Phenotypically Deficient Urinary Elimination of Carboxyphosphamide after Cyclophosphamide Administration to Cancer Patients

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ABSTRACT

The 0-24-h urinary metabolic profile of cyclophosphamide was investigated in a series of 14 patients with various malignancies receiving combination chemotherapy including i.v. cyclophosphamide. This was accomplished using combined thin-layer chromatography-photography-densitometry, which can quantitate cyclophosphamide and its four principal urinary metabolites (4-ketocyclophosphamide, nor-nitrogen mustard, carboxyphosphamide, and phosphoramide mustard). Recovery of drug-related metabolites was 36.5 ± 1.8% (SD) dose, the most abundant metabolites being phosphoramide mustard (18.5 ± 16.1% dose) and unchanged cyclophosphamide (12.7 ± 9.3% dose). The most variable metabolite was carboxyphosphamide, with five patients excreting 0.3% dose or less. These patients were termed low carboxylators (LC) and could be distinguished from high carboxylators (HC) by a carboxylation index (relative percentage as carboxyphosphamide multiplied by 10).

Mean carboxylation indices for the LC and HC phenotypes were 3.4 ± 2.6 and 151 ± 115, respectively. There were no associations between patient age, sex, body weight, tumor type, or concomitant drug therapy and carboxylation phenotype. Neither 4-ketocyclophosphamide nor nor-nitrogen mustard excretion differed between LC and HC phenotypes; however, HC patients had a greater excretion of cyclophosphamide (46.4 ± 15.5 relative percentage) than LC patients (19.4 ± 12.6%). The DNA cross-linking cytotoxic metabolite phosphoramide mustard was elevated more than 2-fold in the LC (76.5 ± 13.9%) compared with the HC (33.0 ± 12.2%) phenotype. It is concluded that these data represent the first evidence of a defect in cyclophosphamide metabolism, and it is proposed that this arises from a hitherto unrecognized aldehyde dehydrogenase genotype.

INTRODUCTION

Metabolic transformation is of paramount importance for the cytotoxic antineoplastic drug cyclophosphamide because it exerts both its beneficial DNA-alkylating effect and its unwanted clinical toxicity via metabolites. Variation between patients in the extent of this metabolic activation would not only be of interest but would also play a significant part in determining the outcome of therapy with cyclophosphamide. Nevertheless, it is the qualitative pattern of metabolism rather than detailed quantitative studies which have preoccupied investigators to date, primarily as an attempt to understand its mode of action and favorable therapeutic index.

As a consequence, the principal metabolic pathways undertaken by cyclophosphamide are now well established (Fig. 1). Cyclophosphamide is first converted to 4-hydroxycyclophosphamide (1-3) by hepatic cytochrome P-450 monooxygenases (4-6). Tautomerization of 4-hydroxycyclophosphamide yields the ring-opened aldehyde metabolite phosphoramide (7-10) which acts as the branching point for either detoxication to carboxyphosphamide (2, 11, 12) or further activation to the cytotoxic species phosphoramide mustard (13, 14), which can itself be further cleaved to bis(2-chloroethyl)amine (nor-nitrogen mustard) (7, 15, 16). Minor metabolites include 4-oxocyclophosphamide (4-ketocyclophosphamide) (2, 12, 14) together with the 4-hydroxycyclophosphamide dehydration product iminocyclophosphamide (not shown in Fig. 1) (17). Finally and importantly, the production of phosphoramide mustard from aldophosphamide yields an equimolar quantity of acrolein (13, 14, 18) thought to be responsible for the urotoxicity of cyclophosphamide (19-22). Considerable evidence has not been accumulated which implicates phosphoramide mustard rather than 4-hydroxycyclophosphamide, aldophosphamide, or nor-nitrogen mustard as the ultimate alkylating and DNA cross-linking metabolite (23). Although cyclophosphamide was originally synthesized (24) as a prodrug for nor-nitrogen mustard release (25) within the tumor, nor-nitrogen mustard is now known only to cause DNA-protein cross-links (26).

Clearly, interindividual differences in the balance of aldophosphamide metabolism to either phosphoramide mustard (activation) or carboxyphosphamide (detoxication) would be of considerable clinical importance. Until now, however, no single and simple method has been available for the quantitative determination of cyclophosphamide metabolites in body fluids. We have recently described (27) a combined TLC-PD method which can determine cyclophosphamide, 4-Ketocyclophosphamide, carboxyphosphamide, phosphoramide mustard, and nor-nitrogen mustard in biological samples and have applied this in a reappraisal of the quantitative nature of cyclophosphamide metabolism in cancer patients. This paper reports the finding of 5 cancer patients, from a series of 14 who were given i.v. cyclophosphamide, with a virtual complete absence in urine of the "major" metabolite carboxyphosphamide with a consequent amplification of the activation pathway that yields phosphoramide mustard.

MATERIALS AND METHODS

Authentic metabolites of cyclophosphamide were the gift of Asta-Werke AG, Bielefeld, Federal Republic of Germany, and of Boehringer Ingelheim Limited, Bracknell, United Kingdom. Each substance was authenticated by 1H-nuclear magnetic resonance and IR spectroscopy at Asta-Werke AG and by elemental analysis in London, details of which are reported elsewhere (27). The TLC-PD method for the determination of cyclophosphamide metabolites has been described in detail (27). Briefly, urinary metabolites are extracted onto a non-ionic polymeric adsorbent, eluted with methanol, and separated with TLC. Visualization of drug-related spots is accomplished by spraying the plates with 4-(4-nitrobenzyl)pyridine, an alkyl acceptor which gives a blue chromophore on alkalization (28). Because the blue spots deteriorate on standing, the TLC plate is photographed within 10 s of spot development and the resultant black and white print is scanned in a densitometer. The method is sensitive down to 1 μg/ml and linear up to

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2 Supported by The British Council and Yarmouk University, Jordan. Present address: Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan.
3 Wellcome Trust Senior Lecturer. To whom requests for reprints should be addressed.

4 The abbreviations used are: TLC-PD, combined thin-layer chromatography-photography-densitometry; LC, low carboxylator; HC, high carboxylator; TLC, thin-layer chromatography; ALDH, aldehyde dehydrogenase.
DEFICIENT URINARY CARBOXYPHOSPHAMIDE EXCRETION

Fig. 1. Metabolic transformations of cyclophosphamide (CP). Boldface arrows, activation pathways; open arrows, detoxication pathways. For abbreviations see Table 2. 4HCP, 4-hydroxycyclophosphamide; KP, 4-ketocyclophosphamide; AP, aldophosphamide; PM, phosphoramide mustard; CX, carboxyphosphamide; NNM, nor-nitrogen mustard.

Table 1 Details of patients studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Wt (kg)</th>
<th>Tumor</th>
<th>CP dose (mg)</th>
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<td>M</td>
<td>53</td>
<td>64</td>
<td>SCLC</td>
<td>1800</td>
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<tr>
<td>2</td>
<td>F</td>
<td>50</td>
<td>60</td>
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<td>92</td>
<td>SCLC</td>
<td>1200</td>
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<td>4</td>
<td>M</td>
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<td>SCLC</td>
<td>1000</td>
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<td>F</td>
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<td>70</td>
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<td>6</td>
<td>M</td>
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<tr>
<td>7</td>
<td>M</td>
<td>64</td>
<td>70</td>
<td>NHL</td>
<td>1200</td>
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<td>8</td>
<td>M</td>
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<td>75</td>
<td>NHL</td>
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<td>14</td>
<td>F</td>
<td>52</td>
<td>53</td>
<td>US</td>
<td>1200</td>
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</tbody>
</table>

* SCLC, small cell lung cancer; NHL, non-Hodgkin's lymphoma; MFH, malignant fibrous histiocytoma; BC, breast carcinoma; US, uterine sarcoma.

RESULTS

The photographic record of a TLC plate which had been visualized with 4-(4-nitrobenzyl)pyridine as described (27) is shown in Fig. 2. The method clearly separates the principal urinary metabolites 4-ketocyclophosphamide, nor-nitrogen mustard, carboxyphosphamide, and phosphoramide mustard from unchanged cyclophosphamide and is specific for the derivatives of cyclophosphamide in the urine, blank urine producing no bands (27). Each urine (typical samples shown in lanes 2–7) contained six bands of variable color intensity and Rf 0.67 (4-ketocyclophosphamide), 0.61 (cyclophosphamide), 0.40 (nor-nitrogen mustard), 0.30 (unidentified metabolite, probably the des-2-chloroethyl compound by comparison with authentic standard), 0.26 (carboxyphosphamide), and 0.02 (phosphoramide mustard). Visual inspection of the photographs of the TLC plates, such as that shown in Fig. 2, revealed that the relative excretion of the metabolites, in particular carboxyphosphamide and phosphoramide mustard, was highly variable between patients. When the black and white photographs of the TLC plates were scanned using a densitometer, a technique referred to as TLC-PD (27), chromatograms were obtained which could be quantitated for each urine specimen. From the concentration of each metabolite in the appropriate urine samples, the percentage of the administered dose excreted as each metabolite was calculated. Table 2 gives these individual data for each of the 14 patients together with the means and SDs. In the 0–24-h urine after i.v. doses of 600–1800 mg cyclophosphamide, the total recovery of drug-related material determined by TLC-PD was 6.5–64.1% (36.2 ± 17.8) dose, in good agreement with results of workers who used radioisotopically labeled cyclophosphamide, 4-ketocyclophosphamide, carboxyphosphamide, and phosphoramide mustard in human urine and has been termed TLC-PD (27).

Fourteen patients with various malignancies were studied, each receiving i.v. cyclophosphamide as part of their combination chemotherapy. The patients comprised eight males and six females, mean age 53.6 ± 8.6 (SD) years, body weight 69.2 ± 13.7 kg, five with small cell lung cancer, four with non-Hodgkin’s lymphoma, two with malignant fibrous histiocytoma, two with breast carcinoma, and one with uterine sarcoma (see Table 1). Patients received 600–1800 mg (1130 ± 260) cyclophosphamide with the following adjuvant drugs (number of patients): promethazine (11), metoclopramide (10), chlorpromazine (10), Adriamycin (9), vincristine (9), paracetamol (8), dextropropoxyphene (8), lorazepam (8), prednisolone (6), etoposide (6), morphine (5), amitriptyline (2), salbutamol (2), prochlorperazine (2), and the following drugs each received by one patient only: nitrazepam, phenytoin, halo-

to 250 μg/ml for cyclophosphamide, 4-ketocyclophosphamide, carboxyphosphamide, phosphoramide mustard, and nor-nitrogen mustard in human urine and has been termed TLC-PD (27).

All patients remained in the hospital for at least 25 h post-cyclophosphamide administration. They collected their bulked urine for various periods as follows: 1 patient 0–2, 2–8, and 8–24 h; 5 patients 0–8 and 8–24 h; 8 patients 0–24 h only. Accordingly, 21 urine samples of recorded volume were frozen at −20°C immediately after collection and then analyzed for their content of cyclophosphamide, 4-ketocyclophosphamide, carboxyphosphamide, phosphoramide mustard, and nor-nitrogen mustard by TLC-PD.
Table 2  Percentage of dose excreted as cyclophosphamide and its various metabolites in the 0–24-h urine in 14 cancer patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>CP%</th>
<th>KP</th>
<th>NNM</th>
<th>CX</th>
<th>PM</th>
<th>Total</th>
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<td>31.4</td>
<td>13.8</td>
<td>4.8</td>
<td>14.1</td>
</tr>
<tr>
<td>2</td>
<td>(21.8)</td>
<td>(20.4)</td>
<td>(48.9)</td>
<td>(40.1)</td>
<td>(9.8)</td>
<td>(46.4)</td>
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<tr>
<td>3</td>
<td>0.8</td>
<td>0.4</td>
<td>0.4</td>
<td>1.0</td>
<td>0.7</td>
<td>1.0</td>
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<td>4</td>
<td>0.5</td>
<td>0.1</td>
<td>0.3</td>
<td>0.6</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>(1.8)</td>
<td>(0.8)</td>
<td>(0.6)</td>
<td>(1.9)</td>
<td>(1.6)</td>
<td>(1.4)</td>
</tr>
<tr>
<td>6</td>
<td>9.4</td>
<td>0.1</td>
<td>4.1</td>
<td>0.1</td>
<td>0.05</td>
<td>0.8</td>
</tr>
<tr>
<td>7</td>
<td>(35.0)</td>
<td>(0.8)</td>
<td>(6.4)</td>
<td>(0.3)</td>
<td>(0.1)</td>
<td>(2.7)</td>
</tr>
<tr>
<td>8</td>
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<td>10.2</td>
<td>27.9</td>
<td>18.8</td>
<td>42.6</td>
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<tr>
<td>9</td>
<td>(38.5)</td>
<td>(75.0)</td>
<td>(43.6)</td>
<td>(54.7)</td>
<td>(87.1)</td>
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<td>13</td>
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<tr>
<td>14</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
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</table>

Mean ± SD 12.7 ± 9.3 (369 ± 19.5) 1.0 ± 0.9 (3.1 ± 2.0) 0.8 ± 0.6 (2.1 ± 1.2) 3.1 ± 3.7 (9.8 ± 11.6) 18.5 ± 16.1 (48.2 ± 24.5) 36.2 ± 17.8

a CP, cyclophosphamide; KP, 4-ketocyclophosphamide; NNM, nor-nitrogen mustard; CX, carboxyphosphamide; PM, phosphoramide mustard.

b (−), nor-nitrogen mustard was below the limit of detection in this patient.
may be of considerable clinical importance and will be returned

to later.

DISCUSSION

The recent development of a method which quantitates all
the principal metabolites of the cytotoxic antineoplastic drug
cyclophosphamide has permitted a more detailed inspection of
the metabolic balance sheet for cyclophosphamide than has been
possible hitherto. While several investigators have re-
ported on the various metabolites in patients, studies that have
yielded good excretion data have invariably relied on the ad-
ministration of radiolabeled drug. The vast majority of deter-
minations of cyclophosphamide “metabolism” or “activation”
have utilized the colorimetric Epstein assay (28) of total alkyl-
ating activity. This method, which is usually calibrated with
the most readily available metabolite bis(2-chloroethyl)amine (nor-
nitrogen mustard), can only give the broadest of indications as
to the quantitative aspects of cyclophosphamide metabolism
and no clue whatsoever as to the qualitative pattern of metab-
olites in the sample analyzed, for the simple reason that the
sine qua non that each metabolite has an identical molar ex-
tinction coefficient in the Epstein assay does not hold. In our

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The detection of genetic polymorphism of the ALDH isozyme responsible for the detoxication of cyclophosphamide would be of great clinical interest, from the point of view of both tolerability of the drug and acquired resistance to it. The low carboxylators who comprised 36% of our small patient cohort excreted over twice the relative amount of cytotoxic phosphoramide mustard and presumably therefore produced twice the molar equivalent of the toxic aldehyde acrolein (see Fig. 1). The TLC-PD method provides a simple means for screening patient populations for ascertainment of the complete metabolic picture after cyclophosphamide administration and the ability to assign carboxylation phenotype. Parallel studies to be reported elsewhere with the isomer of cyclophosphamide, ifosfamide, in non-small cell lung cancer patients reveal a similar proportion of patients with defective excretion of the carboxylic acid metabolite.

ACKNOWLEDGMENTS

The expert technical and photographic guidance of Jane Barker are gratefully acknowledged.

REFERENCES


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