Clinical Pharmacology of Cyclophosphamide

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SUMMARY

The human pharmacology of cyclophosphamide was investigated in 26 patients who received cyclophosphamide-\(^{14}\)C in doses of 6 to 80 mg/kg i.v. Levels of the intact drug in plasma and urine and excretion of \(^{14}\)C label in breath and stools were determined by liquid scintillation counting. Plasma and urine alkylating activity was measured by reaction with 4-(4-nitrobenzyl)pyridine. Protein binding of cyclophosphamide and plasma alkylating metabolites were determined by plasma ultrafiltration.

Injected cyclophosphamide distributed rapidly into 64% of body weight, and plasma cyclophosphamide half-life in patients without prior drug exposure was 6.5 hr. Not more than 20% of injected cyclophosphamide was excreted intact in urine at any dose level.

Plasma alkylating metabolites were 56% bound to plasma proteins. After a 40-mg/kg dose, peak unbound alkylating activity averaged 13.3 \(\mu\)mol/ml, and in most patients at this dose alkylating activity in the plasma was measurable for 24 hr. Sixty-eight % of injected \(^{14}\)C label was excreted in urine. Breath and fecal excretion were negligible.

In a regimen of five consecutive daily cyclophosphamide administrations, cyclophosphamide half-life was shorter and peak alkylating levels were constantly higher on the 5th day than on the 1st day. Prior patient treatment with allopurinol resulted in significantly longer cyclophosphamide half-life, but concomitant prednisolone treatment had no effect. The effect of hepatic metastases on cyclophosphamide metabolism was unclear. Moderate renal failure in one patient resulted in prolonged retention of alkylating materials in plasma and severe toxicity.

Although patients with and without prior exposure to microsomal enzyme-inducing drugs demonstrated marked variation in plasma cyclophosphamide half-life and peak alkylating levels, the total concentration X time product remained relatively constant for a given cyclophosphamide dose, suggesting that alterations in the rate of cyclophosphamide metabolism by drugs or liver metastases in the absence of renal failure will not change toxicity or therapeutic effect.

INTRODUCTION

Cyclophosphamide is an anticancer agent with antitumor effects against a wide variety of human neoplasms for which it is widely used (18). More recently, its immunosuppressive properties have been exploited successfully in the treatment of nonneoplastic diseases such as rheumatoid arthritis, lupus erythematosus, and Wegener’s granulomatosis (19–21, 25). This drug is unique among antitumor drugs in that it requires activation to an alkylating metabolite by the liver. Several investigators have shown that activation occurs on hepatic microsomes and that microsomes from other organs and tumors are largely incapable of activating cyclophosphamide (4, 11, 15).

Hepatic microsomal drug metabolism may be altered by previous and simultaneous exposure to drugs, many of which are commonly used in the care of patients with cancer (7). Although the effects of prior drug therapy on cyclophosphamide activation have been studied in animals, there are few similar studies in man. Likewise, the effect of liver and renal disease, prior cyclophosphamide therapy, and pharmacological differences inherent in p.o. cyclophosphamide administration have not been the subject of most of the published reports. In an attempt to clarify the role of these and other variables, cyclophosphamide metabolism and distribution were studied in patients with the use of radiolabeled compound to follow intact drug levels and the NBP\(^{1}\) assay to determine levels of alkylating activity.

MATERIALS AND METHODS

Patients. Cyclophosphamide-\(^{14}\)C was administered i.v. 43 times and nonradioactive cyclophosphamide 37 times (i.v., 26; p.o., 11) to 26 patients who received cyclophosphamide as therapy for advanced cancers (non-Hodgkin’s lymphoma, 12; carcinoma of ovary, 6; melanoma, 5; others, 3). Whenever possible, patients were studied while on no medication of any sort for in excess of 1 week; but, in most cases, clinical care required the administration of other drugs before and during study. If these drugs were known to affect microsomal metabolism in animals or man, the patients’ data were considered separately and correlations were sought. Prophylactic antiemetics were not given.

Cyclophosphamide doses varied from 6.7 to 80 mg/kg body weight. After labeled drug administration, urine was collected for 1 to 4 days whenever possible, and stools were collected for 4 days in selected patients. Endogenous creatinine clearance and liver function tests were used to evaluate the clinical status of the kidneys and liver. Expired air samples were obtained at intervals over 4 days in 2 patients. After every cyclophosphamide administration, venous blood samples

\(^{1}\)The abbreviations used are: NBP, 4-(4-nitrobenzyl)pyridine; CVP, cyclophosphamide (40 mg/sq m/day for 5 days, p.o. or i.v.), vincristine (1.4 mg/sq m), and prednisone (100 mg/sq m/day for 5 days p.o.); C X T, concentration X time product.
were taken every 1 to 2 hr for 4 hr and then every 4 hr for up to 24 hr in heparinized syringes, and they were centrifuged at once at 4° to obtain plasma. All samples were stored frozen at -10°, unless specified otherwise, and assays were done within 1 week of collection.

Preparation of Cyclophosphamide Dose. Cyclophosphamide uniformly labeled with 14C on the bischloroethyl side chain (specific activity, 9.6 Ci/mole) was obtained from Monsanto Research Corporation, Dayton, Ohio (Lot 131-5(a)) under contract with the National Cancer Institute, Bethesda, Md., and was prepared with sterile water in 5-ml vials by the Radiopharmaceutical Service, National Institutes of Health, Bethesda, Md. Vials remained frozen until use. Purity of cyclophosphamide-14C was determined by ascending chromatography on silica gel plates with the use of 100% ethanol. An aliquot of every patient’s dose was also assayed for purity. For determination of the amount of radioactive nornitrogen mustard in the stock Cyclophosphamide-14C, 2.0 ml of stock solution were acidified to pH 1.30 with HCl and extraction was done. Radioactivity present in the 3rd for purity. For determination of the amount of radioactive nornitrogen mustard in the stock cyclophosphamide-14C, 2.0 ml of stock solution were acidified to pH 1.30 with HCl and extracted twice with 5.0-ml volumes of chloroform. Then the pH was adjusted to 7.50 with NaOH, and a 3rd chloroform extraction was done. Radioactivity present in the 3rd chloroform layer was presumed to be nornitrogen mustard or its hydrolysis products on the basis of these solubility characteristics.

Unlabeled cyclophosphamide for clinical use in 200- or 500-mg vials (Cytoxan; Mead-Johnson Laboratories, Evansville, Ind.) was dissolved in appropriate diluent at a dosage based on the patient’s actual weight. From 30 to 100 μCi of cyclophosphamide-14C were added, these were mixed, and a weighed aliquot was removed for specific activity determination. The drug was then administered i.v. in less than 10 min.

Assay for Cyclophosphamide. Two-ml aliquots of plasma or urine were placed in 45-ml stoppered tubes with subsequent addition of 2 mg unlabeled stock cyclophosphamide (in 0.2 ml) and 5.0 ml chloroform. (The pH of urine samples was adjusted to 7.5 with 0.25 M NaOH, if necessary.) The tubes were shaken mechanically for 15 min and centrifuged to separate layers; the aqueous layer was discarded. Then 1.5 ml of 0.05 M HCl were added, and the mixture was shaken for 15 min, centrifuged, and removed. Two ml of the remaining chloroform layer were dried in counting vials at room temperature and redissolved in 10 ml of dioxane counting fluid. Plasma cyclophosphamide levels were plotted as μmoles/ml on semilog paper, and the half-life and theoretical zero-hr drug level were obtained. In all cases, the cyclophosphamide levels on semilog plot were well fitted by a straight line for at least 8 hr.

Protein Binding Studies. Determination of the fraction of plasma cyclophosphamide bound to protein was done by centrifugation in dialysis tubing, as described by Dixon and Adamson (9) with the use of cyclophosphamide-14C after extraction with 0.05 M HCl to remove impurities. Radioactivity in the original plasma, the filtrate, and the unfiltered residue within the tubing was determined by addition of 0.5 ml of each directly to Aquasol scintillation fluid (New England Nuclear, Boston, Mass.)

For determination of protein binding by urinary metabolites, urine with high alkylating activity was obtained from a patient after cyclophosphamide-14C administration, adjusted to pH 7.5, and extracted exhaustively with chloroform to remove cyclophosphamide and nornitrogen mustard. The extracted urine was then added to 3 heparinized plasma samples to achieve final alkylating activities comparable to those found in vivo, and quadruplicate aliquots of each plasma were placed in dialysis tubing and handled as above.

Protein binding of plasma alkylating activity was determined by comparison of total and free alkylating levels in 4 patients, with correction for solute trapping and for activation of cyclophosphamide during NBP assay (see below).

Assay for Plasma Alkylating Metabolites. For determination of total plasma alkylating metabolite levels, plasma and ascites samples were deproteinized before assay with perchloric acid and KOH neutralization. When titrated water was added to control plasma, 73.8% (range, 72.0 to 75.5%) of tritium label was recovered after this deproteinization procedure. Apparent plasma alkylating metabolite values of plasma samples assayed after deproteinization were corrected by this factor. When plasma from a patient who had received cyclophosphamide was ultrafiltered to remove protein (see below) and then deproteinized, there was no detectable destruction of alkylating ability due to the perchloric acid deproteinization.

Alkylating activity was measured by reaction with NBP, as described by Friedman and Boyer (12), except that ethyl acetate volumes varied from 2 to 5 ml according to anticipated quantity of alkylating activity to improve sensitivity. It was presumed that the plasma alkylating metabolite values determined by this method (with the correction for trapping in protein precipitate) represented the total amount of plasma alkylating metabolites, bound or unbound, in the patient’s plasma.

To determine free plasma alkylating metabolite levels, 3- to 7-ml plasma (or ascites) samples were placed in Centriflo ultrafiltration cones (Amicon Corporation, Lexington, Mass.) and centrifuged at 1000 X g at 4° for 1 to 15 hr. The resultant ultrafiltrate was assayed directly. The filters passed 95+% of plasma alkylating metabolite intact. Because of the large volumes of plasma required at frequent intervals for these assays, only free plasma alkylating metabolite levels were measured in most patients. Urine alkylating levels were measured by the NBP reaction directly.

Radiolabel Excretion Assays. Radiolabel content of stool was determined by combustion with collection of 14CO2 in modified Cardinal solution (Burdick and Jackson Laboratories, Muskegon, Mich.) (22, 23). Total urine 14C content was determined by pipetting 0.50 ml of urine directly into 15 ml Aquasol counting fluid. Timed 1-min breath collections in Douglas bags were obtained at frequent intervals on the 1st day of drug administration and daily thereafter for 4 days. The breath was then aspirated out of the bag through a 14CO2 trap containing 20 ml of the modified Cardinal solution. The breath bubbled through the solution from a fine pipet at a rate that averaged 300 ml/min. For determination of the efficiency of trapping, 5 breath samples were aspirated through 2 such 14CO2 traps in succession; 93.9% (range, 92.8 to 96.5%) of total counts were contained in the 1st trap.
Scintillation Counting. Liquid scintillation counting was done with a Packard Tri-Carb Model 4322 liquid scintillation counter. Quench correction curves were prepared for each scintillation medium with the use of chloroform, nitromethane, plasma, and/or urine for quenching. Counting efficiency for dioxane and Aquasol counting solutions approached 80%, and for the combustion method, it approached 70%.

Variation in results is expressed as ±1 S.D. with range in parentheses. Test for significance of differences was done with the Student t test.

RESULTS

Purity of Cyclophosphamide-\(^{14}\)C. On chromatography in 100% ethanol cyclophosphamide migrated as a single spot (RF, 0.67) containing 86.4% of spotted radioactivity. Assay of an aliquot of each patient’s dose by chloroform extraction yielded an average purity of 87.1 ± 5.6%. Cyclophosphamide specific activity was corrected for this impurity. Nor-nitrogen mustard or its hydrolysis products composed 2.5% (range, 1.3 to 3.3%) of total radioactivity.

Cyclophosphamide and Plasma Alkylating Metabolite Assays. Assay of plasma containing known additions of cyclophosphamide-\(^{14}\)C yielded 94.0% (range, 91.4 to 97.3%) recovery by this method. Chromatography of saline solutions of cyclophosphamide-\(^{14}\)C and of patient’s urine, before and after chloroform extraction, showed complete removal by chloroform of the spot corresponding to cyclophosphamide and appearance of this spot in the chromatograms of the chloroform layers. Other radioactive and NBP-reactive spots in the chromatograms of patient’s urine were not affected by chloroform extraction. Levels of radioactivity in patient’s plasma were too low to be analyzed by radiochromatography.

With weighed standards, the molar extinction coefficients of the alkylated NBP molecule after alkalinization and ethyl acetate extraction were found to be 3.38 × 10\(^3\) (range, 3.3 to 3.45 × 10\(^3\)) cm/mole for nitrogen mustard and 3.42 × 10\(^3\) (range, 3.37 to 3.47 × 10\(^3\)) sq cm/mole for non-nitrogen mustard and were presumed to be identical. Intact cyclophosphamide reacted with NBP to the extent of 0.6 to 1.0%, probably due to hydrolysis to non-nitrogen mustard during assay. Deproteinization and assay of intact cyclophosphamide solutions yielded absorbance equivalent to 1.6% (range, 1.4 to 1.7%) of added drug.

Protein Binding of Cyclophosphamide, Plasma Alkylating Metabolite, and Urinary Metabolites. At plasma cyclophosphamide-\(^{14}\)C concentrations of 10 and 200 \(\mu\)mole/ml, protein bindings were 14 ± 2.5% and 12 ± 5%, respectively. Urinary cyclophosphamide metabolites at concentrations of 2.3 to 110 \(\mu\)mole/ml plasma were not detectably protein bound. Following 5 cyclophosphamide doses of 40 mg/kg to 4 patients, plasma was assayed for both total and free plasma alkylating metabolite levels. At free plasma alkylating metabolite levels of 5.7 to 27 \(\mu\)mole/ml, metabolite was bound 56 ± 4% (range, 52 to 61%) to plasma proteins.

Intact Cyclophosphamide. The characteristics of the patients studied with cyclophosphamide-\(^{14}\)C and results obtained are summarized in Table 1. The levels of cyclophosphamide and plasma alkylating metabolite in plasma of a typical patient are shown in Chart 1.

After i.v. administration, cyclophosphamide-\(^{14}\)C distributed into 64 ± 9% of total body weight within 1 hr. In 3 patients with ovarian carcinoma and ascites, ascites cyclophosphamide levels equilibrated with plasma levels in 6 to 8 hr and exceed plasma levels thereafter.

At the time of cyclophosphamide-\(^{14}\)C administration, 12 of the 25 patients in this study had received no drug therapy known to affect microsomal function in the previous week or more and had no known liver disease. In these patients, the half-life of intact plasma cyclophosphamide was 6.45 ± 1.1 (range, 3.9 to 8.2) hr, and there was no correlation of half-life with the patient’s diagnosis or dosage (6 patients at 9 to 11 mg/kg, 1 patient at 20 mg/kg, 4 patients at 40 mg/kg, and 1 patient at 80 mg/kg). Clinical data in the patients’ records did not suggest that patients with a greater total mass of tumor had a cyclophosphamide half-life different from patients with minimal tumor. The fraction of administered cyclophosphamide excreted unchanged in the urine in the 1st 24 hr after therapy did not correlate with drug dose and was directly proportional to the endogenous creatinine clearance (\(r = 0.71\)), such that a creatinine clearance of 120 ml/min, 15% of cyclophosphamide was excreted unchanged. No patient excreted intact in urine greater than 20% of the administered cyclophosphamide dose. Intact cyclophosphamide excretion after the 1st day was negligible.

Plasma Alkylating Metabolites. A total of 17 i.v. cyclophosphamide studies (12 with labeled and 5 with unlabeled drug) were done in 13 patients who had received no other drugs for over 1 week prior to therapy. Plasma alkylating metabolite was readily measurable in all these studies. In 4 of these patients given 40 mg/kg only total plasma alkylating metabolite levels were assayed, yielding a mean peak level of 22.5 ± 6.1 (range, 15 to 30) \(\mu\)mole/ml. For purpose of estimation of these 4 patients’ free plasma alkylating metabolite levels, total plasma alkylating metabolite levels were corrected for protein binding by multiplying by 0.44 (1 minus percentage of protein bound, as described above).

The mean peak plasma alkylating metabolite levels after doses of 9 to 12 mg/kg was 3.2 ± 1.7 (range, 1.2 to 6.5) \(\mu\)mole/ml (8 studies), and after 40 mg/kg it was 13.3 ± 4.8 (range, 8 to 22) \(\mu\)mole/ml (7 studies). Peak free alkylating metabolite levels occurred 2 to 3 hr after therapy in most patients. In these 17 patients, without prior drug exposure, the plasma alkylating metabolite level 8 hr after therapy averaged 77 ± 15 (range, 45 to 97%) % of the peak (2 hr) value. Twenty-four hr after therapy, plasma alkylating metabolite levels were 18 ± 14% of peak levels but were unmeasurable in most of the patients given doses of 9 to 12 mg/kg.

Peak free plasma alkylating metabolite levels did not show a significant correlation with cyclophosphamide-\(^{14}\)C half-life in those patients without prior drug exposure (Chart 2). In each of 3 patients with ascites, ascites free plasma alkylating metabolite levels rose steadily to a maximum of 50

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### Table 1

**Data from 43 patient studies of cyclophosphamide$^{1,4}C$**

<table>
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<th>Patient</th>
<th>Before study</th>
<th>Dose (mg/kg)</th>
<th>Half-life (hr)</th>
<th>% in urine$^{b}$</th>
<th>Peak free plasma (mumoles/ml)</th>
<th>% in urine$^{b}$</th>
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<td>Cyclophosphamide$^{g}$</td>
<td>Prednisolone 1000</td>
<td>12$^{f}$</td>
<td>4.1</td>
<td>4.7</td>
<td>3.1</td>
</tr>
<tr>
<td>E. J.-1</td>
<td>Cyclophosphamide$^{g}$</td>
<td>None</td>
<td>6.8$^{f}$</td>
<td>4.7</td>
<td>12.6</td>
<td>3.1</td>
</tr>
<tr>
<td>E. J.-2</td>
<td>Cyclophosphamide$^{g}$</td>
<td>Prednisolone 1000</td>
<td>6.8$^{f}$</td>
<td>4.7</td>
<td>8.9</td>
<td>3.1</td>
</tr>
</tbody>
</table>

$^{a}$ Drug and dosage in mg/day.

$^{b}$ Percentage of total cyclophosphamide dose appearing in urine in 1st 24 hr.

$^{c}$ Liver dysfunction at time of study (see text).

$^{d}$ - datum not available. Data from other studies with nonradioactive cyclophosphamide are not shown.

$^{e}$ Calculated from total plasma alkylating metabolite levels assuming 56% plasma binding.

$^{f}$ Received 2nd dose of cyclophosphamide 12 to 24 hr later. Only data from 1st dose considered in this report.

$^{g}$ Receiving CVP (1) regimen. Actual cyclophosphamide dose, 400 mg/sq m.
Chart 1. Plasma levels of cyclophosphamide and of free alkylating activity in patients following injection of cyclophosphamide-14C on Day 1 and again on Day 5 of a daily i.v. cyclophosphamide regimen. Prednisone, 100 mg/sq m/day was given simultaneously with each dose of cyclophosphamide.

to 80% of peak plasma levels and subsequently declined more slowly than did plasma levels.

These patients without prior drug exposure excreted 16.0 ± 5% (8 to 23%) of injected cyclophosphamide as alkylating activity in the urine in 24 hr, without correlation with endogenous creatinine clearance or total dose of cyclophosphamide.

Excretion of Radiolabel. Following cyclophosphamide-14C administration, an average of 62 ± 10% (range, 41 to 82%) of administered 14C appeared in the urine in 2 days, and 68 ± 11% (59 to 82%) appeared in 4 days. A further 1.1% of the dose appeared in urine from the 4th to the 10th day after therapy in the 1 patient thus studied. Six patients had 4-day stool collections, excreting 1.8 ± 0.53% (range, 0.92 to 2.5%) of 14C label by this route. In 2 patients, 4-day breath collections yielded 0.9 and 1.4% of administered 14C label, respectively. Excretion of radiolabel by all routes was maximal in the 1st 24 hr after administration and fell rapidly thereafter.

Effect of Prior or Concomitant Drug Exposure. Nine patients with non-Hodgkin's lymphomas were treated with a combination drug regimen, CVP (1). In 3 of these patients, who had received no medication for over 2 weeks, the regimen was modified, so that cyclophosphamide only was given in the above dosage daily for 5 days as a single morning injection or as a p.o. dose. Labeled drug was given on the 1st and 3rd or 5th days, and no other drugs were allowed during the study. (Vincristine and prednisone were given on the day after completion of study.) Plasma free plasma alkylating metabolite levels were measured daily. In these 3 patients, plasma cyclophosphamide half-life averaged 6.1 ± 2 hr on the 1st day, and the half-life decreased by an average of 31 ± 11% when remeasured on the 3rd or 5th day. Peak plasma alkylating metabolite levels averaged 2.6 ± 1.6 μmole/ml on the 1st day and increased by an average of 73 ± 33% by the 3rd day of therapy, without significant change thereafter. This increase in peak plasma alkylating metabolite levels was not due to accumulation of plasma alkylating metabolite from the doses of the previous day, since in most patients there was no detectable plasma alkylating metabolite 24 hr after each dose; and in the few in which plasma alkylating metabolite was detectable, the difference in base line levels could not wholly account for the higher peak levels on Days 3 to 5 (of Chart 1).

Five patients were treated with the complete CVP regimen, receiving p.o. prednisone daily, simultaneously with each cyclophosphamide dose and other medications were withheld. Three of these patients had received no previous medications for over 2 weeks, and 2 patients had received only allopurinol, 300 mg/day for 5 to 10 days. In these 5 patients, plasma cyclophosphamide half-life averaged 6.4 ± 1 hr and decreased by 41 ± 9% on the 5th day of therapy. Peak plasma alkylating metabolite levels rose by 96 ± 30% by the 3rd day of therapy and did not change thereafter. These changes in cyclophosphamide half-life and plasma alkylating metabolite with 5 days of combined cyclophosphamide and prednisone therapy are not significantly different from those changes found with 5 days of cyclophosphamide alone.

Chart 2. Plasma peak free alkylating activity (μmole/ml) compared to plasma cyclophosphamide half-life (hr) in 12 patients with no prior drug exposure for over 1 week. Each point represents both the cyclophosphamide half-life and peak alkylating activity measured during a single cyclophosphamide-14C study. Actual peak alkylating levels were multiplied by 40/[actual cyclophosphamide dose (mg/kg)] to allow comparison of alkylating levels after different cyclophosphamide doses. In 6 of these patients, after 3 to 5 days of therapy (arrows) with cyclophosphamide or cyclophosphamide and prednisone, cyclophosphamide half-life is shorter and peak alkylating levels are higher.

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For determination of whether large doses of synthetic steroids interfered with cyclophosphamide metabolism, 4 patients were given identical cyclophosphamide-14C doses during each of 2 studies, separated by 2 to 30 days, special precautions being taken to ensure that all patients were receiving the same other medications before and during each administration. At the time of the 2nd study in 3 patients, 1 g of prednisolone was given rapidly i.v., followed in 2 min by the cyclophosphamide-14C dose (6.8, 12, or 40 mg/kg). The 4th patient was given 1 g of prednisolone divided into 5 boluses, 2 hr apart. In each of the 1st 3 patients, plasma cyclophosphamide half-life with prednisolone administration differed by less than 2% from the same patient's earlier control study; in R. S., the half-life decreased by 30%. Peak free plasma alkylating metabolite levels were 15 ± 13% higher with prednisolone administration. The differences are not statistically significant.

Four patients received allopurinol in full dosage for 3 to 10 days prior to cyclophosphamide-14C therapy and during this time received no other medications known to affect microsomal function. Plasma cyclophosphamide half-life in these patients was 8.1 ± 1.4 hr, significantly longer than in patients without prior medication (p < 0.01). However, the fraction of administered doses appearing as intact cyclophosphamide in the urine (8.8 ± 7%) was not different from controls, when the patients' creatinine clearances are considered. Due to technical error, plasma free plasma alkylating metabolite levels are available only on 2 patients. One patient treated at 9.5 mg/kg had a peak level of 2.7 μmoles/ml (versus 3.2 in control patients) and 1 patient had peak levels of 26 μmoles/ml, which is appropriate for his dose of 60 mg/kg. The fraction of injected cyclophosphamide appearing as alkylating metabolites in urine in 24 hr was 17 ± 5%, identical to the value for controls.

Other medications received by patients during or before cyclophosphamide therapy are shown in Table 1. Insufficient studies were done with pretreatment with other drugs to reach firm conclusions regarding their effect on cyclophosphamide metabolism. However, if one considers all studies performed following patient exposure to drugs thought to stimulate microsomal activity (i.e., barbiturates, diphenylhydantoin, cyclophosphamide, and prednisone), the peak plasma alkylating metabolite levels were 70% higher than in the same or similar patients without prior drug exposure, but the 8-hr plasma alkylating metabolite levels showed a more rapid decline (to 44 ± 20% of peak) than in unexposed patients (to 77 ± 15% of peak) (p < 0.01).

In 9 patients receiving a single administration of cyclophosphamide, with or without prior drug exposure, the plasma alkylating metabolite C × T could be calculated with reasonable accuracy. The average value following a dose of 40 mg/sq m was 139 ± 50 μmole hr, and after 80 mg/kg it was 282 ± 211 μmole hr. There was no correlation of C × T with cyclophosphamide half-life (over the range of 1.8 to 8.2 hr) or with prior drug exposure at either dose level.

Cyclophosphamide Therapy p.o. In 6 patients, the cyclophosphamide doses of the CVP regimen was given i.v. on Days 1, 3, and 5 and p.o. [as 50-mg tablets (Mead-Johnson)] on Days 2 and 4. Peak plasma alkylating metabolite levels were determined on every day. The expected rise in peak plasma alkylating metabolite levels from Day 1 to Day 3 was observed, and in every case the Day 2 (p.o.) level was intermediate between Day 1 and Day 3 (i.v.) levels. After p.o. dosage on Day 4, peak plasma alkylating metabolite levels were essentially identical to those of Days 3 and 5 (i.v.). Peak levels occurred 1 to 3 hr after p.o. administration and declined at the same rate as after i.v. administration.

Liver and Renal Disease. Patient D. V., with Hodgkin's disease, filling defects on liver scan, jaundice, and markedly elevated alkaline phosphatase, had the longest cyclophosphamide half-life (8.4 hr) and lowest peak plasma alkylating metabolite level (4.2) of any patient treated with cyclophosphamide, 40 mg/kg. The data were insufficient to calculate his C × T. Patient K. C. had widespread hepatic metastases on liver scan, but liver function tests were normal. She had a normal cyclophosphamide half-life (7.0 hr), but the 2nd lowest plasma alkylating metabolite peak (4.8 μmole/ml) and lowest C × T (95 μmole/hr) of patients receiving 40 mg/kg. Urinary excretion of intact cyclophosphamide and alkylating activity was not different from controls. Patient M. F. had severe jaundice and elevated alkaline phosphatase levels, presumed to be due to extrahepatic duct obstruction with ovarian carcinoma, since a liver scan was normal. Her cyclophosphamide half-life was normal (6.3 hr) and free plasma alkylating metabolite levels (27 μmole/ml) and C × T (600 μmole hr) were the highest found at the 80-mg/kg dose level. Incontinence prevented evaluation of her urinary metabolite excretion. Only 1 patient (A. S.) had severely depressed renal function (due to renal invasion with lymphocytic lymphoma). She was treated with full doses of CVP (cyclophosphamide, 9.7 mg/kg, for 5 days) while her creatinine clearance was 18 ml/min. Initial plasma cyclophosphamide half-life was 3.1 hr (possibly due to the patient's many prior medications since her t1/2 was 6.0 hr when she was restudied later while on no medication). Urinary cyclophosphamide excretion was appropriate for her creatinine clearance. Peak free plasma alkylating metabolite levels (μmole/ml) were: Day 1, 7.8; Day 2, 12.5; Day 3, 17; and Day 5, 23. The plasma alkylating metabolite level 24 hr after Day 1 therapy was 0.5 μmole/ml and after Day 2 it was 2.2 μmole/ml. Unfortunately, 24-hr level assays were not obtained after later doses. Of 7 other patients assayed, who received 9 to 11 mg/kg of drug, and in 2 repeat studies of Patient A. S. after renal function had improved, none had detectable plasma alkylating metabolite remaining 24 hr later.

DISCUSSION

The metabolic pathway for the activation of cyclophosphamide, as proposed by Hill et al. (15), is shown in Chart 3. "Aldophosphamide" inhibited growth of cultured human epidermoid carcinoma No. 2 cells by 50%, at a drug level of 0.2 g/ml (0.7 μmole/ml) and is thought to be the active metabolite of cyclophosphamide (15, 26). Subsequently, aldophosphamide may be oxidized in the liver by the nonmicrosomal enzyme, aldehyde oxidase, to "carboxyphosphamide," which is the major metabolite.
Chart 3. Metabolic pathway of cyclophosphamide leading to formation of the active alkylating metabolite, aldophosphamide, and its subsequent oxidation to carboxyphosphamide, as proposed by Hill et al. (15).

 appearing in the urine (2, 27). Carboxyphosphamide inhibited human epidermoid carcinoma No. 2 cells by 58% at 1.0 g/ml (4 µmole/ml) (27). Metabolism of cyclophosphamide appears to be qualitatively identical in man and animal.

Aldophosphamide, carboxyphosphamide, and nonnitrogen mustard all alkylate NBP (15, 27). However, Hohorst et al. (16) have reported that the cyclophosphamide metabolite with the highest serum levels is uncharged and gives aldehyde reactions. This is compatible with aldophosphamide. Other investigators have shown that, following cyclophosphamide therapy in humans, plasma contains substances capable of completely inhibiting Ehrlich ascites cell cultures and that peak inhibition occurred 2 hr after therapy (28), which is the time when we have most consistently found peak plasma alkylating metabolite levels on NBP assay. Therefore, we feel that plasma alkylating levels, as measured by the NBP assay, give a good indication of the levels of toxic and tumoricidal metabolites resulting from cyclophosphamide administration.

We found negligible cyclophosphamide binding to plasma proteins, as has been reported (6). The half-life of intact plasma cyclophosphamide that we found in 12 patients without prior exposure to drugs capable of inducing microsomal enzymes is longer than previously reported from this laboratory (6.45 versus 5.3 hr) (8). Mellett et al. (20) have described a cyclophosphamide half-life of 4.1 hr in cancer patients. It is possible that patients in some of these earlier studies were receiving medications that were not appreciated as being inducers of microsomal enzymes. A correlation of plasma cyclophosphamide half-life with drug dosage was not seen in this study. The maximal initial cyclophosphamide metabolic rate found in this study (average, 486 mg/hr after 80-mg/kg dose) is similar to that predicted from direct assay of human liver microsomes (440 mg/hr) (3). The fraction of intact cyclophosphamide appearing in urine was also independent of drug dosage.

Peak total plasma alkylating metabolite levels in control patients receiving cyclophosphamide, 40 mg/kg, averaged 22.5 µmole/ml, which is lower than the average levels of 47 µmole/ml found by Brock (3) after 60-mg/kg doses, even when the data are adjusted for the difference in dosage. However, it is not stated whether Brock’s patients were receiving enzyme-inducing drugs.

Our previous studies of patients with Burkitt’s lymphoma in Africa and the United States did not show acceleration of cyclophosphamide metabolism after heavy pretreatment with phenobarbital (8), but Mellett et al. (20) reported shortening of cyclophosphamide half-life from 4.1 to 1.6 hr after 2 weeks of phenobarbital therapy. Two studies with single or multiple doses of cyclophosphamide in rats have suggested that cyclophosphamide itself causes a decrease in microsomal oxidative functions for up to 21 days, which might be expected to inhibit subsequent metabolism of cyclophosphamide (10, 29). However, Sladek found no such inhibition (24). Our data show that, in fact, cyclophosphamide in the doses used here stimulated its own metabolism within the 1st 2 days of consecutive daily therapy and then continued to be metabolized more rapidly until at least the 5th day of treatment. Low-dose daily cyclophosphamide therapy (50 to 200 mg/day) has not been studied and enhanced metabolism may not necessarily occur. Two animal studies have suggested that simultaneous treatment with prednisone inhibits cyclophosphamide metabolism (14, 15), but this has been rebutted by others (13). In our present study, massive single doses of prednisolone just before cyclophosphamide administration did not inhibit cyclophosphamide metabolism. However, we have not studied the effect of prolonged pretreatment with steroids.

Allopurinol has become an essential drug in the prevention of hyperuricemia during therapy of drug-sensitive cancers and has been reported to inhibit microsomal metabolism of other drugs in man, when given for several days prior to testing (30). We found that, although allopurinol pretreatment resulted in longer cyclophosphamide half-life, plasma alkylating activity and urinary metabolite and cyclophosphamide excretion were unchanged. Also allopurinol pretreatment did not prevent self-induction of more rapid metabolism by cyclophosphamide.

Our observations suggest that prior exposure to drugs with an inducing or repressing effect on microsomal oxidase should have little effect on the antitumor and toxic effects of cyclophosphamide. In patients having received inducing drugs, we found higher peak plasma alkylating metabolite levels, which declined more rapidly, and the plasma alkylating metabolite C X T factor, when calculable after a given drug dosage, did not differ significantly over a 4-fold range of cyclophosphamide half-lives in several patients. Since intact cyclophosphamide excretion is slow, it is probable that patients with even greatly prolonged drug half-lives would still be able to metabolize a large fraction of administered drug. Thus, it would appear that cyclophosphamide-alkylating metabolite therapeutic effect and toxicity should be a function of administered dose only and should be largely independent of the rate of metabolic activation. Sladek (24) has made similar observations in rats. Drugs that inhibit aldehyde oxidase, such as chlorpromazine, might be expected to inhibit a aldophosphamide metabolism, increase the C X T product, and result in increased toxicity (17). However, our 1 patient given large doses of the related drug, prochlorperazine, did not have a higher C X T product than other patients.
receiving the same cyclophosphamide dose.

Several investigators have shown that the presence of transplanted tumors in s.c. and i.m. sites results in diminution of microsomal enzyme activity (5, 24). Although we have only 12 non-drug-exposed patients, those with considerable tumor bulk appeared to metabolize cyclophosphamide as rapidly as those with minimal tumor. Our 1 patient with renal function impairment had accelerated cyclophosphamide metabolism on her 1st day of therapy, probably due to prior exposure to a large number of drugs. She subsequently manifested much higher peak plasma alkylating metabolite levels than were seen in any other patient at that dose level. She was the only large number of drugs. She subsequently manifested much

impairment had accelerated Cyclophosphamide metabolism on such patients should receive the drug in low initial dosage with alkylating activity from 8 to 24 hr. Although less active than have a rapid rise and fall in plasma aldophosphamide but that Cyclophosphamide half-life. It is possible that in fact she did have a rapid rise and fall in plasma aldophosphamide but that carboxyphosphamide was retained in plasma for a prolonged weeks following therapy. Until further studies can be done on bulk appeared to metabolize Cyclophosphamide as rapidly in any other patient at that dose level. She was the only

REFERENCES


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Clinical Pharmacology of Cyclophosphamide

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