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Enantiomers of cyclophosphamide and iphosphamide

Cyclophosphamide (CP) (**I**) and iphosphamide (IP) (**II**) are two of the most widely used alkylating agents in cancer chemotherapy. Both compounds are activated by metabolism via their conversion (largely in the liver) by cytochrome P-450 to 4-hydroxy derivatives (**III**, **IV**). These spontaneously decompose to yield the potent alkylating species, phosphoramide mustard (**V**) from CP and iphosphoramide mustard (**VI**) from IP. The 4-hydroxy derivatives **III** and **IV** are also detoxified by oxidation to the 4-keto derivatives **VII** and **VIII** or, via their acyclic tautomers, to the carboxylic acids **IX** and **X**. An additional detoxification pathway is dechloroethylation of the parent drug molecules which yields the monochloroethyl derivatives **XI** from CP or **XI** and **XII** from IP.

The molecules of CP and IP contain a chiral centre at the phosphorus atom, but both drugs were initially administered clinically as their racemic forms. Interest in the possibility that one of the enantiomers of CP might show higher anti-cancer activity and/or lower toxicity was aroused in 1975–1976 both by the first synthesis of the individual enantiomers by Kinas *et al.* [1] and by the observation by Cox *et al.* [2, 3] that patients administered the racemic drug excreted optically active CP in their urine. In fact the latter observation was later shown by a more rigorous method of determining enantiomeric composition (using NMR and chiral shift reagents) to be incorrect [4], but nevertheless studies of the anticancer effectiveness and metabolism of the enantiomers had by that time been initiated and were further stimulated by the discovery that the (−)-enantiomer of CP had *ca.* twice the therapeutic index (LD_{50}/ID_{90}) of the (+)-enantiomer against the ADJ/PC6 plasma cell tumour in mice ((+)-CP 68.9, (−)-CP 128.1, (±)-CP 93.0) [3]. Chemical, crystallographic and pharmacological studies of the CP-enantiomers, and shortly afterwards of IP-enantiomers, rapidly followed and eventually led to limited use of the enantiomers in man.

(a) *Chemical studies of enantiomeric CP and IP*

The first enantiomeric preparation of CP was carried out via the synthesis of diastereoisomeric derivatives bearing α-phenylethyl groups on N-3 [1–5]. Hydrogenolysis of these led to (+)- and (−)-CP with optical rotation α_D 2.3 ± 0.2° [1, 6]. The enantiomeric homogeneity of these was checked by NMR with the use of an optically active shift reagent [6]. Several other approaches to the synthesis of these enantiomers have been reported or patented; the procedure of Sato *et al.* [7] is notable in view of the high stereoselective efficiency of the synthesis. The absolute configuration of the laevorotatory form as *S*- and that of the dextrorotatory form as *R*- was independently determined by X-ray diffraction methods by Adamiak *et al.* [8, 9] and by Karle *et al.* [10]. The oxazaphosphorine ring exists in a chair form with the P=O bond axial.

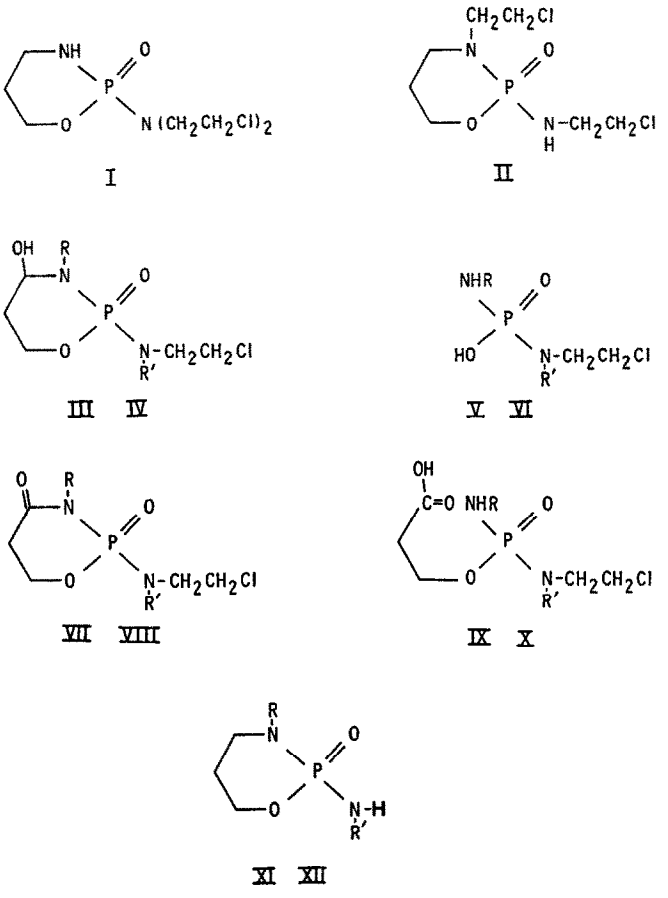
The resolution of racemic CP has also been used as a source of the enantiomers. To achieve this the racemate is reacted with an optically active derivatising reagent. The resulting diastereoisomers are separated chromatographically or by crystallisation and deprotected to yield the enantiomeric CPs. The derivatising agents used have included optically active α-naphthylphenylmethylsilyl chloride (reaction with the lithium salt of CP) [11] and α-phenylbutyric acid (reaction with the N-3 alkylol produced by the reaction of CP with chloral) [12]. The reaction of CP with the optical isomers of 1-phenylethyl alcohol has also been used as the basis of a method using TLC for monitoring enantiomeric composition of CP [13], and resolution of the enantiomers is also possible by chromatography on polyamides with optically active substituents [14].

The synthesis of optically active IP (α_D −39.4°, +39.0°) was achieved by Kinas *et al.* [15] shortly after that of CP, by an analogous route involving diastereoisomeric *N*-α-phenylethyl intermediates. Several modifications of this approach were subsequently published [16, 17]. Racemic IP has also been resolved by chromatography on optically active phases [14], or by forming diastereoisomeric Pt (II) complexes which may be separated and then reconverted to (+) and (−)-IP [18]. The absolute configuration of (−)-IP was determined to be *S*- at the phosphorus atom by X-ray diffraction methods [19] and by stereochemical correlation with CP-enantiomers.

(b) *Metabolism of enantiomers of CP* (in vitro *and in animals)*

Initial metabolic studies on the purified enantiomers of CP, carried out *in vitro* using rat liver microsomes, indicated that *R*- and *S*-CP were metabolised at essentially the same rate [3]. However, later investigations revealed a fascinating species difference in enantiomeric selectivity in metabolism. The nature and rates of metabolism of the enantiomers, while present in a racemic mixture, were studied simultaneously by the use of "pseudoracemates",

Scheme 1. Structures of cyclophosphamide (**I**), iphosphamide (**II**) and their metabolites.



III	R = H, $R^1 = CH_2CH_2Cl$	4-hydroxycyclophosphamide;
IV	$R = CH_2CH_2Cl$, R^1 = H	4-hydroxyiphosphamide;
V	R = H, $R^1 = CH_2CH_2Cl$	phosphoramide mustard;
VI	$R = CH_2CH_2Cl$, R^1 = H	iphosphoramide mustard;
VII	R = H, $R^1 = CH_2CH_2Cl$	4-ketocyclophosphamide;
VIII	$R = CH_2CH_2Cl$, R^1 = H	4-ketoiphosphamide;
IX	R = H, $R^1 = CH_2CH_2Cl$	carboxyphosphamide;
X	$R = CH_2CH_2Cl$, R^1 = H	carboxyiphosphamide;
XI	R = H, $R^1 = CH_2CH_2Cl$	dechloroethylated cyclophosphamide/ iphosphamide;
XII	$R = CH_2CH_2Cl$, R^1 = H	dechloroethylated iphosphamide.

which are racemates containing one stable isotope labelled enantiomer (in this case *S*-d_0/*R*-d_4 and *S*-d_4/*R*-d_0) [20, 21]. Mass spectral determination of the d_0:d_4 ratio for CP recovered after partial metabolism by PB induced rat liver microsomes confirmed the lack of any significant stereoselectivity in this process, and similar results were obtained with mouse microsomes. However, with (uninduced) rabbit liver microsomes the *S*-enantiomer was metabolised considerably faster than the *R*- (ratio *S*:*R* was 4.2 for the *S*-d_0/*R*-d_4 pseudoracemate and 2.6 for the *S*-d_4/*R*-d_0 pseudoracemate).

In vivo experiments with similar pseudoracemates revealed that in mice the formation of urinary 4-keto CP (**VII**) was markedly stereoselective for the R-CP (despite the fact that there was no stereoselectivity for the *in vitro* production of the 4-keto CP precursor, 4-hydroxy CP (**III**)). For the rabbit the stereoselectivity was reversed with the *S*-isomer being in excess of the *R* by 4–11-fold. No stereoselectivity was seen in the rat for 4-keto CP (**VII**) urinary excretion. Carboxyphosphamide (**IX**) was enriched in the *S*-enantiomer for the mouse and rabbit. There was no evidence for stereoselective excretion of unchanged CP by both mouse and rabbit, but the rat excreted 1.3–1.5 as much *R*- as *S*-enantiomer.

In a separate study by Tsui *et al.* [22] using incubation of the separate enantiomers with PB-induced mouse microsomes, the ratio of V_{max} for the production of alkylating equivalents from (−)-CP was found to be 1.34 times that from (+)-CP. The ratio of K_m values was similar (1.35). NMR using optically active shift reagents showed that there was no racemisation of the enantiomers during the *in vitro* metabolism. Interestingly, Tsui *et al.* also reported [22] that the enantiomers showed little difference in activity when tested against the mouse L1210 leukaemia (a result in contrast with that obtained for the PC6 plasma cell tumour).

In summary, it appears that (a) there are considerable species differences in the metabolism of the enantiomers of CP, both in the initial hydroxylation stage and in the

later oxidative detoxification pathways, and (b) that there is no clear indication from the two animal tumour test systems that have been used of a major therapeutic advantage or toxicity decrease for either of the enantiomers.

(c) *Human metabolism of CP and IP enantiomers*

The pharmacokinetics and metabolism of each of the enantiomers of CP were compared with those of the racemate in four patients with squamous cell carcinoma [4]. Following sequential administration of each of the three forms, CP and its metabolites 4-keto CP (**VII**) and carboxyphosphamide (**IX**) were determined by mass spectrometry and stable isotope dilution. Only insignificant inter-individual differences were seen in the $t_{\frac{1}{2}}$ of the three forms of CP (mean values: *R*-7.25, *S*-7.9, *RS*-7.45 hr). The enantiomeric composition of CP recovered from urine after administration of the racemate was determined by NMR; again no evidence for stereoselectivity was observed as only minor enrichments of the *S*-isomer were observed. Urinary output of CP and of carboxyphosphamide (**IX**) were also unaltered by the use of enantiomeric CP. The only evidence for stereoselectivity in man came from the urinary output of 4-keto CP (**VII**) which was higher after administration of *R*-CP. Thus in man (the species which incidentally appears to behave most like the mouse in CP metabolism) there is no reason to believe that there will be a significant advantage in the therapeutic use of either *R*- or *S*-CP in favour of *RS*-CP.

The relative metabolism of the enantiomers of IP has also been studied in two patients treated with racemic IP [23]. The parent drug and four of the metabolites (**VIII**, **X**, **XI** and **XII**) were determined by ^{31}P NMR in urine samples. A chiral shift reagent was also used to determine the enantiometric composition of IP (**II**), and its monodechloroethylated metabolites **XI** and **XII**. IP was slightly enriched in the *R*-enantiomer, whereas **XII** was highly enriched in *S*- (2.7–6.7-fold). One patient yielded an excess of *R*-**XI** and the other *S*-**XI** in the urine. Although detailed conclusions may clearly not be drawn from the results of only two patients, it does appear that more substantial stereoselectivity is occurring for IP metabolism compared to CP metabolism in man.

Conclusion

In the case of a chiral drug, such as CP, where established clinical practice is with the racemic mixture, the choice to change to the use of a single enantiomer should be based on the demonstration of a clear pharmacological and/or toxicological difference between the isomers. Additionally one also may have to consider the chemical feasibility of producing the required enantiomer at an acceptable cost for its routine application. For CP and IP (although only limited human studies have been carried out), there does not seem to be sufficient evidence to support an alteration to the use of an individual enantiomer. However, the causes of the species differences in stereoselectivity in CP-enantiomer metabolism are of considerable interest for further study, as a possible basis for novel drug development.

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