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Metabolism and Pharmacokinetics of Oxazaphosphorines

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Abstract

The 2 most commonly used oxazaphosphorines are cyclophosphamide and ifosfamide, although other bifunctional mustard analogues continue to be investigated. The pharmacology of these agents is determined by their metabolism, since the parent drug is relatively inactive. For cyclophosphamide, elimination of the parent compound is by activation to the 4-hydroxy metabolite, although other minor pathways of inactivation also play a role. Ifosfamide is inactivated to a greater degree by dechloroethylation reactions. More robust assay methods for the 4-hydroxy metabolites may reveal more about the clinical pharmacology of these drugs, but at present the best pharmacodynamic data indicate an inverse relationship between plasma concentration of parent drug and either toxicity or antitumour effect.

The metabolism of cyclophosphamide is of particular relevance in the application of high dose chemotherapy. The activation pathway of metabolism is saturable, such that at higher doses (greater than 2 to 4 g/m^2) a greater proportion

of the drug is eliminated as inactive metabolites. However, both cyclophosphamide and ifosfamide also act to induce their own metabolism. Since most high dose regimens require a continuous infusion or divided doses over several days, saturation of metabolism may be compensated for, in part, by auto-induction. Although a quantitative distinction may be made between the cytochrome P450 isoforms responsible for the activating 4-hydroxylation reaction and those which mediate the dechloroethylation reactions, selective induction of the activation pathway, or inhibition of the inactivating pathway, has not been demonstrated clinically.

Mathematical models to describe and predict the relative contributions of saturation and autoinduction to the net activation of cyclophosphamide have been developed. However, these require careful validation and may not be applicable outside the exact regimen in which they were derived. A further complication is the chiral nature of these 2 drugs, with some suggestion that one enantiomer may have a favourable profile of metabolism over the other.

That the oxazaphosphorines continue to be the subject of intensive investigation over 30 years after their introduction into clinical practice is partly because of their antitumour activity. Further advances in analytical and molecular pharmacological techniques may further optimise their use and allow rational design of more selective analogues.

Cyclophosphamide and ifosfamide are the most widely used oxazaphosphorines and among the first alkylating agents to be used therapeutically. Because phosphoramidase enzymes were thought to be more abundant in tumours compared with normal tissue, cyclophosphamide was designed to be cleaved by these enzymes to deliver nitrogen mustard selectively to malignant cells.[1] Oxazaphosphorines are now known to act as prodrugs, but the pharmacological route to DNA alkylation does not involve phosphoramidase activation and the exact sequence of events is still not clear.

Cyclophosphamide and ifosfamide can have serious toxicities, however, there are interesting differences between the 2 agents.[2] In particular, nephrotoxicity and neurotoxicity are more common with ifosfamide. Nevertheless, they continue to be used in a wide variety of therapeutic settings because of their valuable antitumour activity. Recently, several high dose regimens have been developed which include cyclophosphamide followed by autologous haematological support.

Other oxazaphosphorine agents have been developed (these include trofosfamide,[3,4] mafosfamide,[5] various sugar derivatives[6] and the activated 4-hydroperoxy form of cyclophosphamide[7,8]), but the clinical use of these agents is limited. Trofosfamide, which has 3 chloroethyl groups, may be metabolised to a 4-hydroxy metabolite or either cyclophosphamide or ifosfamide and is a substrate for the same cytochrome P450 (CYP)3A enzymes.[9]

The pharmacokinetics of cyclophosphamide[10] and ifosfamide[11,12] have been reviewed separately and together.[13] The current review will concentrate on recent developments of these 2 agents.

1. Mechanisms of Action and Resistance

Most of the information concerning the mechanisms of action of oxazaphosphorines has been gained by studying cyclophosphamide. However, there are sufficient elements in common that these factors can be considered for the 2 agents together. Following metabolic activation and subsequent formation of mustards (fig. 1), oxazaphosphorines act as bifunctional alkylating agents. The mustards are thought to react with the N^7 atom of purine bases, especially when they are flanked by adjacent guanines.[14] These DNA adducts go on to form cross-links through reaction of the second arm of the mustard.[15] The different intramolecular distance between the

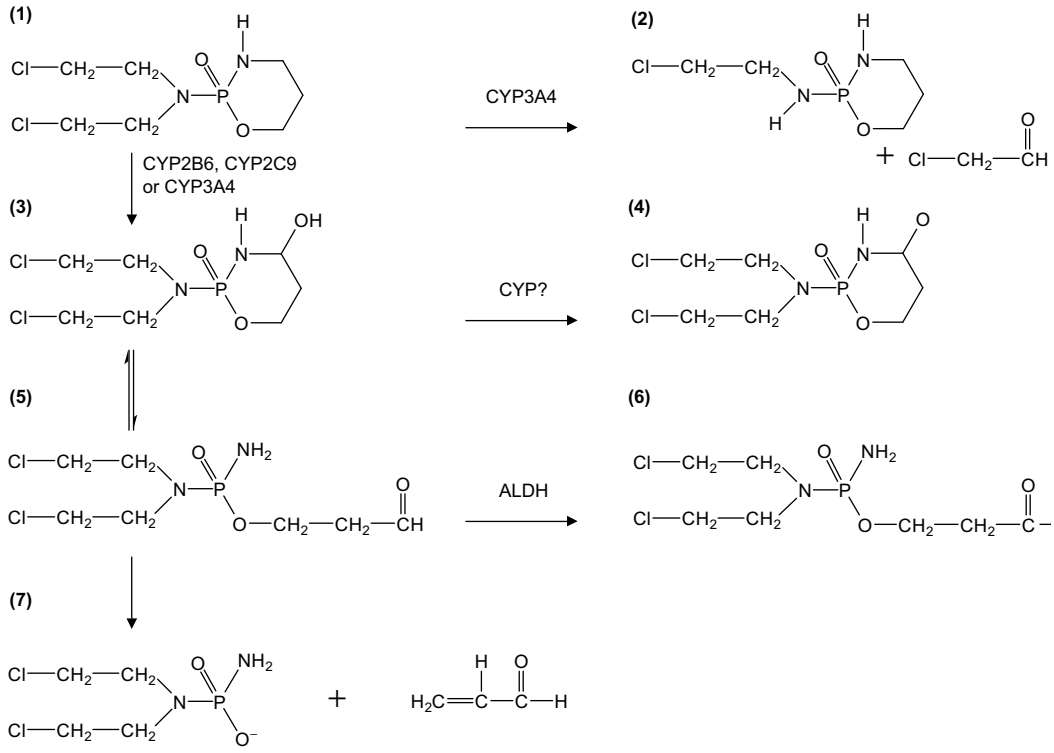

Fig. 1. Metabolism of cyclophosphamide (**1**) by its activation pathway [4-hydroxycyclophosphamide (**3**), aldophosphamide (**5**) and phosphoramide mustard (**7**)] and inactivating pathways [dechloroethylcyclophosphamide (**2**), 4-ketocyclophosphamide (**4**) and carboxyphosphamide (**6**)]. The by-products chloroacetaldehyde and acrolein are also shown.

chloroethyl groups in cyclophosphamide or ifosfamide mustards results in a different range of cross-linked DNA.

Despite an understanding of the chemical reactions between alkylating species and DNA,[16] the nature of the mechanisms linking adduct and cross-link formation with cell death remain unclear.

It is now accepted that cyclophosphamide, as with other alkylating agents, kills tumour cells by apoptosis, a process regulated downstream to DNA damage.[17,18] Several mechanisms of resistance have been proposed including increased levels of intracellular thiols and upregulated glutathione *S*-transferase activity.[19,20] More specifically, cyclophosphamide resistance has been linked to deficient DNA repair[21,22] and increased aldehyde dehydrogenase activity.[23-26] Lastly, transfection of anti-apoptotic proteins belonging to the Bcl2 family inhibits cyclophosphamide-induced cell death *in vitro* confirming the importance of downstream events in determining the outcome of chemotherapy.[27] Many of these elegant systems await confirmation of their clinical importance, prior to the development of strategies to circumvent them.

Recent studies have pointed to a role for the enzyme alkylguanine alkyltransferase (AGAT) in resistance to cyclophosphamide,[28] which opens up the possibility that the O^6 atom of guanine may also be a target for oxazaphosphorines. This has important implications for other possible mechanisms of resistance, as the repair enzymes associated with O^6 lesions differ from those relevant to the N^7 atom. Also, inhibitors of AGAT may be clin-

ically useful to augment the activity of oxazaphosphorines.

2. Cyclophosphamide

2.1 Clinical Use

The oxazaphosphorine cyclophosphamide remains one of the most widely used cytotoxic agents in both adults and children. While its role in the treatment of patients with small cell lung cancer and ovarian cancer is declining, cyclophosphamide continues to be used in the treatment of breast carcinoma where it forms a critical component of the cyclophosphamide, methotrexate, fluorouracil (CMF) regimen[29] and is used in combination with doxorubicin (adriamycin).[30] Both ifosfamide and cyclophosphamide are widely used in the treatment of patients with nonHodgkin's lymphoma and a variety of bone and soft tissue sarcomas.[31] In addition to its role in the treatment of solid tumours, cyclophosphamide is included in many multinational protocols for the treatment of acute lymphoblastic leukaemia.[32]

In comparison with many other anticancer drugs cyclophosphamide exhibits relatively little nonhaematopoeitic toxicity. This feature and experimental reports of a steep dose-response curve[33] have led to the widespread use of escalated doses (up to 7 g/m^2) of cyclophosphamide in an effort to overcome resistance in clinical practice. Other potential advantages of cyclophosphamide include the absence of cross-resistance with other alkylating agents, such as melphalan and nitrosoureas, in addition to potential synergy with topoisomerase II inhibitors.[34,35] High dose therapy forms a component of most bone marrow transplant conditioning regimens in both adults and children.[36]

Cyclophosphamide is usually administered intravenously in either 5% dextrose or 0.9% saline. There is no clear evidence of clinical benefit resulting from more prolonged infusions[37] and the drug is usually given as a single dose over a period of up to 1 hour, although a number of paediatric protocols favour fractionating the total dose over several days. Oral administration of cyclophosphamide is usually restricted to its use as an immunosuppressive and in some adjuvant breast cancer regimens. It is customary to use hydration and simultaneous mesna administration to prevent haemorrhagic cystitis when the total dose of cyclophosphamide per course exceeds 1 g/m^2.

2.2 Analytical Methodology

A range of techniques has been described for the analysis of cyclophosphamide and its metabolites. While gas-chromatography is the most sensitive means of detecting the parent compound,[38,39] dechloroethylcyclophosphamide and ketocyclophosphamide in biological fluids, high performance liquid chromatography (HPLC) has also been employed to determine cyclophosphamide concentrations *in vivo*.[40,41] Assay methods for the more reactive metabolites have been described,[42-44] but these species are unstable and thus require complex derivatisation prior to quantitation. Recently, liquid chromatography-mass spectrometry (LC-MS) methods for parent and metabolites have been described[45] and nuclear magnetic resonance (NMR) has been used to determine concentrations in urine.[46]

Methods for the separate analysis of the stereoisomers of cyclophosphamide have been developed, including chiral derivatisation and chiral chromatography.[47,48]

2.3 Absorption and Distribution

Cyclophosphamide is well absorbed following oral administration,[49-51] although the reported bioavailability of the parent compound may mask differences in the production of individual metabolites following first-pass metabolism. The parent compound is widely distributed throughout the body[52] with a low degree of plasma protein binding (20%).[10] There is some evidence that distribution is increased in obese patients, resulting in a longer half-life of the parent drug.[53]

Several studies have provided differing estimates of the ability of cyclophosphamide to cross the blood-brain barrier with reported cerebrospinal fluid : plasma ratios varying between 0.2 and 4.[54,55] It is likely that the blood-brain barrier is less per-

meable to individual metabolites which are more polar and increasingly bound to plasma proteins.[56] The relevance of these findings is unclear as a proportion of paediatric intracranial tumours are intrinsically sensitive to cyclophosphamide therapy.[57]

2.4 Elimination and Metabolism

Less than 20% of a dose of cyclophosphamide is excreted unchanged in the urine,[58,59] and only 4% is excreted in the bile.[60] The majority of drug elimination occurs by metabolic transformation. Although this may occur predominantly in the liver, activating or inactivating metabolism may occur in other sites, including the erythrocytes[61] and tumour itself. Gene transfer of activating enzymes into tumour cells has been suggested to improve selectivity.[62]

Cyclophosphamide is a prodrug which requires metabolic transformation to generate active alkylating species.[63] The initial activation reaction of hydroxylation at the carbon-4 position of the oxazaphosphorine ring (fig. 1) is mediated by CYP enzymes.[64] This reaction produces 4-hydroxycyclophosphamide, which exists in equilibrium with its tautomer aldophosphamide.[65] Aldophosphamide breaks down by spontaneous β elimination to release phosphoramide mustard and acrolein.[63] While phosphoramide mustard is thought to be the active alkylating species, acrolein is an unwanted by-product responsible for haemorrhagic cystitis.[63] Alternatively, aldophosphamide may be oxidised to inactive carboxyphosphamide by aldehyde dehydrogenase.[66] The other principal inactive metabolite, dechloroethylcyclophosphamide, is produced by a separate oxidative *N*-dealkylation reaction which is also catalysed by CYP3A4.[67,68] Previous studies of urinary metabolites suggested the presence of a polymorphism in aldehyde dehydrogenase catalysed production of carboxyphosphamide.[69,70] However, further investigations indicated that the observed variation may be secondary to altered stability at extremes of urinary pH.[46,71] A minor keto metabolite has also been identified.[46]

The CYP enzymes responsible for cyclophosphamide activation have been identified as CYP2B6, CYP2C family members and CYP3A4.[64] Analysis of CYP2B6 expression in human liver has suggested that this is the major form contributing to cyclophosphamide metabolism *in vivo*,[72] although a role for CYP2C9 has also been reported.[68]

A high degree of interpatient variation in terms of the pharmacokinetics of cyclophosphamide has been reported in both adults[73] and children,[74] which is likely to reflect differences in the expression of the individual CYP enzymes. Variation may also result from concomitant drug therapy with

Table I. Pharmacokinetic parameters for cyclophosphamide. Data are presented as mean ± SD or median (range)

n	Dose	$t_{½β}$ (h)	AUC (mmol/L • h)	CL[a] (L/h/m^2)	Vd[a] (L/kg)	f_e (%)	Reference
12	60 mg/kg	5.0		3.2			10
7	120 mg/kg	5.2 ± 1.8[a]					44
16	150-400 mg/m^2	7.6		2.4	0.52		53
19	60 mg/kg (1.9-2.7 g/m^2)	1st dose 8.7 ± 4.6 2nd dose 3.6 ± 0.9	1st dose 3.1 ± 1.1 2nd dose 1.6 ± 0.7			16	58
23	1.0 g/m^2	6.2	1.6	2.5	0.57	14	59
4	1.6 g/m^2	5.5	2.4	2.6	0.52	12	
38[b]	370-2490 mg/m^2	3.2 (1.1-16.8)		2.9 (1.2-10.6)	0.63 (0.26-1.48)		74
15	4 g/m^2						76
12	500 mg/m^2	4.8	0.7 ± 0.1[a]	2.7	0.49 ± 0.08	19	77
	100 mg/kg		6.0 ± 1.0	2.7	0.45 ± 0.12	30	

a Assuming 70kg or 1.73m^2 as appropriate.

b Ages between 0.17 and 18 years.

AUC = area under the concentration-time curve; **CL** = total body clearance; **f_e** = fraction of the available drug excreted in the urine; **n** = number of participants; **SD** = standard deviation; **$t_{½β}$** = elimination half-life; **Vd** = volume of distribution.

CYP inhibitors, such as systemic antifungals,[75] or prior treatment with inducers including dexamethasone[74] and anticonvulsants.[44] The half-life of cyclophosphamide is between 6 and 9 hours with clearance values of approximately 2.5 to 4.0 L/h/m^2 (table I).[50,53] Cyclophosphamide metabolism is probably more rapid in children as a result of increased CYP activity.[74] Alterations in renal or hepatic function have not been clearly shown to alter the pharmacokinetics of the parent compound *in vivo*,[78] and do not result in any clinically-relevant changes in pharmacology.[79] Autoinduction of cyclophosphamide metabolism is well recognised, providing an increase in clearance and a shortened half-life following repeated administration at 24-hour intervals.[76,80,81] This is because of an increase in CYP enzymes.[82]

The increasing use of 'high dose' cyclophosphamide has led to concerns over the existence of dose-dependent pharmacokinetics, with an increase in the production of inactive metabolites as the predominant activation pathway of metabolism is saturated. The clinical significance of such an effect remains unclear.[76,77] Interestingly, in a study comparing high dose (100 mg/kg) with conventional dose (500 mg/m^2) in the same patients, no difference in overall pharmacokinetics was observed. However, the degree of renal excretion and inactive metabolite formation was increased at the higher dose, associated with a relative decrease in the formation of the active metabolite.[77]

We have recently reported that the clearance of cyclophosphamide is reduced in children with Fanconi's anaemia, possibly as a consequence of altered CYP oxidase-reductase cycling.[83] While hepatic disease might be expected to reduce the activation of oxazaphosphorines, there are few clinical data available to confirm this.[84]

In a comprehensive study of the pharmacokinetics and metabolism of cyclophosphamide administered over 2 days, the area under the concentration-time curve (AUC) of the parent drug was reduced on day 2, compared with day 1, and this was accompanied by an increase in the AUC of the active 4-hydroxy metabolite.[85]

2.5 Drug Interactions

Because the activation and elimination pathways of cyclophosphamide are dependent on drug metabolism, there is wide scope for drug interaction. A large number of drug interactions with cyclophosphamide have been reported in humans. In most cases the underlying mechanism is inhibition of CYP enzymes (allopurinol, chloramphenicol, chlorpromazine,[74] fluconazole,[75] ranitidine,[86] prednisolone[87] and thiotepa[88]). Induction of cyclophosphamide metabolism has also been reported (with dexamethasone[74] and anticonvulsants[44]). Phenytoin induces the *N*-dechloroethylation of the *S*-enantiomer of cyclophosphamide to a greater extent than that of the *R*-enantiomer.[89] The clinical significance of these interactions is unclear. In addition, a clinically significant, schedule-dependent interaction with paclitaxel has been identified.[90]

2.6 Role of Stereochemistry

The oxazaphosphorines possess a chiral centre at the phosphorus atom. The impact of this on pharmacokinetics, and more specifically on the metabolism of cyclophosphamide, has been investigated using enantiospecific assays for the parent and the optically-active metabolites. Small differences in terms of clearance are observed in patients,[91,92] with the *S*-enantiomer being eliminated more rapidly.

2.7 Pharmacokinetic Models

A single compartment, linear model is sufficient to describe the pharmacokinetics of cyclophosphamide following a short intravenous infusion at conventional doses.[73,74] More complex models involving time- and concentration-dependent pharmacokinetics have been proposed for prolonged or high dose administration.[44,76,77,93] While these more complex models apply specifically to the concentration of the parent drug in plasma, they also have implications for the systemic formation of active and inactive metabolites.[94]

The relationship between the pharmacology of cyclophosphamide and treatment outcome is incom-

Table II. Pharmacokinetic parameters for ifosfamide. Data are presented as mean ± SD or median (range)

n	Dose (mg)	$t_{½β}$ (h)	AUC (mmol/L • h)	CL[a] (L/h/m^2)	Vd[a] (L/kg)	f_e (%)	Reference
15	5 g/m^2 (24h)	4.7 ± 2.0	5.4 (3.5-8.7)	3.5 ± 0.9	0.56 ± 0.22	14.4 (5.3-19.4)	101
9	2-3g, IV (20 min) or PO		2.2-2.4	2.1 ± 0.9		5.3 ± 1.6	108
			2.5-3.3	1.8 ± 0.8		4.9 ± 2.7	
7	1.5 g/m^2 (0.5h)	7.0 ± 2.6	2.4 ± 0.7	3.8 ± 0.9	0.62 ± 0.17		112
8	1.8 g/m^2 (60 min)	5.2 (4.4-8.8)	2.0 (1.1-2.7)	3.4 (2.0-6.2)	0.66 (0.41-1.41)		113
12[b]	9 g/m^2 (1h) or	3.2 ± 1.5[c]	6.2 (4.8-12.2)	3.8 ± 1.1[c]	1.1 ± 0.4	20 (7-42)	114
	9 g/m^2 (72h)	2.1 ± 0.7	6.4 (2.3-10.9)	5.5 ± 2.7	0.7 ± 0.4	15 (10-26)	
14	3 g/m^2/day (1h or 24h)		6.9 ± 2.0			21 ± 9	115
			7.0 ± 4.4			25 ± 14	

a Assuming 70kg or 1.73m^2 as appropriate.

b 0.8-16.2 years.

c Day one of treatment.

AUC = area under the concentration-time curve; **CL** = total body clearance; **f_e** = fraction of the available drug excreted in the urine; **IV** = intravenous; **n** = number of participants; **PO** = orally; **SD** = standard deviation; **$t_{½β}$** = elimination half-life; **Vd** = volume of distribution.

pletely understood. This is largely because of difficulties in the measurement of reactive alkylating metabolites and incomplete understanding of which species are important in mediating cytotoxicity. A single study has reported an inverse correlation between the cyclophosphamide AUC and both treatment-related cardiotoxicity and event-free survival in women with breast cancer.[95] A study of cyclophosphamide AUC over 3 days of high dose treatment (1875 mg/m^2/day) reported no relationship between the AUC of the parent drug and either toxicity or relapse-free survival. A high degree of intra-individual variability meant that the AUC on day 1 was not predictive of the overall AUC.[96]

3. Ifosfamide

3.1 Clinical Use

The clinical use of ifosfamide includes adult and paediatric tumours and both haematological and nonhaematological disease. In tumours in adults it is less common in first-line treatment, with the exception of soft-tissue sarcomas. In the treatment of paediatric tumours ifosfamide is usually combined in multi-drug regimens. The clinical use of ifosfamide in paediatric patients is dose-limited by nephrotoxicity, which cannot be easily related to the pharmacology and metabolism of the drug.[97]

Like cyclophosphamide, ifosfamide is usually administered intravenously dissolved in either 5% dextrose or 0.9% NaCl. A short infusion or bolus is often used, and does not differ from more prolonged infusion in terms of side-chain metabolism.[98] Although there is little clinical evidence for any benefit from more prolonged administration, saturation of metabolism at higher doses (greater than 12 mg/m^2) may be of concern.[99] Ifosfamide, must be given with at least an equimolar dose of the uroprotective agent mesna. This prevents haemorrhagic cystitis, thought to be caused by the toxic metabolites acrolein and chloroacetaldehyde. Mesna is usually given intravenously, but an oral formulation is also available.

3.2 Analytical Methodology

Analytical methods for ifosfamide are similar to those for cyclophosphamide, but with a broader range of detectable metabolites. HPLC is a possible method for the detection of most of the stable metabolites, but with ultraviolet detection suffers from the lack of a chromophor. Thin-layer chromatography with photographic densitometry can also be used, but has a relatively limited range of sensitivity.[100] Assay methods for the more relevant active metabolites have been described, but these

species are unstable and so often require complex derivatisation.[11] Given the uncertainty surrounding the site of metabolic activation, the relevance of systemic concentrations of the active metabolites is questionable.[101,102] Assays for the individual enantiomers of ifosfamide have been described, in particular focusing on the dechloroethylation pathways and the precise quantitation of the stereochemistry of this metabolic pathway.[103]

3.3 Absorption

Ifosfamide has been administered orally in clinical studies and is reported to have a high bioavailability.[104-108] However, bioavailability measures based on the parent drug may mask qualitative and quantitative effects, because of metabolism in the gastrointestinal tract or first-pass metabolism,[107,109] and oral administration of ifosfamide is associated with a higher incidence of neurotoxicity.[110,111]

3.4 Distribution

The distribution of ifosfamide in the body is thought to be extensive, with a low degree of protein binding in the plasma.[11] The volume of distribution is hard to estimate because of the complex pharmacokinetics of this drug, but is generally around 0.6 L/kg (table II).[11] There is some evidence that obese patients have a larger volume of distribution, resulting in a longer half-life for the parent drug.[116] In paediatric patients, distribution of the parent drug and metabolites has been determined in the cerebrospinal fluid. Parent drug concentrations were comparable with those seen in plasma, with lower concentrations for the more polar metabolites.[56]

3.5 Metabolism and Excretion

As a prodrug ifosfamide requires metabolic activation to form the DNA reactive mustard species isophosphoramide mustard (fig. 2).[63] As well as the activation pathway, via 4-hydroxy and aldehyde intermediates, ifosfamide predominantly forms inactive dechloroethylated metabolites (fig. 2).[101,117,118] As with cyclophosphamide, the activated form of ifosfamide can be inactivated by aldehyde dehydrogenases to form a carboxy metabolite, with a small amount of inactivation to the keto metabolite.[101,117,118]

The chiral nature of the phosphorous atom in the ifosfamide molecule results in a minor degree of stereoselective metabolism.[119] The pharmacological significance of this is not yet clear, although it has been suggested that one enantiomer of the dechloroethylated metabolites is associated with neurotoxicity,[103] and the optically-pure *R*-isomer is being investigated as a less neurotoxic form.[120]

Investigations of the activation pathway for ifosfamide have indicated that CYP enzymes CYP3A4 and 3A5 are able to catalyse the formation of the 4-hydroxy metabolite.[64,121] Although these enzymes are expressed in hepatic and other host tissues, they may also be expressed in tumours,[122-124] leading to speculation that intratumoral activation may contribute significantly to activity.[62]

Dechloroethylation is also mediated by CYP3A isoforms.[121] This inactivating reaction is much more significant for ifosfamide than for cyclophosphamide, accounting for up to 50% of a dose.[101] Chloroacetaldehyde is formed as a by-product of this reaction and a causative role for chloroacetaldehyde in ifosfamide nephro- or neurotoxicity has been suggested.[125]

When administered intravenously ifosfamide has a half-life of 4 to 6 hours,[11] with clearance values reported to be between 1.8 and 5.5 L/h/m^2 (table II).[11] The majority of elimination is by metabolism, with less than 20% of a dose eliminated unchanged in the urine. There is no difference in pharmacokinetics between paediatric and adult patients.

As with cyclophosphamide, ifosfamide induces its own metabolism following repeated or continuous administration.[126,127] Clearance is increased, half-life decreased and the formation of metabolites increases. Auto-induction of metabolism occurs within 12 to 24 hours of drug administration, but is reversed within 3 weeks.[112]

At high doses, saturation of individual pathways of metabolism may also occur, although the pharmacokinetics of the parent drug have been shown

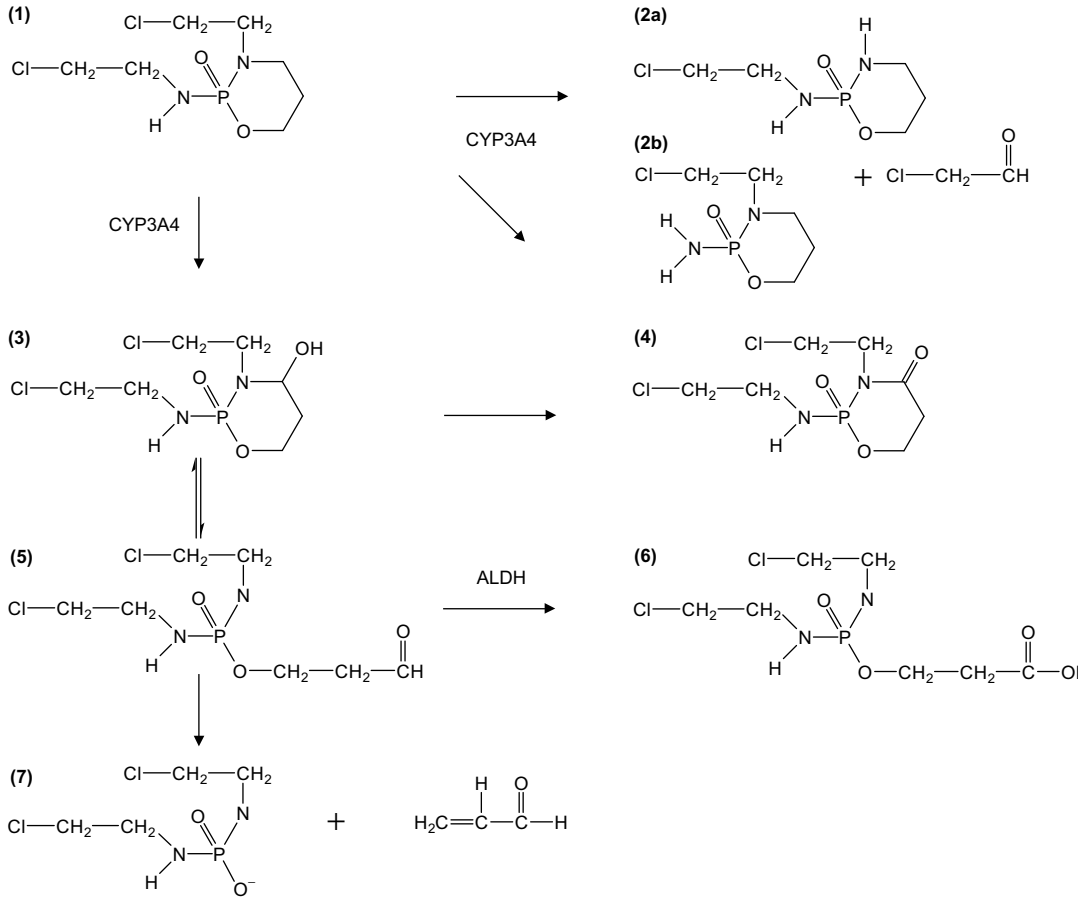

Fig. 2. Metabolism of ifosfamide (**1**) by its activation pathway [4-hydroxyifosfamide (**3**), aldoifosfamide (**5**) and isophosphoramide mustard (**7**)] and inactivating pathways [3-dechloro and 2-dechloroethylcyclophosphamide (**2a** and **b**), 4-ketoifosfamide (**4**) and carboxyifosfamide (**6**)]. The by-products chloroacetaldehyde and acrolein are also shown.

to be dose independent up to 18 g/m^2.[99] The combination of auto-induction and nonlinear metabolism of ifosfamide requires complex models to fully describe its pharmacokinetics.

3.6 Pharmacokinetic Drug Interactions

As with cyclophosphamide, drug interactions resulting in modification of metabolism of ifosfamide are potentially important. Drugs which may inhibit metabolism of ifosfamide include antifungal agents (ketoconazole and fluconazole), allopurinol and chlorpromazine, based on a comparison with the known inhibitors of cyclophosphamide metabolism[74,75] and on *in vitro* studies.[64,121] Conversely, inducers of hepatic metabolising enzymes increase the metabolism of ifosfamide[82] and prolonged administration of corticosteroids and anticonvulsants (phenobarbital and carbamazepine) results in increased clearance.[127] Since these agents are also substrates for the same metabolising enzymes, a complex mixture of competition and induction exists when they are coadministered.

Since administration of ifosfamide induces drug metabolising enzymes, prior administration may increase the rate of elimination of other substrates. However, there are no reports of any clinically significant interactions of this type.

3.7 Pharmacokinetic Models

The pharmacokinetics of ifosfamide following a short, intravenous infusion at conventional doses may be adequately described by a 1-compartment, linear model. However, more complex models involving time- and/or concentration-dependent pharmacokinetics have been proposed for prolonged or high dose administration.[127] Clearance is described as a variable that increases with time[127] or with concentration.[128] While these more complex models apply specifically to concentrations of parent drug in the plasma, they also have implications for the systemic formation of inactive and active metabolites.

3.8 Pharmacodynamic Implications

Because of the prodrug nature of ifosfamide, pharmacodynamic models are difficult to formulate in terms of the parent drug. Furthermore, in multiagent chemotherapy regimens, it is difficult to identify the contribution of any 1 agent to therapeutic or toxic events. Conversely, attempts to determine a relationship between pharmacological effect and systemic concentrations of activated metabolites have been unsuccessful.[101] An application of the comet assay to determine the degree of DNA damage[129] or DNA cross-linking has been described recently and may provide more useful pharmacodynamic information.[130]

4. Conclusion

The oxazaphosphorines have been used since the advent of modern chemotherapy, and have contributed to the effective treatment of many thousands of patients. However, the enigma of the mechanism of action, in terms of antitumour effect and toxicity, persists. Advances in biochemistry, analytical techniques and enzymology have provided further clues, and it is probable that the tools to successfully probe the pharmacology of these drugs are now available.

References

1. Brock N. Oxazaphosphorine cytostatics: past-present-future. Cancer Res 1989; 49: 1-7
2. Colvin M. The comparative pharmacology of cyclophosphamide and ifosfamide. Semin Oncol 1982; 9: 2-7
3. Kusniercysk H, Radzikowski C, Paprocka M, et al. Antitumor activity of optical isomers of cyclophosphamide, ifosfamide and trofosfamide as compared to clinically used racemates. J Immunopharmacol 1986; 8: 455-80
4. Wagner A, Hempel G, Boos J. Trofosfamide: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential int the oral treatment of cancer. Anticancer Drugs 1997; 8: 419-31
5. Schomburg A, Menzel T, Hadam M, et al. Biologic and therapeutic efficacy of mafosfamide in patients with metastatic renal cell carcinoma. Mol Biother 1992; 4: 58-65
6. Veyhl M, Wagner K, Volk C, et al. Transport of the new chemotherapeutic agent beta-D-glucosylisophosphamide mustard (D-19575) into tumor cells is mediated by the Na+-D-glucose cotransporter SAAT1. Proc Natl Acad Sci U S A 1998; 95: 2914-9
7. Phillips PC, Than TT, Cork LC, et al. Intrathecal 4-hydroperoxycyclophosphamide: neurotoxicity, cerebrospinal fluid pharmacokinetics and antitumor activity in a rabbit model of VX2 leptomeningeal carcinomatosis. Cancer Res 1992; 52: 6168-74
8. Passos-Cohellho J, Ross AA, Davis JM, et al. Bone marrow micrometastases in chemotherapy-responsive advanced breast cancer: effect of ex vivo purging with 4-hydroperoxycyclophosphamide. Cancer Res 1994; 54: 2366-71
9. MayManke A, Kroemer H, Hempel G, et al. Investigation of the major human hepatic cytochrome P450 involved in 4-hydroxylation and N-dechloroethylation of trofosfamide. Cancer Chemother Pharmacol 1999; 44: 327-34
10. Moore MJ. Clinical pharmacokinetics of cyclophosphamide. Clin Pharmacokinet 1991; 20: 194-208
11. Kaijser GP, Beijnen JH, Bult A, et al. Ifosfamide metabolism and pharmacokinetics [review]. Anticancer Res 1994; 14: 517-32
12. Wagner T. Ifosfamide clinical pharmacokinetics. Clin Pharmacokinet 1994; 26: 439-56
13. Fleming RA. An overview of cyclophosphamide and ifosfamide pharmacology. Pharmacotherapy 1997; 5 (Pt 2): S146-54
14. Kohn KW, Hartley JA, Mattes WB. Mechanisms of DNA sequence alkylation of guanine-N7 positions by nitrogen mustards. Nucl Acid Res 1987; 14: 10531-45
15. Springer JB, Colvin ME, Colvin OM, et al. Isophosphoramide mustard and its mechanism of bisalkylation. J Org Chem 1998; 63: 7218-22
16. Shulman Roskes EM, Noe DA, Gamcsik MP, et al. The partitioning of phosphoramide mustard and its aziridinium ions among alkylation and P-N bond hydrolysis reactions. J Med Chem 1998; 41: 515-29
17. O'Connor PM, Wassermann K, Sarnag M. Relationship between DNA cross-links, cell cycle and apoptosis in Burkitt's lymphoma cell lines differing in sensitivity to nitrogen mustard. Cancer Res 1991; 51: 6550-7
18. Hickman JA. Apoptosis and cancer chemotherapy. Cancer Metastasis Rev 1992; 11: 121-39

19. Crook TR, Souhami RL, Whyman GD, et al. Glutathione depletion as a determinant of sensitivity of human leukaemia cells to cyclophosphamide. Cancer Res 1986; 46: 5035-8
20. Richardson ME, Siemann DW. DNA damage in cyclophosphamide-resistant tumor cells: the role of glutathione. Cancer Res 1995; 55: 1691-5
21. Andersson BS, Mroue M, Britten RA, et al. The role of DNA damage in the resistance of human chronic myeloid leukaemia cells to cyclophosphamide analogues. Cancer Res 1994; 54: 5394-400
22. Dong Q, Bullock N, Ali-Osman F, et al. Repair analysis of 4-hydroperoxycyclophosphamide-induced DNA interstrand cross-linking in the c-myc gene in 4-hydrocyclophosphamide-sensitive and resistant cell lines. Cancer Chemother Pharmacol 1996; 37: 242-6
23. Maki PA, Sladek NE. Sensitivity of aldehyde dehydrogenases in murine tumor and hematopoietic progenitor cells to inhibition by chloral hydrate to potentiate the cytotoxic action of mafosfamide. Biochem Pharmacol 1993; 45: 231-9
24. Sreerama L, Sladek NE. Identification and characterization of a novel class 3 aldehyde dehydrogenase overexpressed in a human breast adenocarcinoma cell line exhibiting oxazaphosphorine-specific acquired resistance. Biochem Pharmacol 1993; 45: 2487-505
25. Sreerama L, Sladek NE. Identification of the class-3 aldehyde dehydrogenases present in human MCF-7/0 breast adenocarcinoma cells and normal human breast tissue. Biochem Pharmacol 1994; 48: 617-20
26. Magni M, Shammah S, Schiro R, et al. Induction of cyclophosphamide-resistance by aldehyde-dehydrogenase gene transfer. Blood 1996; 87: 1097-103
27. Dole MG, Jasty R, Cooper MJ, et al. Bcl-xl is expressed in neuroblastoma cells and modulates chemotherapy-induced apoptosis. Cancer Res 1995; 55: 2576-82
28. Friedman HS, Pegg AE, Johnson SP, et al. Modulation of cyclophosphamide activity by O-6-alkylguanine-DNA alkyltransferase. Cancer Chemother Pharmacol 1999; 43: 80-5
29. Bonadonna G, Valagussa P, Moliterni A, et al. Adjuvant cyclophosphamide, methotrexate and fluorouracil in node-positive breast cancer. N Engl J Med 1995; 332: 901-6
30. Fisher B, Anderson S, Wickerham DL, et al. Increased intensification and total dose of cyclophosphamide in a doxorubicin-cyclophosphamide regimen for the treatment of primary breast cancer: findings from the national surgical adjuvant breast and bowel project B-22. J Clin Oncol 1997; 15: 1858-69
31. Murphy SB, Bowman WP, Cooper MJ, et al. Results of treatment of advanced stage Burkitts lymphoma and B-cell (Sig+) acute lymphoblastic leukaemia with high dose fractionated cyclophosphamide and co-ordinated high-dose methotrexate and cytarabine. J Clin Oncol 1986; 4: 1732-9
32. Reiter A, Achrappe M, Ludwig W-D, et al. Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. Blood 1994; 84: 3122-33
33. Frei E, Teicher BA, Holden SA, et al. Preclinical studies and clinical correlation of the effect of alkylating dose. Cancer Res 1988; 48: 6417-23
34. Teicher BA, Cucchi CA, Lee JB, et al. In vitro studies of cross-resistance patterns in human cell lines. Cancer Res 1986; 46: 4379-83
35. Lilley ER, Rosenberg MC, Elion GB, et al. Synergistic interactions between cyclophosphamide or melphalan and VP-16 in a human rhabdomyosarcoma xenograft. Cancer Res 1990; 15: 284-7
36. Zecca M, Pession A, Bonetti F, et al. Total body irradiation, thiotepa and cyclophosphamide as a conditioning regimen for children with acute lymphoblastic leukemia in first or second remission undergoing bone marrow transplantation with HLA-identical siblings. J Clin Oncol 1999; 17: 1838-46
37. Klein HO, Wickramanayake PD, Christian E, et al. Therapeutic effects of single-push or fractionated injections or continuous infusion of oxazaphosphorines (cyclophosphamide, Ifosfamide and Asta Z 7557). Cancer 1984; 54: 1193-203
38. Juma FD, Rogers HJ, Trounce JR, et al. Pharmacokinetics of intravenous cyclophosphamide in man, estimated by gas-liquid chromatography. Cancer Chemother Pharmacol 1978; 1: 229-31
39. Jardine I, Fenselau C, Appler M, et al. Quantitation by gas chromatography-chemical ionization mass spectrometry of cyclophosphamide, phosphoramide mustard, and nornitrogen mustard in the plasma and urine of patients receiving cyclophosphamide therapy. Cancer Res 1978; 38: 408-15
40. Motzer RJ, Gulati SC, Tong WP, et al. Phase I trial with pharmacokinetic analyses of high-dose carboplatin, etoposide, and cyclophosphamide with autologous bone marrow transplantation in patients with refractory germ cell tumors. Cancer Res 1993; 53: 3730-5
41. Momerency G, van Cauwenberghe K, Slee PHTJ, et al. The determination of cyclophosphamide and its metabolites in blood plasma as stable trifluoroacetyl derivatives by electron capture chemical ionization gas chromatography/mass spectrometry. Biol Mass Spectrom 1996; 23: 149-58
42. Ludeman SM, Shulman Roskes EM, Wong KKT, et al. Oxime derivatives of the intermediary oncostatic metabolites of cyclophosphamide and ifosfamide: synthesis and deuterium labeling for applications to metabolite quantification. J Pharm Sci 1995; 84: 393-8
43. Kerbusch T, Huitema ADR, Kettenesvanden Bosch JJ, et al. High-performance liquid chromatographic determination of stabilized 4-hydroxyifosfamide in human plasma and erythrocytes. J Chromatogr B 1998; 716: 275-84
44. Slattery JT, Kalhorn TF, McDonald GB, et al. Conditioning regimen-dependent disposition of cyclophosphamide and hydroxycyclophosphamide in human marrow transplantation patients. J Clin Oncol 1996; 14: 1484-94
45. Baumann F, Lorenz C, Jaehde U, et al. Determination of cyclophosphamide and its metabolites in human plasma by high-performance liquid chromatography-mass spectrometry. J Chromatogr B 1999; 729: 297-305
46. Joqueviel C, Gilard V, Martino R, et al. Urinary stability of carboxycyclophosphamide and carboxyifosfamide, two major metabolites of the anticancer drugs cyclophosphamide and ifosfamide. Cancer Chemother Pharmacol 1997; 40: 391-9
47. Reid JM, Stobaugh JF, Sternson LA. Liquid chromatographic determination of cyclophosphamide enantiomers in plasma by precolumn chiral derivatization. Anal Chem 1989; 61: 441-6
48. Masurel D, Wainer IW. Analytical and preparative high-performance liquid chromatographic separation of the enantiomers of ifosfamide, cyclophosphamide and trofosfamide and their determination in plasma. J Chromatogr Biomed Appl 1989; 490: 133-43
49. Struck RF, Alberts DS, Horne K, et al. Plasma pharmacokinetics of cyclophosphamide and its cytotoxic metabolites after intravenous versus oral administration in a randomized, crossover trial. Cancer Res 1987; 47: 2732-26
50. Juma FD, Rogers HJ, Trounce JR. Pharmacokinetics of cyclophosphamide and alkylating activity in man after intravenous and oral administration. Br J Clin Pharmacol 1979; 8: 209-17

51. De Bruijn EA, Slee PHYJ, van Oosteron AT, et al. Pharmacokinetics of intravenous and oral cyclophosphamide in the presence of methotrexate and flourouracil. Pharm Weekbl 1988; 10: 200-6
52. Houghton PJ, Tew KD, Taylor DM. Sudies on the distribution and effects of cyclophosphamide in normal and neoplastic tissues. Cancer Treat Rep 1976; 60: 459-64
53. Powis G, Reece P, Ahmann DL, et al. Effect of body weight on the pharmacokinetics of cyclophosphamide in breast cancer patients. Cancer Chemother Pharmacol 1987; 20: 219-22
54. Hommes OR, Aerts F, Bahr U, et al. Cyclophosphamide levels in serum and spinal fluid of multiple sclerosis patients treated with immunosuppression. J Neurol Sci 1983; 85: 297-303
55. Neuwelt EA, Barnett PA, Frenkel EP. Chemotherapeutic agents' permeability to normal brain and delivery to avian sarcoma virus-induced brain tumours in the rodent: observations on problems of drug delivery. Neurosurgery 1984; 14: 154-60
56. Yule SM, Price L, Pearson ADJ, et al. Cyclophosphamide and ifosfamide metabolites in the cerebrospinal fluid of children. Clin Cancer Res 1997; 3: 1985-92
57. Mahoney DH, Strother D, Camitta B, et al. High dose melphalan and cyclophosphamide with autologous bone marrow rescue for recurrent/progressive malignant brain tumours in children: a pilot pediatric oncology group study. J Clin Oncol 1996; 14: 382-8
58. Fasola G, Lo Greco P, Calori E, et al. Pharmacokinetics of high-dose cyclophosphamide for bone marrow transplantation. Haematologica 1991; 76: 120-5
59. Bailey H, Mulcahy RT, Tutsch KD, et al. A phase I study of SR-2508 and cyclophosphamide administered by intravenous injection. Cancer Res 1991; 51: 1099-104
60. Dooley JS, James CA, Rogers HJ, et al. Biliary elimination of cyclophosphamide in man. Cancer Chemother Pharmacol 1982; 9: 26-9
61. Dockam PA, Sreerama L, Sladek NE. Relative contribution of human erythrocyte aldehyde dehydrogenase to the systemic detoxicification of the oxazaphosphorines. Drug Metab Disp 1997; 25: 1436-41
62. Chen I, Waxman D. Intratumoral activation and enhanced chemotherapeutic effect of oxazaphosphorines following cytochrome P450 gene-transfer – development of a combined chemotherapy cancer gene-therapy strategy. Cancer Res 1995; 55: 581-9
63. Sladek N. Metabolism of oxazaphosphorines. Pharmacol Ther 1988; 37: 301-55
64. Chang TKH, Weber GF, Crespi CL, et al. Differential activation of cyclophosphamide and ifosphamide by cytochromes P-450 2B and 3A in human liver microsomes. Cancer Res 1993; 53: 5629-37
65. Fenselau C, Kan M-NN, Subba Rao S, et al. Identification of aldophsophamide as a metabolite of cyclophosphamide in vitro and in vivo in humans. Cancer Res 1977; 37: 2538-43
66. Dockham PA, Lee M-O, Sladek NE. Identification of human liver aldehyde dehydrogenases that catalyze the oxidation of aldophosphamide and retinaldehyde. Biochem Pharmacol 1992; 43: 2453-69
67. Bohnenstengel F, Hofmann U, Eichelbaum M, et al. Characterization of the cytochrome p450 involved in side-chain oxidation of cyclophosphamide in humans. Eur J Clin Pharmacol 1996; 51: 297-301
68. Ren S, Yang JS, Kalhorn TF, et al. Oxidation of cyclophosphamide to 4-hydroxycyclophosphamide and deschloroethylcyclophosphamide in human liver microsomes. Cancer Res 1997; 57: 4229-35
69. Boddy AV, Furtun Y, Sardas S, et al. Individual variation in the activation and inactivation metabolic pathways of cyclophosphamide. J Natl Cancer Inst 1992; 84: 1744-8
70. Hadidi A-HFA, Coulter CEA, Idle JR. Phenotypically deficient urinary elimination of carboxyphosphamide after cyclophosphamide administration to cancer patients. Cancer Res 1988; 48: 5167-71
71. Yule SM, Boddy AV, Cole M, et al. Cyclophosphamide metabolism in children. Cancer Res 1995; 55: 803-9
72. Roy P, Yu LJ, Crespi CL, et al. Development of a substrate-activity based approach to identify the major human liver P-450 catalysts of cyclophosphamide and ifosfamide activation based on cDNA-expressed activities and liver microsomal profiles. Drug Metab Disp 1999; 27: 655-66
73. Moore MJ, Ehrlichman C, Thiessen JJ, et al. Variability in the pharmacokinetics of cyclophosphamide, methotrexate and 5-fluorouracil in women receiving adjuvant treatment for breast cancer. Cancer Chemother Pharmacol 1994; 33: 472-6
74. Yule SM, Boddy AV, Cole M, et al. Cyclophosphamide pharmacokinetics in children. Br J Clin Pharmacol 1996; 41: 13-9
75. Yule SM, Walker D, Cole M, et al. The effect of fluconazole on cyclophosphamide metabolism in children. Drug Metab Disp 1999; 27: 417-21
76. Chen T-L, Passos-Coelho J, Noe D, et al. Nonlinear pharmacokinetics of cyclophosphamide in patients with metastatic breast cancer receiving high-dose chemotherapy followed by autologous bone marrow transplantation. Cancer Res 1995; 55: 810-6
77. Busse D, Busch FW, Bohnenstengel F, et al. Dose escalation of cyclophosphamide in patients with breast cancer: Consequences for pharmacokinetics and metabolism. J Clin Oncol 1997; 15: 1885-96
78. Bramwell V, Calvert RT, Edwards G, et al. The disposition of cyclophosphamide in a group of myeloma patients. Cancer Chemother Pharmacol 1979; 3: 253-9
79. Juma FD, Rogers HJ, Trounce JR. Effect of renal insufficiency on the pharmacokinetics of cyclophosphamide and some of its metabolites. Eur J Clin Pharmacol 1981; 19: 443-51
80. Schuler U, Ehninger G, Wagner T. Repeated high-dose cyclophosphamide administration in bone marrow transplantation: exposure to activated metabolites. Cancer Chemother Pharmacol 1987; 20: 248-52
81. D'Incalci M, Bolis G, Facchinetti T, et al. Decreased half life of cyclophosphamide in patients under continual treatment. Eur J Cancer 1979; 13: 7-10
82. Chang TKH, Yu L, Maurel P, et al. Enhanced cyclophosphamide and ifosfamide activation in primary human hepatocyte cultures: response to cytochrome P-450 inducers and autoinduction by oxazaphosphorines. Cancer Res 1997; 57: 1946-54
83. Yule SM, Price L, Cole M, et al. Cyclophosphamide metabolism in children with Fanconi's anaemia. Bone Marrow Transplant 1999; 24: 123-8
84. Juma FD. Effect of liver failure on the pharmacokinetics of cyclophosphamide. Eur J Clin Pharmacol 1984; 26: 591-3
85. Ren S, Kalhorn KF, McDonald GB, et al. Pharmacokinetics of cyclophosphamide and its metabolites in bone marrow transplantation patients. Clin Pharmacol Ther 1998; 64: 289-301
86. Alberts DS, Mason-Liddil N, Plezia PM, et al. Lack of ranitidine effects on cyclophosphamide bone marrow toxicity or metabolism: a placebo-controlled clinical trial. J Natl Cancer Inst 1991; 83: 1739-43
87. Faber OK, Mouridsen HT, Skovsted L. The biotransformation of cyclophosphamide in man: influence of prednisone. Acta Pharmacol Toxicol 1974; 35: 195-200

88. Anderson L, Chen T-L, Colvin OM, et al. Cyclophosphamide and 4-hydroxycyclophosphamide/aldophosphamide kinetics in patients receiving high-dose cyclophosphamide chemotherapy. Clin Cancer Res 1996; 2: 1481-7
89. Williams ML, Wainer IW, Embree L, et al. Enantioselective induction of cyclophosphamide metabolism by phenytoin. Chirality 1999; 11: 569-74
90. Kennedy MJ, Zahurak ML, Donehower RC, et al. Sequence-dependent hematological toxicity associated with the 3-hour paclitaxel/cyclophosphamide doublet. Clin Cancer Res 1998; 4: 349-56
91. Holm KA, Kindberg CG, Stobaugh JF, et al. Stereoselective pharmacokinetics and metabolism of the enantiomers of cyclophosphamide. Preliminary results in humans and rabbits. Biochem Pharmacol 1990; 39: 1375-84
92. Williams ML, Wainer IW, Granvil CP, et al. Pharmacokinetics of (R)- and (S)-cyclophosphamide and their dechloroethylated metabolites in cancer patients. Chirality 1999; 11: 301-8
93. Busse D, Busch FW, Schweizer E, et al. Fractionated administration of high-dose cyclophosphamide: influence on dose-dependent changes in pharmacokinetics and metabolism. Cancer Chemother Pharmacol 1998; 43: 263-8
94. Huitema ADR, Kerbusch T, Tibben MM, et al. Modelling of the pharmacokinetics (PK) and auto-induction of high-dose cyclophosphamide (CP) and 4-hydroxycyclophosphamide (HCP) using NONMEM [abstract 2536]. American Association for Cancer Research; 1999 Apr 10-14; Philadelphia, 40
95. Ayash LJ, Wright JE, Tretyakov O, et al. Cyclophosphamide pharmacokinetics: Correlation with cardiac toxicity and tumor response. J Clin Oncol 1992; 10: 995-1000
96. Nieto Y, Xu XH, Cagnoni PJ, et al. Nonpredictable pharmacokinetic behavior of high-dose cyclophosphamide in combination with cisplatin and 1,3-bis(2-chloroethyl)-1-nitrosurea. Clin Cancer Res 1999; 5: 747-51
97. Boddy AV, English M, Pearson ADJ, et al. Ifosfamide nephrotoxicity: limited influence of metabolism and mode of administration during repeated therapy in pediatrics. Eur J Cancer 1996; 32A: 1179-84
98. Boos J, Silies H, Hohenlochter B, et al. Short-term versus continuous infusion: no influence on ifosfamide side-chain metabolism. Eur J Cancer 1995; 31A: 2417-8
99. Cerny T, Leyvraz T, von Briel A, et al. Saturable metabolism of continuous high dose ifosfamide with Mesna and GM-CSF: a pharmacokinetic study in advanced sarcoma patients. Ann Oncol 1999; 10: 1087-94
100. Boddy AV, Idle JR. Combined thin-layer chromatography-photography-densitometry for the quantification of ifosfamide and its principal metabolites in urine, cerebrospinal fluid and plasma. J Chromatogr Biomed Appl 1992; 575: 137-42
101. Boddy AV, Proctor M, Simmonds D, et al. Pharmacokinetics, metabolism and clinical effect of ifosfamide in breast cancer patients. Eur J Cancer 1995; 31A: 69-76
102. Petros WP and Colvin OM. Metabolic jeopardy with high-dose cyclophosphamide?: not so fast. Clin Cancer Res 1999; 5: 723-4
103. Wainer IW, Ducharme J, Granvil CP, et al. Ifosfamide stereoselective dechloroethylation and neurotoxicity. Lancet 1994; 343: 982-3
104. Cerny T, Margison JM, Thatcher N, et al. Bioavailability of ifosfamide in patients with bronchial carcinoma. Cancer Chemother Pharmacol 1986; 18: 261-4
105. Wagner T, Drings P. Pharmacokinetics and bioavailability of oral ifosfamide. Arzniettelforschung 1986; 36: 878-80
106. Lind MJ, Margison JM, Cerny T, et al. Comparative pharmacokinetics and alkylating activity of fractionated intravenous and oral ifosfamide in patients with bronchogenic carcinoma. Cancer Res 1989; 49: 753-7
107. Kurowski V, Cerny T, Küpfer A, et al. Metabolism and pharmacokinetics of oral and intravenous ifosfamide. J Cancer Res Clin Oncol 1991; 117 Suppl. 4: S148-S53
108. Aeschlimann C, Kupfer A, Schefer H, et al. Comparative pharmacokinetics of oral and intravenous ifosfamide/mesna/methylene blue therapy. Drug Metab Disp 1998; 26: 883-90
109. Lind MJ, Roberts HL, Thatcher N, et al. The effect of route of administration and fractionation of dose on the metabolism of ifosfamide. Cancer Chemother Pharmacol 1990; 26: 105-11
110. Cerny T, Küpfer A. The enigma of ifosfamide encephalopathy. Ann Oncol 1992; 3: 679-81
111. Cerny T, Lind M, Thatcher N, et al. A simple outpatient treatment with oral ifosfamide and oral etoposide for patients with small cell lung cancer (SCLC). Br J Cancer 1989; 60: 258-61
112. Lewis LD. A study of 5 day fractionated ifosfamide pharmacokinetics in consecutive treatment cycles. Br J Clin Pharmacol 1996; 42: 179-86
113. Comandone A, Leone L, Oliva C, et al. Pharmacokinetics of ifosfamide administered according to three different schedules in metastatic solft tissue and bone sarcomas. J Chemotherapy 1998; 10: 385-93
114. Boddy AV, Yule SM, Wyllie R, et al. Comparison of continuous infusion and bolus administration of ifosfamide in children. Eur J Cancer 1995; 31A: 785-90
115. Singer J, Hartley J, Brennan C, et al. The pharmacokinetics and metabolism of ifosfamide during bolus and infusional administration: a randomised cross-over study. Br J Cancer 1998; 77: 978-84
116. Lind MJ, Margison JM, Cerny T, et al. Prolongation of ifosfamide elimination half-life in obese patients due to altered drug distribution. Cancer Chemother Pharmacol 1989; 25: 139-42
117. Boddy AV, Yule SM, Wyllie R, et al. Pharmacokinetics and metabolism in children of ifosfamide administered as a continuous infusion. Cancer Res 1993; 53: 3758-64
118. Hartley JM, Hansen L, Harland SJ, et al. Metabolism of ifosfamide during a 3 day infusion. Br J Cancer 1994; 69: 931-6
119. Corlett SA, Parker D, Chrystyn H. Pharmacokinetics of ifosfamide and its enantiomers following a single 1-h intravenous infusion of the racemate in patients with small-cell lung carcinoma. Br J Clin Pharmacol 1995; 39: 452-5
120. Roy P, Tretyakov O, Wright J, et al. Stereoselective metabolism of ifosfamide by human P450s 3A4 and 2B6: favorable metabolic properties of the R-enantiomer. Drug Metab Disp 1999; 27: 1309-18
121. Walker D, Flinois J-P, Monkman SC, et al. Identification of the major human hepatic cytochrome P450 involved in activation and N-dechloroethylation of ifosfamide. Biochem Pharmacol 1994; 47: 1157-63
122. Murray GI, McKay JA, Weaver RJ, et al. Cytochrome P450 expression is a common molecular event in soft tissue sarcomas. J Pathol 1993; 171: 49-52
123. Murray GI, Weaver RJ, Paterson PJ, et al. Expression of xenobiotic metabolizing enzymes in breast cancer. J Pathol 1993; 169: 347-53
124. Janot F, Massaad L, Ribrag V, et al. Principal xenobiotic-metabolizing enzyme systems in human head and neck squamous cell carcinoma. Carcinogenesis 1993; 14: 1279-83
125. Goren MP, Wright RK, Pratt CB, et al. Dechloroethylation of ifosfamide and neurotoxicity. Lancet 1986; II: 1219-20

126. Kurowski V, Wagner T. Comparative pharmacokinetics of ifosfamide, 4-hydroxyifosfamide, chloroacetaldehyde and 2- and 3-dechloroethylifosfamide in patients on fractionated intravenous ifosfamide therapy. Cancer Chemother Pharmacol 1993; 33: 36-42
127. Boddy AV, Cole M, Pearson ADJ, et al. The kinetics of the auto-induction of ifosfamide metabolism during continuous infusion. Cancer Chemother Pharmacol 1995; 36: 53-60
128. Hassan M, Svensson U, Ljungman P, et al. A mechanism-based pharmacokinetic model for cyclophosphamide autoinduction in breast cancer patients. Br J Clin Pharmacol 1999; 48: 669-77
129. Johnstone EC, Lind MJ, Siddiqui N, et al. Assessment of DNA damage caused by ifosfamide and metabolites [abstract 17]. Annual Conference of the American Association for Cancer Research; 1997 Apr 12-16; San Diego, 38: 3
130. Hartley JM, Spanswick VJ, Gander M, et al. Measurement of DNA cross-linking in patients on ifosfamide therapy using the single cell gel electrophoresis (comet) assay. Clin Cancer Res 1999; 5: 507-12

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