusion, 17 CSF samples were collected within 10 min after the end of IFO infusion. In 8 of these patients, the CSF was obtained in up to 5 sequential 2-ml portions to detect a potential caudocranial concentration gradient. Additionally, blood was collected before treatment and immediately following IFO infusion.
Results IFO was detected in all 17 CSF samples at a median concentration of 79.24 μmol/l (39.27–176.73) and a median CSF/plasma ratio of 0.38 (0.18–0.72). 4-OH-IFO was detected in 11 CSF samples from 7 patients at a median concentration of 4.1 μmol/l (2.44–36.03) and a median CSF/plasma ratio of 3.07 (0.62–29.12). 4-OH-IFO was undetectable in 6 CSF samples from 5 patients and in one plasma sample. Both CSF drug concentrations and their CSF/plasma quotients neither correlated with steroid comedication nor with albumin quotients (QAlb).
Conclusions Both IFO and 4-OH-IFO can penetrate into the CSF of human adults without a correlation to CSF turnover. In contrast to IFO, 4-OH-IFO CSF penetration is not reliable with levels ranging between undetectable and exceeding those in the corresponding plasma.

Keywords Ifosfamide · 4-OH-ifosfamide · Cerebrospinal fluid · Blood–CSF barrier · CNS malignancies · CSF penetration

Introduction

The inability of cytotoxic agents to enter the CNS protected by the intact blood–brain barrier (BBB) and blood–CSF barrier (BCB) represents a major limitation on the treatment of CNS malignancies. Only a few cytotoxic agents are able to cross the barriers and enter the CNS. Ifosfamide (IFO), an alkylating oxazaphosphorine derivative, is active against a variety of malignant diseases [1, 2]. It acts as a prodrug requiring hydroxylation at the cyclic carbon-4 position by hepatic cytochrome *P*-450 isoenzymes, mainly CYP3A4 resulting in the formation of a moderately active metabolite, 4-hydroxy-ifosfamide (4-OH-IFO) [3, 4]. 4-OH-IFO itself is unstable, and its tautomer aldophosphamide spontaneously decomposes to the highly active isophosphoramide mustard and acrolein [5].

IFO is assumed to penetrate through the BBB/BCB due to its lipid solubility, small molecular size, and minimal binding to plasma and tissue proteins. IFO metabolites are less likely to enter the CNS because of their greater polarity [6]. Moreover, IFO and its metabolites have been described not to be

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substrates of p-glycoprotein (PGP) and multiple resistance protein 1 (MRP1) [7]—both transport molecules provide an important mechanism within the BBB for drug efflux.

Data on pharmacokinetic investigation of IFO and its metabolites in the CNS are limited. This is the first study to examine the penetration of IFO and its active metabolite 4-OH-IFO into the CSF of human adults. Since a free exchange between the brain interstitial fluid and CSF is assumed, CSF drug levels are likely to reflect the corresponding concentration in the brain.

Patients and methods

Patients and treatment

The study was approved by the institutional ethics committee, and informed consent was obtained from all patients before study entry. Twelve patients (six men and six women), median age 63.5, treated with HDMTX 4,000 mg/m^2 over 4 h on day 1 and IFO 1,300–2,000 mg/m^2 over 3 h intravenously on days 3–5 were included. Nine patients had intracerebral lymphoma (six primary and three secondary CNS lymphoma) with meningeal involvement on cytomorphologic CSF examination proven and suspected in one patient each. Three patients had metastatic breast cancer, all with malignant cells in the CSF (Table 1). All patients received intensive intravenous hydration, mesna (20% of the IFO dose before the start of infusion and 4 + 8 h thereafter) and leucovorin rescue. None of the patients experienced treatment-related neurotoxicity.

CSF and blood sampling

Seventeen CSF samples were obtained by lumbar puncture (LP) within 10 min after the end of IFO infusion. In eight patients, 4–5 sequential 2-ml CSF portions were collected without interruption during the same LP to assess the influence of BCB functions on CSF drug concentrations of IFO and 4-OH-IFO. Ten CSF samples were obtained without concomitant steroid treatment, and seven samples on dexamethasone (median 12 mg/d) (Table 1).

CSF samples were evaluated within 30 min of collection for cell count, cytomorphology, albumin and immunoglobulin G (IgG) concentrations (with a simultaneous serum sample) using a Behring nephelometer analyzer (Dade Behring, Marburg, Germany), and intrathecal IgG synthesis using isoelectric focusing on MacroPAG with silver staining. The albumin quotient (QAlb) of the sequential CSF portions was used as parameter for lumbar CSF turnover [8], and the IgG Index (QIgG/QAlb) as parameter for the permeability of the BCB [9].

Eleven plasma samples from 10 patients were obtained before IFO infusion, and sixteen from 11 patients immediately after IFO infusion.

All samples for 4-OH-IFO detection were immediately ('bedside') spiked with trichloroacetate (10% dilution) for acidification, shaken vigorously to avoid rapid decay of the 4-OH metabolites, and frozen at −80°C until analysis as has previously been validated for plasma [10]. CSF samples for IFO detection were immediately frozen at −80°C, plasma was frozen after centrifugation. All samples were stored at −80°C for up to 3 months. IFO concentrations

Table 1 Patient/sample characteristics

No.	Age (years)/sex	Diagnosis	IFO dosage (mg/m^2)	Sampling time: cycle (day)	Sequential 2-ml CSF portions	Dexamethasone
1	59/m	PCNSL	2,000	5 (1)	1	None
2	80/m	SCNSL	2,000	4 (3)	1	None
3a	76/m	SCNSL	2,000	1 (1)	1	3 × 4 mg
3b			2,000	3 (1)	4	None
4a	63/m	SCNSL	1,500	1 (1)	1	3 × 8 mg
4b			2,000	3 (1)	4	None
5a	64/f	PCNSL	1,300	1 (1)	1	3 × 2 mg
5b			1,500	2 (1)	4	None
6a	62/f	BC	2,000	1 (1)	4	3 × 2 mg
6b			1,500	8 (1)	1	None
7a	66/f	BC	1,500	1 (3)	4	3 × 8 mg
7b			2,000	7 (2)	1	None
8	60/f	BC	1,500	1 (2)	1	3 × 2 mg
9	76/f	PCNSL	2,000	2 (3)	1	3 × 4 mg
10	42/f	PCNSL	2,000	6 (3)	5	None
11	64/m	PCNSL	2,000	2 (3)	5	None
12	52/m	PCNSL	2,000	4 (1)	5	None

IFO ifosfamide, *CSF* cerebrospinal fluid, *m* men, *f* women, *PCNSL* primary central nervous system lymphoma, *SCNSL* secondary central nervous system lymphoma, *BC* breast cancer

were analyzed by gas chromatography, and 4-OH-IFO by high-performance liquid chromatography as previously described [11, 12]. CSF/plasma quotients for IFO concentration were determined for 16 sample sets, including the sequential CSF portions.

Statistics

Descriptive statistics were used for data presentation. Mean values of independent groups were compared with Student's *t* test, variances were compared with the Levene-test. For all non-parametric correlations, Spearman's rank correlation coefficient was calculated. The level of significance was 0.05 (two-sided).

Results

IFO and 4-OH-IFO plasma concentrations

Both drugs were not detectable in pre-treatment plasma on day 1 before IFO infusions. IFO was detected in all five samples obtained before the infusion on day 2 or 3 at a median concentration of 25 µmol/l (9.27–95.74), whereas 4-OH-IFO was only detectable in one of five samples (1.72 µmol/l) before IFO infusion on day 3. In all 16 post-infusion samples, IFO was detected at a median concentration of 223.76 µmol/l (154.25–290.99), whereas 4-OH-IFO was detected in 15/16 samples at a median concentration of 2.2 µmol/l (0.17–7.41) (Table 2).

No significant difference was found for mean IFO or 4-OH-IFO plasma concentrations or their variances with respect to IFO dose (2,000 mg/m^2 vs. ≤1,500 mg/m^2) and concomitant dexamethasone therapy.

IFO and 4-OH-IFO CSF concentrations and CSF/plasma quotients

IFO was detected in all samples at a median concentration of 79.24 µmol/l (39.27–176.73); 4-OH-IFO in 11/17 samples at a median concentration of 4.1 µmol/l (2.44–36.03), whereas no 4-OH-IFO was detectable in 6/17 samples despite considerable IFO concentrations and 4-OH-IFO detection in the corresponding plasma samples (Table 2). Median CSF/plasma quotient for IFO was 0.38 (0.18–0.72) and for 4-OH-IFO (calculated for the 9 samples with 4-OH-IFO detection) was 3.07 (0.62–29.12).

Mean IFO concentration in samples obtained after infusion of 2,000 mg/m^2 was 98.05 ± 15.1, after infusions of ≤1,500 mg/m^2 74.99 ± 4.92 µmol/l; this difference was not significant ($P = 0.29$) (Fig. 1a). The concentration variance was significantly greater in the 2,000 mg/m^2 dose group ($P = 0.018$). No significant differences were found for CSF 4-OH-IFO concentrations and both IFO and 4-OH-IFO CSF/plasma quotients with respect to IFO dose (Fig. 1b) and the use of corticosteroids.

Table 2 Individual sample data

No.	$c[IFO]_{plasma}$ (µM)	$c[4\text{-OH-IFO}]_{plasma}$ (µM)	$c[IFO]_{CSF}$ (µM)	$c[4\text{-OH-IFO}]_{CSF}$ (µM)	$c[IFO]_{CSF}/c[IFO]_{plasma}$	$c[4\text{-OH-IFO}]_{CSF}/c[4\text{-OH-IFO}]_{plasma}$	CSF cell count (/µl)	Albumin quotient	IgG index
1	167.67	1.30	42.44	3.99	0.25	3.07	ND	14.6	0.55
2	ND	ND	90.77	3.04	NE	NE	ND	ND	ND
3a	221.24	4.69	39.27	9.24	0.18	1.97	25	27.8	0.57
3b	261.87	1.62	104.05	5.80	0.40	3.58	9	26.1	0.51
4a	198.83	1.28	71.21	4.09	0.36	3.20	5	39.5	0.47
4b	285.91	1.09	62.31	2.79	0.22	2.56	2	44.3	0.42
5a	164.33	n.d.	79.24	5.27	0.48	NE	30	8.3	0.56
5b	216.89	2.61	94.14	4.00	0.43	1.53	10	5.8	0.49
6a	252.08	2.15	179.10	34.49	0.71	16.04	44	31.4	0.46
6b	200.06	2.17	58.75	n.d.	0.29	NE	1	46.7	0.5
7a	154.25	5.88	69.10	4.46	0.45	0.76	2	13.2	0.73
7b	218.77	0.17	156.80	4.95	0.72	29.12	9	706	0.68
8	226.27	2.47	85.63	n.d.	0.38	NE	ND	ND	ND
9	290.99	7.41	68.51	n.d.	0.24	NE	4	6.8	0.57
10	265.81	6.19	140.68	n.d.	0.53	NE	0	6.6	0.51
11	255.39	2.20	158.93	n.d.	0.62	NE	1	10.1	0.46
12	257.03	3.19	86.77	n.d.	0.34	NE	3	6.5	0.46

c concentration, *IFO* ifosfamide, *4-OH-IFO* 4-hydroxy-ifosfamide, *CSF* cerebrospinal fluid, *IgG* immunoglobulin G, *NE* not evaluable, *ND* not done, *n.d.* not detectable

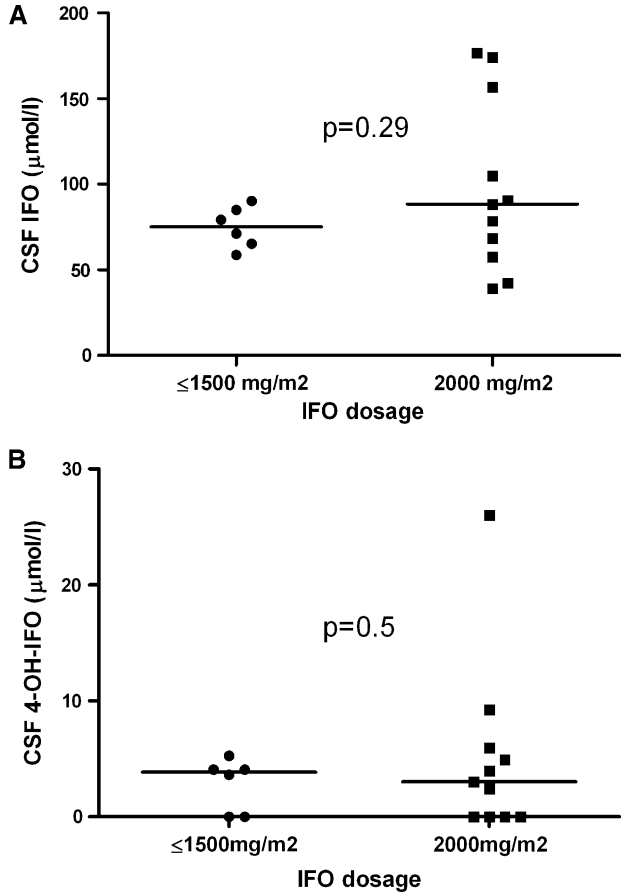

Fig. 1 IFO and 4-OH-IFO CSF concentrations according to IFO dosage. CSF concentrations of IFO (**a**) and 4-OH-IFO (**b**) according to dosage of prior IFO infusion in 17 samples of 12 patients. Median concentrations are indicated by *horizontal lines*. There was no significant difference between IFO or 4-OH-IFO CSF concentrations between the two dose groups (*t* test, $P = 0.29$ and 0.5), however, the variance of IFO concentrations was higher in the 2,000 mg/m^2 group compared with ≤1,500 mg/m^2 (Levene-test, $P = 0.018$)

QAlb was pathologic (>8) in 10/15 evaluated CSF, suggesting a slowed lumbar CSF turnover. Neither drug concentrations nor their CSF/plasma quotients correlated with QAlb.

Sequential CSF sampling

In all sequential CSF portions, albumin concentration showed a decreasing caudocranial gradient, indicating a caudally slowing CSF turnover (Table 3). IFO CSF concentration increased in the cranial direction in 6 patients, decreased in one (No. 11), and remained stable in one patient (6a). 4-OH-IFO was detectable in 5 of these patients, with increasing concentration in 2 patients (4b and 7a) and decreasing in three. Five patients with sequential sampling had no intrathecal IgG synthesis and were thus eligible for the evaluation of a potential influence of a disturbed BCB permeability and CSF turnover on the CSF drug concentrations. In 3 of them IFO concentration was increasing in the caudocranial direction (3b, 5b, and 12), and in two, it was decreasing or stable (6a and 11). 4-OH-IFO was detectable in 3 of these patients (3b, 5b, and 6a) with decreasing craniocaudal concentration (Fig. 2).

Table 3 Measurements of sequential CSF portions collected in caudocranial sequence

Sample set	QAlb	CSF IFO	CSF 4-OH-IFO	IgG index
3b*	26.7	88.47	5.96	0.51
	27.3	103.47	5.94	0.5
	26.1	124.14	5.67	0.51
	24.4	100.11	5.62	0.53
4b	46	57.57	2.44	0.4
	44	61.3	2.76	0.42
	44.5	62.78	2.95	0.4
	42.7	67.58	2.99	0.44
5b*	6.1	85.06	4.1	0.47
	5.8	98.28	4.04	0.5
	5.6	100.61	3.96	0.5
	5.6	92.59	3.88	0.5
6a*	35.8	176.73	36.03	**0.41**
	32	179.29	35.98	**0.47**
	29.7	180.94	33.1	**0.48**
	28.2	179.38	32.86	**0.48**
7a	16.1	65.34	3.63	0.78
	14.4	65.82	4.95	0.74
	11.7	71.08	4.84	0.76
	10.4	74.15	4.41	0.65
10	8.6	105.12	Not detectable	0.51
	6.5	118		0.48
	6.1	121.69		0.5
	6	156.79		0.51
	5.6	201.79		0.55
11*	10.3	174.27	Not detectable	**0.5**
	10.1	166.33		**0.46**
	10.3	152.95		**0.43**
	10.3	151.8		**0.44**
	9.4	149.29		**0.47**
12*	6.6	78.57	Not detectable	0.47
	6.5	78.25		0.47
	6.4	95.58		0.45
	6.4	86.86		0.48
	6.4	94.58		0.45

QAlb albumin quotient, *CSF* cerebrospinal fluid, *IFO* ifosfamide, *4-OH-IFO* 4-hydroxy-ifosfamide, *IgG Index* immunoglobulin G index

* Samples without intrathecal immunoglobulin G (IgG) synthesis. In sample sets 6a and 11. IgG Index changed by 16–17% (*bold*) suggesting a disturbed blood–CSF barrier permeability for IgG/albumin

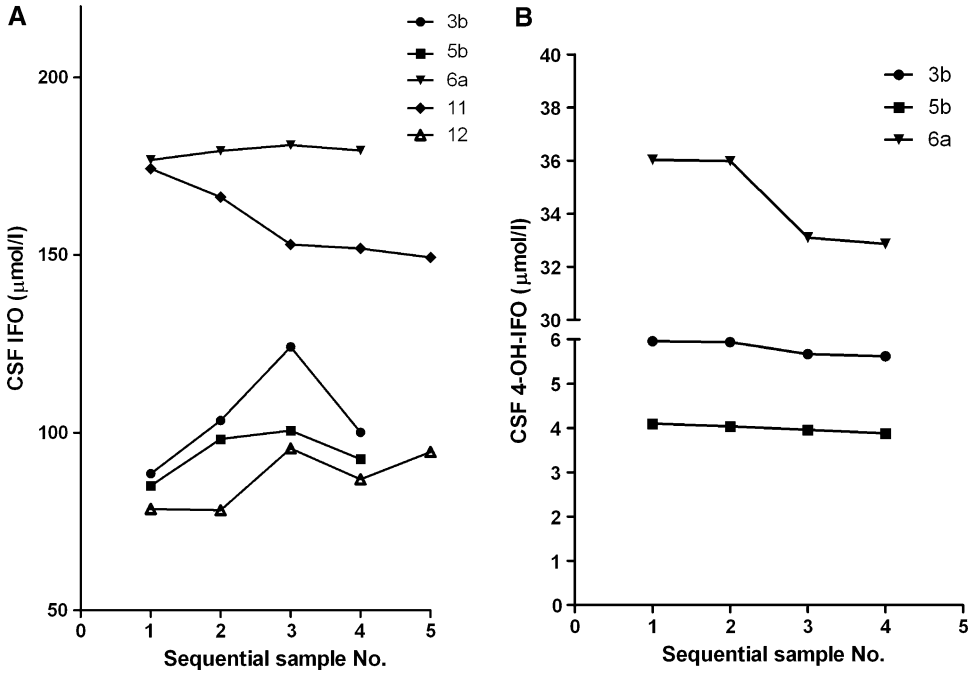

Fig. 2 IFO and 4-OH-IFO CSF concentration in sequential 2-ml portions collected without interruption in 5 patients without intrathecal IgG synthesis. IFO concentrations (**a**) increased in the caudocranial sequence in 3 patients with an intact BCB permeability and decreased or remained unchanged in 2 patients with a disturbed BCB permeability for IgG/albumin. 4-OH-IFO was detected in 3 of these patients (**b**) with decreasing concentrations irrespective of the BCB IgG/albumin permeability

In 2 patients without intrathecal IgG synthesis (No. 6a and 11), the IgG Index changed by 16–17%, suggesting a disturbed BCB permeability for IgG/albumin. In these patients, IFO concentrations were 174.3 and 176.7 µmol/l, when compared to 78.6–88.5 µmol/l in the 3 patients with a stable IgG Index; this difference was significant (*t* test, $P = 0.006$). 4-OH-IFO was detectable in only one of these patients (6a), however, with a high CSF/serum ratio of 16.8.

In the 3 patients with a stable IgG Index, CSF IFO concentrations increased in the caudocranial direction. 4-OH-IFO was detectable in one of these patients with an increasing caudocranial gradient.

Discussion

With a median CSF/plasma quotient for IFO of 0.38, our findings are in line with the results obtained by others. Creaven et al. [13] found CSF IFO at levels of 23–49% of the corresponding plasma levels 2–3 h following 45-min infusions of 3.8–5 g/m^2 IFO in three patients. CSF/plasma ratios of median 1.2 were determined by Yule et al. [14] in four children receiving a 72-h continuous infusion of 9 g/m^2 IFO. Another small study in children determined CSF/plasma ratios for IFO of 0.5–1.7 [15]. Similar to the results published by Yule and colleagues, we found a fourfold interpatient variability of CSF/plasma ratios. This may explain differences in effectivity and toxicity among patients.

The failure to detect 4-OH-IFO in 35% of CSF sample sets in our study remains without sufficient explanation. 4-OH-IFO is extremely instable in blood necessitating an immediate ("bedside") spiking with trichloroacetate and deep-freezing to obtain meaningful results. However, 4-OH-IFO has a greater stability in low-protein fluids with an in vitro half-life without trichloroacetate in full blood of 27 min but of 220 min in CSF (data not shown). Thus, measurement or sampling errors seem unlikely in our patients with undetectable CSF 4-OH-IFO because the plasma 4-OH-IFO concentrations were within the expected range. Interestingly, in samples with 4-OH-IFO detection, a high median CSF/plasma ratio of 3.07 (with a higher interpatient variability when compared to IFO) was found. An independent IFO metabolism within the CSF seems unlikely because activation by mixed function oxidases (mainly CYP3A4) is known to occur only in the liver. The half-life of 4-OH-IFO within the CSF compartment is longer, presumably due to increased sulfhydryl-binding and consecutively a greater instability in the blood. Additionally, a "trapping" of more polar metabolites like isophosphoramide mustard within the CSF may contribute to this phenomenon.

The drug concentration in the CSF is affected by the permeability of the BCB and the CSF turnover (bulk flow). Both can be altered in malignant leptomeningeal disease. Sequential CSF portions allow in the absence of intrathecal IgG synthesis to use intraindividual changes in the IgG index as parameter for a disturbed BCB permeability for IgG/albumin and possibly for other substances and to use intraindividual changes in the QAlb as parameter of a changing CSF turnover if the BCB permeability is stable. The higher CSF IFO concentrations in 2 patients with IgG

Index changes >10% when compared to 3 patients with a stable IgG Index suggest that a disturbed BCB permeability for IgG/albumin may also represent an increased BCB permeability for IFO. The caudocranial increase in the CSF IFO concentrations despite decreasing QAlb values in the latter 3 patients furthermore allows to assume that IFO should have entered the upper spinal and/or cranial CSF spaces more readily than the lumbar space, e.g., by an increased BCB permeability or by active transport, and that the caudally slowing CSF turnover may have little or no influence on the CSF concentration of IFO. 4-OH-IFO was detectable in one of these patients with a caudocranial concentration decrease possibly suggesting an influence of CSF turnover. The validity of the assumptions, however, is limited by the very small number of patients.

The conflicting data concerning penetration of IFO metabolites into the CSF correspond to findings by other investigators. Yule et al. [14] detected another active IFO metabolite, isophosphoramide mustard, in 8 of 9 samples with a median CSF/plasma ratio of 3.2 during 72-h continuous IFO infusion. In another study, assessing alkylating activity as a measure of drug activity in the CSF and plasma of four children after bolus infusion of 3 g/m^2, a mean CSF/plasma ratio of 0.53 was found [16]. Kaijser et al. [15] found CSF 4-OH-IFO concentrations slightly lower than in the corresponding plasma in three samples and were unable to detect 4-OH-IFO in either plasma or CSF in one sample set from two children after a 24-h continuous IFO infusion. Contrary to these findings, Creaven et al. [13] found negligible amounts of alkylating activity in the CSF after a 45-min IFO infusion. In rhesus monkeys receiving 1 g/m^2 IFO as 30-min infusion, a 4-OH-IFO peak concentration of 2 μM was found corresponding to a CSF/plasma ratio for 4OH-IFO of only 0.13 [17]. However, continuous sampling from an Ommaya reservoir for up to 240 min following the start of the infusion showed AUCs of 4-OH-IFO to be 400 μM*min, equaling those AUCs of 4-OH-cyclophosphamide known to be cytotoxic in vitro in cell lines of lymphoblastic leukemia, rhabdomyosarcoma, and breast cancer [18]. In vitro IC_{50} for 4-OH-IFO has previously determined to be 10.8 μM in breast cancer cell line MX1 after 4-h incubation with additional cytotoxicity observed after longer incubation [19]. Thus, it seems likely that the 4-OH-IFO levels measured in our study were cytotoxic.

Dexamethasone is known to reduce the permeability of normal cerebral blood vessels [20, 21]. In the study of Yule et al. [14], the only patient with no detectable isophosphoramide mustard in CSF was on dexamethasone. In our sample series, however, we did not find significant differences in the concentration of either IFO or 4-OH-IFO in the CSF with respect to the concomitant use of corticosteroids.

In summary, our results indicate that both IFO and 4-OH-IFO can penetrate into the CSF, however, in contrast to IFO, the penetration of 4-OH-IFO is not reliable with levels ranging between undetectable and high (exceeding the corresponding plasma level). This can contribute to inter-patient differences in treatment response. Both CSF IFO and 4-OH-IFO seem to be independent from the local CSF turnover, but both substances might be influenced by disturbed BCB permeability. These results should be verified in a larger series before definite conclusions concerning 4-OH-IFO penetration into the CNS and the utility of IFO for treatment of CNS malignancies can be drawn.

Conflict of interest statement None.

References

1. Rodeghiero F, Elice F (2003) Ifosfamide in hematological malignancies of adults. Oncology 65(Suppl 2):85–93
2. Sneller V, Armitage J (2003) Current role and future perspectives for ifosfamide in the treatment of malignant non-Hodgkin's lymphoma—results from an expert meeting. Ann Oncol 14(Suppl 1):i1–i3
3. Tascilar M, Loos WJ, Seynaeve C, Verweij J, Sleijfer S (2007) The pharmacologic basis of ifosfamide use in adult patients with advanced soft tissue sarcomas. Oncologist 12:1351–1360
4. Brüggemann SK, Radike K, Braasch K et al (2006) Chloroacetaldehyde: mode of antitumor action of the ifosfamide metabolite. Cancer Chemother Pharmacol 57:349–356
5. Zhang J, Tian Q, Yung Chan S et al (2005) Metabolism and transport of oxazaphosphorines and the clinical implications. Drug Metab Rev 37:611–703
6. Lokiec F (2006) Ifosfamide: pharmacokinetic properties for central nervous system metastasis prevention. Ann Oncol 17(Suppl 4): iv33–iv36
7. Andersson BS, Mroue M, Britten RA, Farquhar D, Murray D (1995) Mechanisms of cyclophosphamide resistance in a human myeloid leukemia cell line. Acta Oncol 34:247–251
8. Reiber H (1994) Flow rate of cerebrospinal fluid (CSF)—a concept common to normal blood-CSF barrier function and to dysfunction in neurological diseases. J Neurol Sci 122:189–203
9. Seyfert S, Quill S, Faulstich A (2009) Variation of barrier permeability for albumin and immunoglobulin G influx into CSF. Clin Chem Lab Med 47:955–958
10. Kurowski V, Wagner T (1993) Comparative pharmacokinetics of ifosfamide, 4-hydroxyifosfamide, chloroacetaldehyde, and 2- and 3-dechloroethylifosfamide in patients on fractionated intravenous ifosfamide therapy. Cancer Chemother Pharmacol 33:36–42
11. Brinker A, Kisro J, Letsch C, Brüggemann SK, Wagner T (2002) New insights into the clinical pharmacokinetics of trofosfamide. Int J Clin Pharmacol Ther 40:376–381
12. Kaijser GP, Ter Riet PG, de Kraker J et al (1997) Determination of 4-hydroxyifosfamide in biological matrices by high-performance liquid chromatography. J Pharm Biomed Anal 15:773–781
13. Creaven PJ, Allen LM, Alford DA, Cohen MH (1974) Clinical pharmacology of isophosphamide. Clin Pharmacol Ther 16:77–86
14. Yule SM, Price L, Pearson AD, Boddy AV (1997) Cyclophosphamide and ifosfamide metabolites in the cerebrospinal fluid of children. Clin Cancer Res 3:1985–1992
15. Kaijser GP, De Kraker J, Bult A, Underberg WJ, Beijnen JH (1998) Pharmacokinetics of ifosfamide and some metabolites in children. Anticancer Res 18:1941–1949
16. Ninane J, Baurain R, de Kraker J, 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 1):S2–S6
17. Arndt CA, Balis FM, McCully CL, Colvin OM, Poplack DG (1988) Cerebrospinal fluid penetration of active metabolites of cyclophosphamide and ifosfamide in rhesus monkeys. Cancer Res 48:2113–2115
18. Arndt CA, Colvin OM, Balis FM, Lester CM, Johnson G, Poplack DG (1987) Intrathecal administration of 4-hydroperoxycyclophosphamide in rhesus monkeys. Cancer Res 47:5932–5934
19. Brüggemann SK, Kisro J, Wagner T (1997) Ifosfamide cytotoxicity on human tumor, renal cells: role of chloroacetaldehyde in comparison to 4-hydroxyifosfamide. Cancer Res 57:2676–2680
20. Hedley-Whyte ET, Hsu DW (1986) Effect of dexamethasone on blood-brain barrier in the normal mouse. Ann Neurol 19:373–377
21. Narang VS, Fraga C, Kumar N et al (2008) Dexamethasone increases expression and activity of multidrug resistance transporters at the rat blood-brain barrier. Am J Physiol Cell Physiol 295: C440–C450