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Determination of the urinary excretion of ifosfamide and its phosphorated metabolites by phosphorus-31 nuclear magnetic resonance spectroscopy*

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Summary. Phosphorus-31 nuclear magnetic resonance spectroscopy was used to analyze urine samples obtained from patients treated with ifosfamide (IF). This technique allows the individual assay of all phosphorated metabolites of IF in a single analysis without the need for prior extraction. In addition to the classic IF metabolites 2-dechloroethylifosfamide (2DECIIF), 3-dechloroethylifosfamide (3DECIIF), carboxyifosfamide (CARBOXYIF), and isophosphoramide mustard (IPM), several signals corresponding to unknown phosphorated compounds were observed. Four of them were identified: one is alcoifosfamide (ALCOIF), two come from the degradation of 2,3-didechloroethylifosfamide (2,3-DECIIF), and one results from the decomposition of 2DECIIF. The total cumulative drug excretion as measured over 24 h in nine patients was 51% of the injected IF dose; 18% of the dose was recovered as unchanged IF. The major urinary metabolites were the dechloroethylated compounds, with 3DECIIF excretion (11% of the injected dose) always being superior to 2DECIIF elimination (4% of the injected dose). Degradation compounds of 2DECIIF and 2,3DECIIF represented 0.4% of the injected dose. The metabolites of the dechloroethylation pathway always predominated over those of the activation pathway (CARBOXYIF, ALCOIF, and IPM, representing 3%, 0.8%, and 0.2% of the injected dose, respectively). In all, 14% of the injected dose was excreted as unknown phosphorated compounds. The interpatient variation in levels of IF metabolites was obvious and involved all of the metabolites. Renal excretion was not complete at 24 h, since 11% of the injected dose was recovered in the 24- to 48-h urine samples.

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Introduction

Ifosfamide (IF), a structural isomer of the oxazaphosphorine cyclophosphamide, has significant activity against numerous human malignancies. Like cyclophosphamide, IF is a prodrug that requires biotransformation to become cytotoxic (Fig. 1) [3–6, 19]. Hydroxylation of the oxazaphosphorine ring system at the C4 position leads to the formation of 4-hydroxyifosfamide (OHIF), which can then be either oxidized to form 4-ketoifosfamide (KETOIF), an inactive metabolite, or converted into aldoifosfamide (ALDOIF) by ring opening. ALDOIF can be oxidized to yield carboxyifosfamide (CARBOXYIF), an inactive metabolite. ALDOIF can also undergo spontaneous elimination of acrolein to produce the alkylating compound isophosphoramide mustard (IPM). In addition to this ring oxidation, *N*-dealkylation of the chloroethyl side chains can occur, leading to the formation of two inactive metabolites, 2-dechloroethylifosfamide (2DECIIF) and 3-dechloroethylifosfamide (3DECIIF), and to the elimination of chloroacetaldehyde, a compound that may be responsible for the neurotoxic and nephrotoxic effects of IF [9–11, 20, 22].

We have previously demonstrated the value of the phosphorus-31 nuclear magnetic resonance ([^{31}P]-NMR) technique in the analysis of the body fluids of patients who have been treated with IF [14]. Indeed, as can be seen in Fig. 1, nearly all IF metabolites contain the phosphorus atom and are therefore easily detected and quantified by [^{31}P]-NMR in a single analysis. Moreover, since [^{31}P]-NMR enables the direct study of intact body fluid, the problems encountered in extraction, recovery, and chemical derivatization as well as those stemming from the pH sensitivity of many metabolites are avoided through its application. The main limitation of [^{31}P]-NMR is its low sensitivity. As determined using a currently available spectrometer (7 T), the detection threshold in a 10-mm-diameter NMR tube is 10 μM for a 24-h recording period. The use of this technique has enabled us to show that the metabolism of IF is certainly more complex than reported. Indeed, in addition to the classic metabolites of IF, several

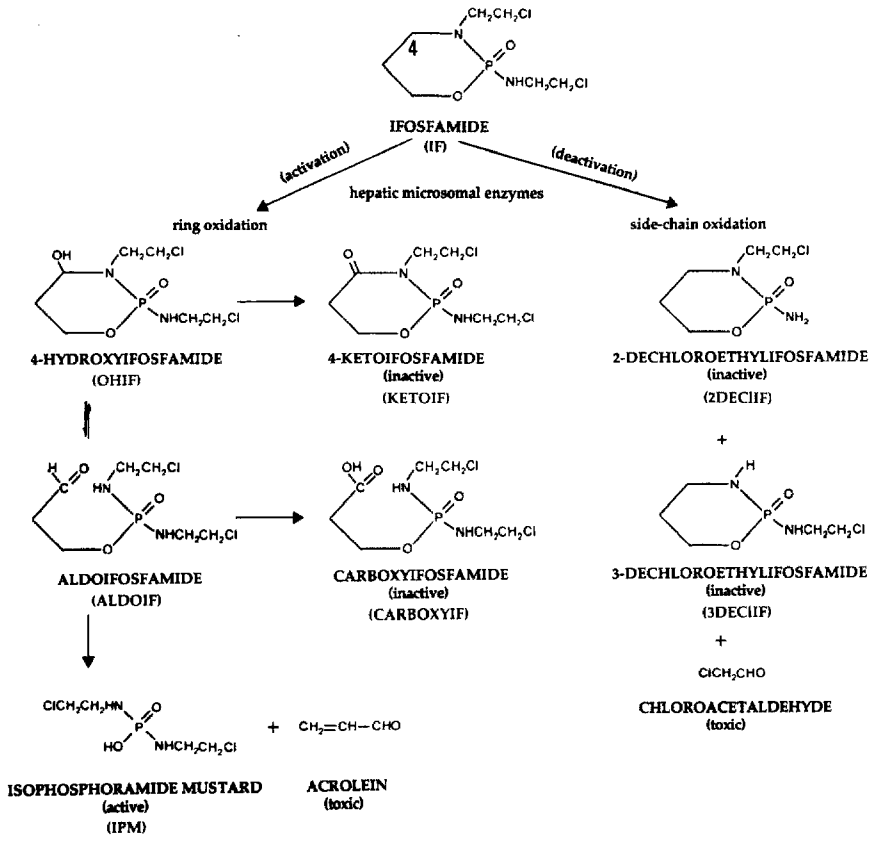


Fig. 1. Metabolic pathways of IF

signals corresponding to unknown phosphorated compounds were observed in the [^{31}P]-NMR spectra of urine samples obtained from patients treated with IF.

This paper describes the identification of four unknown phosphorated compounds detected in the urine of patients treated with IF as well as the quantification of the urinary excretion of IF and its metabolites by nine patients.

Patients and methods

Patients and urine sampling. Nine patients with metastatic soft-tissue sarcoma were investigated. Their median age was 53.5 years (range, 21–79 years). They received IF i.v. at a dose of 3 g/m^2 as a 3-h perfusion in 1 l 5% glucose. The uroprotector mesna was given at a dose of 3 g/m^2 in 1 l 5% glucose as a 24-h infusion, which began 30 min before the injection of IF. None of the patients received any other phosphorated drug during the study. The accompanying cytostatic was Adriamycin (for seven patients) or Eldesine (for one patient).

Urine was collected over 8-h intervals for 24 (seven patients) or 48 h (two patients) after the beginning of the infusion. The volume of micturition was noted, and the samples were immediately frozen and stored at –80° C until analysis. For the identification of metabolites, some urine samples were concentrated (from 50 to 3 ml) by lyophilization. For quantitative purposes crude (i.e., unconcentrated) urine samples were analyzed except for those from one patient who had severe diuresis. In this case, 20 ml urine was lyophilized and resuspended in a known volume of H_2O sufficient to redissolve the whole pellet (≈5 ml); otherwise, the quantification would have been incorrect [14].

Materials. IF, CARBOXYIF (cyclohexylamine salt), KETOIF, IPM, 2DECIIF, 3DECIIF, 2,3-didechloroethylifosfamide (2,3-DECIIF), and *cis*-4-hydroperoxyifosfamide (OOHIF) were generously supplied by Asta Medica AG (Frankfurt, FRG). Chromium (III) acetylacetonate [Cr(acac)$_3$] was obtained from Spectrométrie Spin Techniques (Paris, France). Methylphosphonic acid (MPA) and hexachlorocyclotriphosphazene (HCCP) were purchased from Aldrich (Strasbourg, France), and phenylphosphinic acid (PPA) was supplied by Fluka (Mulhouse, France).

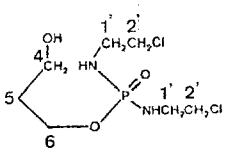
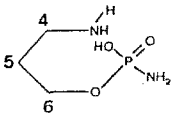
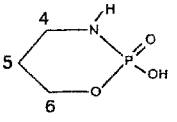
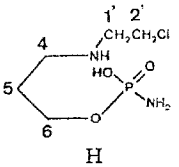
Alcoifosfamide (ALCOIF) was prepared by treating a solution of 0.036 mmol OOHIF dissolved in 0.3 ml CH_3OH with 0.4 mmol dimethylsulfide at room temperature for 1 min. This solution was then slowly added to a solution of $NaBH_4$ (0.4 mmol) in 2 ml CH_3OH. The mixture was stirred for 1.5 h at room temperature. The CH_3OH was evaporated in vacuo and the residue was redissolved in 2 ml H_2O. This solution was loaded onto Sep-Pak cartridges (Waters-Millipore, Saint-Quentin-les-Yvelines, France) containing 500 mg sorbent, which had previously been activated by washing with 3 ml CH_3OH and then with 9 ml H_2O. The column was washed with 1.5 ml H_2O and then with 1 ml acetonitrile. The retained ALCOIF eluted with acetonitrile. The solvent was removed in vacuo. ALCOIF was identified by [^{13}C]-NMR and mass spectrometry (Table 1).

A solution of 0.08 mmol 2,3DECIIF in 2 ml H_2O was maintained at pH 4.9 for 3 h at room temperature. The substance obtained (compound H′) was identified by [^{13}C]-NMR and mass spectrometry (Table 1). To get compound J, a solution of compound H′ (0.08 mmol in 2 ml H_2O) was maintained at pH 8–9 for 7 days at room temperature. The spectral characteristics of compound J are given in Table 1.

A solution of 0.03 mmol 2DECIIF in 2 ml H_2O was maintained at pH 3.5 for 2 h at room temperature. The substance obtained (compound H) was identified by [^{13}C]-NMR and mass spectrometry (Table 1).

Identification of the unknown signals observed at 10.91, 10.88, 6.50, and 19.21 ppm in urine samples. We had noticed that when 2,3DECIIF was added to a urine sample, a transient signal was detected at 17.74 ppm. Its intensity rapidly decreased in favor of a signal at 10.88 ppm and a signal at 6.50 ppm that appeared more slowly. Similarly, in urine samples at a pH of <5, we have previously observed a significant decrease in the intensity of the 2DECIIF signal in favor of a signal at 10.91 ppm [14]. We thus synthesized compounds H′, J, and H as described above. Spiking

Table 1. Spectroscopic characteristics of ALCOIF and compounds H, H′, and J

	 ALCOIF	 H′	 J	 H
FAB-positive mass--spectral data (m/z)	$[MH]^+$ 279,281,283	$[MH]^+$ 155	$[MH]^+$ 138	$[MH]^+$ 217,219
$[^{13}C]$-NMR δ (ppm)[a] (multiplicity, J_{CP} in Hz)				
C4	60.5	40.4	45.0 (d, 4.4)	42.2
C5	34.6 (d, 7.6)	30.4 (d, 7.2)	29.8 (d, 6.7)	29.3 (d, 7.0)
C6	65.7 (d, 5.6)	65.1 (d, 5.1)	70.8 (d, 5.5)	65.0 (d, 5.4)
C1′	48.1 (d, 5.1)			51.7
C2′	45.0			48.2

[a] Chemical shifts are related to external 3-trimethylsilyl propane sulfonic acid. Spectra were recorded at 25° C in D_2O
FAB, Fast atom bombardment

Table 2. Accuracy and reproducibility of the $[^{31}P]$-NMR assay[a]

Concentration (M)	Number of experiments	Mean concentration measured (M), accuracy (%)	Reproducibility (%)
9.14×10^{-4}	12	8.39×10^{-4}, − 8.2	5.6
4.77×10^{-4}	8	4.84×10^{-4}, + 1.6	8.4
9.73×10^{-5}	12	8.51×10^{-5}, −12.5	9.7
4.91×10^{-5}	6	5.34×10^{-5}, + 8.9	3.0
1.97×10^{-5}	6	2.07×10^{-5}, + 5.0	17.6
9.85×10^{-6}	4	3.82×10^{-6}, −61.2	12.7

[a] The validity of the assay was determined using IF solutions at known concentrations ranging from 10^{-5} to 10^{-3} M and two standards, an external one (HCCP) that had previously been calibrated [14] and an internal one (PPA)

of several concentrated urine samples with the authentic standards H, H′, and J led to an increase in the signals at 10.91, 10.88, and 6.50 ppm, respectively.

The signal at 19.21 ppm was attributed to ALCOIF in two sets of experiments. First, two lyophilized urine samples were spiked with the authentic standard obtained by chemical synthesis. This led to an increase in the signal at 19.21 ppm. Second, four urine samples obtained from two patients [one was taken immediately before the beginning of the IF infusion (U_0) and one was collected at 8–16 h after the beginning of the IF infusion (U_2) for each patient] were extracted according to the method of Goren [8]. After their reconstitution in 2 ml H_2O, the extracts were analyzed using $[^{31}P]$-NMR. No signal could be detected in the two U_0 samples except for the signal of inorganic phosphate. The signals of IF, 2DECIIF, and 3DECIIF as well as the unknown signal at 19.21 ppm were the only peaks observed in the two U_2 samples. Spiking of the U_2 extracts with authentic ALCOIF led to an increase in the signal at 19.21 ppm.

The same extracts were analyzed by high-performance liquid chromatography (HPLC) on a Waters liquid chromatograph using a Merck Lichrosorb RP Select B column (5 µm, 250×4 mm). A photodiode-array detector (Waters model 991) was set at 200 nm. From 0 to 30 min after the injection, the mobile phase consisted of 90% H_2O and 10% acetonitrile. From 30 to 40 min, the proportion of acetonitrile was increased to 30%. The proportion of acetonitrile was then decreased to 10% and the column was reequilibrated prior to the next sample injection. The flow rate was 1 ml/min. These analytical conditions allowed the separation of 2DECIIF (7.7 min), 3DECIIF (9.4 min), ALCOIF (24.8 min), and IF (38.4 min). The peak of ALCOIF was not detected in the U_0 extracts but was observed in the U_2 samples. It increased following spiking of the extracts with authentic ALCOIF.

$[^{31}P]$-NMR analysis. The NMR recording conditions and the methodology of the NMR assay have been described in detail in a previous publication [14]. In the present work, PPA was used instead of MPA as an internal standard for the quantification of some urine samples.

Due to the amount of time required for the quantitation of metabolites in unconcentrated urine samples (15–24 h), NMR data were acquired in 2-h blocks. These blocks were then compared to check the stability of the phosphorated metabolites over the duration of the NMR recording. In some samples, CARBOXYIF slightly degraded with time due to the pH and the ionic strength. Nevertheless, the quantification of IF and its metabolites was performed on the sum spectrum resulting from the addition of the blocks.

Under the experimental conditions used, the accuracy and reproducibility of the $[^{31}P]$-NMR assay was determined using IF solutions at known concentrations ranging from 10^{-5} to 10^{-3} M and two standards, an external one (HCCP) that had previously been calibrated [14], and an internal one (PPA). The quantification was acceptable at concentrations ranging from 2×10^{-5} to 10^{-3} M (Table 2). However, the error was high for the lowest concentration used ($\approx 10^{-5}$ M), which is the detection limit for the spectrometer employed. The quantification of IPM, ALCOIF, compound J, and unknown compounds corresponding to the signals detected at 18.98, 13.01, and 12.77 ppm was thus subject to a large error in only 2, 2, 1, 1, 1, and 3 of the 33 urine samples analyzed, respectively.

Results

Identification of four new IF metabolites detected in the urine of patients treated with IF

A representative $[^{31}P]$-NMR spectrum of a concentrated urine sample obtained from a patient treated with IF is shown in Fig. 2C. CARBOXYIF (B, 19.09 ppm), 2DECIIF (C, 17.71 ppm), IF (D, 15.89 ppm), 3DECIIF (E, 15.80 ppm), IPM (F, 13.83 ppm), and KETOIF (G, 11.27 ppm, observed only in some samples) were detected. 2,3DECIIF was never found; however, two of its degrada-

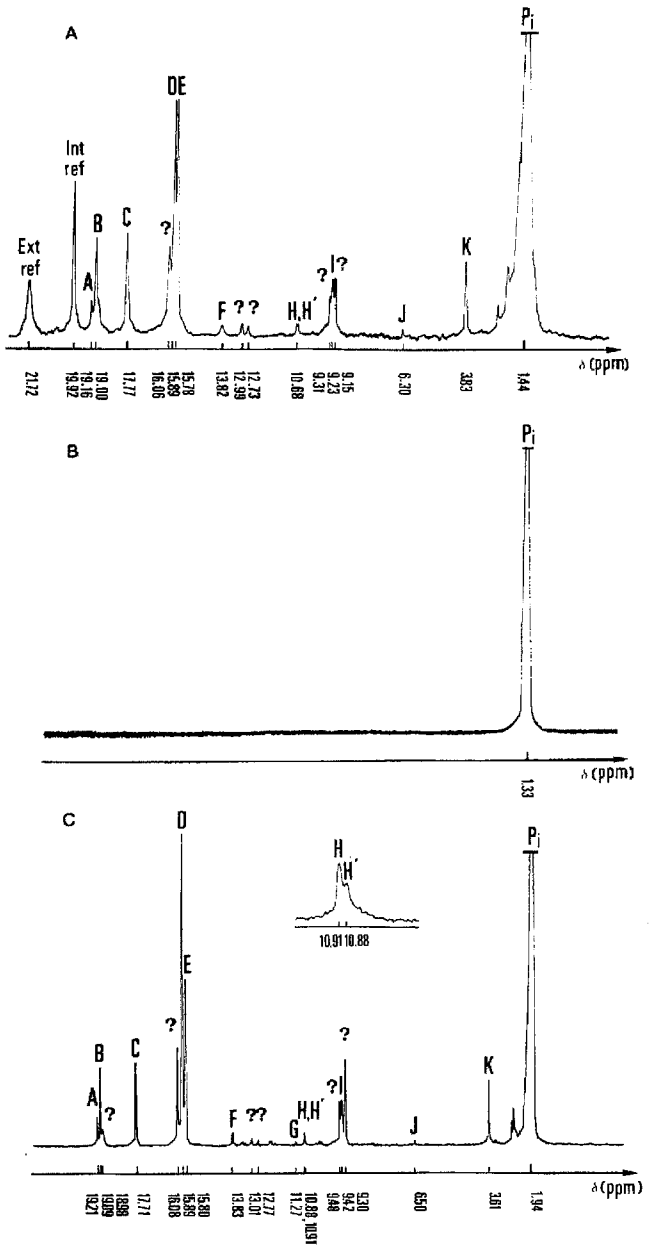


Fig. 2A–C. [^{31}P]-NMR spectra of urine samples obtained from patients treated with IF (3 g/m^2 given by i.v. infusion over 3 h). The urine samples were collected before therapy (**B**) and at 16–24 (**A**) or 8–16 h (**C**) after the beginning of the infusion, were frozen immediately after their collection, and were either left unconcentrated (**A**) or subjected to 17-fold concentration by lyophilization (**B, C**). **A** pH 6.1; number of scans, 10,000. **B** pH 5.7; number of scans, 1,000. **C** pH 5.9; number of scans, 1,000. *Ext/int ref*, External (HCCP) and internal (PPA) standards used for quantification: *A*, ALCOIF; *B*, CARBOXYIF; *C*, 2DECIIF; *D*, IF; *E*, 3DECIIF; *F*, IPM; *G*, KETOIF; *H*, degradation product of 2DECIIF (structure shown in Fig. 3); *H'*, degradation product of 2,3-DECIIF (Fig. 3); *I* (9.42 ppm), degradation product of CARBOXYIF; *J*, degradation product of 2,3DECIIF (Fig. 3); *K*, degradation product of IPM; P_i, inorganic phosphate; *?*, unknown compounds. *Insert*: Expansion of H, H' signal region. Chemical shifts (δ) are reported relative to the resonance peak of 85% H_3PO_4 as an external chemical-shift reference

tion products (compounds H', 10.88 ppm, and J, 6.50 ppm) were detected. The structures of compounds H' and J have been determined (Fig. 3). H' is the compound obtained by hydrolysis of the endocyclic P-N bond of 2,3DECIIF and J is the product resulting from hydrolysis of the exocyclic P-N bond. The structure of compound H (10.91 ppm) has also been determined; it comes from hydrolysis of the endocyclic P-N bond of 2DECIIF (Fig. 3). Compounds I and K are degradation products of CARBOXYIF and IPM, respectively [14], but their exact structures have not yet been determined. Using [^{31}P]-NMR and HPLC analysis, we demonstrated that the compound resonating at 19.21 ppm corresponds to ALCOIF. Several other signals from phosphorated metabolites of yet unknown structure were also detected. We have previously shown that they do not correspond to conjugates of IF or its metabolites with the uroprotector mesna [more exactly, to the compounds resulting from a nucleophilic attack of mesna on the chloroethyl group(s) of IF, 2DECIIF, 3DECIIF, CARBOXYIF, or IPM] or to the activated metabolites of IF (ALDOIF, OHIF) [14].

In crude (natural) urine samples, the same signals described above were observed, except that the signals of compounds H and H' could not be separated due to the low signal-to-noise ratio in unconcentrated urine samples and the signal of KETOIF was too low to be detected (Fig. 2A). Inorganic phosphate was the only phosphorated compound detected by [^{31}P]-NMR in control (natural) or concentrated urine samples (Fig. 2B).

Urinary excretion of IF and its metabolites

Table 3 summarizes the data obtained on the 24-h urinary excretion of IF and its phosphorated metabolites in the nine patients given 3 g/m^2 IF. The measured values for the 8-h aliquots were summed to give the total amount of unchanged IF and metabolites excreted over the 24-h period. The total urinary excretion of IF and its phosphorated metabolites as recovered from urine over 24 h was 50.6% ± 10.6% (range, 38.6%–72.3%). A mean of 17.9% ± 4.7% (range, 11.4%–28.5%) of the delivered IF dose was excreted unmetabolized. The total recovery of phosphorated metabolites was 32.7% ± 8.7% (range, 19.3%–47.8%) of the injected dose.

The most abundant urinary metabolites were the dechloroethylated compounds, i.e., 2DECIIF, 3DECIIF, and 2DECIIF and 2,3DECIIF degradation products (compounds H, H', and J), with the median excretion value being 14.9% ± 3.6% (range, 8.5%–20.4%) of the delivered IF dose. 3DECIIF represented 10.5% ± 2.7% (range, 5.5%–15.8%), of the injected dose, 2DECIIF corresponded to 3.9% ± 1.3% (range, 1.0%–5.7%) of the original dose, and 2DECIIF and 2,3DECIIF degradation products represented 0.4% ± 0.6% (range, 0–1.8%) of the injected dose. 3DECIIF excretion always predominated over 2DECIIF excretion, with 3DECIIF reaching levels of up to 9-fold (mean, 3.3 ± 2.2; range, 1.4–9.0) those of 2DECIIF.

Urinary excretion of the metabolites of the activation pathway, i.e., CARBOXYIF, IPM, and ALCOIF, was about 3 times lower than that of the metabolites of the

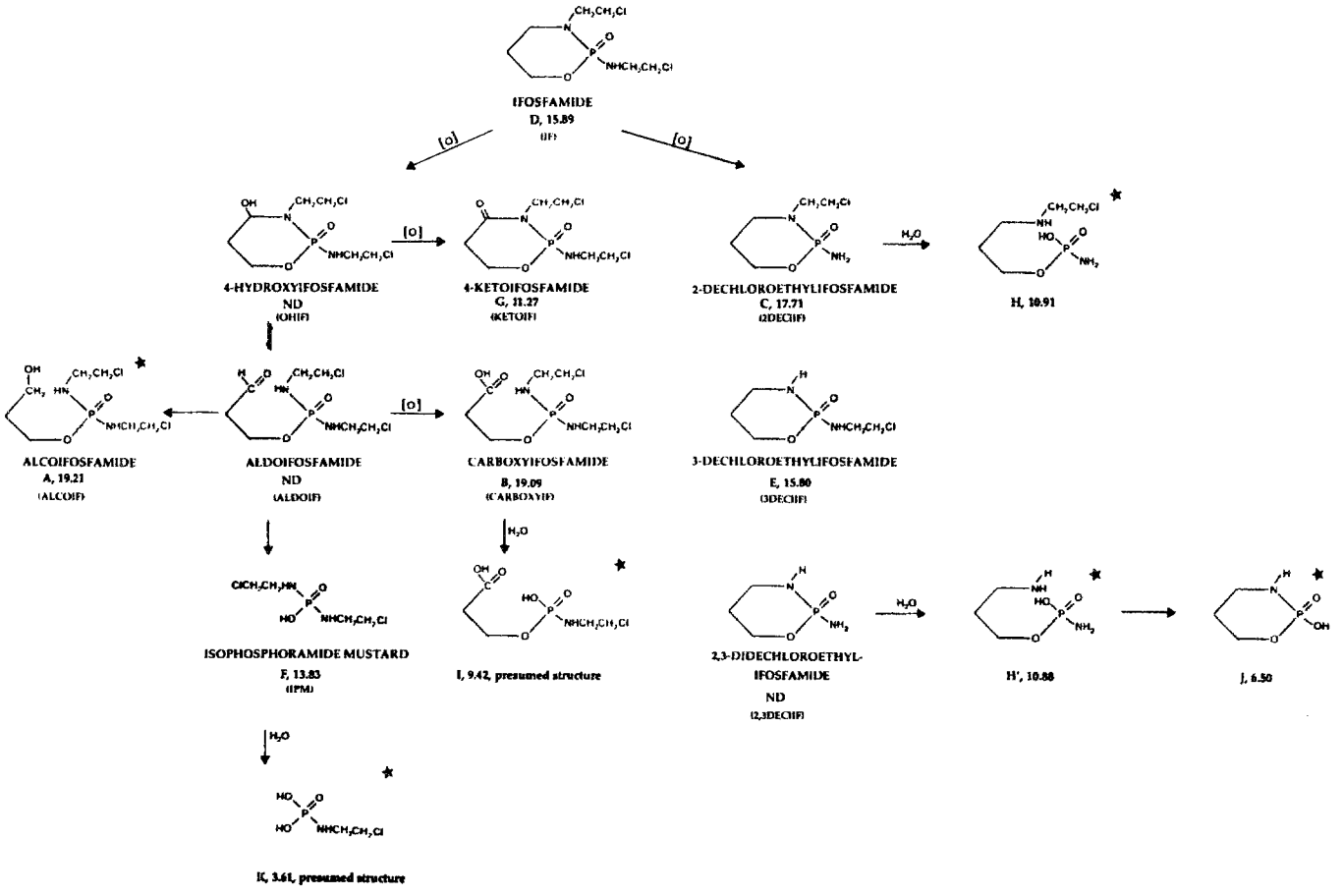


Fig. 3. Structures of the IF derivatives detected in concentrated urine samples using [^{31}P]-NMR. Chemical shifts are given for a pH of 5.9. *ND*, Not detected

dechloroethylation pathway, with the median value being 4.4%±2.7% (range, 2.4%–11.4%) of the delivered IF dose. Excretion of CARBOXYIF showed interpatient variability, with the median value being 3.4%±2.5% (range, 1.3%–9.6%) of the injected dose. Urinary levels of IPM and ALCOIF were low, with the median values being 0.2%±0.2% (range, 0–0.5%) and 0.8%±0.5% (range, 0.1%–1.7%) of the injected IF dose, respectively.

The unknown compound detected at 16.08 ppm represented a non-negligible fraction of the drug excreted in the urine (mean, 6.3%±2.1% of the injected IF dose; range, 3.0%–9.9%), which was higher than that of CARBOXYIF. Excretion of the other unknown compounds (18.98 ppm, 15.52 ppm, 13.01 ppm, 12.77 ppm, and 9.3–9.5 ppm as well as compounds with a δ of <5 ppm) amounted to 7.3%±4.8% (range, 1.1%–18.2%) of the injected IF dose.

The excretion of IF and its phosphorated metabolites was nearly equal in the 0- to 8-h (17.3%±8.7% of the delivered IF dose) and 8- to 16-h (21.3%±10.6% of the injected dose) fractions but was lower in the 16- to 24-h fraction (12.1%±3.9% of the original dose). The same phosphorated compounds were found in each 8-h fraction (Fig. 4), except that the unknown metabolite resonating at 15.52 ppm appeared in the 16- to 24-h fraction of urine obtained from eight of the nine patients studied. In one patient only, it could have been detected in the 8- to 16-h fraction. The proportions of IF and of the unknown compound resonating at 16.08 ppm decreased with time, whereas those of the dechloroethylated compounds and of CARBOXYIF increased. 3DECLIF was the main metabolite detected in each 8-h fraction. In the 0- to 8-h fraction, the unknown metabolite resonating at 16.08 ppm also represented an important part of the excretion.

The urinary excretion of IF and its metabolites was not complete at 24 h. In all, 13.6% and 9.0% of the injected IF dose, respectively, was recovered in the 24- to 48-h urine samples obtained from the two patients in whom sampling was continued for an additional 24-h, with the respective values for each patient amounting to 6.7% and 3.9% of the injected IF dose in the 24- to 32-h fraction, 5.1% and 4.2% of the delivered dose in the 32- to 40-h fraction, and 1.8% and 0.9% of the injected dose in the 40- to 48-h fraction. Unmetabolized IF was still detected and represented ≈20% of the excreted dose. The excretion mostly involved the dechloroethylated metabolites 3DECIIF (≈40% of the excreted dose) and 2DECIIF (≈12%). CARBOXYIF and the unknown metabolites resonating at 15.52 and 16.08 ppm represented ≈6%, 10%, and 8% of the excreted dose, respectively.

Table 3. Cumulative urinary excretion of IF and its phosphorated metabolites as measured over 24 h after the beginning of the 3-h i. v. infusion of 3 g/m^2 IF

	Mean	SD	Range
Total	50.6%	10.6%	38.6%–72.3%
Unmetabolized IF	17.9%	4.7%	11.4%–28.5%
Total of metabolites	32.7%	8.7%	19.3%–47.8%
DECLIF[a]	14.9%	3.6%	8.5%–20.4%
3DECLIF	10.5%	2.7%	5.5%–15.8%
2DECLIF	3.9%	1.3%	1.0%–5.7%
DECLIF degradation compounds[b]	0.4%	0.6%	0–1.8%
Ratio of 3DECLIF/2DECLIF	3.3	2.2	1.4–9.0
CARBOXYIF+IPM+ALCOIF	4.4%	2.7%	2.4%–11.4%
CARBOXYIF	3.4%	2.5%	1.3%–9.6%
IPM	0.2%	0.2%	0–0.5%
ALCOIF	0.8%	0.5%	0.1%–1.7%
18.98 ppm	0.2%	0.3%	0–1.0%
16.08 ppm	6.3%	2.1%	3.0%–9.9%
15.52 ppm	0.5%	0.5%	0–1.2%
13.01 ppm	0.6%	0.5%	0–1.6%
12.77 ppm	0.3%	0.4%	0–0.6%
9.3–9.5 ppm	2.8%	3.1%	0.9%–11.5%
Others[c]	2.8%	1.9%	0.2%–6.6%
Total of unknown compounds[d]	7.3%	4.8%	1.1%–18.2%

Data are expressed as a percentage of the injected dose and represent the mean of the values obtained in nine patients

[a] Includes 2DEClIF, 3DEClIF, and compounds H, H′, and J

[b] Includes compounds H, H′, and J

[c] Compounds with a δ of <5 ppm

[d] Except the compound resonating at 16.08 ppm

Discussion

This paper describes a simple method for assaying the urinary excretion of IF and its phosphorated metabolites. Its main advantage is that it allows the individual assay of all of the phosphorated metabolites of IF in a single analysis without the need for prior extraction.

In addition to the classic metabolites of IF, four new phosphorated compounds detected in urine samples were identified. The signal at 19.21 ppm corresponded to ALCOIF. To our knowledge, the presence of ALCOIF in human biofluids has not previously been reported; ALCOIF has been detected only in the blood of mice and in the urine of dogs [21]. The signals at 10.91, 10.88, and 6.50 ppm corresponded to compounds H, H′, and J, respectively (Fig. 3). These compounds are degradation products of 2DEClIF and 2,3DEClIF. Spontaneous decomposition of these metabolites may thus occur during the storage of urine before freezing and/or in the bladder of humans.

Table 4 summarizes the data obtained for the urinary excretion of IF and its metabolites by several groups of investigators and in the present study using various analytical techniques. In the present study, unmetabolized IF was the major compund recovered in the urine (mean, 18% of the injected dose). This amount agrees with the values reported by several authors for similar doses [2, 10, 14–16] but is higher than those obtained by Lind et al. [13].

The major urinary metabolites detected in the present study were the dechloroethylated compounds. The levels recovered are similar to those we previously found in a preliminary [^{31}P]-NMR study in three patients [14] but are much higher than the values obtained by Norpoth [18] and Lind et al. [13] using thin-layer chromatography plates on which 2DEClIF and 3DEClIF are not separated. In contrast, we found lower amounts of dechloroethylated compounds than did Boos et al. [2] or Goren [8]. This difference is reflected in the detected levels of 3DEClIF, which were lower in our study than in the two previously cited, rather than in the recovery of 2DEClIF, which in the present study was identical to that reported by Boos et al. [2] but was slightly lower than that reported by Goren [8]. However, it should be pointed out that the studies of these authors concerned children, whereas ours involved adult patients. Like Goren [8] and Boos et al. [2], we also ob-

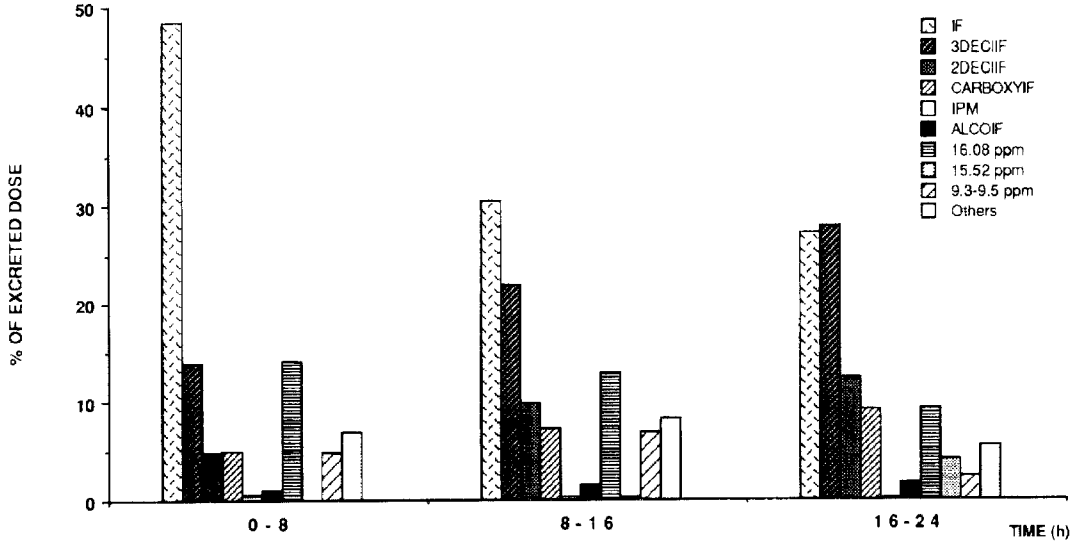


Fig. 4. Proportions of IF and its metabolites detected in urine samples, expressed as the mean of values obtained in nine patients treated with 3 g/m^2 IF infused i. v. over 3 h. The histogram entitled *2DEClIF* includes the proportions of 2DEClIF and those of compounds H, H′, and J. The histogram entitled *Others* includes the proportions of the unknown compounds detected at 18.98, 13.01, and 12.77 ppm as well as the proportions of those with a δ of <5 ppm

Table 4. Comparison of the urinary excretion of IF and its metabolites as reported in the literature and as determined in the present study

Author(s)	Creaven et al. [7]	Norpoth [18]	Allen et al. [1]	Nelson et al. [16]	Misiura et al. [15]	Ninane et al. [17]	Lind et al. [13]	Goren [8]	Boos et al. [2]	Martino et al. [14]	Present study
Number and age of patients	5, 42–63 years	5	2	3	1, 19 years	8 children	10, 44–71 years	5 children	11 children	3	9, 21–79years
Dose	5 g/m^2	5 g	9.5 or 7.75 g	1.6 or 2.4 g/m^2	2 g/day	3 g/m^2	1.5 g/m^2/daily	1.6 g/m^2	0.8–3 g/m^2	3 g/m^2	3 g/m^2
Administration	45-min i. v. infusion		short i. v. infusion	30-min i. v. infusion	i. v. infusion	1-h i. v. infusion	30-min i. v. infusion	15-min i. v. infusion	1-h or continuous i. v. infusion	3-h i. v. infusion	3-h i. v. infusion
Urine sampling	24-h urine	18-h urine	24-h urine	24-h urine	24-h urine on day 3[d]	24-h urine	24-h urine	24-h urine	up to 24 h after end of therapy	24-h urine	24-h urine
Analytical method	radioactivity; GC[a] for IF	TLC (PBH)[a, b]	GC[a] for IF; NBP[b] and colorimetry	GC[a] for IF	[^{31}P]-NMR	NBP[b] and colorimetry	TLC (NBP)-PD[a, b]	HPLC[a]	CGC[a]	[^{31}P]-NMR	[^{31}P]-NMR
Unmetabolized IF	32%–37%	8%	50% (9.5 g) 39% (7.75 g)	14% (1.6 g/m^2) 15.5% (2.4 g/m^2)	17.4%		2.58% (0–6.37%)	18% (11%–30%)	21% (14%–34%)	16.6%	18% (11%–29%)
2DECIIF +3DECIIF		6%			17.2%		1.73% (0.75%–3.91%)	23% (15%–31%)	18% (12%–31%)	15.0%	14% (8%–20%)
3DECIIF					13.0%			16% (11%–21%)	14% (9%–29%)	10.4%	11% (6%–16%)
2DECIIF					4.2%			7% (3%–10%)	4% (2%–8%)	4.6%	4% (1%–6%)
Ratio of 3DECIIF/ 2DEC1IF					3.1[c]			2.6[c] (1.9– 4.2)[c]	3.9 (2–11)	2.3[c]	3.3 (1.4–9.0)
CARBOXYIF		9%			3.1%		1.30% (0.04%–3.9%)			3.3%	3.4% (1.3%–9.6%)
IPM					0		0 (0–1.47%)				0.17% (0–0.5%)
KETOIF					2.1%		0.064% (0–0.32%)[e]			0	0
Total metabolites	19%–29%	15%[c] alkylating metabolites	18% (9.5 g) 16% (7.75 g) alkylating metabolites		22.4%	18.7% (13.7%–26.1%) alkylating metabolites	3.45% (1.18%–7.8%) DECIIF+CARBOXYIF +IPM	23%[c] DECIIF	18%[c] DECIIF		33% (19%–48%)

Data are expressed as a percentage of the delivered dose

[a] GC, Gas chromatography; TLC, thin-layer chromatography; TLC-PD, thin-layer chromatography with photographic densitometry; HPLC, high-performance liquid chromatography; CGC, capillary gas chromatography

[b] Reagents used for the measurement of alkylating activity: PBH, 4-pyridinealdehyde-2-benzothiazolylhydrazone; NBP, 4-(4′-nitrobenzyl)-pyridine

[c] Values not reported by the authors but calculated by us from their data

[d] First day of evaluation by the authors during a 10-day treatment period

[e] Values from Lind et al. [12]

served a predominance of 3DECIIF excretion over 2DECIIF elimination.

The excretion of dechloroethylated compounds in the present study was subject to interpatient variability, as was that previously reported by Norpoth [18], Lind et al. [13], and Goren [8]; however, this variability was more moderate than that observed for CARBOXYIF excretion. Norpoth [18] and Lind et al. [13] also noted great interpatient variability in urinary levels of CARBOXYIF. In our series of nine patients, the excretion of dechloroethylated derivatives was always higher than that of CARBOXYIF, in contrast to the observations of Norpoth [18], who reported the same finding in only 14 of the 25 patients he studied. The levels of IPM recovered from the urine were low but are in concordance with those reported by Lind et al. [13]. The absence of [^{31}P]-NMR-detectable levels of KETOIF in unconcentrated urine samples in the present study is not surprising, since Lind et al. [12] measured very low levels of this metabolite in the urine of ten patients. The percentage of dose metabolized in our study is clearly higher than that reported by other authors; however, it is not readily comparable with other published results, as the [^{31}P]-NMR assay includes all of the phosphorated IF metabolites, which may not be the case for the methods used to measure global alkylating activity. The excretion of ALCOIF, compounds H, H′, and J, and all of the unknown phosphorated compounds of IF represented 14.8% of the injected dose, i.e., about half of the total excretion of metabolites. The interpatient variation in levels of IF metabolites was obvious, even in the homogeneous series of patients studied (adults, same dose, same protocol of administration). This variability, which is typical of the oxazaphosphorines, has been established by several authors [13, 17, 18].

The urinary excretion of IF and its metabolites was not complete at 24 h, since ≈11% of the injected dose was recovered in the 24- to 48-h urine samples obtained from two patients, mainly as dechloroethylated metabolites and unchanged IF. This observation contrasts with the findings of Ninane et al. [17], who previously reported that the renal elimination of alkylating metabolites of IF was almost complete after the first 24 h of treatment in children receiving 3 g/m^2 IF as a 1-h infusion.

References

1. Allen LM, Creaven PJ, Nelson RL (1976) Studies on the human pharmacokinetics of isophosphamide (NSC-109724). Cancer Treat Rep 60: 451
2. Boos J, Welslau U, Ritter J, Blaschke G, Schellong G (1991) Urinary excretion of the enantiomers of ifosfamide and its inactive metabolites in children. Cancer Chemother Pharmacol 28: 455
3. Brock N (1979) The special position of ifosfamide in the series of cytostatically active oxazaphosphorines. In: BW Fox (edn) Advances in medical oncology, research and education, vol 5. Basis for cancer therapy 1. Pergamon, Oxford New York, p 39
4. Brock N (1989) Oxazaphosphorine cytostatics: past-present-future. Seventh Cain Memorial Award lecture. Cancer Res 49: 1
5. Brock N, Hohorst HJ (1977) The problem of specificity and selectivity of alkylating cytostatics: studies on *N*-2-chloroethylamidooxazaphosphorines. Z Krebsforsch 88: 185
6. Brock N, Hilgard P, Peukert M, Pohl J, Sindermann H (1988) Basis and new developments in the field of oxazaphosphorines. Cancer Invest 6: 513
7. Creaven PJ, Allen LM, Alford DA, Cohen MH (1974) Clinical pharmacology of isophosphamide. Clin Pharmacol Ther 16: 77
8. Goren MP (1991) Determination of urinary 2- and 3-dechloroethylated metabolites of ifosfamide by high-performance liquid chromatography. J Chromatogr Biomed Appl 570: 351
9. Goren MP, Wright RK, Pratt CB, Pell FE (1986) Dechloroethylation of ifosfamide and neurotoxicity. Lancet II: 1219
10. Goren MP, Pratt CB, Viar MJ (1989) Tubular nephrotoxicity during long-term ifosfamide and mesna therapy. Cancer Chemother Pharmacol 25: 70
11. Lewis LD, Meanwell CA (1990) Ifosfamide pharmacokinetics and neurotoxicity. Lancet 335: 175
12. Lind MJ, Margison JM, Cerny T, Thatcher N, Wilkinson PM (1989) Comparative pharmacokinetics and alkylating activity of fractionated intravenous and oral ifosfamide in patients with bronchogenic carcinoma. Cancer Res 49: 753
13. Lind MJ, 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
14. Martino R, Crasnier F, Chouini-Lalanne N, Gilard V, Niemeyer U, Forni M de, Malet-Martino MC (1992) A new approach to the study of ifosfamide metabolism by the analysis of human body fluids with ^{31}P nuclear magnetic resonance spectroscopy. J Pharmacol Exp Ther 260: 1133
15. 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
16. Nelson RL, Allen LM, Creaven PJ (1977) Pharmacokinetics of divided-dose ifosfamide. Clin Pharmacol Ther 19: 365
17. 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]: S2
18. Norpoth K (1976) Studies on the metabolism of isophosphamide (NSC-109724) in man. Cancer Treat Rep 60: 437
19. Sladek NE (1988) Metabolism of oxazaphosphorines. Pharmacol Ther 37: 301
20. Smeitink J, Verreussel M, Schröder C, Lippens R (1988) Nephrotoxicity associated with ifosfamide. Eur J Pediatr 148: 164
21. Struck RF, Dykes DJ, Corbett TH, Suling WJ, Trader MW (1983) Isophosphoramide mustard, a metabolite of ifosfamide with activity against murine tumours comparable to cyclophosphamide. Br J Cancer 47: 15
22. Watkin SW, Husband DJ, Green JA, Warenius HM (1989) Ifosfamide encephalopathy: a reappraisal. Eur J Cancer Clin Oncol 25: 1303