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SHORT COMMUNICATIONS

Differences of cyclophosphamide and 6-mercaptopurine metabolic rates in perfused liver of normal and tumour-bearing animals

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Tumour-bearing animals dispose of drugs in a different manner compared to normal animals [1–4]. For example, pentobarbital [5] and zoxazolamine [6] disappear more slowly from plasma of Walker 256 carcinoma bearing rats compared to normal animals; and hydrocortisone [7] and 6-mercaptopurine [8] are eliminated more rapidly. This may be related to the fact that several factors playing an important role in drug metabolism—such as plasma protein pattern [7], amount of adipose tissue [9], renal excretion [10] and liver microsomal enzymes [1, 11]—are altered in tumour-bearing animals.

Utilizing the technique of isolated perfused liver it has been established that pentobarbital disappears more slowly in experiments with the liver of tumour-bearing animals than in normal liver [12]. Blood of tumour-bearing animals also impaired the metabolic capacity of liver of normal animals. This reduction of activity of liver microsomal enzymes in tumour-bearing animals may be due to an "inhibitory factor" [13] produced by the tumour and present in the blood.

This note reports similar studies performed with two antitumoural drugs, cyclophosphamide and 6-mercaptopurine.

In the *in vivo* experiments lower concentrations of the alkylating metabolites of cyclophosphamide were found in blood of tumour-bearing than in control Sprague–Dawley rats after administration (80 mg/kg body wt, intravenously) of cyclophosphamide. This difference is particularly evident considering the areas under the curve (AUC) (see Table 1).

It has been shown [14] by using the technique of perfusion of isolated liver that the hepatic tissue is responsible for the major part of cyclophosphamide transformation in the organism.

In our experiments with the perfusion of isolated liver the accumulation of cyclophosphamide metabolites in the medium (see Table 2) was higher when blood and liver were obtained from normal control rats than in any other combination. The impairment of biotransformation of cyclophosphamide is quite evident by utilizing the liver of

Table 1. Concentration of cyclophosphamide metabolites in rat serum after i.v. administration (80 mg/kg)

Time after treatment (min)	nmoles of nor-HN_2 equivalents/ml	
	Controls	Walker
1	32·0 ± 4·3	47·6 ± 10·0
5	73·9 ± 5·1	47·6 ± 7·8*
10	97·0 ± 4·3	62·5 ± 2·2†
15	103·5 ± 2·8	73·1 ± 3·3†
20	131·5 ± 12·4	78·1 ± 7·8*
40	119·9 ± 2·2	82·2 ± 13·1*
60	93·7 ± 12·4	60·8 ± 7·1
120	18·9 ± 1·6	22·2 ± 2·5
AUC	8574 ± 746	5530 ± 364*

* $P < 0.05$ with respect to controls.
† $P < 0.01$ with respect to controls.
AUC—area under the curve (between 1 and 120 min).
Results are given as mean ± S.E.

Table 2. Cyclophosphamide metabolites in perfusion medium

Liver Blood	Normal* Normal	Tumour† Tumour	Normal* Tumour	Tumour† Normal
Perfusion (min)	nmoles of nor-HN_2 equivalents/ml			
10	32·0 ± 2·4	9·3 ± 2·4	14·0 ± 2·1	9·0 ± 0·5
20	75·9 ± 5·9	13·8 ± 1·3	38·0 ± 4·2	25·3 ± 3·9
30	109·8 ± 11·0	35·6 ± 3·4	52·6 ± 6·5	44·3 ± 6·4
60	138·0 ± 7·0	77·4 ± 6·8	93·5 ± 8·7	75·8 ± 11·0
120	153·8 ± 5·5	152·0 ± 8·0	143·9 ± 3·2	105·2 ± 12·4
180	160·9 ± 4·2	173·4 ± 5·2	150·1 ± 4·9	137·9 ± 6·0
$K_1 \times 10^{-3}$ min^{-1}	30·4 ± 3·5	12·6 ± 0·9	18·2 ± 1·3	11·4 ± 0·8

Mean of * eight or † six experiments. At the beginning of the experiment cyclophosphamide was added at a concentration of 150 μg/ml of medium. The alkylating metabolites were not detectable.

The K_1 is calculated for the period 0–60 min as the constant rate of accumulation of alkylating metabolites in the perfusion medium.

Results are given as mean ± S.E.

Table 3. Serum levels of 6-mercaptopurine in rats after i.v. administration (80 mg/kg)

Time after treatment (min)	6-Mercaptopurine concn (μg/ml)	
	Controls	Walker
1	95·1 ± 2·1	41·6 ± 1·4*
5	23·6 ± 1·6	10·1 ± 0·9*
15	15·9 ± 2·6	1·2 ± 0·5*
30	3·3 ± 0·7	0·5 ± 0·3

* $P < 0.01$ with respect to controls.
Each figure represents an average of five determinations.
Results are given as mean ± S.E.

tumour-bearing animals. Also, however the blood of these animals diminishes the formation of cyclophosphamide metabolites in a normal liver.

It should be stressed that the results express the total amount of alkylating metabolites of cyclophosphamide. The identification of the single metabolites and of their pattern is currently being studied.

The levels of 6-mercaptopurine in the serum of Walker carcinosarcoma bearing rats *in vivo* are consistently lower as compared to the control animals after i.v. administration of 6-MP (80 mg/kg body wt; see Table 3). It seems that the volume of distribution rather than the metabolism of the drug may be affected in tumour-bearing animals because of lower initial level of 6-MP (1 min).

On the other hand the experiments carried out with the isolated perfused liver indicate that the disappearance of 6-MP in the perfusion medium is comparable for the liver of tumour-bearing and normal animals (see Table 4). This suggests that the biotransformation of 6-MP by the liver is not the major factor that may explain the differences *in vivo* between tumour-bearing and normal animals (see Table 3). The accumulation of 6-MP in the tumour tissue might influence the serum levels of 6-MP *in vivo*.

It is concluded that the use of liver perfusion technique may be helpful in establishing the role of the liver and the blood in the alteration of drug disposition occurring in tumour-bearing animals.

Table 4. Concentration of 6-mercaptopurine in perfusion medium

Liver Blood	Normal* Normal	Tumour† Tumour
Perfusion (min)	μg of 6-MP/ml	
0	41·6 ± 0·8	39·6 ± 1·2
10	25·6 ± 1·1	26·5 ± 1·6
20	20·6 ± 1·1	20·3 ± 1·2
30	16·6 ± 1·2	14·8 ± 1·0
60	9·9 ± 1·1	7·3 ± 1·0
120	4·3 ± 0·5	2·8 ± 0·2
$t_{1/2}$	42·5 ± 5·6	34·6 ± 3·6

The half-life ($t_{1/2}$) of 6-MP in both experimental groups does not differ significantly ($P > 0.05$).
Mean of * seven or † five experiments.
The concentration of 6-MP added to the perfusion medium was 50 μg/ml.
Results are given as mean ± S.E.

Methods

Male Sprague–Dawley rats (CD-COBS Charles River, Italy) with or without Walker 256 carcinosarcoma were used for the *in vivo* experiments and as donors of liver and blood for the perfusion experiments. The tumour-bearing animals were used 12 days after subcutaneous transplantation of the carcinosarcoma.

The average weight of the liver of normal rats was 10·1 ± 0·4 g (S.E.) and that of tumour-bearing animals was 12·8 ± 0·8 g (S.E.). The average weight of Walker 256 carcinosarcoma tumours was 36·1 ± 2·3 g (S.E.) at the time of the experiments.

The conditions of liver isolation and the perfusion apparatus were described before [15, 16]. The perfusion medium contained 1/3 heparinized defibrinated blood, 1/3 homologous serum and 1/3 Krebs–Ringer–bicarbonate buffer pH 7·4. The flow of the medium was 1 ml/min per g of liver and the total volume of the medium was proportional to the liver weight, i.e. 5 ml/g of liver.

The initial concentration of 6-mercaptopurine in the medium was 50 μg/ml. Its levels were assayed by a fluorimetric method [18].

The initial concentration of cyclophosphamide was 150 μg/ml of the perfusion medium. The total amount of the alkylating cyclophosphamide metabolites was measured according to the method of Friedman and Boger [17] and the values were expressed as bis-(β-chlorethyl)amine equivalents.

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SHORT COMMUNICATIONS

Identification of 2-methyl-4-(5-amino-2-furyl)thiazole as the reduced metabolite of 2-methyl-4-(5-nitro-2-furyl)thiazole

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The reduction of a number of 5-nitrofurans by mammalian tissues and bacteria has been investigated. Due to the lability of the reduced products, only a few metabolites have been identified. 5-Nitro-2-furaldehyde semicarbazone solution incubated with mammalian tissues [1], xanthine oxidase [1, 2] or aldehyde oxidase [3] in the presence of electron donors resulted in a metabolite suggested to be the corresponding hydroxylamine. Incubation of 5-nitro-2-furaldehyde semicarbazone with *Aerobacter aerogenes* provided a metabolite identified as an aminofuran [4]. When 5-nitro-2-furaldehyde acetylhydrazone was fed to rabbits, 5-acetamidofuraldehyde acetylhydrazone was isolated as a urinary metabolite [5].

A number of 5-nitrofurans have been reduced to the corresponding amines by H_2 with palladium on charcoal as catalyst [6]. After hydrogenation of 5-nitro-2-furaldehyde semicarbazone with Raney nickel, glyoxylpropionitrile was isolated, and was believed to be formed by ring opening of 5-amino-2-furaldehyde semicarbazone [7].

Certain 4-(5-nitro-2-furyl)thiazole derivatives were carcinogenic for experimental animals [8]. The nitro group was implicated in the carcinogenicity [8–11]. Unlike those tested [8–12], 2-methyl-4-(5-nitro-2-furyl)thiazole (MNFT) does not possess an amino substitute at the 2-position of the thiazole ring. It is more soluble in organic and inorganic solvents than the others. These properties make it a potentially useful compound for metabolic investigations of the structurally related carcinogenic 4-(5-nitro-2-furyl)thiazole deriva-

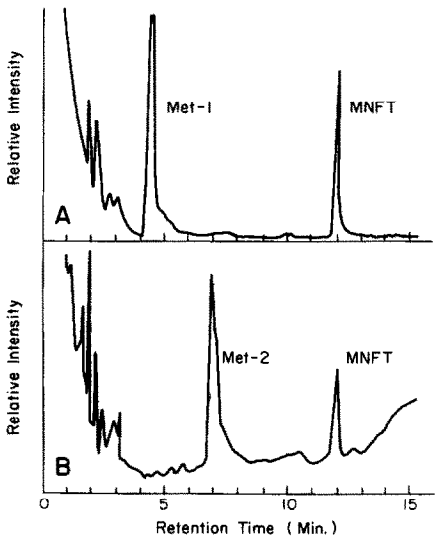

Fig. 1. GLC of methylenechloride extract of MNFT incubated with NADPH and NADPH–cyt. *c* reductase (A), and that of MNFT incubated with NADPH and NADPH–cyt. *c* reductase followed by incubation with mouse liver cytosol (B).

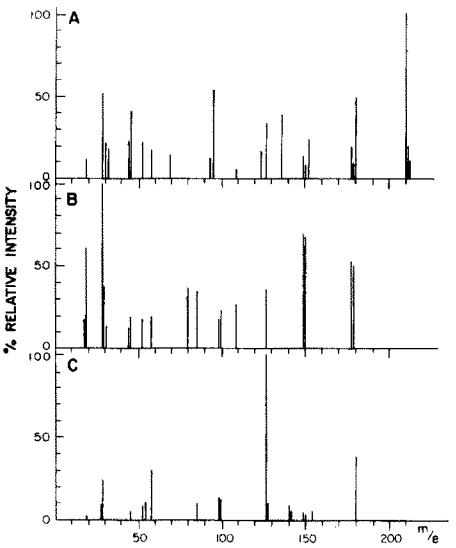

Fig. 2. MS of MNFT (A), Met-1 (B) and Met-2 (C). MS of MNFT was obtained from direct insertion; the other two were from GLC–MS.