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ORIGINAL ARTICLE

Variations in schedules of ifosfamide administration: a better understanding of its implications on pharmacokinetics through a randomized cross-over study

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Abstract
Purpose The metabolism of ifosfamide is a delicate balance between a minor activation pathway (4-hydroxylation) and a mainly toxification pathway (N-dechloroethylation), and there remains uncertainty as to the optimal intravenous schedule.
Methods This study assesses ifosfamide pharmacokinetics (PK) according to two standard schedules. Using a 1:1 randomized trial design, we prospectively evaluated ifosfamide PK on two consecutive cycles of 3 g/m²/day for 3 days (9 g/m²/cycle) given in one of two schedules either by continuous infusion (CI) or short (3 h) infusion. Highly sensitive analytical methods allowed determination of concentrations of ifosfamide and the key metabolites 4-hydroxy-ifosfamide, 2- and 3-dechloroethyl-ifosfamide.
Results Extensive PK analysis was available in 12 patients and showed equivalence between both schedules (3 h versus CI) based on area under the curves (μmol/l × h) for ifosfamide, 4-hydroxy-ifosfamide, 2- and 3-dechloroethyl-ifosfamide (9,379 ± 2,638 versus 8,307 ± 1,995, 152 ± 59 versus 161 ± 77, 1,441 ± 405 versus 1,388 ± 393, and 2,808 ± 508 versus 2,634 ± 508, respectively, all $P > 0.2$). The classical auto-induction of metabolism over the 3 days of infusion was confirmed for both schedules.
Conclusion This study confirms similar PK for both active and toxic metabolites of ifosfamide in adult cancer patients when 9 g/m² of ifosfamide is administered over 3 days by CI or daily 3-h infusions.

Keywords Alkylating agents · Ifosfamide · Pharmacodynamics · Pharmacokinetics

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Introduction

Ifosfamide is an anti-cancer alkylating agent widely used in daily oncology practice. It belongs to the class of oxazaphosphorines, whose leading compound is its congener cyclophosphamide [6]. Although both drugs share the same chemical structure and final mechanism of action, they have repeatedly demonstrated different pharmacological properties including non-overlapping spectra of toxicity and efficacy. These observations derive partly from important differences in the metabolism of these cytotoxic agents.

Both are prodrugs, requiring in vivo activation by liver cytochromes P450 (CYP) to exert their cytotoxic activity. Their metabolism is best described as a fragile balance between a true activation pathway and a presumed deactivation or toxification pathway [6, 30]. The former relies on a hydroxylation reaction forming a 4-hydroxylated metabolite (4-hydroxy-ifosfamide or 4-hydroxy-cyclophosphamide) and its ring-opened aldo tautomer, which ultimately decomposes within the cell to yield the corresponding and therapeutically beneficial alkylating mustard, releasing at the same

time acrolein by β elimination. The final mustard being highly ionized is unable to cross the cell membrane; both 4-hydroxy-ifosfamide and 4-hydroxy-cyclophosphamide are considered as the transport forms for "activated" oxazaphosphorines. The second pathway consists of an N-dechloroethylation reaction and yields metabolites considered either inactive [dechlorethyl-cyclophosphamide or 2- and 3-dechloroethyl-ifosfamide] or potentially toxic (chloroacetaldehyde) in a 1:1 molar ratio [14, 29, 35], although some preclinical works confer also part of ifosfamide anti tumour activity to chloroacetaldehyde [9].

Both reactions are catalysed by different subsets of liver microsomes depending on the drug and pathway studied. In human, CYP 2B6 is the main enzyme responsible for cyclophosphamide 4-hydroxylation [12, 27], with a final reported rate of activation reaching up to 90%, making easy the use of such compound in the clinic [6]. Conditions are far from being similar with ifosfamide, CYP 3A4 being the major actor of metabolism, but contributing at the same time to both activation and toxification pathways [12, 27]. Moreover CYP 3A4 low catalytic affinity for its substrate gives metabolic importance to other CYP subsets (CYP 2B6 and 2B1), which may result in a rate of ifosfamide toxification exceeding 50% [6].

In order to improve the narrow therapeutic ratio of ifosfamide, several strategies have been investigated including (1) the use of specific inducers and inhibitors of liver enzymes to divert ifosfamide metabolism towards the beneficial 4-hydroxylation pathway [19], (2) the utilization of the well-established autoinduction mechanism observed when doses of oxazaphosphorines are fractionated over several days [2, 6, 22], and (3) the use of different schedules of intravenous administration, continuous infusion (CI) or short (3–4 h) infusion [6, 17, 23]. There remains controversy with regard to this third option, with some authors reporting fewer side effects with CI, and others advocating short infusions to improve efficacy [1, 10, 26]. Although different groups have tried to answer this question with pharmacokinetic (PK) studies in both children and adults [3, 7, 20, 28], they have used various analytical techniques with low sensitivity [4] and the investigated schedules have mostly involved administration of ifosfamide in combination with other cytotoxic agents. Furthermore data exploring the activation pathway have often been incompletely reported or have focused on debatable PK endpoints such as ifosfamide mustard [20] rather than the transport form of "activated ifosfamide", namely 4-hydroxy-ifosfamide [30].

The aims of this study were therefore to compare the PK of ifosfamide and its main metabolites in a randomized cross-over study using the same dose of ifosfamide (9 g/m^2) monotherapy fractionated on 3 days, delivered either by CI or short (3-h) daily infusion, in an homogeneous patient population and employing recently developed highly sensitive analytical methods [22, 33].

Materials and methods

Study design

This was a single centre phase II PK study, enrolling patients with advanced solid tumours. Eligible patients were at least 18 year old, with Eastern Cooperative Oncology Group performance status of 0–2 and adequate end organ function: neutrophils count ≥1,500/mm^3, platelets count ≥100,000/mm^3, serum creatinine ≤120 μmol/l and/or creatinine clearance ≥80 ml/min/1.73 m^2 (estimated by Cockroft-Gault formula). Patients were randomly assigned to receive ifosfamide 3 g/m^2 × 3 days (total dose 9 g/m^2/cycle) (HOLOXAN®, Baxter Oncology, France) as either a daily 3-h infusion dose (3-h schedule) or a 72-h CI (CI schedule) for the first cycle. The same total dose of sodium-2-mercaptoethane-sulphonate (MESNA®, Baxter Oncology France) was administered, either mixed in the same vial as ifosfamide for the CI schedule or split over three short bolus injections for the 3-h schedule (just before ifosfamide, 4, then 8 h after start of ifosfamide infusion). On the second cycle, patients were crossed-over to the alternative schedule. Cycles were given every 4 weeks, provided the same criteria were met as at inclusion. Comedications were carefully screened. When showing potential interferences with CYP3A4 as inducers (e.g. steroids, phenobarbital) or inhibitors (e.g. erythromycin, diltiazem, verapamil, ketoconazole), they were to be avoided to the extent of possible. From the third cycle onwards, all patients were to be treated using the short infusion schedule unless a true difference of side effects had been detected between cycle 1 and 2. Hyper-hydration included systematically 500 ml of Na·HCO_3^- 1.4%. Emesis was prevented with standard anti-HT3 treatment and alizapride, but excluding steroids. Primary prophylaxis for febrile neutropenia was not permitted. The primary endpoint of the study was to compare the PK profiles of ifosfamide and its main metabolites (4-hydroxy-ifosfamide, 2- and 3-dechloroethyl-ifosfamide) across both the first and second cycles, using a full sampling strategy. Toxicity and efficacy data were secondary endpoints, and collected during cycles 1 and 2. Patients having received only one cycle of either schedule were therefore inevaluable and were to be replaced.

The trial was sponsored by René Huguenin Cancer Centre. Study investigations were performed after approval by the ethics committee of Saint-Germain en Laye (78 Yvelines) and by the institutional review board. All patients provided written informed consent. The trial was conducted according to Good Clinical Practice, International Conference on Harmonization rules and "Loi Huriet".

Pharmacokinetic points

For the 3-h schedule, blood samples (3 ml) were drawn daily immediately before treatment, then at the following intervals: end of infusion (3 h), then 5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 5 h, 7 h and 21 h following the end of infusion. For the CI schedule, fewer samples were drawn: before treatment on day 1, then 6, 12, 24, 32, 48, 56 and 72 h following the start of infusion, then 1, 6 and 24 h after the end of infusion.

Analytical methods

Hydroxylation pathway was investigated through the determination of 4-hydroxy-ifosfamide in blood, according to a method originally developed for the analysis of 4-hydroxy-cyclophosphamide and based on the fluorometric detection of 7-hydroxyquinoline resulting from the condensation of 4-hydroxy-ifosfamide and acrolein [33]. Immediately following venesection, a 1 ml aliquot of blood was added to 3 ml of aqueous 12% perchloric acid, 500 µl of 10 mg/ml 3-aminophenol, 12 mg/ml hydroxylamine hydrochloride in 1.0 M HCl, and 40 µl of aqueous 10 µM methyl vinyl ketone (internal standard). After vortex and addition of 100 µl of 10% sodium tungstate, samples were centrifuged for 15 min, heated at 100°C in a boiling water bath for 15 min, then cooled in the dark. Derivatized samples were analysed by an high performance liquid chromatography system including two Waters 515 pumps, an isocratic mobile phase (0.1 M ammonium acetate pH 4.0 ± glacial acetic acid + 9% acetonitrile, flow rate 1 ml/min), a Waters µBondapak phenyl guard-pak and a post-acidification column flowed by 0.3 M trifluoroacetic acid delivered at 0.5 ml/min. The fluorescent hydroxyquinoline derivatives were quantified with a Kontron 5FM25 fluorescence detector at excitation and emission wavelengths of 350 and 500 nm, respectively. Standards were prepared with 0–140 µM of 4-hydroperoxy-ifosfamide or acrolein (gifts from Dr Pohl, ASTA MEDICA, Germany). Retention times were 9 min for 4-hydroxy-ifosfamide and 14 min for internal standard (4-methyl-7-hydroxyquinoline). Lower limits of quantification and detection for 4-hydroxy-ifosfamide were 0.2 and 0.07 µmol/l, respectively. Recovery rate was 77 ± 6% in total blood.

Gas chromatography with selective nitrogen-phosphorus detection was used for the investigation of the dechloroethylation pathway, allowing the simultaneous determination of ifosfamide, 2- and 3-dechloroethyl-ifosfamide in plasma as described previously [22]. Standards were prepared with 2- and 3-dechloroethyl-ifosfamide (gifts from Dr Pohl, ASTA MEDICA, Germany). Values for lower limits of quantification and detection were, respectively, 0.5 and 0.15 µmol/l for dechloroethylated metabolites and 0.4 and 0.11 µmol/l for ifosfamide.

Statistical methods

Plasma concentrations (µmol/l for ifosfamide, 2-dechloroethyl-ifosfamide, 3-dechloroethyl-ifosfamide and 4-hydroxy-ifosfamide were plotted as a function of time. Area under the curve (AUC) was calculated by the trapezoidal method. Non-compartmental PK analysis included maximal concentration (C_{max}, µmol/l) and AUC for ifosfamide and each metabolite of interest. Terminal half-life ($T_{1/2}$, h) and clearance (l/h) were calculated for the parental drug. Analyses of variance (ANOVA) were made using the R statistical package [16] and included the role of sequence (group 1: cycle 1, 3-h schedule, cycle 2, CI schedule; group 2: reverse sequence). Effects were regarded as statistically significant for $P < 0.05$.

Results

Population

Amongst 17 patients included in the study, five stopped chemotherapy following first cycle for early tumour progression or decline (1 patient in CI group and four patients in 3-h group), and were not considered for this PK report. Baseline characteristics for the 12 patients with available extensive PK analysis are described in Table 1.

Toxicity and efficacy

Table 2 reports main side effects recorded during cycles 1 and 2 according to schedule of ifosfamide administration. At least one episode of grade 4 neutropenia was observed in each patient, with no significant difference of rate of febrile neutropenia across groups (25% with the 3-h schedule versus 33% with the CI schedule).

Table 1 Baseline characteristics in 12 patients having completed cycles 1 and 2

Characteristic	No.
Sex ratio (male/female)	6/6
Age (years), median	50.5 (35–63)
Body surface area (m^2)	1.70 (1.39–1.89)
Primary tumour	
Head and neck	7
Sarcoma	3
Breast	1
Uterus	1
Metastatic setting	7
Locoregional disease	5
Prior radiotherapy	10
Prior surgery	7
Prior chemotherapy	
1 line	11
2 lines	1
Previous exposition to ifosfamide	3

Table 2 Main side effects (National Cancer Institute Common Toxicity Criteria) according to schedule of administration in 12 patients and 24 cycles

Characteristic	3-h infusion (n = 12) no. (%)	Continuous infusion (n = 12) no. (%)
Neutropenia		
Grade 3	1 (8)	3 (25)
Grade 4	11 (92)	8 (67)
Febrile neutropenia	3 (25)	4 (33)
Anaemia		
Grade 3	1 (8)	0 (0)
Grade 4	1 (8)	1 (8)
Thrombopenia		
Grade 2	3 (25)	0 (0)
Grade 3	0 (0)	2 (17)
Creatinine grade 2	0 (0)	2 (17)
Encephalopathy	1 (8)	0 (0)

One 63-year-old man with head and neck tumour developed generalized epilepsy at the end of the ifosfamide 3-h infusion on day 2 of the second cycle. He was found to be chronically treated "over the counter" with carbamazepine, a well-known inducer of CYP 450 enzymes [21]. The oxazaphosphorine might have affected with 48-h delay the metabolism of the central nervous system agent, lowering the observed plasma concentration (7 μM/l) below therapeutic range (17–51 μM/l). The patient having recovered within a few hours, day 3 of ifosfamide was administered as planned after adjustment of oral dose for carbamazepine and without any further evidence of epilepsy or encephalopathy.

All the 12 patients are evaluable for response, as they have completed the 2 PK investigational cycles of ifosfamide. Partial response was achieved in one woman with metastatic breast cancer (1/1) and in three patients with advanced head and neck carcinoma (3/7).

Pharmacokinetics

During the 3-h schedule, the peak of ifosfamide concentration was observed immediately after the end of infusion on day 1. The rise was more gradual for the CI schedule and peaked at the end of first day. Only the patient on chronic carbamazepine therapy showed lower ifosfamide concentrations reflecting an upfront twofold increase in ifosfamide clearance.

There was no significant effect on PK parameters due to sequence (group 1: cycle 1, 3-h schedule, and cycle 2, CI schedule versus group 2: reverse sequence), as assessed by the ANOVA. Integration of concentrations obtained in the patient chronically treated with carbamazepine did not affect the results of ANOVA. Therefore, data for all cycles with same schedule (i.e. 12 patients for each schedule) have been pooled in Table 3, with no significant difference between 3-h and CI schedules except, as expected, for ifosfamide C_{max} (431 ± 88 versus 163 ± 42 μmol/l, $P = 0.03$).

Auto-induction of ifosfamide metabolism was observed for both schedules as illustrated by the evolution of AUC over time in Fig. 1. The gradual decrease of AUC_{IF} over the 3 days of infusion reflected the rise of ifosfamide clearance by a factor 1.7 from day 1 to day 3 and was compensated by a concomitant increase in the AUC of the metabolites (all $P \leq 0.0001$). This increase was almost maximal by the second day with little further change between day 2 and day 3, especially for dechloroethylated metabolites.

Table 3 Pharmacokinetics and metabolism of ifosfamide 9 g/m^2 given on 3 successive days according to different intravenous schedules: 3-h versus continuous infusion (mean ± SD)

Parameter	3-h infusion (n = 12)	Continuous infusion (n = 12)	P**
Clearance (l/h/m^2)	4.3 ± 2.7	4.6 ± 2.2	0.09
$T_{1/2}$ (h)	4.1 ± 2*	4.2 ± 1.1	0.92
AUC_{IF} (μmol/l × h)	9,379 ± 2,638	8,307 ± 1,995	0.32
$AUC_{2\text{-DC-IF}}$ (μmol/l × h)	1,441 ± 405	1,388 ± 393	0.57
$AUC_{3\text{-DC-IF}}$ (μmol/l × h)	2,808 ± 508	2,634 ± 508	0.27
$AUC_{4\text{-OH-IF}}$ (μmol/l × h)	152 ± 59	161 ± 77	0.59
IF C_{max} (μmol/l)	431 ± 88	163 ± 42	0.03
2-DC-IF C_{max} (μmol/l)	39 ± 12	28 ± 8	0.84
3-DC-IF C_{max} (μmol/l)	69 ± 12	53 ± 10	0.75
4-OH-IF C_{max} (μmol/l)	6 ± 3	3 ± 2	0.98

*Value on day 3; **no effect of sequence for all parameters; 2-DC-IF, 2-dechloroethyl-ifosfamide; 3-DC-IF, 3-dechloroethyl-ifosfamide; 4-OH-IF, 4-hydroxy-ifosfamide; *AUC* area under the curve; C_{max}, maximal concentration; *IF* ifosfamide; *SD* standard deviation; $T_{1/2}$, terminal half-life

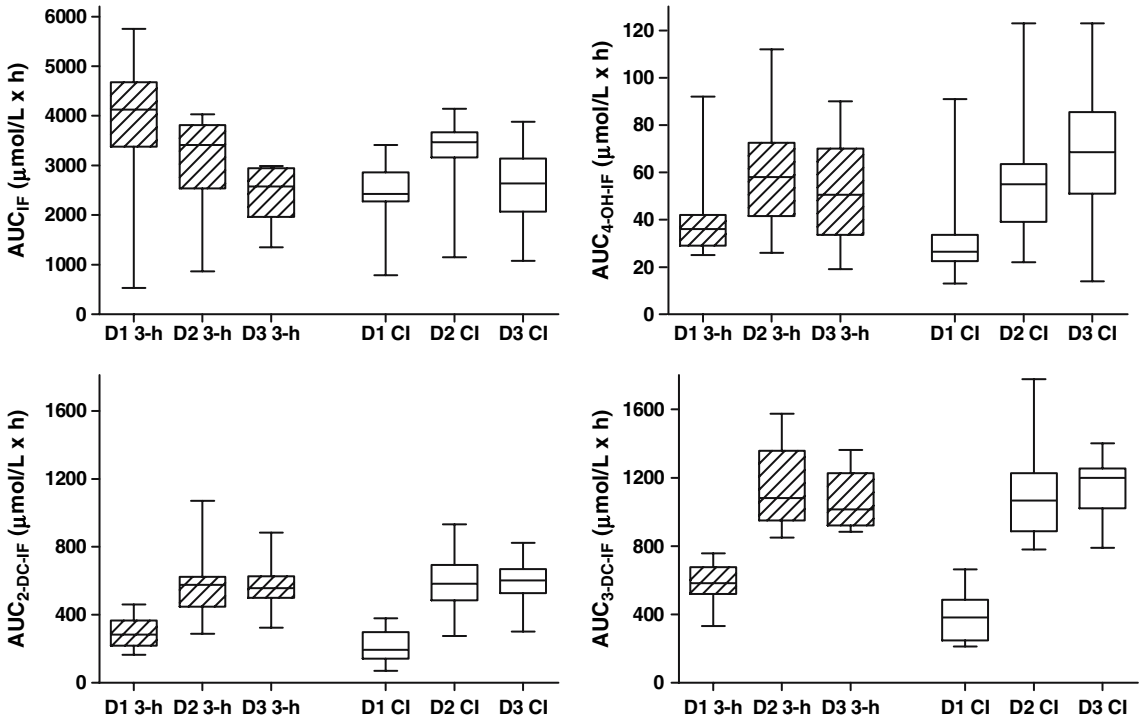

Fig. 1 Box and whiskers graphs for area under the curves of ifosfamide and main metabolites according to ifosfamide schedule of administration along 3 days

Discussion

Although ifosfamide was first introduced into the clinic in the late 1960s, its optimal schedule of administration is still questioned [6]. In contrast to the easy dosing of its sister compound cyclophosphamide, which is mainly metabolized via the hydroxylation pathway, ifosfamide prescription is more complex. This is due to an over-representation of dechloroethylation producing metabolites potentially responsible for the specific spectrum of toxicity seen with this compound [2, 5–7, 25], although some of these dechloroethylated metabolites might also play an important role in the therapeutic efficacy of ifosfamide [9]. This unbalance leads to a theoretical unfavourable therapeutic ratio, which has been the basis for the exploration of a large number of alternate schedules including oral, subcutaneous or long-term intravenous administration [11, 17, 23]. Early clinical reports suggested that CI would be better tolerated [1, 10] but this approach was challenged later by others warning against loss of efficacy [26].

Our study confirms the equivalence of these schedules based on AUC, the most valid measure of drug exposure [13], showing no beneficial influence of either schedule on the production of the therapeutically useful 4-hydroxy-ifosfamide, the transport form of "activated ifosfamide" [30]. The same lack of difference between schedules was also found for the production of presumed toxic dechloroethylated metabolites and for the release of the well-known phenomenon of ifosfamide auto-induction [6]. Although these results are in agreement with other previously published studies, with similar AUC values for ifosfamide and metabolites [3, 7, 28], they differ in several ways from these earlier studies and provide meaningful additional data on this complex metabolic phenomenon.

Firstly, previous works have often included paediatric populations [3, 7] which are not comparable with adults for drug metabolism [15]. In these trials, ifosfamide was co-administered with other cytotoxics, and the cycles compared for schedules of administration (CI versus short infusion) were often administered several months apart. The relevance of the reported significant decrease in the production of 3-dechloroethyl-ifosfamide in case of short infusion [AUC 1,640 (570–4,560) versus 1,960 (810–7,170) µmol/l × h, $P < 0.02$] can be questioned given the wide confidence interval of values [7]. Of note, in a paper related to the same series focusing on 8 of the 17 initial subjects, the occurrence of acute and chronic nephrotoxicity was correlated to a lower ifosfamide dechloroethylation, which is contradictory with the classical pharmacological hypothesis conferring nephrotoxicity and neurotoxicity to chloroacetaldehyde produced by this pathway [3]. This might indirectly revive the debate about the designation of dechloroethylation as a "true" toxification pathway, as

suggested by some authors who have shown recently that chloroacetaldehyde may cause also DNA-strand-breaks and strong inhibition of DNA-synthesis [9].

A separate English study enrolled 11 adult patients in a PK program very similar to the design of ours (with intrapatient cross-over). However, patients were also treated with polychemotherapy including ifosfamide, and according to different schedules (2 or 3 days) [28]. Some declined venesection, while others had more than 2 cycles explored. Furthermore, the thin-layer chromatography analytical technique used in this study to analyse ifosfamide mustard [4] has a much lower sensitivity and plasma recovery rate than in ours for the identification of 4-hydroxy-ifosfamide [33] (lower limit of detection 4.6 versus 0.07 μmol/l, respectively). Consequently, the values of ifosfamide mustard in the serum were regarded as too unreliable and were disregarded, precluding any solid conclusion on the influence of schedule on ifosfamide activation pathway [28].

Finally, in both discussed studies [3, 7, 28], investigation of hydroxylation pathway was attempted through the determination of ifosfamide mustard, whereas our study selected 4-hydroxy-ifosfamide since it is considered by many to be the best plasma reflection of the amount of ifosfamide mustard reaching the tumour cell nucleus [30]. However, as reported, that AUC values for ifosfamide mustard tend to be an order of magnitude higher than those for 4-hydroxy-ifosfamide in our study (1,190–2,080 μmol/l × h depending on schedule and population versus 152–161 μmol/l × h, respectively) [5, 7] does not close the debate regarding the best PK surrogate marker for ifosfamide alkylating activity. That only strenghtens the high unsteadiness of the hydroxylated metabolite.

However, our data cannot exclude potentially different pharmacodynamic effects for the two schedules, as no interpretation regarding toxicity or efficacy can be drawn from such a small heterogeneous patient population. Peak plasma drug concentrations may contribute to final cytotoxicity although this is less likely to be the case for cell cycle-independent compounds such as alkylating agents [24]. Nevertheless, preclinical work has suggested an increased cytotoxicity with low concentrations of 4-(*S*-ethanol)-sulfido-cyclophosphamide, a prodrug for 4-hydroxy-cyclophosphamide, when maintained above a threshold for prolonged time with repeated intraperitoneal injections, as compared to a single intravenous injection of the same cumulative total dose [31]. Although the only significant difference between the schedules was the C_{max} for ifosfamide, preclinical data that is important in the alkylating effects of ifosfamide are lacking and therefore warrant further investigation.

It is of interest to note that the observed increase in the ifosfamide clearance was seen in the patient pretreated with carbamazepine as it stresses the theoretical impact of chronically coadministered CYP modulators on ifosfamide PK, despite a discrepancy between attractive results at the preclinical level [8, 32, 34] and disappointing attempts of application in patients [19, 28]. Since there is a possibility of increased metabolism and consequent decreased ifosfamide efficacy upon chronic coadministration of CYP 3A4 inducers such as steroids widely used in the clinic for anti-emetic purposes, care needs to be taken with regard to concomitant medication when administering such cytotoxic agents.

In conclusion, this extensive study of the key ifosfamide metabolites provides the most definitive positive answer to the question of equivalence between CI and short infusion schedules based on AUC, when ifosfamide is administered as a monotherapy, with an identical dose (9 g/m²) given over 3 days, in an adult population. Further work will focus on the development of new PK models to compare with those already published [18], adding pharmacodynamic assessments to allow simulations of less toxic schedules and prior investigations in the clinic.

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