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Comparative pharmacokinetics of ifosfamide, 4-hydroxyifosfamide, chloroacetaldehyde, and 2- and 3-dechloroethylifosfamide in patients on fractionated intravenous ifosfamide therapy

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Abstract. The initial metabolism of the oxazaphosphorine cytostatic ifosfamide (IF) consists of two different pathways: ring oxidation at carbon-4 forms the cytostatically active metabolite 4-hydroxyifosfamide (4-OH-IF, "activated ifosfamide"), whereas side-chain oxidation with liberation of the presumably neurotoxic compound chloroacetaldehyde (CAA) that may also be responsible for IF-associated nephrotoxicity results in the formation of the cytostatically inactive metabolites 2-dechloroethylifosfamide (2-DCE-IF) and 3-dechloroethylifosfamide (3-DCE-IF). The pharmacokinetics of IF and its metabolites were investigated in 11 patients with bronchogenic carcinoma receiving IF on a 5-day divided-dose schedule (1.5 g/m^2 daily). Blood samples were drawn on days 1 and 5 for up to 24 h after the start of the IF infusion. IF, 2-DCE-IF, and 3-DCE-IF were simultaneously quantified by gas chromatography (GC) with an NIP flame-ionization detector (NPFID), CAA was determined by GC with an electron-capture detector (ECD), and the highly unstable compound 4-OH-IF was measured using a high-performance liquid chromatography (HPLC) assay with fluorometric detection of 7-OH-quinoline, which is formed by the condensation of 4-OH-IF-derived acrolein with *m*-aminophenol. As compared with the values obtained on day 1, on day 5 the terminal half-life and AUC values determined for IF were reduced by 30% (6.36 vs 4.06 h and 1781 vs 1204 nmol h ml^{-1}, respectively), whereas the maximal concentration (C_{max}) values were not affected significantly (199.1 vs 181.1 nmol ml^{-1}). This known phenomenon is explained by autoinduction of hepatic IF metabolism and was paralleled by increased metabolite levels. The mean C_{max} values determined for 4-OH-IF, CAA, 3-DCE-IF, and 2-DCE-IF (on day 1/on day 5) were 1.51/2.59, 2.69/4.85, 12.9/26.5, and 8.6/16.7 nmol ml^{-1}, respectively. The corresponding AUC values were 11.3/16.5, 30.3/34.3, 146/354, and 111/209 nmol h ml^{-1}, respectively. As calculated by intraindividual comparison, the mean C_{max} (day 5):C_{max} (day 1) ratios for 4-OH-IF, CAA, 3-DCE-IF, and 2-DCE-IF were 1.94*, 2.05*, 2.52*, and 2.33*, respectively; the corresponding AUC (day 5):AUC (day 1) ratios were 1.51*, 1.29, 2.34*, and 2.23*, respectively (* $P < 0.05$). These data reveal that during fractionated-dose IF therapy the cancerotoxic effect of the drug increases. If the assumed role of CAA in IF-associated neurotoxicity and nephrotoxicity is a dose-dependent phenomenon, the probability of developing these side effects would also increase during prolonged IF application.

Introduction

The oxazaphosphorine derivative ifosfamide (IF), a structural analogue of the well-established alkylating agent cyclophosphamide (CP), is of proven value in the treatment of a variety of malignancies [3]. Like CP, IF is a prodrug that requires activation by the hepatic microsomal mixed-function oxygenase system before it can exert its cancerotoxic effects [21]. The initial step of IF activation (ring oxidation at carbon-4) results in the formation of 4-hydroxyifosfamide (4-OH-IF, "activated" IF) [4]. In contrast to CP, for IF the competitive initial metabolic pathway of side-chain oxidation at the cyclic (position 3) and the extracyclic (position 2) nitrogen with liberation of the presumably neurotoxic metabolite chloroacetaldehyde (CAA) [6, 10] that may also be responsible for the nephrotoxicity observed after IF (but not after CP) treatment [19] and the formation of the cytostatically inactive metabolites 3-dechloroethylifosfamide (3-DCE-IF) and 2-dechloroethylifosfamide (2-DCE-IF) has been described [16, 21]. As clinical and experimental studies have shown that fractionated doses of IF have a better therapeutic index than a

Abbreviations: IF, ifosfamide; CAA, chloroacetaldehyde; CP, cyclophosphamide; 4-OH-IF, 4-hydroxyifosfamide ("activated ifosfamide"); 2-DCE-IF, 2-dechloroethylifosfamide; 3-DCE-IF, 3-dechloroethylifosfamide

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single bolus injection [7, 14], the drug is usually given on divided-dose schedules over a period of several days [3]. It has repeatedly been shown that fractionation of the IF dose over several days produces a time-dependent decrease in the IF half-life [8, 11, 12, 15, 17, 23]. This phenomenon has been explained by autoinduction of hepatic IF metabolism and has been shown to be paralleled by an increase in urinary excretion of IF metabolites [12, 13] and by higher AUCs of plasma alkylating activity [11, 12]. Due to methological problems (instability, low concentrations), very limited information exists about the pharmacokinetics of the IF metabolites.

As we have now established improved analytical methods for the determination of 4-OH-IF and CAA as well as an assay that allows for simultaneous quantification of IF and its dechloroethyl metabolites, the present study was designed to investigate the pharmacokinetics of IF and its metabolites and the changes occurring during a 5-day divided-dose schedule of IF.

Patients and methods

Patients and study design. A group of 11 patients (8 men and 3 women aged 43–71 years; median, 59 years) with bronchogenic carcinoma (9, small-cell; 2, non-small-cell) were included in the study after having given their written consent and after approval of the study design by the local ethics committee was available. The patients did not have severe hepatic or renal dysfunction as measured by standard biochemical parameters prior to the commencement of therapy (creatinine, <150 µmol/l; bilirubin, <20 µmol/l; serum cholinesterase, >2.5 kU/l; GOT/GPT, <40 U/l), and the Karnofsky performance status of all patients was >70%. None of the patients had received cytostatics within 4 weeks prior to their inclusion in the study. The chemotherapeutic regimen consisted of a combination of IF and etoposide that has been shown to be effective in small-cell and non-small-cell lung cancer [5, 26]. IF was applied i. v. as a short infusion (60 min) on days 1–5 at a daily dose of 1.5 g/m^2. Etoposide was also given as a short infusion (30 min) on days 2–4, the daily dose being 120 mg/m^2. Mercaptoethanesulfonic acid (mesna) was given i. v. at 0, 4, and 8 h after the start of the IF infusion, the single dose being 20% of the daily IF dose. During the entire 5-day study interval, all patients received both standard antiemetic prophylaxis with metoclopramide (10 mg/h) and a continuous infusion of Ringer's solution (125 ml/h) to guarantee sufficient diuresis.

Blood samples were drawn at 0, 0.5, 1, 1.5, 2, 4, 6, 9, and 24 h following the start of the IF infusion. For the assay of IF and its dechloroethyl metabolites (3-DCE-IF and 2-DCE-IF), blood samples (4 ml) were drawn into heparinized syringes. The samples were centrifuged and the plasma was stored at –25° C until analysis. For the determination of 4-OH-IF (activated IF) and CAA, whole blood samples were immediately ("bedside") processed as described below.

Assay for IF and its dechloroethyl metabolites 3-DCE-IF and 2-DCE-IF. After thawing of the frozen samples, 100 µl of patient plasma was spiked with 10 µl of the internal standard solution containing the oxazaphosphorine derivative trofosfamide (3.5 mg dissolved in 10 ml of ethyl acetate); 2 ml of dichloromethane was added and the samples were shaken vigorously for 3 min. After centrifugation, 1.9 ml of the organic layer was transferred to conical glass tubes and the extraction procedure was repeated by adding another 2 ml of dichloromethane to the plasma samples. After centrifugation, the 1.9-ml dichloromethane aliquot derived from the second extraction was combined with that obtained by the first extraction step and the organic layer was evaporated to dryness under a gentle stream of nitrogen. The residue was redissolved in 200 µl of ethyl acetate and 1–2 µl was subsequently used for analysis.

Simultaneous determination of IF, its dechloroethyl metabolites, and trofosfamide was performed by means of N/P flame-ionization gas chromatography (GC/NPFID) using a fused-silica capillary column (Hewlett Packard PAS 1701) under isothermal conditions, with N_2 serving as the carrier gas. The temperatures of the oven, injector, and detector were set at 195°, 300° C and 300° C, respectively. The retention times of 2-DCE-IF, 3-DCE-IF, IF, and trofosfamide were 1.9, 2.4, 4.3, and 7.9 min, respectively.

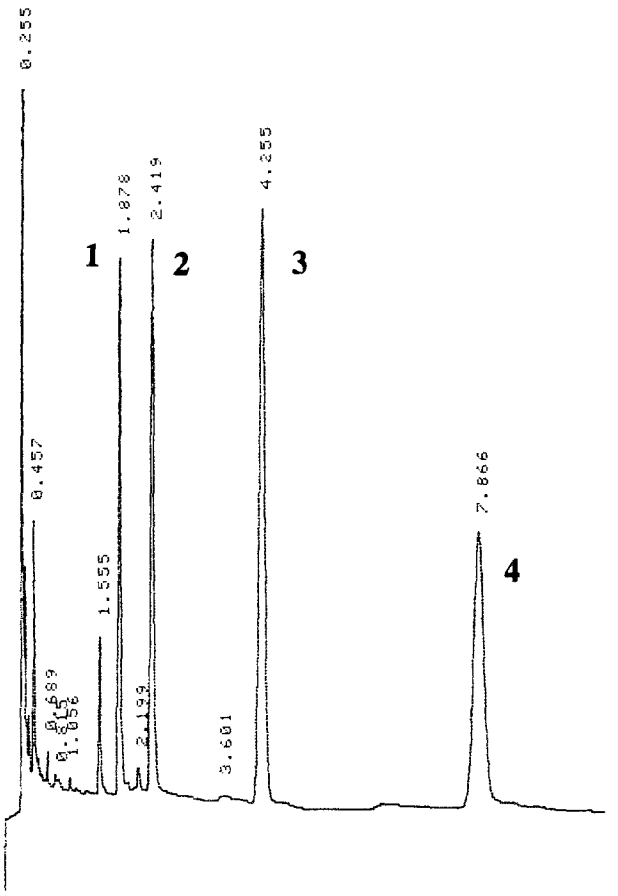

Fig. 1. Chromatogram (GC/NPFID) derived from a processed patient sample at 4 h after the infusion of IF (1.5 g/m^2). *Peak 1* (1.9 min), 2-DCE-IF; *peak 2* (2.4 min), 3-DCE-IF; *peak 3* (4.3 min), IF; *peak 4* (7.9 min), trofosfamide (internal standard)

Standards were prepared by adding known amounts of IF, 3-DCE-IF, and 2-DCE-IF (which were kindly supplied by Asta Medica, Frankfurt, Germany) to plasma samples obtained from a healthy volunteer. The concentration ranges used for calibration that gave rise to linear peak area versus concentration curves were 0.5–30 nmol ml^{-1} for 3-DCE-IF and 2-DCE-IF and 5–200 nmol ml^{-1} for IF. Each calibration curve consisted of a total of six values. All patient samples and standards were run in duplicate. Quantitative estimation of drug and metabolite concentrations in the patient samples was carried out according to the internal-standard ratio.

The lower limits of detection at a peak-height to baseline-noise ratio of 3 : 1 for 3-DCE-IF, 2-DCE-IF, and IF were 0.2, 0.2, and 0.4 nmol ml^{-1}, respectively. The extraction recovery rates for 3-DCE-IF, 2-DCE-IF, IF, and trofosfamide were 84%, 86%, 97%, and 94%, respectively. The intraassay coefficients of variation at average concentrations that were measured at 2–6 h after IF application for IF (50 nmol ml^{-1}), 3-DCE-IF (10 nmol ml^{-1}), and 2-DCE-IF (10 nmol ml^{-1}) were 2.1%, 6.4%, and 7.6%, respectively. The corresponding values at lower concentrations as measured at 9–24 h after IF infusion for IF (5 nmol ml^{-1}), 3-DCE-IF (1 nmol ml^{-1}), and 2-DCE-IF (1 nmol ml^{-1}), were 9.2%, 16.8%, and 17.6%, respectively. IF and the dechloroethyl metabolites remained stable in the frozen plasma samples for at least 3 months but were generally analyzed within 1 week after sampling. A representative chro-

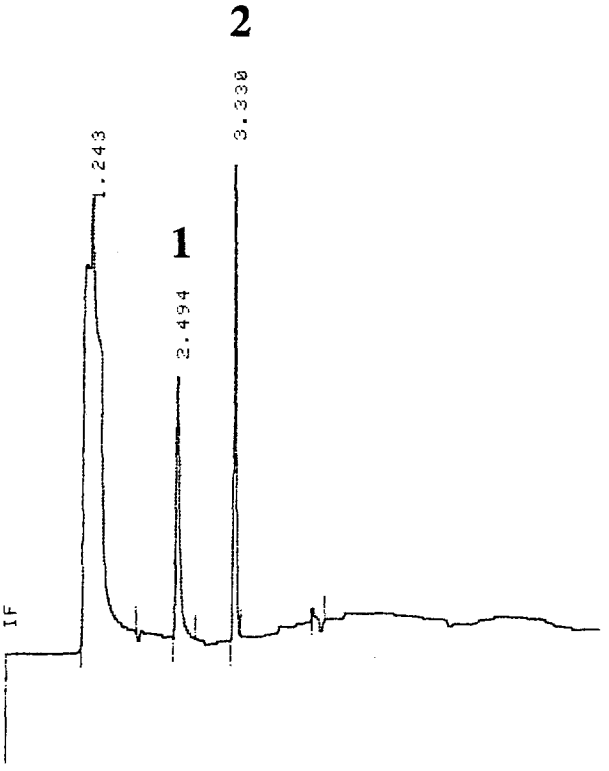

Fig. 2. Chromatogram (GC/ECD) derived from a processed patient sample at 2 h after the infusion of IF (1.5 g/m^2). *Peak 1* (2.5 min), CAA; *peak 2* (3.3 min), chloroform (internal standard)

matogram derived from a processed patient plasma sample at 4 h after the IF infusion is shown in Fig. 1.

Assay for 4-OH-IF. The terms 4-OH-IF and "activated IF" as used synonymously in this paper denote the sum of all IF derivatives that give rise to liberation of acrolein, i.e., 4-OH-IF, its acyclic tautomer aldoifosfamide, and 4-S,R-sulfidoifosfamide metabolites constituting reversible detoxification metabolites with thiols. The cytostatically inactive metabolites ketoifosfamide and carboxyifosfamide formed by further oxidation of 4-OH-IF and aldoifosfamide, respectively [4], do not release acrolein and are therefore not determined by this assay.

Immediately after being drawn, patient blood samples (1 ml) were deproteinized by adding 1 ml of 10% trichloroacetic acid. The samples were shaken vigorously and centrifuged. A 1.5-ml aliquot of the supernatant was heated at 95° C for 20 min, resulting in the release of acrolein from the "activated" IF metabolites [25]. Acrolein subsequently reacted with 3-aminophenol under acidic conditions to form 7-OH-quinoline (7-OH-QU), which can be detected fluorometrically [1]. Methological details of the acid hydrolysis and derivatization procedure are given in a previous publication [25]. For the elimination of interfering fluorescence, the acrolein-derived 7-OH-QU had to be extracted from the derivatization mixture and was subsequently quantified by high-performance chromatography (HPLC) with fluorescence detection. Standards were prepared by adding known amounts of the cyclohexylamine salt of mafosfamide (ASTA Z 7557; ASTA Medica, Frankfurt, Germany) to deproteinize blood samples obtained from a healthy volunteer. The procedure as described above resulted for ASTA Z 7557 in the release of 4-hydroxycyclophosphamide, which was further hydrolyzed for the release of acrolein that could be derivatized as IF-derived acrolein.

The concentration range used for calibration was 0.1 – 4.0 nmol ml^{-1}, generating a linear peak area versus concentration curve. The calibration curves for every single assay performance consisted of six values. All patient samples and standards were run in duplicate. The lower limit of detection at a 3:1 peak-height to baseline-noise ratio was 0.05 nmol ml^{-1}. The recovery rate for the whole assay procedure (hydrolysis, derivatization, and extraction of 7-OH-QU) as calculated from the comparison of known amounts of ASTA Z 7557 processed as described above with equimolar amounts of 7-OH-QU was 72%. The extraction recovery rate of 7-OH-QU was 85%. The intraassay coefficients of variation calculated for ASTA Z 7557 concentrations of 1 and 0.2 nmol ml^{-1} were 8.2% and 13.9%, respectively. Details of the extraction procedure, the standard preparation, and the HPLC assay of fluorescent 7-OH-QU as well as representative chromatograms have been published recently [8].

Assay for CAA. Immediately after being drawn, 1.8 ml of blood was added to 0.2 ml of a 3.7% formaldehyde solution. The mixture was shaken vigorously for 2 min, resulting in complete lysis of the erythrocytes and other blood cells. The samples were centrifuged to spin down cell fragments and the supernatant was put on ice until subsequent analysis, which was always performed within 24 h after sampling. Before determination, 20 µl of the internal standard solution (1 µl of chloroform in 100 ml of methanol) was added to 200 µl of the supernatant derived from the formaldehyde hemolysis procedure. The samples were mixed and 1 – 2 µl was directly injected onto the GC system using a fused-silica capillary column (Hewlett Packard-5, cross-linked 5% phenyl methyl silicone), with helium serving as the carrier gas. CAA and the internal standard were detected by a ^{63}Ni electron-capture detector (ECD) at 300° C. The injector temperature was set at 200° C, and the oven temperature was held at 35° C for 3 min, followed by a linear increase of 45° C/min up to a final temperature of 120° C. The retention times for CAA and chloroform were 2.5 and 3.3 min, respectively.

Standards (six calibration samples for every assay run) were prepared by adding known amounts of CAA (0.2 – 10 nmol ml^{-1}) to blood samples obtained from a healthy volunteer that had to be processed immediately as described above. Quantitative estimation was carried out according to the internal-standard ratio.

Formaldehyde had to be added to the blood samples instantly after drawing to stabilize CAA by an excess to aldehyde groups. In the absence of formaldehyde, 50% and 100% deterioration of CAA in blood can be observed after 6 and 20 min, respectively. When formaldehyde is added to the blood samples and the supernatant resulting from centrifugation is stored at 4° C, CAA remains relatively stable for up to 24 h, the deterioration rate being in the range of 5% – 10%.

The recovery rate of CAA under the assay conditions described above was 79%. The lower limit of detection at a peak-height to noise ratio of 3:1 was 0.06 nmol ml^{-1}. The intraassay coefficients of variation at CAA concentrations of 5 and 0.5 nmol ml^{-1} were 13.7% and 18.2%, respectively. A typical chromatogram derived from a processed patient blood sample at 2 h after the IF infusion is shown in Fig. 2.

Pharmacokinetics and calculation. From the concentration/time profiles following distribution, the terminal half-lives of IF were calculated. The areas under the concentration/time curves (AUC) of all compounds examined in the study were calculated by the trapezoidal rule. For intraindividual comparison of the pharmacokinetic parameters derived on days 1 and 5 of the study interval, the Wilcoxon test for paired data ($P < 0.05$) was used.

Results

Pharmacokinetics of IF

The concentration/time curves generated for IF plasma levels on days 1 and 5 of the 5-day divided-dose schedule (1.5 g/m^2 daily) are shown in Fig. 3. The corresponding maximal concentration (C_{max}) and AUC values are summarized in Table 1. Large interindividual variation was observed in the elimination half-lives calculated on day 1 [geometric mean ($\bar{x}_{geo}$, 6.4 h; range, 1.9 – 10.3 h and on day 5 ($\bar{x}_{geo}$, 4.1 h; range, 1.7 – 6.6 h)]. The maximal IF

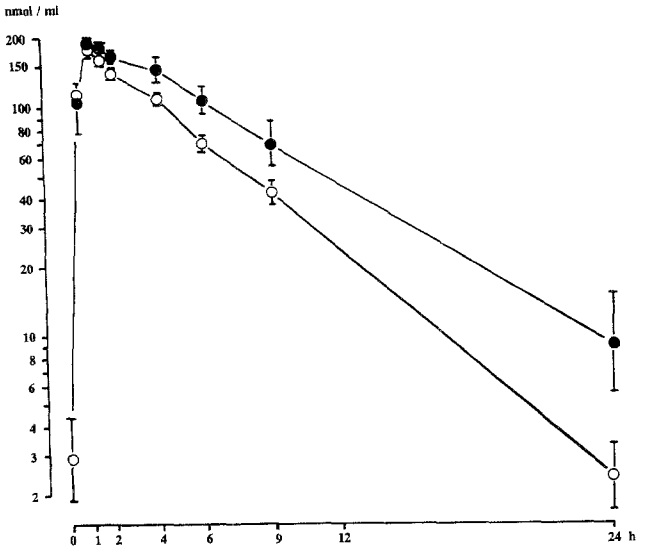

Fig. 3. Comparative pharmacokinetics (plasma concentration vs time) of IF determined on day 1 (●) and on day 5 (○) in patients under fractionated i.v. IF therapy (1.5 g/m² given daily for 5 days). Data points represent geometric mean values ± SEM ($n = 11$)

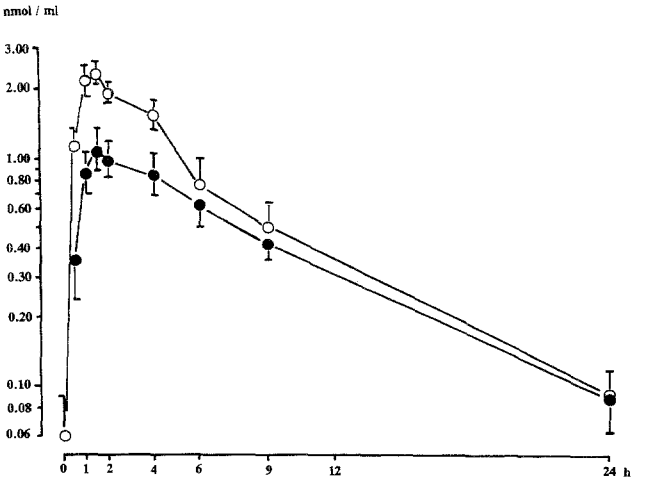

Fig. 4. Comparative pharmacokinetics (blood concentration vs time) of 4-OH-IF (activated IF) determined on day 1 (●) and on day 5 (○) in patients under fractionated i.v. IF therapy (1.5 g/m² given daily for 5 days). Data points represent geometric mean values ± SEM ($n = 11$)

levels were determined on both days in the samples drawn at the end of the short IF infusion (t_{max}, 1 h). The mean ratios that were calculated from the ratios obtained by intraindividual comparison of pharmacokinetic parameters for the single patients (day 5 vs day 1) are given in Table 2. Whereas the C_{max} values determined on day 5 were not significantly lower than those measured on day 1, a substantial decrease (approximately 30%) in the half-lives and in the corresponding AUCs was found in 10 of 11 patients.

Pharmacokinetics of 4-OH-IF

Figure 4 shows the blood concentration/time curves constructed for 4-OH-IF. In addition, Table 1 gives the corresponding C_{max} and AUC values. As compared with the IF plasma levels, the concentrations determined for the activated metabolite ranged 2 orders of magnitude lower. The mean t_{max} values for 4-OH-IF as calculated from the times of maximal blood levels after the start of the IF infusions on days 1 and 5 were 1.7 and 1.5 h, respectively. The decrease in 4-OH-IF concentrations paralleled the reduction in those of the parent compound IF. Since in some patients, metabolite concentrations in the 9- and 24-h samples were measured close to the lower limit of sensitivity of the assay, reliable calculation of the mean elimination half-life of 4-OH-IF could not be performed. In the concentration/time curves shown in Fig. 4, the $t^1/_2$ value recorded on day 5 appears to be somewhat lower than that obtained on day 1. As indicated in Table 2, the C_{max} values determined for 4-OH-IF were found to have nearly doubled over the 5-day period of IF administration. This enhancement was also accompanied by a significant increase in AUC values.

Table 1. C_{max} and AUC values determined for IF and its metabolites on days 1 and 5 of a 5-day fractionated i.v. IF therapy

Drug	Day	C_{max} (nmol ml^{-1})		AUC (nmol ml^{-1} h)	
		$\bar{x}_{geo}$[a]	Range	$\bar{x}_{geo}$[a]	Range
IF	1	199	158 – 253	1780	630 – 2740
	5	181	132 – 217	1200	720 – 1680
4-OH-IF	1	1.5	0.9 – 3.7	11.3	4.1 – 21.9
	5	2.6	1.7 – 4.0	16.5	4.9 – 34.4
CAA	1	2.7	0.5 – 7.3	30.3	7.4 – 86.7
	5	4.9	1.7 – 10.3	34.2	13.0 – 114.3
3-DCE-IF	1	12.9	2.0 – 31.3	146	25 – 513
	5	26.5	9.8 – 51.4	354	72 – 923
2-DCE-IF	1	8.6	4.0 – 38.9	111	26 – 369
	5	16.7	7.1 – 28.4	209	79 – 469

Patients were given IF at a daily dose of 1.5 g/m²

[a] Geometric means ($n = 11$)

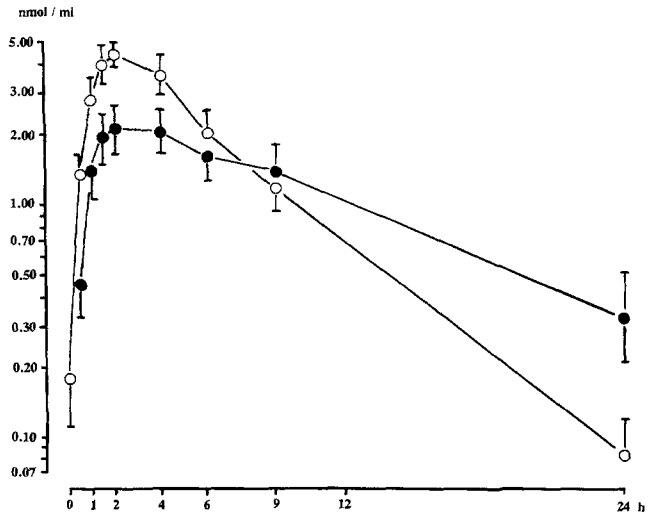

Fig. 5. Comparative pharmacokinetics (blood concentration vs time) of CAA determined on day 1 (●) and on day 5 (○) in patients under fractionated i. v. IF therapy (1.5 g/m^2 given daily for 5 days). Data points represent geometric mean values ± SEM (n = 11)

Table 2. Intraindividual comparison of the pharmacokinetic parameters of IF and its metabolites on days 1 and 5 of a 5-day fractionated i. v. IF therapy

Drug	Parameters
IF	$t_{1/2}$ (day 5): $t_{1/2}$ (day 1) = 0.70 ± 0.12
	C_{max} (day 5): C_{max} (day 1) = 0.92 ± 0.04
	AUC (day 5): AUC (day 1) = 0.73 ± 0.12*
4-OH-IF	C_{max} (day 5): C_{max} (day 1) = 1.94 ± 0.28*
	AUC (day 5): AUC (day 1) = 1.51 ± 0.11*
CAA	C_{max} (day 5): C_{max} (day 1) = 2.05 ± 0.28*
	AUC (day 5): AUC (day 1) = 1.29 ± 0.17
3-DCE-IF	C_{max} (day 5): C_{max} (day 1) = 2.52 ± 0.52*
	AUC (day 5): AUC (day 1) = 2.34 ± 0.50*
2-DCE-IF	C_{max} (day 5): C_{max} (day 1) = 2.33 ± 0.43*
	AUC (day 5): AUC (day 1) = 2.23 ± 0.44*

Data represent geometric mean values ± SEM (n = 11)
* Significantly different from 1.0 (P <0.05)

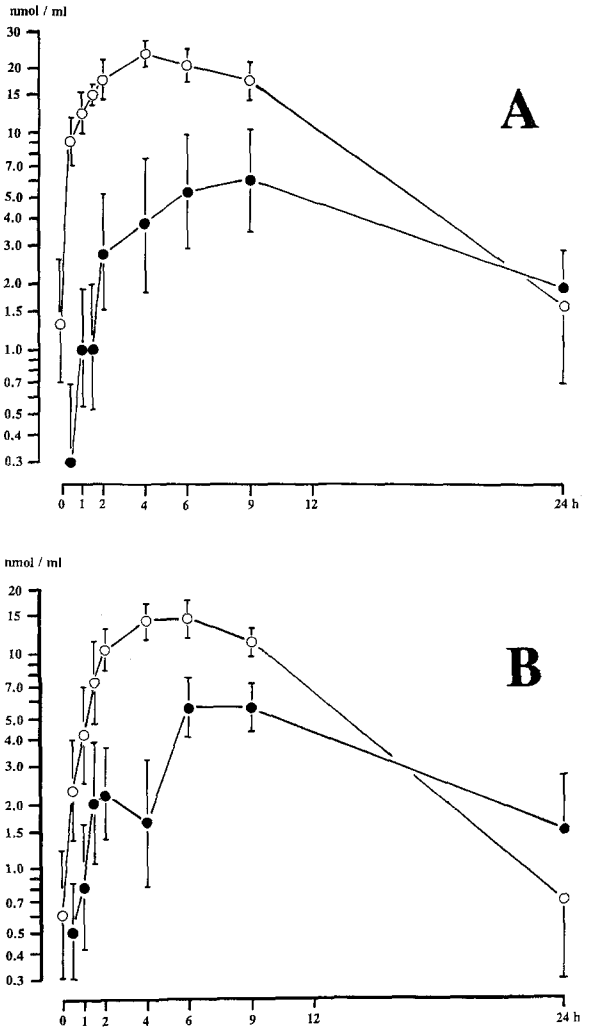

Fig. 6 A, B. Comparative pharmacokinetics (plasma concentration vs time) of **A** 3-DCE-IF and **B** 2-DCE-IF determined on day 1 (●) and on day 5 (○) in patients under fractionated i. v. IF therapy (1.5 g/m^2 given daily for 5 days). Data points represent geometric mean values ± SEM (n = 11)

Pharmacokinetics of CAA

The blood concentration/time curves plotted for CAA derived from side-chain oxidation of IF are shown in Fig. 5, and the C_{max} and AUC values are given in Table 1. The CAA concentrations were in the range of those measured for 4-OH-IF. As compared with 4-OH-IF, which is formed by the alternate initial metabolic pathway of ring oxidation, an even more pronounced interpatient variability (see the range of C_{max} values shown in Table 1) and a slightly slower increase in CAA concentrations were found. The t_{max} values recorded on days 1, 5 were 2.5 and 2.2 h, respectively. As calculated by intraindividual comparison, the C_{max} values determined for CAA on day 5 were twice as high as those measured on day 1 (see Table 2). Since high CAA concentrations were measured for up to 24 h after IF application in one patient on day 1, the increase in AUC values (day 5 vs day 1) did not reach statistical significance (Table 2).

Pharmacokinetics of 3- and 2-DCE-IF

The concentration/time curves generated for the two dechloroethyl metabolites 3-DCE-IF and 2-DCE-IF as formed by side-chain oxidation (*N*-dealkylation) of IF are shown in Fig. 6, and Table 1 gives the corresponding values for C_{max} and AUC. Approximately 50% higher concentrations were measured for 3-DCE-IF than for 2-DCE-IF. The sum of the concentrations recorded for the two dechloroethyl metabolites exceeded the levels measured for the corresponding metabolite CAA, which is formed in the side-chain oxidation pathway in equimolar amounts, by 1 order of magnitude. In contrast to 4-OH-IF and CAA, the plasma pharmacokinetics of 3- and 2-DCE-IF did not virtually parallel the concentration/time profile of the parent compound IF (Figs. 3, 6). 3-DCE-IF and 2-DCE-IF seemed to accumulate for up to 4–9 h after the

start of the IF infusion. The mean t_{max} values calculated for 3-DCE-IF on days 1 and 5 were 5.5 and 4.0 h, respectively; the corresponding values obtained for 2-DCE-IF were 7.3 and 4.0 h, respectively. As calculated by intraindividual comparison, the 5-day period of fractionated IF application resulted for both dechloroethyl metabolites in a statistically significant doubling of the C_{max} and AUC values (Table 2).

Discussion

This paper focuses on the pharmacokinetics of IF and its metabolites as formed by ring oxidation (yielding 4-OH-IF) and by the competitive initial metabolic pathway of side-chain oxidation (yielding 3-DCE-IF or 2-DCE-IF and CAA, respectively) as well as on the changes occurring in the metabolic pattern during fractionated IF infusion over a 5-day period, which is the duration of IF application often used in chemotherapeutic regimes [3]. The reduction in IF half-life noted during fractionated administration (see Fig. 3), which is explained by autoinduction of hepatic IF metabolism, is a known phenomenon and has repeatedly been demonstrated [8, 11, 12, 15, 17, 23]. In contrast, comparatively little information exists about the pharmacokinetics of the IF metabolites and the possible changes caused by the assumed increase in IF metabolism following fractionated administration of the drug, predominantly because the activated oxazaphosphorine metabolites are highly instable and, thus, their in vivo detection requires great methodological efforts [20, 24]. The same is true for CAA. We have recently improved the assay of 4-OH-IF [25] by subsequent extraction and HPLC separation of the fluorescent acrolein-derived compound 7-hydroxyquinoline [8]. Whereas in former studies the pharmacokinetic parameters of activated oxazaphosphorine metabolites in blood could be obtained only after the application of high doses [18, 22], the accuracy and sensitivity of the present assay allows for reliable pharmacokinetic investigations using standard IF doses in humans.

The pharmacokinetic data recorded for 4-OH-IF (Table 1, Fig. 4) is in accordance with the results we previously obtained in patients receiving comparable IF doses [8, 25]. The large interindividual variation in 4-OH-IF blood levels and the increase in C_{max} and AUC values over the 5-day period of IF administration (Fig. 4, Table 2) have been predicted by previous findings of a substantial variability and a time-dependent increase in plasma alkylating activity [11, 12] or by an increase in urinary excretion of isophosphoramide mustard, which is thought to be the 4-OH-IF-derived ultimate alkylating agent [13]. The higher concentrations of 4-OH-IF measured on day 5 might be assumed to have been due to a change in elimination by further metabolism, e. g., a decrease in the inactivation of 4-OH-IF by the formation of carboxyifosfamide. However, as the pharmacokinetics of the unstable compound 4-OH-IF virtually paralleled the concentration/time curves constructed for the parent drug IF and the time-dependent decrease in IF half-life was also accompanied by an increase in side-chain oxidation (formation of CAA and the dechloroethyl metabolites), the rise in 4-OH-IF levels observed on day 5 was in all probability caused by a higher degree of generation of the metabolite from IF due to an induction of the metabolic pathway of ring oxidation, too. If it is assumed that the C_{max} value and/or the AUC of activated IF (4-OH-IF) reflects the cytostatic activity of a given IF dose, it can be concluded from our results that fractionated IF therapy produces an increase in the drug's cancerotoxic effect.

CAA, which is released by the alternate initial metabolism of IF (side-chain oxidation), is of clinical importance, since it has been associated with the CNS toxicity occasionally observed during IF treatment [6, 10]. This observation seems reasonable with respect to the structural similarity of CAA to the ethanol metabolite acetaldehyde and the hypnotic chloralhydrate. In addition, CAA has been shown to be neurotoxic in rats [9]. Most recently the role of CAA in the development of nephrotoxicity related to IF application has been discussed [19]. It might well be that IF-induced tubular toxicity, which is not seen after treatment with cyclophosphamide (CP), where side-chain oxidation is of comparatively minor importance, is caused by CAA, since this compound has been shown to deplete substantially intracellular glutathione levels at clinically relevant concentrations and may thus predispose to cellular damage [19].

The CAA assay introduced in this paper is, in contrast to our own previous efforts [8], well applicable for pharmacokinetics studies. The increase in CAA blood levels observed on day 5 as compared with day 1 (Fig. 5) was not accompanied by any neurotoxic or nephrotoxic side effects at the doses used in this study. However, Goren and coworkers [6] have reported on CAA levels measured in two pediatric patients who developed CNS toxicity during IF infusion following a regimen comparable with that used in our study (1.6 g/m^2 daily for 5 days). The CAA levels associated with neurotoxic side effects (maximum, 109 nmol ml^{-1}) were 1 order of magnitude higher than those measured in our adult patients. Also, in the children, who did not present with neurotoxic symptoms, the CAA levels were higher than those determined by us. In agreement with our results, the data reported for two of the children [6] reveal a time-dependent increase in CAA concentrations. As it has been observed that IF-related encephalopathy usually develops on or after the 3rd day of treatment [13], the use of higher IF doses in adult cancer patients may also lead to higher CAA levels and, subsequently, neurotoxicity might become clinically evident. If the proposed role of CAA in IF-induced nephrotoxicity is a dose-dependent phenomenon, the risk of developing this side effect would increase during prolonged fractionated-bolus dose or continuous-infusion schedules of IF administration. Measurement of blood and urinary CAA concentrations and their relation to parameters indicating tubular damage should be the subject of further investigations and may be helpful in identifying patients who are susceptible to IF-induced nephrotoxicity.

Corresponding to the release of CAA, the metabolites formed by IF side-chain oxidation at the cyclic or extracyclic nitrogen are 3-DCE-IF or 2-DCE-IF, respectively [16]. Whereas some data exist concerning their urinary excretion [2, 13, 16], the plasma pharmacokinetics of the

dechloroethyl metabolites have thus far not been thoroughly investigated. In accordance with the range of the corresponding CAA levels, the 3- and 2-DCE-IF plasma concentrations were subject to a high degree of interindividual variability (Table 1). Figure 6 shows that side-chain oxidation "prefers" the cyclic (position 3) nitrogen. These findings agree with the results obtained in studies on urinary excretion, which also reveal higher concentrations for 3-DCE-IF than for 2-DCE-IF as well as a large interpatient variability in the levels of both metabolites [2, 13, 16]. Whereas the concentration/time curves of the unstable intermediates 4-OH-IF and CAA are mainly determined by the generation of the latter from IF rather than by elimination characteristics and thus parallel IF pharmacokinetics, 3-DCE-IF and 2-DCE-IF are more stable compounds, are therefore measured in concentrations higher than those recorded for the corresponding metabolite CAA, and reveal some accumulation for up to 4–9 h after IF application (Fig. 6). The question may arise as to whether the time-dependent increase observed in the C_{max} and AUC values for 4-OH-IF, CAA, 3-DCE-IF, and 2-DCE-IF could simply have been due to metabolite accumulation. Judging from the levels measured before the start of the IF infusion on day 5 (see the 0-h values in Figs. 4–6), we conclude that the relative contribution of accumulation to the total increase in metabolite AUCs is rather negligible. The average trough levels of 4-OH-IF, CAA, 3-DCE-IF, and 2-DCE-IF accounted for only 2.5%, 4.0%, 5.6%, and 4.3%, respectively, of the subsequent maximal values.

In the present study the complete pharmacokinetic profiles of IF and the metabolites formed by the initial steps of IF metabolism were investigated for the first time. Our data reveal a time-dependent increase in IF metabolism without any evidence of a shift in the metabolic pattern during the 5-day observation period; the degree of increase in C_{max} and/or AUC values (Table 2) is virtually the same for both of the initial metabolic pathways of the parent compound IF. The questions as to the exact time course of enzyme induction within the 5-day study interval and as to whether the end point of autoinduction of hepatic IF metabolism might have been reached after 5 days of fractionated IF administration remain unanswered and should be the subject of further investigations.

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