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Ifosfamide plasma clearance in relation to polymorphic debrisoquine oxidation

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Summary. Ifosfamide (IF) pharmacokinetics and the plasma (NBP)-alkylating activity were determined in 33 patients with different tumours after the administration of IF as single-agent chemotherapy. All subjects had been phenotyped for debrisoquine oxidation. There is a lack of correlation between the debrisoquine metabolic ratio (DMR) and either the total plasma clearance of IF (CL_{IF}) or the AUC of the plasma NBP-alkylating activity.

Introduction

Patients undergoing chemotherapy for cancer are exposed to potentially toxic drugs and their metabolite(s). Definite data pertaining to dose-related response or toxicity is lacking. It is not unreasonable to suggest that plasma drug and metabolite concentrations may reflect tumour and tissue levels and indirectly correlate with the magnitude of response and/or toxicity.

One of the major determinations of the plasma concentration of a drug and its metabolite is the rate of metabolic clearance of the drug and the formation of metabolite. Large interindividual variation exists in metabolic rates due to environmental and genetic factors [13, 24].

Debrisoquine (D) 4-hydroxylation capacity is bimodally distributed in the population [16]; 8.9% of British Caucasians are poor metabolisers, the rest are extensive metabolisers [9]. The debrisoquine metabolic ratio (DMR) is the closest index for D oxidation capacity [21] and is represented by the ratio of D to 4-hydroxydebrisoquine (D/4-OHD) excreted in urine over 8 hours following dosing. This ratio offers a good correlative tool with several drugs [5, 8], which indicates the operation of identical isoenzymes or a common genetic regulation.

Ifosfamide is an alkylating agent chemically related to cyclophosphamide. In animals and man it shows a broad spectrum of anti-tumour activity [2]. It is metabolised by the mixed-function oxidase system and a number of highly reactive metabolites are generated [6]. Isophosphoramide mustard appears to be the most reactive metabolite, and acrolein, another product of IF metabolism, is incriminated in urothelial toxicity [23].

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This study explores the possibility of predicting IF disposition in vivo by using a marker drug exhibiting polymorphic oxidation.

Methods

Subjects. The study was conducted on 33 non-consecutive patients of both sexes and mixed ethnic origins, with various advanced malignant tumours, who were being treated at Guy's Hospital, London. The age range was 23–75 years (mean, 51.5 ± 13.3). All subjects gave informed consent to participate in the study, which was approved by the Committee on Ethical Practice of Guy's Hospital. A creatinine clearance of < 50 ml/min excluded entry into this study.

Protocol of the study. (a) Debrisoquine oxidation testing: D oxidation testing was carried out by the method of Mahgoub et al. [16]. After the bladder was emptied, 10 mg D (Declinax, Roche) was given orally. Urine was collected over the subsequent 8 h and an aliquot was stored at $-20°$ C pending analysis by gas chromatography [14].

(b) IF administration and blood sampling: After a minimal interval of 24 h, IF (Mitoxan, Asta) was given as a single agent by IV infusion (in 1 l 5% dextrose) by two major regimens: (1) 1.5 g/m^2 over 0.5 h daily for 3 or 5 days, and (2) 5.0 g/m^2 over 24 h. Mesna (Uromitoxan, Boehringer-Ingelheim) was given concurrently as recommended by the manufacturer to reduce urothelial damage.

At suitable intervals between 0.25 h and up to 48 h after the end of the infusion, 6–10 ml blood was collected from a contralateral arm vein. Plasma was immediately separated, divided into two tubes, and stored at $-20°$ C.

(c) IF plasma assay: IF in plasma was quantified by the gas chromatographic method described by Talha and Rogers [22].

(d) Plasma NBP-alkylating activity: Plasma NBP-alkylating activity was estimated by a modification of the colorimetric method of Friedman and Boger [19] and Juma et al. [12].

Pharmacokinetic analysis. The plasma elimination half-life ($T_{1/2}$) of IF was calculated by the least-squares linear regression analysis of the log plasma concentration-time curve. The area under the plasma concentration curve (AUC) was calculated by the trapezoidal rule and extrapolated to infinity using the Wagner-Nelson correction. Total

Table 1. Study results

Subject	Age	Sex	Diagnosis	IF Dose (g)	DMR	IF $T_{1/2}$ (h)	IF $AUC_{(0-\infty)}$ (μg/ml per h)	CL_{IF} (l/h)	Alkylating activity (NBP) $AUC_{(0-\infty)}$
1	23	M	Osteosarcoma	2.4	1.2	4.9	445.6	5.4	1.6
2	72	M	Oat cell carcinoma	9.0	2.9	5.2	2528.5	3.6	48.2
3	45	F	Squamous cell carcinoma	2.25	0.5	4.7	611.9	3.7	7.6
4	38	F	Adenocarcinoma	4.0	0.6	10.0	1332.4	3.0	39.7
5	70	M	Large cell carcinoma	8.9	0.9	6.2	2964.8	3.0	176.7
6	56	M	Squamous cell carcinoma	8.75	94.0	3.4	1980.4	4.4	44.8
7	58	M	Leiomyosarcoma	2.7	2.6	20.6	1945.0	1.4	68.2
8	60	M	Squamous cell carcinoma	2.25	0.4	11.0	939.9	2.4	0.9
9	60	M	Oat cell carcinoma	2.6	0.9	4.3	1420.8	1.8	N.D.
10	31	F	Squamous cell carcinoma	2.1	63.0	7.2	342.8	6.1	4.3
11	75	M	Non-Hodkin's lymphoma	1.8	0.4	10.9	730.3	2.5	8.4
12	67	M	Squamous cell carcinoma	1.5	0.4	7.6	501.5	3.0	39.7
13	40	F	Squamous cell carcinoma	2.5	5.6	9.4	464.7	5.4	2.3
14	52	F	Squamous cell carcinoma	2.4	0.9	6.9	489.9	4.9	32.2
15	52	F	Adenocarcinoma	2.5	2.0	7.4	671.2	3.7	16.5
16	54	M	Adenocarcinoma	2.0	0.5	9.5	854.1	3.5	41.9
17	41	F	Thymoma	2.60	3.9	7.9	643.7	4.0	74.4
18	45	M	Adenocarcinoma	9.15	7.73	9.1	1452.4	6.3	49.2
19	26	M	Thymoma	2.5	1.2	6.0	635.9	3.9	105.6
20	51	F	Squamous cell carcinoma	2.0	0.3	5.8	641.8	3.1	34.7
21	55	M	Large cell carcinoma	2.9	1.6	7.4	1309.2	2.2	35.2
22	63	M	Squamous cell carcinoma	2.6	0.4	5.1	286.4	9.1	N.A.
23	35	M	Adenocarcinoma	2.6	0.3	9.7	731.8	3.6	N.A.
24	40	F	Squamous cell carcinoma	2.6	0.9	7.6	504.0	5.2	N.A.
25	35	M	Non-Hodgkin's lymphoma	1.85	24.0	4.2	211.5	8.7	74.7
26	42	M	Squamous cell carcinoma	2.0	1.2	8.5	329.7	6.1	60.0
27	41	F	Adenocarcinoma	2.25	2.2	3.3	228.4	9.9	132.3
28	66	M	Squamous cell carcinoma	2.2	1.0	8.9	278.6	7.9	147.8
29	60	M	Squamous cell carcinoma	2.8	0.5	6.0	486.6	5.8	44.5
30	62	M	Squamous cell carcinoma	2.4	1.7	3.9	252.5	9.5	80.5
31	54	F	Squamous cell carcinoma	2.7	4.0	12.3	1024.3	2.6	78.0
32	62	F	Squamous cell carcinoma	2.0	0.8	9.0	876.1	2.3	100.1
33	59	M	Squamous cell carcinoma	2.5	0.9	8.3	596.6	4.2	57.8

N.D., not detected; N.A., not available

plasma clearance (CL_{IF}) was obtained by dividing the dose by the $AUC_{(0-\infty)}$. The $AUC_{(0-\infty)}$ of NBP-alkylating activity in plasma was also calculated by the trapezoidal rule method.

Statistical methods. The non-parametric Kendall's rank correlation (T) [20] was used to explore the relation between the DMR and the CL_{IF} and AUC of plasma NBP-alkylating activity. A P value of 0.05 or less was considered to be statistically significant.

Results

Table 1 gives the diagnoses, IF doses, pharmacokinetic parameters, DMRs, and AUC NBP-alkylating activities resulting from this study. The mean $T_{1/2}$ and CL_{IF} were 7.7 ± 3.3 h and 4.6 ± 2.3 l/h, respectively. Three patients

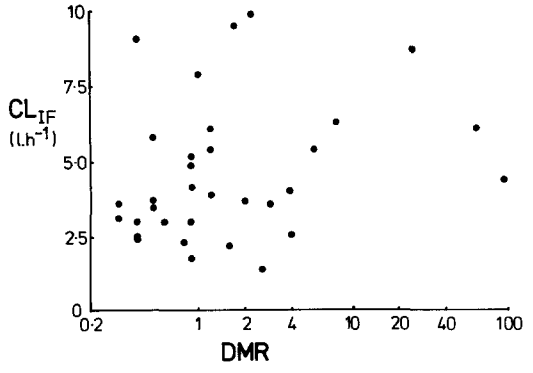

Fig. 1. Plot of plasma ifosfamide clearance against DMR

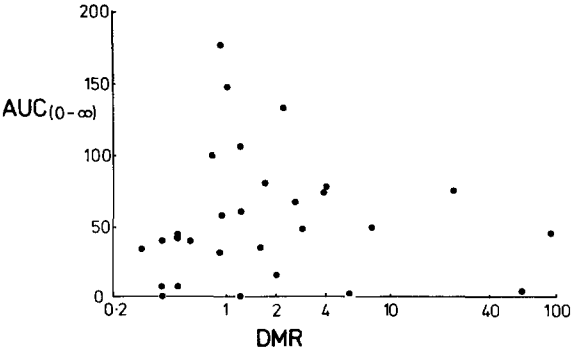

Fig. 2. Plot of the AUC for plasma-alkylating activity against DMR

were poor metabolisers with DMRs of 24, 94, and 63. The Kendall's rank correlation between the CL_{IF} and DMR was poor ($T = 1.86$; $P > 0.10$) (Fig. 1). In particular, the three poor metabolisers had CL_{IF} values within the range of values of the whole group. The realtionship between the DMR and plasma-alkylating activity is depicted in Fig. 2. There was no correlation between the two ($T = 0.15$; $P > 0.10$).

Discussion

IF has wide and expanding therapeutic applications in cancer chemotherapy. It is chiefly metabolised by the cytochrome P-450 enzymes and partly excreted unchanged by the kidneys [1]. Metabolism appears to be a prerequisite for its cytotoxic activity as well as tissue toxicity. 4-Hydroxylation is an initial, important step generating 4-hydroxy-ifosfamide, which is in equilibrium with aldo-ifosfamide, which spontaneously degrades into the directly alkylating ifosfamide mustard and acrolein [4, 7, 11]. 4-Hydroxyifosfamide possesses a higher cytotoxic specifity than other oxazaphosphorine metabolites and alkylating agents.

Norpoth [18] suggested that there was a wide interindividual variation in IF metabolism when the unchanged IF/converted IF ratios in urine were determined, as well as when differential metabolic analyses were undertaken. Therefore, one can expect wide interindividual variations in the therapeutic response and adverse reactions to IF.

This study aims at exporing the possibility of predicting IF disposition in vivo using one of the available probes for microsomal oxidation. Such an approach has increasingly been used in recent years to assess the individual capacity for oxidation [19]. Information obtained may have a predictive role for response and the occurrence of toxic reaction. In other words, it aims at rationalising drug treatment according to individual characteristics.

The results of this study suggest that the DMR will not predict the CL_{IF}. The occurrence of multiple isoenzymes of cytochrome P-450 is well established, entailing substrate specificity and separate genetic control [15]. It has been noted that most of the drugs known to be substrates of polymorphic D oxidation have a basic nitrogen centre, an asymmetrical centre, and a lipophilic domain where oxidation occurs [17]. Nevertheless, it cannot be categorically excluded that the presence of some oxidation steps in IF metabolism could closely relate to the DMR. To explore this further, the production and clearance of individual IF metabolites should be measured [3]. However, IF metabolites are highly reactive and unstable in vitro, posing difficulties in quantification. An approximation to the total activity of those metabolites in plasma is the plasma NBP-alkylating activity [10, 12]. Unfortunately, this assay is nonspecific and does not tell us the individual contribution of the metabolites. No significant correlation was discovered between the DMR and NBP-alkylating activity in this study. Of note was the low AUC of the plasma-alkylating activity in the group as a whole and in some patients in particular. It is possible that results may vary according to the precise methodology and sample treatment used in measuring the alkylating activity.

In conclusion, the DMR cannot predict IF plasma clearance. In the future, other marker drugs for oxidation that are substrates of other cytochrome P-450 isoenzymes should be investigated e.g. antipyrine. In the same manner, the rate of metabolite(s) formation can be correlated with the disposition of the probe drug.

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