Evaluation of the autoinduction of ifosfamide metabolism by a population pharmacokinetic approach using NONMEM

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Aims This study investigated the population pharmacokinetics of ifosfamide in 15 patients treated for soft tissue sarcoma with 9 or 12 g m⁻² ifosfamide by means of a 72 h continuous i.v. infusion.

Methods A model was developed using nonlinear mixed effects modelling (NONMEM) to describe the nonlinear pharmacokinetics of ifosfamide by linking the ifosfamide plasma concentrations to the extent of the autoinduction.

Results The proposed model revealed the effect of autoinduction on the disposition of ifosfamide. The initial clearance, volume of distribution, rate constant for enzyme degradation, induction half-life of the enzyme and the ifosfamide concentration at 50% of the maximum inhibition of enzyme degradation were estimated at 2.94 ± 0.27 l h⁻¹, 43.5 ± 2.9 l, 0.0546 ± 0.0078 h⁻¹, 12.7 h and 30.7 ± 4.8 µM, respectively. Interindividual variabilities of initial clearance, volume of distribution, rate constant for enzyme degradation were 24.5, 23.4 and 22.7%, respectively. Proportional and additive variability not explained by the model were 13.6% and 0.0763 µM, respectively.

Conclusions The absence of a lag time for the autoinduction of ifosfamide metabolism could be the result of an immediate inhibition of the enzymatic degradation of CYP3A4 by ifosfamide. By application of the autoinduction model individual pharmacokinetic profiles of patients were described with adequate precision. This model may therefore be used in the future development of a model to individualize dose selection in patients.

Keywords: autoinduction, ifosfamide, NONMEM, population pharmacokinetics

Introduction

Ifosfamide ((chloro-2-ethyl)-3[(chloro-2-ethyl)amino]-2-tetrahydro-2-H-oxazaphosphorine, Holoxan[®]) is an alkylating oxazaphosphorine widely applied as an anti-cancer agent in the treatment of various solid and haematological malignancies in adults and children [1, 2]. Ifosfamide is a prodrug requiring activation to 4-hydroxyifosfamide as depicted in Figure 1. After β-elimination of the urotoxic acrolein from 4-hydroxyifos-

famide, the alkylating ifosforamide mustard is formed [3]. Deactivation of ifosfamide leads to 2- and 3-dechloroethylifosfamide and the release of the potentially neurotoxic chloroacetaldehyde. Both activation (hydroxylation) and deactivation (dechloroethylation) are mediated by the cytochrome P450 3A4 (CYP3A4) iso-enzyme [4, 5]. Metabolism of ifosfamide is an autoinducible enzymatic process, resulting in increased clearance over time [6].

During the last decades several methods have been proposed for the description of the pharmacokinetics of ifosfamide and its metabolites [7]. Allen & Creaven and Lind *et al.* used a two-compartment pharmacokinetic model for describing the pharmacokinetics of ifosfamide [6, 8]. Nelson *et al.* proved a one-compartment pharmacokinetic model to be superior for 1.5–2.4 g m⁻² in 30 min i.v. infusion but not when

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Figure 1 Metabolism of the prodrug ifosfamide. Ifosfamide is activated through 4-hydroxyifosfamide to the ultimate alkylating agent ifosforamide mustard by β -elimination of urotoxic acrolein. Ifosfamide is deactivated to 2-and 3-dechloroethylifosfamide yielding neurotoxic chloroacetaldehyde.

dosed $3.8\text{--}5\text{ g m}^{-2}$ [9]. Boddy *et al.* used a model independent approach for describing ifosfamide pharmacokinetics [1]. All these methods were developed to describe concentration-time profiles of infusions of short duration (1–3 h) and did not take into account the effect of the autoinduction on the pharmacokinetics of ifosfamide. Prasad *et al.* and Boddy *et al.* were the first to report models, which enabled an adequate description of the concentration-time data for ifosfamide infusions of long duration of up to 72 h [10, 11]. Their models included a lag time before the development of autoinduction started and described the increase of clearance of ifosfamide over time.

Large interpatient variability in clinical toxicity and response rates are observed during ifosfamide treatment [12]. This could possibly be explained by interpatient differences in ifosfamide pharmacokinetics, e.g. autoinduction. In this study we aimed to develop a nonlinear pharmacokinetic model for ifosfamide, by which all relevant pharmacokinetic parameters including the rate and extent of autoinduction over time could be estimated. Our future aim is to investigate the possibility of therapeutic drug monitoring (TDM) of ifosfamide. Therefore, a population pharmacokinetic approach was selected, because this allowed assessment of interindividual and residual variability, necessary for TDM. Furthermore, a simulation was performed to assess the influence of autoinduction on the pharmacokinetics of ifosfamide.

Methods

Patient population and treatment

Patients treated for various types of soft tissue sarcoma with single agent high-dose ifosfamide in an open nonrando-

mised phase II trial, were subjected to pharmacokinetic sampling after written informed consent was obtained. Six patients received 12 g m^{-2} ifosfamide as a continuous i.v. infusion in 72 h once every 4 weeks. Due to unacceptable central neurotoxicity in this group the following nine patients received a decreased dose of $9\text{ g m}^{-2}\text{ 72 h}^{-1}$. This resulted in individual total doses ranging from 10.3 to 23.5 g ifosfamide. Supportive care consisted of mesna and extensive hydration to prevent haemorrhagic cystitis and bicarbonate to prevent acidosis. Anti-emetics and methylene blue (a neurotoxicity antidote) were given when indicated. Additional comedication consisted of 21 different drugs with a mean of 7 per patient, including anticoagulants, H_2 receptor antagonists, glucocorticosteroids and tricyclic antidepressants.

Pharmacokinetic sampling and analysis

Whole blood samples were drawn at 0, 3, 10, 20, 24, 34, 48, 58, 68, 72, 73, 76, 80 and 92 h after start of the infusion. Following plasma separation the samples were stored at -70°C pending analysis. After alkalized liquid-liquid extraction with ethyl acetate, ifosfamide was determined using gas-chromatography with nitrogen-phosphorous detection [13]. The method was validated resulting in an accuracy of 103.2, 99.9, 104.5, 102.0 and 93.3%, an intra-assay precision of 5.5, 0.8, 1.8, 2.0 and 2.9% and an interassay precision of 3.5, 2.9, 3.6, 2.6 and 5.1% at concentrations of 0.192, 0.383, 0.958, 38.3 and $383\text{ }\mu\text{M}$ ifosfamide, respectively.

Model development

Ifosfamide plasma concentration-time profiles were described by a one-compartment model as depicted in

Figure 2 Pharmacokinetic model of ifosfamide with autoinduction of its metabolism. The time profile of ifosfamide amount (A_1 , μmol) in compartment 1 was described by an influx of dose and an efflux calculated by an apparent clearance (CL_{app} , l h^{-1}) divided by volume of distribution (V , l). The time profile of the relative amount of enzyme (A_2) in the hypothetical compartment 2 was determined by a rate constant for enzyme formation ($K_{enz,in}$) and a rate constant for enzyme degradation ($K_{enz,out}$). The dashed arrows represent the effect of the enzyme (A_2) on the elimination rate of ifosfamide (CL_{app}/V) and the effect of ifosfamide (A_1) on the degradation rate ($K_{enz,out}$) of the enzyme.

Figure 2. The change of the amount of ifosfamide in the central compartment (A_1 , μmol) over time was described by equation 1,

$$\frac{dA_1}{dt} = R - (CL_{app} \times C_p) \quad (\text{Equation 1})$$

in which R ($\mu\text{mol h}^{-1}$) is the infusion rate of ifosfamide, CL_{app} (l h^{-1}) is the apparent ifosfamide clearance and C_p ($\mu\text{mol l}^{-1}$) is the plasma concentration in compartment 1, which is equal to A_1/V with V (l) as the volume of distribution of ifosfamide. CL_{app} is defined by equation 2.

$$CL_{app} = CL \times A_2 \quad (\text{Equation 2})$$

In this equation CL (l h^{-1}) is the initial clearance of ifosfamide and A_2 is an amount of enzyme in the hypothetical compartment 2 as depicted in Figure 2. The change in A_2 over time is described by equation 3,

$$\frac{dA_2}{dt} = K_{enz,in} - K_{enz,out} \times A_2 \times \left(1 - \frac{C_p}{C_p + IC_{50}}\right) \quad (\text{Equation 3})$$

in which $K_{enz,in}$ (mol h^{-1}) is the zero-order rate constant for enzyme formation/activation, $K_{enz,out}$ (h^{-1}) is the first-order rate constant for enzyme degradation/inactivation

and IC_{50} (μM) is the ifosfamide concentration at 50% of the maximum inhibition of enzyme degradation. Before treatment with ifosfamide ($t=0$) steady-state levels of the enzyme were assumed. Hence, the change in enzyme amount was zero. $K_{enz,in}$ was equal to $K_{enz,out}$ times the amount of enzyme at $t=0$ ($A_{2,t=0}$). By defining $A_{2,t=0}$ at a value of 1, $K_{enz,in}$ could be substituted for $K_{enz,out}$, yielding equation 4,

$$\frac{dA_2}{dt} = K_{enz,out} - K_{enz,out} \times A_2 \times \left(1 - \frac{C_p}{C_p + IC_{50}}\right) \quad (\text{Equation 4})$$

in which A_2 is the relative amount of enzyme. The induction half-life of the enzyme ($t_{1/2,enz}$, h) was calculated by dividing $\ln(2)$ by $K_{enz,out}$. Data analysis was performed using the nonlinear mixed effects modelling programme (NONMEM) (double precision, version V, level 1.0) [14] operated on an MS-DOS computer under FORTRAN90 Powerstation (version 4.0). Population and individual estimates for CL , V , $K_{enz,out}$ and IC_{50} were obtained using a general nonlinear population pharmacokinetic program of NONMEM with first-order processes and nonstiff differential equations (ADVAN6 TOL=5) [15]. The interindividual variability for all pharmacokinetic parameters was estimated with a proportional error model. The residual variability was modelled using a combined additive plus proportional error model. The POSTHOC function of NONMEM enabled estimation of the individual pharmacokinetic parameters using a Bayesian approach taking both individual observations and population effects into account. The predictive performance of the model was assessed by plotting the population predicted (PRED) and individual Bayesian predicted estimates (IPRED) *vs* the observed concentrations (OBSERVATION).

The effect of autoinduction on the dose-area under the curve (AUC) relationship was investigated by simulation. The profiles were simulated based upon the population parameters found.

Results

The patient population consisted of nine males and six females with a mean age of 49 years (ranging from 23 to 72 years) and a mean weight of 59 kg (ranging from 49 to 82 kg). Tumour types were rhabdomyosarcoma ($n=3$), neurofibrosarcoma ($n=1$), osteosarcoma ($n=3$), synoviumsarcoma ($n=2$), leiomyosarcoma ($n=4$) and endometriumsarcoma ($n=2$).

Pharmacokinetic assessment of the 15 patients resulted in 176 concentration-time points. The predictive performance is represented in Figure 3. By including individual

Figure 3 The relationships between estimates (PRED), individual Bayesian estimates (IPRED) of and observed (OBS) ifosfamide concentrations.

estimates the predictive performance was increased. The individual predictions (IPRED) were symmetrically distributed around the line of unity, suggesting adequate model prediction. No outliers or trends could be observed in the concentration range or time range.

The observed and Bayesian estimated pharmacokinetic profiles of all patients are represented in Figure 4. Most patients received 72 h infusions and showed initial build-up of ifosfamide plasma concentrations in the first 24 h (reaching maximum concentrations), followed by a gradual decrease due to autoinduction. After 72 h the infusion was stopped and elimination pharmacokinetics could be observed. The infusion in one patient was

Figure 4 The observed (dots) and Bayesian estimated (lines) ifosfamide concentrations *vs* time for all patients ($n = 15$). The open circles represent a patient who suffered from a neurotoxicity episode. The drug infusion for this patient was stopped after 48 h.

stopped after 48 h, due to acute severe neurotoxicity. Therefore, the patient received only 66% of the planned dose.

The predicted relative amount of enzyme in the hypothetical compartment 2 is described in Figure 5. The relative amount of enzyme increased over time during the infusion and decreased after cessation. Notably, during the first 24 h for all patients the model predicted an average doubling of the relative enzyme amount. Estimates of pharmacokinetic population parameters, their standard error, their interindividual variability and the residual variability are presented in Table 1. The effect of autoinduction on the AUC after doubling of the dose is simulated in Figure 6 and represented in Table 2. This simulation shows that after increasing the dose from 10 to 20 g ifosfamide, the AUC was only 56% larger.

Discussion

A population model of the pharmacokinetics of ifosfamide has been presented, in which autoinduction was modelled by inhibition of the degradation of the enzyme responsible for ifosfamide metabolism. The exact physiological mechanism of the autoinduction of ifosfamide is unknown, but several mechanisms of drug induced metabolic changes have been reported for other compounds. For most of these drugs the amount of enzymes performing the metabolic breakdown is increased via *de novo* synthesis. For instance, the barbiturate pentobarbital induces nortriptyline metabolism by increasing translation of the DNA coding for the enzyme and thereby increasing clearance of systemic nortriptyline. *De*

novo synthesis of cytochrome P450 (CYP) by pentobarbitone was observed after 2 days [16]. Rapid autoinduction of carbamazepine metabolism due to *de novo* synthesis was observed between 12–24 h rising to a maximum after 6 weeks [17]. Autoinduction of ifosfamide has been observed within 24 h after the start of the treatment [18]. However, it is unlikely that an alkylating agent like ifosfamide (which reduces DNA/RNA and protein synthesis) increases enzyme protein synthesis of CYP. Non-transcriptional induction mechanisms have been demonstrated to produce drug induced metabolic changes as well. Troleandomycin produced no increase in the rate of CYP3A4 protein synthesis, but it decreased the rate of the CYP3A4 protein degradation by interacting with specific catalytic enzymes [19]. This resulted in an increased pool of the CYP3A4 protein. An uncommon interaction of a drug with its metabolizing enzyme has been demonstrated for phenobarbitone [20]. This did not result in an increased synthesis of the enzyme, but was achieved by direct influence of the drug on the enzyme. By binding of the drug on a noncatalytic site of the enzyme, the catalytic ability increased due to an altered spin state of the haemoprotein of the CYP.

Ifosfamide has been shown to reduce protein synthesis [21] and could have a similar effect on the enzymes responsible for CYP3A4 degradation/inactivation, thus increasing ifosfamide clearance. CYP3A4 performs both a desired hydroxylation and an undesired dechloroethylation of ifosfamide, making it a target for the development of a specific inhibition or heteroinduction regimens and thereby directing ifosfamide metabolism to a more favourable toxicity-efficacy profile.

Although comedication consisted of a total of 24 different drugs, none have been shown to be inhibitors or

Figure 5 Individual time profiles of the relative amount of enzyme (A_2) for all patients ($n=15$). The profiles were obtained on basis of the individual Bayesian parameter estimates provided by NONMEM. At the initiation of the therapy A_2 was 100%.

inducers of CYP3A4. Furthermore, no metabolic interactions between ifosfamide and any of these drugs have been reported.

Figures 3 and 4 indicate that the proposed population pharmacokinetic model described the observed data well. Although programmes such as NONMEM were designed to describe populations using sparse data, they require fewer subjects if the pharmacokinetic data are rich. Figure 4 indicates that the patient suffering from severe neurotoxicity had the highest AUC between 0 and 48 h. The CL and $K_{enz,out}$ of this patient were 2.25 l h^{-1} and 0.0471 h^{-1} , respectively. Population values ranged from 2.22 to 4.96 l h^{-1} and from 0.0439 to 0.0697 h^{-1} , respectively. Curiously, this patient had the second lowest $K_{enz,out}$ (a slow onset of autoinduction) and the second lowest CL of the subjects receiving a dose of 12 g m^{-2} . The relative low clearance of ifosfamide in this patient should theoretically result in a lower AUC of neurotoxic metabolites and less neurotoxicity. The latter is in contrast with our observations. It can therefore be concluded that the development of neurotoxicity cannot be predicted or explained on basis of the pharmacokinetic parameters of ifosfamide alone.

Previously reported models of ifosfamide pharmacokinetics used a lag time before the start of autoinduction, because it was first noticed only after 24 h [10, 11]. Our model described the autoinduction as a progressively developing effect with a half-life of 12.7 h. Figure 5 shows that our data could be modelled without a lag time and the autoinduction of ifosfamide metabolism could be the result of direct inhibition of the enzymatic degradation of CYP3A4 by the drug, rather than its *de novo* synthesis, which requires time. However, activation of CYP3A4 activity can not be excluded. The use of a lag time model results in overestimation of the initial CL of ifosfamide compared with our model. Thus, an initial CL of

Table 1 Estimates of pharmacokinetic population parameters for ifosfamide with their standard error, interindividual variability and residual variability.

Parameter	Mean	Standard error	Interindividual variability	Residual variability
CL (l h^{-1})	2.94	0.27	24.5%	
V (l)	43.5	2.9	23.4%	
$K_{enz,out}$ (h^{-1})	0.0546	0.0078	22.7%	
$t_{1/2,enz}$ (h)	12.7			
IC_{50} (μM)	30.7	4.8		
P.E.				13.6%
A.E. (μM)				0.0763

CL = initial ifosfamide clearance, V = volume of distribution of ifosfamide, $K_{enz,out}$ = first-order rate constant for enzyme degradation, $t_{1/2,enz}$ = induction half-life of the enzyme, IC_{50} = ifosfamide concentration at 50% of the maximum inhibition of enzyme degradation, P.E. = proportional residual error, A.E. = additive residual error.

Figure 6 The simulated pharmacokinetic profiles of ifosfamide after 10 g (dashed lines) and 20 g (solid lines) ifosfamide in a patient receiving a 72-h continuous i.v. infusion, based upon population parameters found.

$3.48 \pm 0.88 \text{ l h}^{-1} \text{ m}^{-2}$ was reported in 15 breast cancer patients after $5 \text{ g m}^{-2} 24^{-1} \text{ h}$ continuous i.v. infusion using a model with a lag time [2], compared with our findings of $1.77 \text{ l h}^{-1} \text{ m}^{-2}$ (2.94 l h^{-1}) after 9 and $12 \text{ g m}^{-2} 72^{-1} \text{ h}$ continuous i.v. infusion.

The observed interindividual variability in population parameters was small (less than 24.5%). With respect to IC_{50} , the inclusion of interindividual variability did not improve the fit. This should not be interpreted as an absence of interindividual variability; the data were not sufficiently rich to estimate it. A proportional error of 13.6% and an additive error of 0.0763 µM accounted for the residual variability not explained by the model. The residual variability may in part be explained by errors in the bio-analytical assay and errors in drug administration (e.g. infusion duration) and sampling times.

In clinical studies with ifosfamide, considerable variability has been observed in its efficacy and toxicity [12]. Interpatient pharmacokinetic variability could be one of the factors that contributes to these differences in response. Our observations demonstrate that pharmacokinetic variability in our population was only modest. However, since the metabolites of ifosfamide are responsible for its pharmacological and toxicological effects, the pharmacokinetic variability of these metabolites should be characterized when defining dose-response relationships.

The increase in AUC of ifosfamide after doubling of the

Table 2 The simulated effect of different doses of ifosfamide on the area under the plasma concentration-time curve (AUC).

Ifosfamide dose (g/72 h)	AUC (µM h)
10	1.7
20	2.7
increase	56%

dose was only 56%. This emphasizes the importance of the integrating autoinduction into the modelling of nonlinear ifosfamide pharmacokinetics.

In conclusion, the proposed model allowed quantification of the effect of autoinduction on the concentration-time profiles of ifosfamide. The model demonstrated no necessity for a lag time in onset of induction. Although the small number of patients studied prevented the use of techniques such as data-splitting, cross-validation and bootstrapping for testing the predictive performance, the model was able to describe the individual pharmacokinetic profile of patients with adequate precision. Future utilization of this model may include investigating the relationship between the pharmacodynamics (e.g. bone marrow suppression, neurotoxicity) and the pharmacokinetics of ifosfamide. In order to achieve this plasma 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide metabolite data will have to be included in the model, since they are thought to be responsible for specific toxicities. Secondly, covariables could also be studied for their possible predictiveness of the pharmacokinetics and pharmacodynamics of ifosfamide.

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