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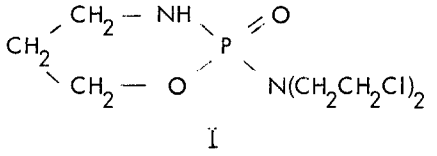
OBSERVATIONS ON THE DIFFERENTIAL METABOLISM AND BIOLOGICAL ACTIVITY OF THE OPTICAL ISOMERS OF CYCLOPHOSPHAMIDE

P.J.Cox, P.B.Farmer, M.Jarman* and M.Jones
Chester Beatty Research Institute, Institute of Cancer Research:
Royal Cancer Hospital, Fulham Road, London SW3 6JB, England

and

W.J.Stec and R.Kinas
Centre for Molecular and Macromolecular Studies, Polish Academy of Sciences,
90-362 Lodz, Boczna 5, Poland

Cyclophosphamide (I), a widely used anticancer drug, is a dissymmetric molecule because it contains a chiral phosphorus atom and optical isomers are therefore possible. Studies of metabolism (1,2) and the clinical use of the drug have hitherto involved the racemic (±) form of cyclophosphamide produced by the conventional method of synthesis (3). The observation (4) that, following the administration of racemic cyclophosphamide to patients, the drug recovered from the urine was laevorotatory, indicated that stereoselective metabolism had occurred. The recent synthesis (5) of the optical isomers of cyclophosphamide has allowed an assessment of the degree of stereoselective metabolism of racemic cyclophosphamide in man and a demonstration of markedly different antitumour effects of the enantiomers in animal tests.



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Total 24-hour urine samples were collected from 3 patients each treated i.v. with racemic cyclophosphamide (1 g). Each urine sample was extracted with chloroform (3 x 500 ml) and cyclophosphamide was recovered from the extracted material by elution from a column (25 x 2 cm) of silica gel (Merck, Kieselgel 60) with chloroform followed by thin-layer chromatography on silica gel (Merck, Kieselgel G) using chloroform-methanol (19:1). The products were crystallized from water and the data in Table I show that two of the samples were markedly laevorotatory thereby demonstrating stereoselective metabolism of the dextrorotatory form.

* Biology of Human Cancer Unit, Ludwig Institute for Cancer Research at the Institute of Cancer Research

Table I

Patient	Cyclophosphamide recovered (24-hour urine)	$[\alpha]_D^{28-29}$ (1 dm cell)
1 (ref.4)	9.5 mg	$-1.5^\circ \pm 0.1^\circ$ (c 0.95, MeOH)
2	16.9 mg	$-0.3^\circ \pm 0.1^\circ$ (c 0.84)
3	17.5 mg	$-1.9^\circ \pm 0.1^\circ$ (c 0.88)

The enantiomers of cyclophosphamide were synthesized (5) by condensation of (+)- or (-)-N-(3-hydroxypropyl)-1-phenylethylamine with N,N-bis(2-chloroethyl)phosphoroamidic dichloride and fractionation of each resulting pair of diastereoisomers of 3N-(1-phenylethyl)cyclophosphamide, followed bycatalytic hydrogenation to yield (+)- and (-)-cyclophosphamides having $[\alpha]_D^{25}$ $+2.3 \pm 0.1^\circ$ (c 12.2 in MeOH, 1 dm cell) and $-2.3 \pm 0.1^\circ$ (c 4.6), respectively. Further studies on the optical purity of these enantiomers are in progress, but, on the basis of the foregoing $[\alpha]_D$ values, the two most laevorotatory cyclophosphamide samples reported in Table I are calculated to contain 83 and 91%, respectively, of the (-)-isomer.

The enantiomers of cyclophosphamide were tested (two-fold dose spacing) against the ADJ/PC6 plasma cell tumour in mice (6) (Table 2)

Table 2

PC6 Tumour Test Results

	LD_{50} (mg/Kg)	ID_{90} (mg/Kg)	Therapeutic index (LD_{50}/ID_{90})
(+)-cyclophosphamide	365	5.3	68.9
(-)-cyclophosphamide	365	2.85	128.1
Racemic cyclophosphamide	335	3.6	93.0

The (-)-isomer is considerably more effective (lower ID_{90}) in killing the tumour cells in this test system than is the (+)-isomer. Preliminary experiments using a bioassay technique (2) showed that the (-)-isomer had greater toxicity towards tumour cells in tests involving in vitro incubation of TLX 5 cells with the drug in the presence of a microsomal activating system and subsequent injection into mice. Measurement of the inhibition of growth of Walker ascites cells in culture after microsomal activation of the drugs (7) showed that when metabolism of the racemate and the (+)- and (-)- forms was 98% complete, the metabolic products were of equal toxicity (8).

The metabolism of the racemic, (+)- and (-)- forms of cyclophosphamide was monitored by quantitating the unchanged drug by stable isotope dilution-mass spectrometry (9) using racemic cyclophosphamide-4,5,6-d_6 as internal standard. The initial rates for the three forms were virtually identical, as was the extent of metabolism (45%) after 15 minutes.

The rates and patterns of metabolism of the enantiomers of cyclophosphamide are being further investigated in relation to an assessment of the clinical implications of differential metabolism and the correlation between these rates and anti-tumour activity in experimental animals. It has long been known (10) that the enantiomers of drugs having a dissymmetric structure may show marked differences in biological activity and that certain enantiomers undergo different metabolic transformations (11).

Assessment of the differential metabolism of the enantiomers in racemic cyclophosphamide on the basis of optical rotation data requires milligram quantities of carefully purified material. We are presently investigating an alternative approach involving microgram quantities and analysis by mass spectrometry utilising racemic cyclophosphamide in which one enantiomer is labelled with deuterium. A variety of deuterated derivatives of cyclophosphamide is now available (4, 9,12, 13).

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