Phenobarbital Effects on Cyclophosphamide Pharmacokinetics in Man

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SUMMARY

Plasma concentrations and urinary excretion rates of cyclophosphamide and its total metabolites were measured after i.v. administration of 500- to 1000-mg doses of radiolabeled cyclophosphamide to four patients. The data obtained before and after phenobarbital treatment were analyzed pharmacokinetically with the use of a two-compartment open model. Phenobarbital had no quantitatively important effects on the distribution and renal excretion of cyclophosphamide. The rate of biotransformation of the inactive precursor to its total metabolites was increased 2- to 3-fold by phenobarbital. However, because biotransformation is the predominant pathway of cyclophosphamide disposition, the total quantity of metabolites formed was only increased slightly by phenobarbital. These observations, together with comprehensive data in the literature showing that phenobarbital has little effect on the chemotherapeutic properties of cyclophosphamide in several animal tumor systems, indicate that phenobarbital treatment should modify only slightly the efficacy and toxicity of cyclophosphamide in man.

INTRODUCTION

The kinetic aspects of cyclophosphamide distribution, metabolism, and excretion in man have been studied recently in detail (6). It has been established in animals that cyclophosphamide is biotransformed into active alkylating metabolites by NADPH-dependent microsomal enzymes of the liver (2, 5, 15). The effects of phenobarbital on cyclophosphamide activation by this system has been reported extensively in animals (5-10, 16), but detailed data from man have not appeared. This report describes the effects of phenobarbital pretreatment on the pharmacokinetics of cyclophosphamide in patients with neoplastic disease.

MATERIALS AND METHODS

The volunteers for this study were 4 male patients; 3 had inoperable bronchogenic carcinoma and 1 had lymphosarcoma. The patients had normal renal (Table 3) and hepatic functions as shown by standard clinical tests. During the study, the patients were essentially asymptomatic and were taking no other drugs. Each patient was studied 1 day prior to and following treatment with phenobarbital. Such treatment consisted of p.o. doses of 30 mg of phenobarbital 3 times daily and 90 mg before bedtime for 10 consecutive days. This regimen was discontinued the day before cyclophosphamide administration. Previous studies had demonstrated that cyclophosphamide had no effect on its own pharmacokinetics even after multiple courses.

Cyclophosphamide-14C (New England Nuclear, Boston, Mass.) was mixed with nonradioactive drug to produce dosages of 5 μCi of activity and 500 to 1000 mg of cyclophosphamide. Details of administration, sample collection, and analysis have been previously published (6).

RESULTS

It has been previously determined that the decline in plasma cyclophosphamide level after i.v. injection is biexponential with an initial rapid distributive (α) phase and a subsequent lower (β) phase during which the drug is primarily metabolized and excreted. The concentration (A,B) and rate (α,β) parameters of the equation

\[ C_p = A_p e^{-αt} + B_p e^{-βt} \]  

were obtained from such data by digital computer least-squares iteration (14) and are listed in Table 1 for all 4 cross-over studies. These kinetic parameters were used in equations characterizing the 2-compartment open model shown in Scheme 1 to calculate the distribution rate and volume constants of cyclophosphamide (6). The average values for the studies before and after phenobarbital treatment are listed in Table 2. No consistent change in the volume of the central compartment \( V_1 \) or in the intercompartmental transfer rate constants \( k_{12} \) and \( k_{21} \) occurred when phenobarbital was given to the patients. The graphical parameters from Equation A were also used to derive the rate constant for elimination \( k_{el} \) of cyclophosphamide (Table 3). The change in \( k_{el} \) values shows that predadministration of phenobarbital increased the rate of elimination of cyclophosphamide by 2- to 3-fold. This effect is also reflected partly by the change in values and half-life (Table 1).

The relative contribution of renal excretion and biotransformation to the overall elimination of cyclophosphamide can

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2The mathematical treatment of pharmacokinetic parameters of cyclophosphamide has previously been published (6).
Table 1  
Pharmacokinetic parameters of cyclophosphamide plasma level decline before and after phenobarbital treatment

<table>
<thead>
<tr>
<th>Subject</th>
<th>Dose (mg)</th>
<th>Treatment</th>
<th>Least-square biexponential parameters</th>
<th>β half-life (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>µg/ml hr⁻¹</td>
</tr>
<tr>
<td>1</td>
<td>800</td>
<td>Control</td>
<td>16.3</td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phenobarbital</td>
<td>38.5</td>
<td>6.20</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>Control</td>
<td>20.4</td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phenobarbital</td>
<td>12.9</td>
<td>4.26</td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
<td>Control</td>
<td>52.8</td>
<td>8.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phenobarbital</td>
<td>62.6</td>
<td>9.60</td>
</tr>
<tr>
<td>4</td>
<td>900</td>
<td>Control</td>
<td>16.7</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phenobarbital</td>
<td>32.0</td>
<td>4.83</td>
</tr>
</tbody>
</table>

Table 2  
Pharmacokinetic distribution constants of cyclophosphamide before and after phenobarbital treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume of central compartment (1)</th>
<th>Transfer rate constants (hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>k₁₂</td>
</tr>
<tr>
<td>Control</td>
<td>21.4 ± 8.7°</td>
<td>2.57 ± 1.83</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>18.8 ± 6.4</td>
<td>1.89 ± 0.69</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

Table 3  
Pharmacokinetic elimination constants and renal clearance of cyclophosphamide before and after phenobarbital treatment

<table>
<thead>
<tr>
<th>Subject</th>
<th>Treatment</th>
<th>Renal clearance (ml/min)</th>
<th>Cyclophosphamide</th>
<th>Creatinine</th>
<th>Elimation constants (hr⁻¹)</th>
<th>kₑl</th>
<th>kₑ</th>
<th>kₑm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>13.8</td>
<td>74.4</td>
<td>0.284</td>
<td>0.030</td>
<td>0.254</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenobarbital</td>
<td>13.8</td>
<td>9.6</td>
<td>0.623</td>
<td>0.051</td>
<td>0.572</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>20.8</td>
<td>89.6</td>
<td>0.672</td>
<td>0.047</td>
<td>0.625</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenobarbital</td>
<td>8.4</td>
<td>94.1</td>
<td>0.327</td>
<td>0.038</td>
<td>0.289</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>17.6</td>
<td>10.9</td>
<td>0.999</td>
<td>0.087</td>
<td>0.912</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenobarbital</td>
<td>17.9</td>
<td>102.7</td>
<td>0.705</td>
<td>0.051</td>
<td>0.655</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>Control</td>
<td>10.7</td>
<td>74.4</td>
<td>0.297</td>
<td>0.031</td>
<td>0.266</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenobarbital</td>
<td>17.5°</td>
<td>102.7</td>
<td>0.750°</td>
<td>0.059°</td>
<td>0.691°</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Statistically significant difference (p < 0.05) by method of paired comparisons.

be determined from the measurements of the excretion of unchanged cyclophosphamide in the urine. As shown by the cumulative urinary excretion data as a function of time for Subject 4 in Chart 1, only a small fraction of the dose of cyclophosphamide is excreted unchanged in the urine (about 10%). Phenytoin pretreatment did not appreciably affect the urinary recovery of cyclophosphamide in any of the patients (Table 4). The renal clearance of cyclophosphamide...
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was estimated from actual or interpolated plasma concentrations (Equation A) at the midpoints of the urinary excretion intervals. Average renal clearance values of about 11 ml/min in the 4 subjects (Table 3), together with the knowledge that cyclophosphamide is only slightly bound to plasma proteins (12), indicates that extensive renal tubular reabsorption of the drug occurs. Pretreatment with phenobarbital appeared to increase the mean renal clearance (Clr) of cyclophosphamide in 3 out of 4 subjects, but this effect is not of quantitative importance. An estimate of the renal excretion rate constant (k_e) can be obtained from the ratio of Clr/Vc. The data listed in Table 3, in comparison with the much larger k_urt values, further reflect the minor contribution of renal clearance to removal of drug from the body. Since the lipid solubility of cyclophosphamide results in relatively little or no extrarenal excretion of the drug (6), the difference of k_urt - k_e provides an approximation of the rate constant for biotransformation (k_m) of cyclophosphamide. Comparison of the k_m values obtained before and after phenobarbital treatment reveals a significant difference (p < 0.05) which indicates that the primary effect of the barbiturate was enhancement of biotransformation of cyclophosphamide.

The analysis of the formation and excretion of the numerous (1) cyclophosphamide metabolites is, of necessity, limited by the lack of identification of the precise constituents of the metabolite pool in man. However, some general observations can be made. Plasma levels of the biotransformation products of cyclophosphamide before and after phenobarbital treatment are shown in Table 4. Almost immediately after cyclophosphamide administration, a plateau-like plasma level of metabolites was reached which was maintained for at least 6 hr. A similar pattern in total plasma metabolite levels was noted previously by Brock et al. (3). Phenobarbital treatment caused a large increase in this early plasma metabolite level in all 4 patients.

The data in Chart 1 for Subject 4 and in Table 4 for the other patients show the important fact that the total (48-hr) urinary excretion of cyclophosphamide metabolites was not appreciably altered by phenobarbital. Phenobarbital pretreatment produced an increase only in the initial rate of urinary excretion of the metabolites (Table 5; Chart 1). This increase in the early appearance of metabolites in the urine reflects the corresponding increase in the rate of their formation. It thus appears that phenobarbital pretreatment enhances the initial biotransformation rate of cyclophosphamide to its metabolites but not the total quantity of metabolites eventually formed.
DISCUSSION

It was determined previously (6) that the pharmacokinetics of cyclophosphamide in man can be characterized by a 2-compartment open model as shown in Scheme 1. The same kinetic model was applied in the present study which was designed to investigate the effect of phenobarbital upon the measurable pharmacokinetics of cyclophosphamide. The experimental data show that the administration of phenobarbital increases greatly the rate of disappearance of cyclophosphamide from the plasma. Although phenobarbital seemed to produce an increase in the renal clearance of cyclophosphamide, this is quantitatively insignificant and was probably caused by variability in the data. The primary effect appears to be an increase in the rate of metabolism \( (k_m) \) of cyclophosphamide. In view of the known inductive effect of phenobarbital on drug metabolism, the data suggest that the metabolism of cyclophosphamide in man, as in animals, is mediated by the mixed-function oxidases of the liver. These drug-metabolizing enzymes are induced or inhibited by a large number of agents used in clinical medicine (4). Whereas changes in the rate of metabolism of other drugs, for example, bishydroxycoumarin (4), can be directly correlated with therapeutic effectiveness, it is not known for certain whether modifying cyclophosphamide metabolism in man would alter its antitumor activity and toxicity.

The issue seems to be particularly confusing because of the unusual pharmacokinetic behavior of cyclophosphamide. The present study shows clearly that the initial rate of formation of total metabolites of cyclophosphamide is increased by phenobarbital. However, because its lipid solubility results in the very slow renal excretion of only a small amount of unchanged cyclophosphamide, most of the drug is metabolized eventually, regardless of its rate of metabolism. Therefore, the total amount of metabolites formed in the body is only slightly increased in the phenobarbital experiments. This is shown by the mean total recovery of the dose as urinary metabolites of 55% in the control study and 62% in the phenobarbital study.

In essence, the effect of phenobarbital treatment is analogous to a change from a slower to a more rapid rate of i.v. infusion of active drug. However, the behavior of individual metabolites and particularly the chemotherapeutically active compound is uncertain and may be quite different from the pattern of total metabolites. Because as many as 8 (1) biotransformation products may be formed from cyclophosphamide, the differences in time course of total metabolite level may also reflect a change in metabolite composition where either more or less of an active product may be formed. This question should be resolvable by examination of data in the literature concerning the effect of phenobarbital on the effects of a given dose of cyclophosphamide on animal tumor systems.

It can be predicted theoretically (13) and has been argued intuitively (16) that the antitumor effect of a cell cycle-nonspecific chemotherapeutic agent is expected to correlate with the plasma concentration \( \text{versus} \) time area of the active drug. On this basis, the therapeutic efficacy of cyclophosphamide should either remain relatively unchanged or increase slightly when phenobarbital is given if the total amount of alkylating agent formed is only slightly altered. This appears to be the case, at least in several animal systems (7–9, 11, 16). It can therefore be expected that, in man, the phenobarbital-induced changes in formation rate of cyclophosphamide metabolites should be accompanied by only a small increase in therapeutic efficacy of the drug and a similar change in toxicity. This, however, remains to be proven with clinical measures of cyclophosphamide efficacy and toxicity.

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REFERENCES

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