Short Communications

Pharmacokinetics of cyclophosphamide in man

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1. The pharmacokinetics of cyclophosphamide were investigated in cancer patients. The data can be characterized by a two-compartment open model. The half life of the elimination phase of the drug ranged between 3 and 11 hours.

2. Extensive tubular reabsorption of the drug resulted in the excretion of only a small percentage of the administered dose. The mean renal clearance was 10·7 ml/minute.

3. The calculated fraction of the dose of drug which was metabolized averaged 88%.

Since its introduction to clinical medicine more than a decade ago, cyclophosphamide has become a well established agent in the management of neoplastic disease. In addition, the drug is gaining increased use for other therapeutic problems such as autoimmunological and immunological disorders (Cooperating Clinics Committee of the American Rheumatism Association, 1970; White, Cameron & Trounce, 1966). In view of the toxicological problems encountered in the use of an alkylating agent and the present and potential use of cyclophosphamide in a wide spectrum of diseases, it is of considerable importance to further elucidate the pharmacokinetics of this compound. An evaluation of the pharmacokinetics of cyclophosphamide was thus undertaken in man to provide a basis for more rational therapeutic use of this agent.

Methods.—The pharmacokinetics of cyclophosphamide were investigated in seven male patients with various types of neoplastic disease. At the time of the studies, each patient had normal hepatic and renal function as shown by standard clinical tests. Six of the seven patients were not receiving any other drugs at the time of the study. The seventh patient (No. 7), had been taking quinalbarbitone (100 mg) daily, and pentazocine (300 mg) daily, for several months before the investigation. The regimen was maintained during the period of this study.

Cyclophosphamide-^{14}C, labelled at the side chain, was obtained from New England Nuclear Corporation. Its radiopurity was confirmed by thin-layer chromatography in a system of *n*-butanol:acetic acid:water (4:1:1) followed by radioautography. Five microcuries of cyclophosphamide-^{14}C mixed with 200–1,000 mg of unlabelled cyclophosphamide were administered to the patients by intravenous injection over 2 minutes.

An indwelling catheter in the antecubital vein was filled with heparin saline (0·1 ml approx) containing 1,000 U.S.P. units/ml. Because of the small volume of the anticoagulant solution and the large volume of the sample collected, it was not considered necessary to remove the anticoagulant. Sample dilution was kept constant by flushing the cannula with the heparin solution after the withdrawal of each sample. Blood samples (10 ml) were usually withdrawn at 5, 10, 20, 30, 60, 120, 180, 240, 300 and 360 min after drug administration. Urine samples were collected hourly during the initial 6–8 hours. Thereafter, samples were collected at approximately 6 h intervals when possible, for up to an additional 40 hours.

The method of analysis of cyclophosphamide and cyclophosphamide metabolites in plasma and urine is based on the differential extraction of cyclophosphamide with methylene chloride (Graul, Schaumloffel, Hundeshogen, Wilmans & Simon, 1967).

Results.—The time course of unchanged cyclophosphamide concentration in plasma after intravenous injection shows a bi-exponential decline with an initial rapid distribution phase followed by a slower elimination phase with a half life of 3–11 hours. The plasma concentration (Cp) versus time (t) data were analysed by digital computer using the non-linear least-square regression programme NLIN of Marquardt (Marquardt, 1966). The concentration (A,B) and rate (α,β) parameters of the equation:

$$Cp = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

along with the doses given to the patient-volunteers are summarized in the table. Based on these data, the volume of distribution (central compartment volume) of cyclophosphamide was calculated from:

$$V_c = \text{Dose}/A + B \quad (2)$$

and was found to average about 22 l. or 32% body weight for the seven patients.

The distribution, metabolism, and excretion pattern of unchanged cyclophosphamide are represented by a two-compartment open model. Bio-transformation is assumed to take place within the central compartment since the relatively large volume of distribution is likely to encompass the liver where oxidation of cyclophosphamide occurs. The distribution rate constants, k_{12} and k_{21}, and overall elimination rate constant, k_{e1}, of cyclophosphamide can be generated (Gibaldi, 1969; Rescigno & Serge, 1966) from the parameters of equation 1 using the following relationships:

$$k_{21} = \frac{A\beta + B\alpha}{A \quad B} \quad (3)$$

$$k_{e1} = \alpha\beta/k_{21} \quad (4$$

and

$$k_{12} = \alpha+\beta-k_{21}-k_{e1} \quad (5)$$

The distribution and elimination constants thus obtained for the seven subjects based on the least-square parameters are listed in the table.

Only a small fraction of the administered dose appears in the urine as unchanged cyclophosphamide. The mean for the group was 14·2% (S.D.±5·2) of the total dose. Although the urinary excretion rates of unchanged cyclophosphamide tended to be somewhat variable, it was possible to estimate the renal clearances (Cl_c) of the drug. Based on actual or interpolated plasma concentrations at the midpoints of the urinary excretion intervals, six–eight clearance values were obtained with each set of data. Average clearance values of about 11 ml/min were found as shown in the table. Since cyclophosphamide is apparently not bound to plasma proteins (Israels and Linford, 1963) these data suggest that the observed low renal clearance of cyclophosphamide is the result of extensive renal tubular reabsorption.

Using the values obtained for the renal clearance and volume of the central compartment, it is possible to estimate (Jusko & Levy, 1970) the renal excretion rate constant (k_e) for cyclophosphamide from:

$$k_e = C1_c/V_c \quad (6)$$

Since we are unable to find measurable amounts of unmetabolized cyclophosphamide in the stool (unpublished observations in two additional patients), it appears that the only significant excretion route is renal. This allows an estimate of the rate constant for metabolism (k_m) of cyclophosphamide by:

$$k_m = k_{e1}-k_e \quad (7)$$

and the fraction of the dose of drug metabolized is then obtained from the ratio of $k_m : k_{e1}$. Calculations based on the results of this study indicate that 70–95% (average 88%) of the usual 200–1,000 mg doses of cyclophosphamide is biotransformed. These data, as well as the respective elimination rate constants suggest that there is little, if any, dose-dependency in the biotransformation of cyclophosphamide in the dose range investigated. The distribution parameters also show no inter-or-intra-subject dependence on dose. The data, though limited, indicate that prior treatment with cyclophosphamide does not appear to effect the kinetics of the drug when studied in subsequent weeks.

Discussion.—The pharmacokinetics of cyclophosphamide were investigated in seven male patients with neoplastic disease, and plasma concentration and urinary excretion data were evaluated using a two-compartment open model. Several aspects of the distribution and elimination of cyclophosphamide could then be considered in relation to this model. The central compartment volume which was obtained for the drug is approximately the volume of the 'easily diffusible' extracellular water space of the body. This factor primarily reflects the lipid solubility and lack of plasma protein binding of the drug which permit rapid diffusion out of the plasma.

The mechanism of elimination of cyclophosphamide involved renal excretion as a minor route. The renal clearance estimates for cyclophosphamide yielded extremely low values which, consistent

TABLE 1. *Pharmacokinetics of cyclophosphamide subjects*

	1	2	3	4	5	6*			7*		Mean† (S.D.)
Dose (mg/kg)	10·1	7·8	15·8	11·5	12·1	13·8	6·9	2·8	4·3	10·9	21·8	
Least-square biexponential parameters												
A (μg/ml)	16·3	20·4	52·8	16·7	29·4	9·46	10·7	32·4	11·1	6·08	4·74	
α (h^{-1})	3·09	12·6	8·86	2·12	11·1	8·73	3·80	3·76	15·4	2·36	5·35	
B (μg/ml)	13·1	13·2	23·5	13·3	17·0	4·16	9·78	20·9	5·71	12·6	24·2	
β (h^{-1})	0·134	0·178	0·103	0·062	0·104	0·096	0·070	0·068	0·218	0·206	0·203	
β half life (h)	5·2	3·9	6·7	11·3	6·6	7·2	10·0	10·2	3·2	3·4	3·4	
Distribution constants												
V_c (l)	27·2	14·9	13·1	30·4	21·6	14·7	24·2	18·7	11·9	26·7	34·5	21·6 (6·3)
k_{21} (h^{-1})	1·45	5·06	2·80	0·97	4·14	2·73	1·85	1·52	5·37	1·66	4·51	2·90 (1·51)
k_{12} (h^{-1})	1·49	7·27	5·84	1·08	6·81	5·79	1·88	2·15	9·64	0·62	0·81	4·20 (2·49)
Elimination constants												
k_{el} (h^{-1})	0·284	0·442	0·327	0·134	0·280	0·306	0·143	0·168	0·626	0·293	0·241	0·294 (0·105)
Cl_c (ml/min)	13·8	9·6	8·4	10·9	5·2	15·9	12·6	15·5	15·8	8·4	13·4	10·7 (3·3)
k_e (h^{-1})	0·030	0·035	0·038	0·021	0·014	0·065	0·031	0·050	0·080	0·019	0·023	0·32 (0·12)
k_m (h^{-1})	0·254	0·407	0·289	0·113	0·266	0·241	0·112	0·118	0·546	0·274	0·218	0·262 (0·102)
Calculated fraction of dose metabolized	0·89	0·92	0·88	0·84	0·95	0·79	0·78	0·70	0·87	0·94	0·90	0·88 (0·06)

* Doses in order of administration. † Average data for subjects 6 and 7 were used for calculation of overall arithmetic mean values.

with the non-ionized nature of the drug, were indicative of extensive renal tubular reabsorption. This appears to result in biotransformation of most of the drug.

The biotransformation of cyclophosphamide is of prime interest since one or more of the products function as alkylating agents and are therefore responsible for its pharmacological properties (Brock, 1967; Foley, Friedman & Drolet, 1961). The liver is the principal site of biotransformation of cyclophosphamide as shown by Brock & Hohorst (1963) in studies with animal systems. Recently, Cohen & Jao (1970) have further demonstrated that the activation of cyclophosphamide is mediated by the NADPH-dependent mixed function oxidase system of hepatic microsomes.

Barbiturates enhance the activity of certain microsomal drug metabolizing enzymes in man as well as animals. Cohen & Jao (1970) have recently demonstrated an induction of cyclophosphamide-activating enzyme by phenobarbitone in rats. In an extension of the investigation described here, we have found a similar effect in man which appears to account for the results found with subject 7. Since patients with neoplastic disease and other disorders which might require cyclophosphamide therapy are likely to receive a variety of other drugs concomitantly, concern about enhancement of alkylating activity and toxicity of cyclophosphamide is warranted.

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