Cancer Chemother Pharmacol (1982) 8: 311–313

Cancer Chemotherapy and Pharmacology

Short Communication

Metabolism of High Doses of Cyclophosphamide

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Summary. *The excretion of cyclophosphamide and the enzymatically derived metabolites 4-ketocyclophosphamide and carboxyphosphamide has been measured in four patients after the administration of cyclophosphamide (5 g). At this dose the enzymes responsible for the biotransformation and detoxification of cyclophosphamide are not saturated. In two patients the metabolite profile was unaffected by a previous high dose of cyclophosphamide and in one patient a small primary dose did not alter metabolism.*

Introduction

Cyclophosphamide is a widely used antitumour agent which undergoes biotransformation to toxic metabolites in vivo (Fig. 1). In view of the increasing use of large doses (5 g+) of this drug with and without autologous marrow support, it was decided to investigate whether the metabolism of a large bolus of the drug differed from that of a more conventional dose. The amounts of unchanged drug and enzymatically derived metabolites in the urine were determined following the administration of 5 g cyclophosphamide to four patients with disseminated breast cancer.

The pharmacokinetics of cyclophosphamide in man has been previously studied using ^{14}C-labelled cyclophosphamide [3]. The data fitted a two-compartment open model. The half-life of the elimination phase ranged from 3 to 11 h. Owing to extensive tubular reabsorption only a small fraction of the dose was excreted by the kidneys, whilst an average of 88% of the dose was metabolised. The maximum dose administered in the study was 1 g.

Pharmacokinetic studies of the cyclophosphamide analogue, ifosfamide [1], indicated that single high doses (3.8–5 g/m^2) were less efficiently metabolised in man than the same total dose given individed doses (1.6–2.4 g/m^2/day × 3 days). After single high doses, the half-life of the β-phase was 15 h compared to 6.9 h for the lower doses. Also only 49% of the drug was metabolised compared to 80% with the divided dose regime. These findings indicate either a saturable biotransformation process or inhibition of metabolism by high doses of ifosfamide.

In a study of cyclophosphamide pharmacology in man, there was no correlation between drug dosage and either plasma half-life or the fraction of unchanged drug excreted in the urine [2]. Cyclophosphamide was measured as chloroform-extractable radioactivity. Metabolites were measured by 4-(4-nitrobenzyl)pyridine assay of alkylating activity, which was felt to give a good indication of the levels of toxic and tumoricidal metabolites. However, the alkylation values obtained are the sum of values for individual metabolites. More important, the alkylating activity does not necessarily indicate antitumour activity [12]. At high doses the enzymatic detoxification pathways affording 4-ketocyclophosphamide and carboxyphosphamide (Fig. 1) might also be saturated. This could result in greater toxicity and possibly loss of selectivity [5]. Measurements of total alkylating activity would not discriminate between the various metabolites. For this reason, unchanged drug and enzymatically derived metabolites carboxyphosphamide and 4-ketocyclophosphamide were measured individually in the urine of patients receiving high doses (5 g) of cyclophosphamide.

Previous reports indicated that cyclophosphamide administered to mice prior to a further dose of cyclophosphamide or other alkylating agents could protect against death due to bone marrow suppression [9, 10]. Enhancement of haemopoietic recovery has since been demonstrated in patients given cyclophosphamide prior to high dose melphalan therapy [8]. In one patient, metabolism of a 5-g dose was studied after a priming dose of 0.5 g given 3 days previously.

Materials and Methods

The four patients were females with disseminated carcinoma of the breast. All had normal hepatic and renal function as determined by standard biochemical tests. They received cyclophosphamide (5 g) by intravenous infusion during 0.5 h, followed by forced diuresis with intravenous fluids and frusemide diuretic.

Urine was collected for 24 h from the start of the infusion at hourly intervals and stored at −30° C. Two patients (FC, JE) received a second 5-g dose 3 weeks later. In one patient (FC) this was preceded 3 days earlier by a 0.5-g dose. The unchanged cyclophosphamide excreted between 24 and 48 h following a 5-g dose was also measured in this patient.

Quantification of Cyclophosphamide, 4-Ketocyclophosphamide and Carboxyphosphamide in Urine. The methods for assaying cyclophosphamide and its metabolites using mass spectrometry and deuterium-labelled internal standards, and comparing peak intensities for $[M-CH_2Cl]^+$ ions following methylation, have been described elsewhere [6, 7]. Because of the high doses used in this study, chromatographic separation

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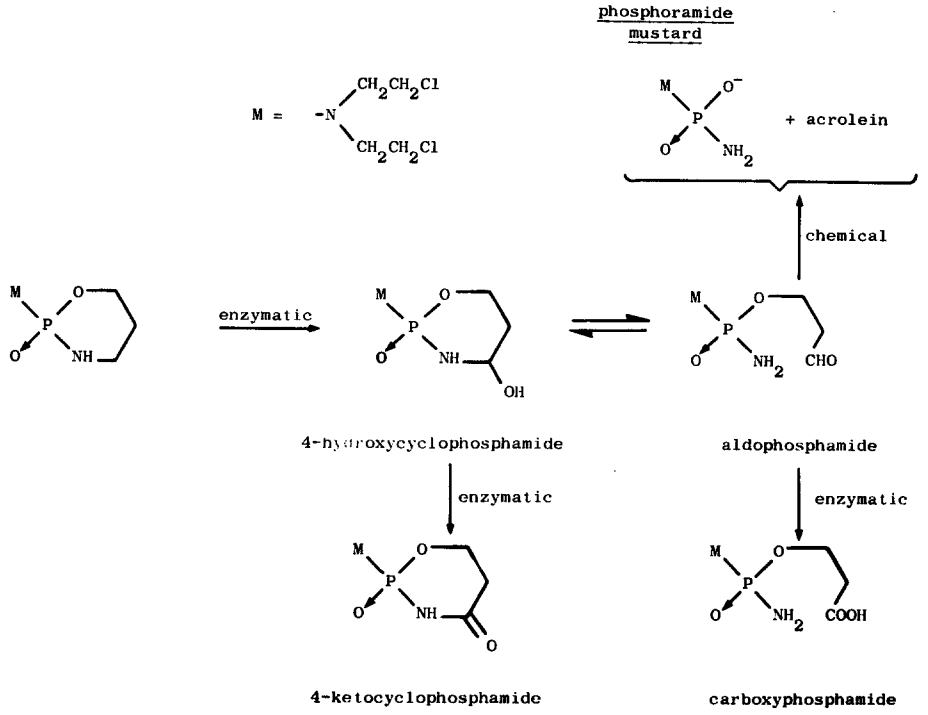

Fig. 1. Metabolism of cyclophosphamide

Table 1. Urinary excretion of cyclophosphamide and two metabolites (5-g dose)

	Patient					
	FC_1	FC_2	JE_1	JE_2	DF	SE
	(recovery as % of administered dose)					
Cyclophosphamide	7.4	5.7	5.3	7.9	21.9	23.5
Carboxycyclophosphamide	5.8	5.6	3.2	–	33.9	–
4-Ketocyclophosphamide	1.4	0.8	0.8	–	1.5	–

Table 2. Urinary excretion of cyclophosphamide and two metabolites: effect of priming dose (patient FC)

	Dose		
	5 g	0.5 g	5 g
Cyclophosphamide	7.4	7.8	5.7
Carboxyphosphamide	5.8	–	5.6
4-Ketocyclophosphamide	1.4	1.6	0.8

of the metabolites, usually necessary when 1-g doses were employed [7] was unnecessary here and the procedure followed was therefore that of Griggs and Jarman [6] with the following changes: (1) the volumes of standard solutions were 100 µl for cyclophosphamide and carboxyphosphamide; (2) deuterated carboxyphosphamide was the d_6-derivative [4].

Results

Results are presented in Tables 1 and 2.

Discussion

The fraction of the dose excreted unchanged showed the wide inter-patient variation found in previous studies [2, 11]. For six administrations the range was 5.3%–23.5%, with a mean of 12.0%. These findings are in agreement with previous studies using lower doses [3] and with results from patients receiving 80 mg/kg cyclophosphamide [2]. At the 5-g dose at least 75% of the drug is metabolised. The fraction of the dose excreted as carboxyphosphamide also varied between patients, but with one exception (patient DF), was similar to that seen at lower doses [7]. The data, although limited, suggest that cyclophosphamide metabolism is not saturated at the 5-g dose.

Patient FC had a very similar metabolite profile for both 5-g doses. Metabolism of the second dose was not altered by the 0.5-g dose administered 3 days earlier. Only 0.2% of the first 5-g dose was excreted unchanged between 24 and 48 h after administration. This accords with a previous study using smaller doses [11].

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Received June 7, 1982/Accepted June 8, 1982