ELSEVIER

Journal of Chromatography A, 694 (1995) 169–179

JOURNAL OF CHROMATOGRAPHY A

Using chirality as a unique probe of pharmacological properties

Irving W. Wainer*, Julie Ducharme, Camille P. Granvil, Heli Parenteau, Sami Abdullah

Department of Oncology, McGill University, Montreal, Quebec, Canada

Abstract

The development of enantioselective chromatographic techniques has made it feasible to routinely follow the metabolism and disposition of the separate enantiomers of a chiral drug. These studies are a source of data about in vivo pharmacological processes. The key question is recognition of the fundamental information contained within the results and the application of this data to the development of a deeper understanding of the clinical consequences of stereochemistry. This manuscript presents two examples of how chirality can be used as a unique probe of basic pharmacological properties.

1. Introduction

"*The difficulty in science is often not so much how to make the discovery but to know that one has made it. In all experiments there are a number of effects, produced by all kinds of extraneous causes, which are not in the least significant, and it requires a certain degree of intelligence or intuition to see which of them really means anything*" (J.D. Bernal [1]).

The pharmacological importance of stereoisomerism has been established by numerous in vitro studies involving ligand-biopolymer and substrate–enzyme interactions. At the same time, the in vivo fate of chiral compounds has not received the same attention. This has been primarily due to the lack of adequate analytical methodology.

In the past few years this situation has dramatically changed with the rapid increase in commercially available chromatographic chiral stationary phases (CSPs) for both HPLC and GC. These phases have formed the backbone of many enantioselective bioanalytical methods which have been used in pharmacokinetic and metabolic studies of chiral drugs.

These technological advances have resulted in an increased interest in the in vivo disposition of the enantiomers of chiral substances; particularly from the drug regulatory agencies. This, in turn, has produced a steady rise in enantioselective pharmacokinetic and metabolic studies which are now standard procedures in the development and testing of new chiral drugs, for both racemic and single isomer formulations.

The routine determination of enantioselective pharmacokinetics represents a positive scientific advance and the realization that pharmacology is a complex three-dimensional phenomenon. However, as this pharmacokinetic approach be-

* Corresponding author. Address for correspondence: Montreal General Hospital, Room B7113, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4, Canada.

0021-9673/95/$09.50
SSDI 0021-9673(94)00749-7

comes routine, the possibility arises that the innovative and extraordinary data provided by enantioselective analysis will be lost in the day-to-day activities. Indeed, pharmacokinetic and metabolic studies of a chiral compound contain an abundance of unique information. For example, since the physicochemical properties of enantiomers are the same, an enantioselective difference in distribution can be used to probe for specific ligand–biopolymer binding interactions or a distinct enantiomer-related metabolic pattern may lead to the identification of multiple enzyme systems. These possibilities will be illustrated by recent studies with the drugs hydroxychloroquine (HCQ) and ifosfamide (IFF).

In the first example, the results from pharmacokinetic studies of HCQ enantiomers in the human and in the rabbit suggested that the observed differences might be due to an enantioselective sequestration. This led to a further study of the enantioselective ocular distribution of HCQ in the rabbit which confirmed that enantioselective accumulation occurs in ocular tissues. The data also indicated that the observed enantioselectivity is a result of binding to an as of yet unidentified biopolymer.

In a similar manner, the relative enantiomeric metabolite patterns of the anticancer agent IFF were determined during a clinical pharmacokinetic study. Addressing metabolite stereoselectivity becomes critical if the metabolite is pharmacologically active, either contributing to the parent drug's activity or to its toxicity. Through a comparison of the different urinary excretion patterns from patients, it was concluded that at least two microsomal isoenzymes are responsible for the metabolism of this drug. These results suggest that treatment-limiting neurotoxicity may be linked to a particular metabolic phenotype which reflects an overexpression of one or both of these enzymes.

Neither of these conclusions could have been drawn from the experimental data without using chirality "to see which of them (i.e. results) really means anything". These studies are presented below.

2. Distribution of the enantiomers of hydroxychloroquine in ocular tissues: observing a new ligand–biopolymer interaction

HCQ is an aminoquinolone used in the treatment of malaria, extraintestinal amebiasis, rheumatoid arthritis, discoid and systemic lupus erythematosus. Ocular toxicity, in particular pigmentary retinopathy, has been associated with the clinical use of HCQ [2,3]. The mechanism producing retinal damage is unknown.

HCQ is an enantiomeric compound which is administered as a racemic mixture. HCQ undergoes hepatic metabolism and after chronic oral administration, the plasma and whole blood contain significant levels of HCQ and of three metabolites, desethylchloroquine (DCQ), desethylhydroxychloroquine (DHCQ) and bisdesethylchloroquine (BDCQ). Since DCQ, DHCQ and BDCQ are also enantiomeric, the plasma and whole blood can contain up to eight distinct chemical entities.

2.1. Pharmacokinetics and metabolism of HCQ enantiomers

The clinical pharmacokinetics and metabolism of HCQ enantiomers, *R*-HCQ and *S*-HCQ, have been studied after multiple [4–6] and single [7] doses of the racemate, *rac*-HCQ. These studies have demonstrated that the distribution, elimination and metabolism of HCQ is enantioselective. After administration of *rac*-HCQ, the systemic exposure, calculated from the plasma and whole blood concentration–time plots as area-under-the-curve (AUC), was greater for *R*-HCQ than for *S*-HCQ. Conversely, for the metabolites, the levels of the *S*-enantiomers were always higher. In addition, *S*-HCQ renal clearance was also higher than that of *R*-HCQ.

The enantioselective pharmacokinetics of *R*- and *S*-HCQ in the rabbit has also been investigated [8]. Both albino (New Zealand White, NZW) and pigmented rabbits were used in the study which involved oral dosing of *rac*-HCQ and the separate enantiomers. Following the administration of *rac*-HCQ, the whole blood

levels of *R*-HCQ always surpassed those of *S*-HCQ, while the opposite situation was found in plasma. In whole blood, *R*/*S* ratios varied from 1.3 to 3 [8], similar to the 1.6–2.9 range found in human blood [6]. No significant difference was observed between the HCQ whole blood or plasma levels in the NZW and the pigmented rabbits. In addition, no stereochemical interconversion was detected after separate administrations of *R*-HCQ and *S*-HCQ [8].

2.2. Determination of ocular concentrations of HCQ enantiomers

The results from the rabbit studies indicated that this species could be a useful model for the investigation of the distribution of the enantiomers of HCQ and its metabolites in ocular tissues. This study was undertaken and the results have been recently reported [9]. In this study, both NZW and pigmented animals were used and single and multiple daily oral doses of *rac*-HCQ or the separate enantiomers were administered. In addition, NZW and pigmented animals were dosed with *rac*-HCQ for 8 days and washed out for an additional 7 days. At the end of the study periods, plasma and whole blood samples were collected and the rabbits were sacrificed. The eyes were collected, the aqueous humor removed and the eyes separated into the cornea, lens, vitreous body, iris, retina/choroid, sclera and conjunctiva. The concentrations of *R*-HCQ, *S*-HCQ and their respective metabolites were determined using a validated enantioselective liquid chromatographic assay [10] (Fig. 1).

The mean HCQ levels in the cornea, sclera, iris, retina/choroid and conjunctiva determined at the time of sacrifice for the NZW rabbits dosed daily for 8 days are presented in Table 1. The plasma levels of HCQ were significantly lower than those found in the ocular tissues indicating that the drug had accumulated in these tissues. The mean concentrations of HCQ in the cornea, sclera, iris, retina/choroid and conjunctiva after daily oral dosing with *rac*-HCQ for 8 days followed by a 7-day washout are also presented in Table 1. The results from the washout study indicate that the accumulations of HCQ in these ocular tissues were reversible. After the 7-day washout period, the levels of HCQ in these tissues decreased by an average of 74%. The average *R*-HCQ:*S*-HCQ ratio fell from approximately 60:40 to 52:48 at the end of the 7-day washout, indicating that there was no preferential sequestration of one enantiomer.

The mean HCQ levels in the cornea, sclera, iris, retina/choroid and conjunctiva determined at the time of sacrifice for the pigmented rabbits dosed daily for 8 days are presented in Table 1. The plasma levels of HCQ were significantly lower than those found in the ocular tissues indicating that the drug had accumulated in these tissues. The mean concentrations of HCQ in the cornea, sclera, iris, retina/choroid and conjunctiva after daily oral dosing with *rac*-HCQ for 8 days followed by a 7-day washout are also presented in Table 1. Unlike the NZW rabbits, the HCQ levels either remained unchanged during the washout period, cornea and conjunctiva, or increased; the HCQ concentrations in the sclera and iris doubled and the levels in the retina/choroid increased by 78%. The data suggest that melanin, found in the iris and retina/choroid, continues to concentrate HCQ and acts as a intraocular source of the drug for the non-pigmented tissues, cornea, sclera and conjunctiva. The observed enantioselectivity of the non-melanin containing tissues, 55:45 *R*-HCQ:*S*-HCQ, versus the 50:50 *R*-HCQ:*S*-HCQ ratios determined in the iris and retina/choroid indicates that HCQ-melanin binding is not enantioselective.

2.3. Summary and conclusions: the identification of a HCQ-binding biopolymer

The data from this study indicates that after systemic administration of *rac*-HCQ, *R*-HCQ and *S*-HCQ accumulate in the ocular tissues of the rabbit. Comparison of the enantioselectivities of the accumulations in albino and pigmented rabbits demonstrates that HCQ binds to two biopolymers —melanin which is found in the

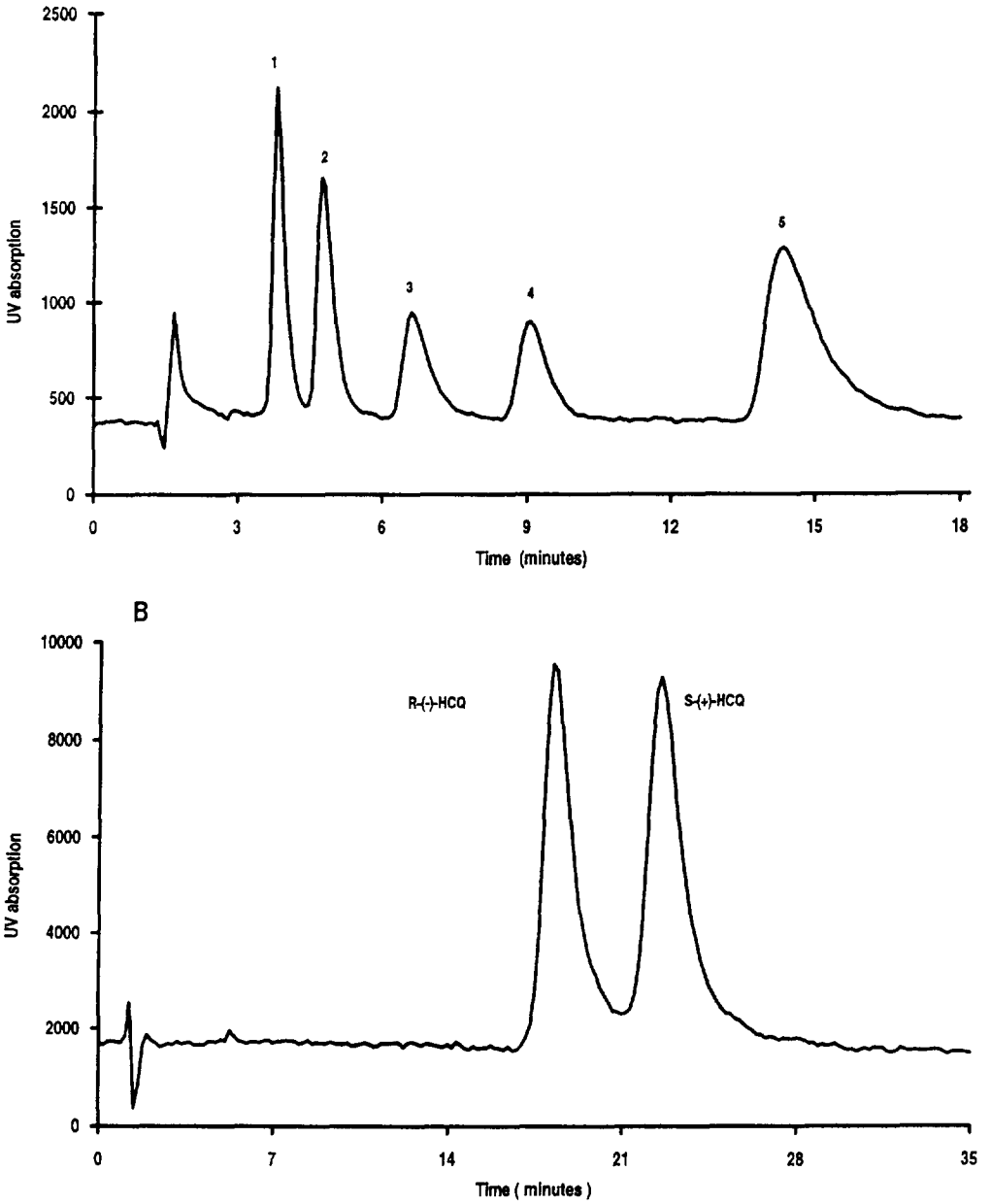

Fig. 1. (A) The chromatographic analysis of a urine sample spiked with 100 ng/ml each of HCQ and its major metabolites, where 1 = BDCQ, 2 = DHCQ, 3 = DCQ, 4 = HCQ and 5 = chloroquine (internal standard). (B) The chromatographic separation of a racemic (50:50 mixture) of the enantiomers of HCQ; (−)-HCQ and (+)-HCQ. See Ref. [10] for experimental conditions.

pigmented iris and retina/choroid and a currently unidentified biopolymer (BP-X) which is present in the cornea, sclera, iris, retina/choroid and conjunctiva. These conclusions are based upon the following observations:

(1) The accumulation of HCQ in non-melanin containing ocular tissues is enantioselective: in the non-pigmented ocular tissues, the mean *R*-HCQ:*S*-HCQ ratio was 60:40. The observed enantioselectivity mirrored the relative distribu-

Table 1
Ocular tissue concentrations of hydroxychloroquine in the rabbit presented as ng/g tissue after daily oral dosing of *rac*-HCQ, 20 mg/kg

Tissue	Pigmented dosing schedule			Albino dosing schedule		
	8 days	22 days	8 days + 7-day washout	8 days	15 days	8 days + 7-day washout
Cornea	644 ± 716	165 ± 80	436 ± 91	6 361 ± 4 416	1 211 ± 665	1 364 ± 1 177
Sclera	2 960 ± 1 905	1 342 ± 1 494	6 735 ± 1 578	3 028 ± 462	1 298 ± 253	593 ± 64
Iris	154 029 ± 13 555	80 923 ± 15 415	314 154 ± 386 326	8 362 ± 4 244	679 ± 145	2 239 ± 335
Retina/choroid	51 911 ± 33 161	45 455 ± 13 537	92 191 ± 17 501	5 225 ± 630	544 ± 143	884 ± 95
Conjunctiva	1 678 ± 1 274	621 ± 35	1 490 ± 21	1 204 ± 311	0	977 ± 178

tion of *R*-HCQ and *S*-HCQ in whole blood where the average *R*-HCQ:*S*-HCQ ratio was also 60:40 (range 54:46 to 67:33). The opposite result was observed for the total plasma concentration of HCQ where the average *R*-HCQ:*S*-HCQ ratio is 40:60 (range 47:53 to 39:61).

(2) *R*- and *S*-HCQ do not compete for transport or binding: when the albino animals were dosed with the separate enantiomers of HCQ, the resulting average tissue concentrations were similar to those attained after dosing with the racemate. This indicates that *R*-HCQ and *S*-HCQ do not compete with each other for transport into the ocular tissues and in binding to BP-X. These results were also similar to the relative whole blood concentrations achieved after dosing with the separate enantiomers; after 5 days of dosing with either *R*-HCQ or *S*-HCQ, the whole blood concentrations of *R*-HCQ were two-fold higher than the corresponding concentrations of *S*-HCQ. HCQ was stereochemically stable in the rabbit and an enantioselective interconversion between *R*-HCQ and *S*-HCQ was not observed [8].

(3) The enantioselective ocular accumulation is not primarily due to plasma protein binding: the in vitro human plasma protein binding of *R*-HCQ and *S*-HCQ has been studied and found to be enantioselective; the binding of *S*-HCQ was almost two-fold higher than *R*-HCQ, 64 and 37%, respectively [11]. Since only the free fraction of a drug can pass through biological membranes and distribute into tissues, *R*-HCQ, with a 63% unbound fraction, would be expected to accumulate to higher levels in ocular tissues. Thus, the observed enantioselectivity may only be a reflection of the enantioselectivity of the protein binding.

However, the low proportion of protein binding and the of loss of enantioselectivity in the melanin binding suggests that the difference between the free fractions of *R*-HCQ and *S*-HCQ in the plasma is probably not the determinant influence on the ocular distribution of these enantiomers. The principal determinant appears to be enantioselective binding to BP-X and the importance of this interaction will only be ascertained when the biopolymer is isolated, identified and its binding properties elucidated.

(4) The binding of HCQ to melanin is not enantioselective: When melanin is present in the iris and retina/choroid, the enantioselective accumulations observed in these tissues in the albino rabbits is lost. The presence of melanin in the iris and retina/choroid of the pigmented rabbits resulted in an 18-fold increase in the HCQ concentration in the iris and a 13-fold increase in the retina/choroid; the average concentration in the iris of pigmented rabbits was 154.029 ng/g tissue which was more than 50-fold higher than the mean of the concentrations found in the non-pigmented tissues. The increases in HCQ concentrations in the iris and retina/choroid were accompanied by a loss of enantioselectivity as the *R*-HCQ:*S*-HCQ ratio fell from 65:35 (albino rabbits) to 50:50 (pigmented rabbits).

These results indicate that melanin has a relatively higher affinity for HCQ than BP-X. However, confirmation of this supposition also

awaits the isolation and identification of BP-X and elucidation of its binding properties.

(5) The distribution of HCQ metabolites into ocular tissues: the distribution of the enantiomers of DCQ and DHCQ into the ocular tissues studied in this program follows the same patterns as HCQ. The accumulations are reversible and enantioselective. The presence of melanin in the retina/choroid and iris results in a significant increase in the quantity of metabolite accumulated in the tissue relative to the non-pigmented tissues. The affinity of DCQ and DHCQ binding to BP-X and the enantioselectivity of this process remain to be described.

3. Enantioselective N-dechloroethylation of ifosfamide: uncovering a multiple-enzyme metabolic pathway

IFF is an alkylating agent which has demonstrated activity against a wide range of tumor types [12] including Ewings sarcoma, osteosarcoma and rhabdomyosarcoma. IFF is a chiral molecule which contains an asymmetrically substituted phosphorous atom and exists in two enantiomeric forms, *R*-IFF and *S*-IFF. In clinical practice, IFF is administered as a racemic (50:50) mixture of the two enantiomers and the majority of reported pharmacological studies have been carried out without consideration of the metabolic and pharmacokinetic fate of *R*- and *S*-IFF.

In its original form, IFF is not an active antitumor agent. The compound must be metabolically transformed into the cytotoxic agent, isophosphoramide mustard (IPM, Fig. 2). IPM is an active alkylating agent responsible for the cross-linking of DNA produced by IFF [13,14]. The initial metabolic step in the transformation of IFF to IPM is the oxidation of the 4-carbon of the oxazaphosphorine ring by hepatic microsomal enzymes to form 4-hydroxy-IFF, efficacious pathway (Fig. 2). Oxidation also occurs (up to 48% of the dose) at one of the two β-chloroethyl side chains, toxic pathway (Fig. 2). This pathway produces the N-dechloroethylated metabolites, 2-dechloroethyl-IFF (2-DCE-IFF) and 3-dechloroethyl-IFF (3-DCE-IFF) which are not active antitumor agents, and chloroacetaldehyde, a central nervous system (CNS) toxin [13,14].

3.1. Quantitation of the enantiomeric composition of IFF, 2-DCE-IFF and 3-DCE-IFF

In order to follow the in vivo and in vitro metabolism and distribution of IFF, an enantioselective GC–MS method was developed for the quantitation of the enantiomers of IFF and 2-DCE-IFF and 3-DCE-IFF in biological matrices [15]. IFF and the two dechloroethylated metabolites were extracted into chloroform, enantioselectively resolved on a GC CSP based upon heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin and quantitated using mass selective detection with selective ion monitoring (Fig. 3). The limits of quantitation for the enantiomers of IFF, 2-DCE-IFF and 3-DCE-IFF in plasma were 250 and 500 ng/ml, respectively. In urine, the limits of quantitation for the enantiomers of IFF, 2-DCE-IFF and 3-DCE-IFF were 500 ng/ml. The method is able to detect concentrations as low as 250 ng/ml of each enantiomer of 2-and 3-DCE-IFF in plasma and urine. The intra- and inter-day relative standard deviations for this method were with one exception less than 8%. The assay was validated for enantioselective pharmacokinetic studies in humans and rats and was the first reported enantioselective assay for the measurement of the enantiomers of 2- and 3-DCE-IFF in plasma.

3.2. Enantioselective metabolism and distribution of IFF

Initial studies by Misiura et al. [16] indicated that there were stereochemical differences in the human metabolism of IFF. Urine samples contained more *R*-IFF than *S*-IFF and the production of 2-DCE-IFF from *S*-IFF was 2.7- to 6.7-fold higher than from the corresponding *R*-isomer. Boss et al. [17] and Wainer et al. [18] have confirmed these results.

The plasma concentration vs. time profiles

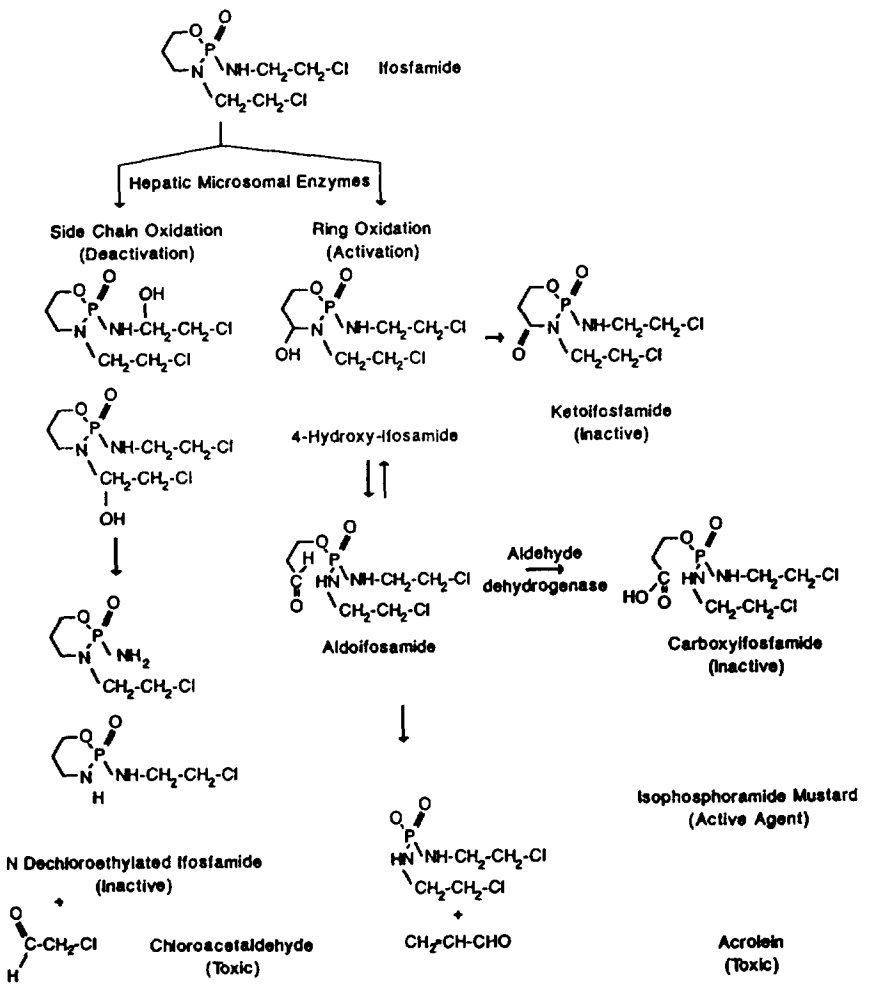

Fig. 2. The metabolic pathways of ifosfamide.

(AUCs) of *R*- and *S*-IFF and their metabolites have been recently measured in women receiving IFF for treatment of pelvic carcinomas [19] and the data are presented in Table 2. The plasma AUC for *R*-IFF was significantly greater than the AUC of *S*-IFF while the AUCs for the metabolites arising from *S*-IFF were greater than those of the metabolites produced from *R*-IFF. It is important to note that *S*-IFF gives rise to *S*-2-DCE-IFF and *R*-3-DCE-IFF while *R*-2-DCE-IFF and *S*-3-DCE-IFF are produced from *R*-IFF; the apparent change in stereochemical configuration is a result of the Cahn–Ingold–Prelog nomenclature system. The results indicate that enantioselective dechloroethylation occurs and favors *S*-IFF.

3.3. Neurotoxic side-effects associated with IFF

Neurotoxic side-effects such as cerebellar dysfunction, seizures and changes in mental status have been reported in up to 30% of patients on high-dose IFF [20] and as a dose-limiting toxicity with oral IFF in up to 50% of

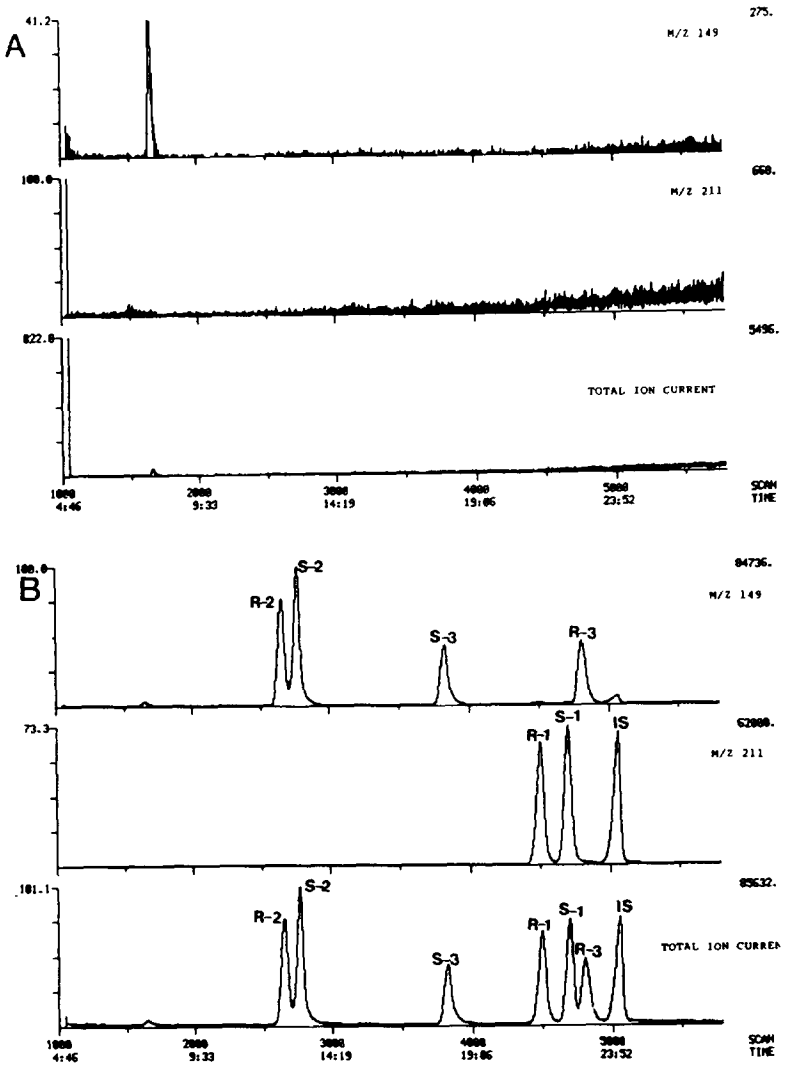

Fig. 3. Typical selected ion chromatograms and total ion current of *rac*-IFF and its metabolites. (A) Blank samples (urine or plasma). (B) Samples (urine or plasma) spiked with 20 μg/ml IFF and its metabolites. R-2 = *R*-2-DCE-IFF; S-2 = *S*-2-DCE-IFF; S-3 = *S*-3-DCE-IFF; R-1 = *R*-IFF; S-1 = *S*-IFF; R-3 = *R*-3-DCE-IFF; IS = cyclophosphamide (CP). See Ref. [17] for experimental conditions.

the patients [21]. This neurotoxicity has been attributed to the chloroacetaldehyde produced by the metabolic pathway involving side chain oxidation, toxic pathway (Fig. 2). Studies in patients undergoing treatment with IFF have associated CNS toxicity with high blood levels of chloroacetaldehyde [21].

Work in this laboratory has described for the first time a relationship between CNS toxicity and urinary excretion of the dechloroethylated metabolites of IFF [22]. In the study of gynecological cancer described above, the patient with the highest levels of *R*-3-DCE-IFF in urine (28% dose) experienced severe neuro-

Table 2
Plasma area-under-the-curves (AUCs) for *R*- and *S*-ifosfamide (IFF) and their 2- and 3-N-dechloroethylated metabolites (2-DCE-IFF, 3-DCE-IFF) after a 3-h infusion of *rac*-IFF (3 g/m^2)

Compound	AUC (mg/l · h)
R-IFF	688 ± 244
S-IFF	515 ± 158
R-2-DEC-IFF	24 ± 8
S-2-DCE-IFF	61 ± 62
R-3-DCE-IFF	121 ± 63
S-3-DCE-IFF	63 ± 46

Data obtained fron Ref. [19].

toxicity, precluding any further IFF administration (Table 3, patient 5). When *R*-2-DCE-IFF was elevated, so was *R*-3-DCE-IFF (e.g. patient 5). The same trend was observed for *S*-2-DCE-IFF and *S*-3-DCE-IFF (e.g. patient 4).

3.4. Identification of multiple metabolic pathways for IFF

The results from the patient study indicate that at least two microsomal enzymes, i.e. cytochrome P450 isoenzymes (CYP), are responsible for the N-dechloroethylation of IFF. One CYP catalyzes the transformation of *S*-IFF and *R*-IFF to *S*-2-DEC-IFF and *S*-3-DCE-IFF, respectively, while the other is responsible for the production of *R*-3-DCE-IFF from *S*-IFF and *R*-2-DCE-IFF from *R*-IFF. The existence of at least two CYPs could not have been determined without a careful examination of the enantioselectivity of the N-dechloroethylation process.

The results from the patient studies are consistent with in vivo and in vitro studies in the rat of the effect of phenobarbital induction on the microsomal N-dechloroethylation of IFF. In the in vivo studies [23], the rats were treated with phenobarbital for one-week and then administered a single i.v. dose of IFF. Phenobarbital pretreatment induced the N-dechloroethylation of IFF in an enantioselective manner relative to the controls; in particular, there was a statistically significant increase in the formation of *S*-2-DCE-IFF from *S*-IFF at the apparent expense of the other metabolite of IFF, *R*-3-DCE-IFF. These results suggest that the phenobarbital inducible CYPs are responsible for the observed increases in *S*-2-DCE-IFF and *S*-3-DCE-IFF, but had no effect on *R*-2-DCE-IFF and *R*-3-DCE-IFF.

In order to determine the source of the in vivo effects on IFF metabolism observed after phenobarbital induction an in vitro study was conducted utilizing microsomes prepared from a series of rats pretreated with known inducers of cytochrome P450 isoenzymes [24]. The inducers were: clofibric acid, 2,4-dichlorophenoxyacetic acid and perfluorodecanoic acid (which induce the CYP4A family); phenobarbital (which in-

Table 3
Total 24-h urinary excretion of the enantiomers of 2- and 3-N-dechloroethylated ifosfamide (2-DCE-IFF, 3-DCE-IFF) as per cent of administered dose (% dose)

Patients and observed CNS toxicity	*S*-2-DCE (% dose)	*R*-2-DCE (% dose)	*S*-3-DCE (% dose)	*R*-3-DCE (% dose)
Mean of 8 patients	6	4	5	13
Patient 5	7	8	6	28
Patient 4	11	4	10	6

duces mainly CYP2Bs but also CYP2Cs and CYP3As); β-naphthoflavone (which inhibits the CYP1A family); and dexamethasone (which induces the CYP3A family). Liver microsomes were prepared using standard techniques from experimental and control animals and the production of DCE-IFF metabolites was monitored by enantioselective GC–MS.

The results from these initial experiments are presented in Fig. 4. From these data it appears that pretreatment with perfluorodecanoic acid (PFDA), clofibric acid (CLOF), 2,4-dichlorophenoxyacetic acid (2,4D) and dexamethasone (DEX) had no significant effect on the dechloroethylation of IFF. However, phenobarbital (PB) induction increased the production of *S*-2-DCE-IFF and *S*-3-DCE-IFF relative to control while having no significant effect on the metabolism of *S*-IFF to *R*-3-DCE-IFF and *R*-IFF to *R*-2-DCE-IFF. These results are consistent with the observations from the in vivo studies with phenobarbital induced rats and indicate that one isoenzyme, PB-inducible (CYPs 2B, 2C or 3A), is responsible for the production of *S*-2-DCE-IFF and *S*-3-DCE-IFF while a second isoenzyme, BF-inhibited (CYP1A), controls the metabolism of IFF to *R*-3-DCE-IFF and *R*-2-DCE-IFF. In humans, CYP3As appear to be implicated [25], but a second CYP has not been identified.

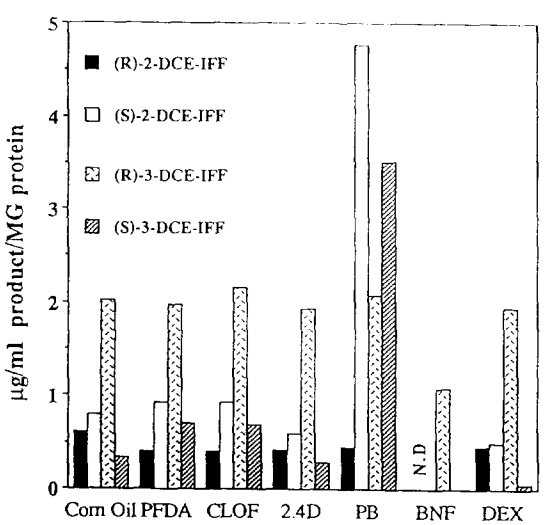

Fig. 4. Effect of microsomal inducers on the in vitro N-dechloroethylation of ifosfamide enantiomers. See Ref. [24] for experimental details. N.D. = Not detected.

4. Conclusions

The existence of stereoisomeric forms of a chemical has been a recognized fact for almost 150 years. However, the clinical consequences of symmetry and asymmetry are only just beginning to be considered. The next few years should see a growth in the realization that within the three-dimensional structures of the human body lie tremendous potentials for differential drug actions and, perhaps, new keys to the treatment of cancer and other diseases. The challenge will be to know when we have found these keys.

References

[1] J.D. Bernal, in *Science in History*, Vol. 2, M.I.T. Press, Cambridge, MA, 1971, p. 608.
[2] H. Hobbs and C. Calnan, *Lancet*, i (1958) 1207.
[3] M. Easterbrook, *Semin Arthritis Rheum.*, 23 (Suppl 2) (1993) 62.
[4] A.J. McLachlan, S.E. Tett and D.J. Cutler, *J. Chromatogr.*, 570 (1991) 119.
[5] S.E. Tett, A.J. McLachlan, R.O. Day and D.J. Cutler, *Agents Actions Suppl.*, 44 (1993) 145.
[6] A.J. McLachlan, S.E. Tett, D.J. Cutler and R.O. Day, *Br. J. Clin. Pharmacol.*, 36 (1993) 78.
[7] J. Ducharme, I.W. Wainer, H. Fieger and S.K.W. Khalil, *Br. J. Clin. Pharmacol.*, in press.
[8] J. Ducharme I.W. Wainer, H.I. Parenteau and J.H. Rodman, *Chirality*, 6 (1994) 337.
[9] I.W. Wainer, J.C. Chen, H. Parenteau, S. Abdullah, J. Ducharme, H. Fieger and J. Iredale, *Chirality*, 6 (1994) 347.
[10] J. Iredale and I.W. Wainer, *J. Chromatogr.*, 573 (1992) 253.
[11] A.J. McLachlan, D.J. Cutler and S.E. Tett, *Eur. J. Clin. Pharmacol.*, 44 (1993) 401.
[12] H. Jurgens, J. Treuner, K. Winkler and U. Gobel, *Seminars Oncol.*, 16 (Suppl. 3) (1989) 46.
[13] L.M. Allen and P.J. Craven, *Cancer Chemother. Rep.*, 56 (1972) 603.
[14] M. Colvin, *Seminars Oncol.*, 9 (Suppl. 1) (1982) 2.
[15] C.P. Granvil, B. Gehrcke, W.A. Konig and I.W. Wainer, *J. Chromatogr.*, 622 (1993) 21.

[16] K. Misiura, A. Okruszek, K. Pankiewick, W.K. Stec, Z. Czownicki, and B. Utracka, *J. Med. Chem.*, 26 (1983) 674.
[17] J. Boss, U. Welslau, J. Ritter, G. Blaschke, and G. Schellong, *Cancer Chemother. Pharmacol.*, 28 (1991) 455.
[18] I.W. Wainer, C.F. Stewart, C.L. Young, D. Measurel, and H. Frank, *Proc. Am. Soc. Clin. Oncol.*, 7 (1988) 72.
[19] J. Ducharme, C.P. Granvil, B. Leyland-Jones, M. Trudeau and I.W. Wainer, *Proc. Am. Assoc. Cancer Res.*, 35 (1994) 423.
[20] L.D. Lewis and C.A. Meanwell, *Lancet*, 335 (1990) 175.
[21] M.J. Lind, H.L. Roberts, N. Thatcher and J.R. Idle, *Cancer Chemother. Pharmacol.*, 26 (1990) 105.
[22] I.W. Wainer, J. Ducharme, C.P. Granvil, B. Leyland-Jones and M. Trudeau, *Lancet*, 343 (1994) 982.
[23] C.P. Granvil, T. Wang, G. Batist and I.W. Wainer, *Drug Metab. Dispos.*, 22 (1994) 165.
[24] I.W. Wainer, J. Ducharme, C.P. Granvil, U. Sanzgiri and A. Parkinson, *Proc. Am. Assoc. Cancer. Res.*, 35 (1994) 423.
[25] D. Walker, J.P. Flinois, S.C. Monkman, C. Beloc, A.V. Boddy, S. Cholerton, A.K. Daly, M.J. Lind, A.D.J. Pearson, P.H. Beaune and J.R. Idle, *Biochem. Pharmacol.*, 47 (1994) 1157.