Mechanisms of Cyclophosphamide Action on Hepatic P-450 Expression

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ABSTRACT

Cyclophosphamide was administered to adult male rats (130 mg/kg, single i.p. injection) and its effects on the P-450 enzymes that contribute to the activation of this drug in rat liver were then assessed. P-450-mediated cyclophosphamide 4-hydroxylase activity in isolated rat liver microsomes decreased by approximately 70% over a 9-day period following drug treatment. This decrease was due to the loss of cytochrome P-450 form 2c (IIC11), a major contributor to cyclophosphamide 4-hydroxylation in untreated male rat liver, while the other major hepatic cyclophosphamide 4-hydroxylase, P-450 PB-1 (IIC6), was largely unaffected. The loss of P-450 2c activity did not result from a decrease in P-450 reductase or from direct inactivation of the P-450 protein by cyclophosphamide or its metabolites, but rather was due to a reduction in hepatic P-450 2c protein and mRNA levels. Hepatic P-450 2a (III2A2) and P-450 RLM2 (IIA2) were also suppressed by cyclophosphamide treatment. Serum testosterone, which contributes to the expression of P-450s 2c, 2a, and RLM2, was severely depleted in the cyclophosphamide-treated rats; however, this loss was not the direct cause of the decreases in these hepatic P-450s, since the decreases were not reversed upon restoration of normal testosterone levels by human chorionic gonadotropin stimulation of testicular androgen production. In contrast to the suppression of these testosterone-dependent P-450s, P-450 3 (IIIC11), P-450 (IIIE1), and the P-450-independent microsomal enzyme steroid 5a-reductase were each elevated in rat liver following cyclophosphamide administration. In contrast to P-450 3 and steroid 5a-reductase, however, the elevation of P-450j protein was transient and was not accompanied by an increase in P-450j-associated hepatic microsomal aniline hydroxylase activity. In vitro experiments revealed that P-450j was several fold more susceptible to inactivation by the cyclophosphamide metabolite acrolein as compared with P-450 3. These observations suggest that P-450j protein is induced by cyclophosphamide treatment but that the protein is inactivated by the cyclophosphamide metabolite acrolein. These findings establish that cyclophosphamide treatment can modulate hepatic P-450 activities through multiple mechanisms and in a manner that may alter metabolism of cyclophosphamide and perhaps other anticancer drugs that undergo bioactivation in the liver.

INTRODUCTION

The alkylating agent cyclophosphamide is one of a group of anticancer drugs that are administered as inactive prodrugs, and are activated in vivo via one or more metabolic steps (1). The initial step in the bioactivation of cyclophosphamide involves cytochrome P-450-mediated hydroxylation at C-4; this is followed by an equilibration of the 4-hydroxy metabolite with aldo- phosphamide, and spontaneous decomposition of aldo- phosphamide to yield acrolein as well as phosphamide mustard, the therapeutically significant metabolite of cyclophosphamide (2, 3). Recent studies conducted in this laboratory have demonstrated that the primary contributors to cyclophosphamide 4-hydroxylation in uninduced adult male liver are P-450 form 2c (IIC11), a constitutively expressed, male-specific testosterone 2α/16α-hydroxylase, and P-450 PB-1 (IIC6), a P-450 form that is expressed constitutively but is also inducible by phenobarbital (6). P-450 PB-1 (IIB1), which is also phenobarbital-inducible, exhibits a high level of cyclophosphamide 4-hydroxylase activity (6); however, this form is not expressed at a significant level in uninduced rats. Although it is well established that cyclophosphamide treatment of adult rats leads to a significant decrease in hepatic P-450 levels and enzymatic activities (7–9), more than a dozen distinct P-450 enzymes have been isolated from rat liver (10, 11), and it is currently not known which of these P-450s are susceptible to suppression by cyclophosphamide. Studies with other anti-cancer drugs have revealed that while an overall decrease in total P-450 may occur, some individual P-450 forms can actually be elevated (12).

Changes in the level of expression of hepatic P-450s can affect the level of cyclophosphamide metabolism and accordingly its efficacy. Sladek (13) observed that administration of the P-450 modulators phenobarbital and 3-methylcholanthrene increased and decreased, respectively, the rate of cyclophosphamide metabolism in male rats. However, although phenobarbital administration had no effect on the overall efficacy of cyclophosphamide, 3-methylcholanthrene reduced its effectiveness. Studies with phenobarbital-treated patients established that phenobarbital does not increase drug efficacy due to the more rapid drug excretion that accompanies the increase in drug metabolism (14). The effect of cyclophosphamide on P-450 levels might not only be important with respect to its own activation, but could also influence the efficacy of drugs administered in combination with cyclophosphamide and that are also subject to P-450-mediated activation or deactivation (3). The success of such drug combinations could, in principle, be affected by the level of expression of individual P-450 enzymes. Thus, studies on the responses of individual P-450s to cyclophosphamide treatment may provide further understanding of the effects of cyclophosphamide on its own metabolism and on the metabolism of other drugs administered with it in combination chemotherapy regimens.

The present study assesses the effects of cyclophosphamide treatment on individual rat hepatic P-450 enzymes and examines the mechanisms by which this drug alters hepatic drug metabolism. These studies were carried out using the rat as a model system, since individual hepatic P-450s have been thoroughly characterized in this species, and several of these enzymes appear to be orthologous to human P-450s that have recently been identified (15).

MATERIALS AND METHODS

Animal Treatments. Adult male Fischer 344 rats (Harlan-Sprague Dawley, Inc., Frederick, MD) were used in this study to minimize interindividual differences in P-450 activities and enzyme levels that appear to be more prominent in outbred strains such as Sprague-Dawley.

* P-450 protein designations used in this study are summarized elsewhere (4). Systematic P-450 gene product designations, indicated by Roman numerals, are based on data described previously (5).
(e.g., Ref. 16). The animals were 9 weeks old at the initiation of the experiments and were housed under a 12-h photoperiod with food and water freely available. Cyclophosphamide (Sigma), freshly prepared in 0.9% NaCl, was administered as a single injection (130 mg/kg, i.p., unless indicated otherwise) with rats sacrificed at defined times thereafter. Rats were sacrificed by cervical dislocation after brief asphyxiation under CO2. Livers were rapidly excised, perfused with ice-cold 1.15% KCl (w/v), then used for microsome preparation from individual livers using a calcium precipitation method detailed elsewhere (12). Final microsomal pellets were resuspended in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.1 mM EDTA and 20% glycerol (v/v) and stored in aliquots at -80°C.

Enzyme Assays. Cyclophosphamide 4-hydroxylation catalyzed by isolated liver microsomes was assayed using 14C-labeled drug (0.5 mM at 0.5 mCi/mmol; Du Pont New England Nuclear) in a bovine serum albumin-binding assay described elsewhere (6). Microsomal aniline hydroxylase activity was determined by measuring the rate of 4-aminophenol generation in incubations containing 8 mM aniline (17). Microsomal testosterone hydroxylase and steroid 5α-reductase activity were assayed as summarized previously (12).

Fig. 1. Hepatic microsomal cyclophosphamide 4-hydroxylase activity (CPA 4-OHase) in cyclophosphamide-treated rats. Animals were given a single injection of 130 mg/kg cyclophosphamide on day 0, and enzyme activity measured in liver microsomes prepared from rats sacrificed 3, 6, or 9 days later. Activity values represent the mean ± SE for n = 4 individual rat liver microsome preparations.

Fig. 2. Response of hepatic P-450s 2c and PB-1 to cyclophosphamide treatment. A, P-450 2c protein (○) and testosterone 2α-hydroxylase activity (T-2αOHase; □); B, P-450 PB-1 protein levels in rat liver microsomes following administration of 130 mg/kg cyclophosphamide on day 0. Values represent the mean ± SE for n = 4 rats sacrificed at each time point. Hydroxylase activities are presented as relative values, where 100 = 2.32 nmol/min/mg. Protein values are relative to the protein levels present in the untreated adult male rat group (day 0 time point), which is arbitrarily set at 100.

Table 1 Influence of cyclophosphamide treatment on hepatic microsomal P-450 reductase activity, steroid 5α-reductase activity, and serum testosterone levels and responses to chorionic gonadotropin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P-450 reductase activity (nmol/min/mg)</th>
<th>Serum testosterone (ng/ml)</th>
<th>5α-Reductase activity (nmol/min/mg)</th>
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<tr>
<td>Experiment 1</td>
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<tr>
<td>Untreated male rat</td>
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<tr>
<td>+ Cyclophosphamide</td>
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<td>(3 days)</td>
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<td></td>
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<tr>
<td>+ Cyclophosphamide</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(6 days)</td>
<td>207 ± 28 (81)</td>
<td>4.6 ± 2.4</td>
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<tr>
<td>+ Cyclophosphamide</td>
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<tr>
<td>(9 days)</td>
<td>167 ± 13 (66)</td>
<td>12.8 ± 3.1</td>
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<tr>
<td>Experiment 2</td>
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<tr>
<td>Untreated male rat</td>
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<tr>
<td>+ Cyclophosphamide</td>
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<tr>
<td>(7 days)</td>
<td>19 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td></td>
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<tr>
<td>+ hCG (7 days)</td>
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* Liver microsomes were isolated from untreated or cyclophosphamide-treated male rats sacrificed 3, 6, or 9 days after a single drug injection at 120 mg/kg (Experiment 2) or 130 mg/kg (Experiment 1). Microsomal P-450 reductase was assayed by the reduction of cytochrome c (monitored at 550 nm) in 0.3 M potassium phosphate, pH 7.7, at 30°C. Steroid 5α-reductase was assayed by the reduction of [4-14C]androst-4-ene-3,17-dione (Experiment 1) or by the reduction of [4-14C]17β-hydroxyandrost-4-ene-3-one to [4-14C]17β-hydroxy-5α-androstan-3-one (Experiment 2). Data are expressed as mean ± SD for n = 4 (Experiment 1) or n = 3 individuals per treatment group (Experiment 2). Typical steroid 5α-reductase activities catalyzed by untreated adult female rat liver microsomes are 15-18 nmol/min/mg (data not shown).

RESULTS

Effects of Cyclophosphamide Treatment in Vivo on the Microsomal Cyclophosphamide 4-Hydroxylases P-450 2c and P-450 PB-1. Administration of a single dose of cyclophosphamide to adult male rats resulted in a progressive loss of microsomal cyclophosphamide 4-hydroxylase activity during a 9-day period subsequent to the injection.
Cyclophosphamide suppression of hepatic P-450s

A.

Fig. 3. Alkylation of microsomal proteins by [14C]cyclophosphamide. A. Radiolabeling of rat liver microsomal proteins (1.8 mg/ml of 0.1 M potassium phosphate buffer, pH 7.4, 0.1 mM EDTA) during incubation with [14C]cyclophosphamide (0.37 mM, 1 mCi/nmol) in the presence or absence of 0.8 mM NADPH. Triplicate samples were incubated for 90 min at 37°C, then analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and autoradiography (1 day exposure). Western blot analysis of the gel with anti-P-450 antibody after transfer to nitrocellulose revealed that the major radiolabeled band in the + NADPH lanes comigrated with this P-450 form, indicating that the major radiolabeled band shown corresponds to a protein in the molecular weight range of cytochrome P-450. B. Time course for [14C]cyclophosphamide radiolabeling of the major band revealed in A. Relative band intensities were determined by laser densitometry of an autoradiograph similar to the one shown in A.

following drug treatment (Fig. 1). This suggests that cyclophosphamide treatment leads to the suppression of the activity of one or more P-450 forms that contribute to the bioactivation of this drug by the 4-hydroxylase pathway. The effect of cyclophosphamide treatment on the primary enzymatic contributors to cyclophosphamide activation in uninduced adult male rats (P-450 2c, a male-specific hepatic P-450, and P-450 PB-1, a sex-independent, constitutive enzyme; Ref. 6) was therefore evaluated to further define the mechanism by which cyclophosphamide suppresses its own activation. Western blot analysis revealed that cyclophosphamide reduced P-450 2c protein and its associated testosterone 2α-hydroxylase activity by approximately 75% 9 days after dosing (Fig. 2A), while having no suppressive effect on P-450 PB-1 protein levels (Fig. 2B). These results indicate that the decrease in microsomal cyclophosphamide 4-hydroxylase activity following cyclophosphamide treatment in vivo is primarily due to the loss of P-450 2c protein and its contribution to this activity.

Cyclophosphamide treatment also led to a 20–35% reduction

B.

Fig. 4. Northern blot analysis of P-450 2c and P-450 PB1 mRNA in cyclophosphamide-treated rats. Total liver RNA was prepared from untreated (UT) male rats, from rats sacrificed 7 days after cyclophosphamide treatment (120 mg/kg (+CPA) or from cyclophosphamide-treated rats administered hCG by daily injection for 7 days (see Fig. 6). Last three lanes, RNA isolated from untreated female rat liver, which does not express the male-specific P-450 2c. Shown is an autoradiograph of a Northern blot analyzing RNA samples isolated from 2 or 3 individual rats in each treatment group. The blot was probed sequentially with oligonucleotides complementary to P-450 2c mRNA and P-450 PB1 mRNA (see "Materials and Methods").

Fig. 5. Suppression of P-450 2a and P-450 RLM2 in cyclophosphamide-treated rats. A. P-450 2a protein (•) and testosterone 6β-hydroxylase activity (T-6βOHase; O); B. P-450 RLM2 protein (•) and testosterone 15α-hydroxylase activity (T-15αOHase; O) following administration of 130 mg/kg cyclophosphamide. Values represent the mean ± SE for n = 4 rats sacrificed at each time point. Enzyme activities are presented as relative values, where 100 = 0.66 nmol/min/mg for testosterone 6β-hydroxylase and 100 = 0.18 nmol/min/mg for testosterone 15α-hydroxylase activity.
in hepatic microsomal P-450 reductase activity at 6–9 days (Table 1). This microsomal flavoenzyme catalyzes an obligatory electron transfer from NADPH to all microsomal P-450 enzymes, including P-450 2c. Supplementation of the liver microsomes prepared from cyclophosphamide-treated rats with exogenous, purified P-450 reductase (cf., Ref. 18) did not, however, alter the percentage loss of P-450 2c-dependent testosterone 2α-hydroxylase activity at any of the time points shown in Fig. 2A (data not shown). Thus, the partial loss of hepatic P-450 reductase activity does not make a major contribution to the P-450 2c activity losses observed in Figs. 1 and 2.

The decrease in total hepatic P-450 in cyclophosphamide-treated rats has been previously ascribed to the binding of the cyclophosphamide metabolite acrolein to P-450 protein, resulting in denaturation of the cytochrome (20). Indeed, in vitro incubation of [14C]cyclophosphamide with isolated liver microsomes resulted in an NADPH-dependent binding of radiolabel, probably representing acrolein, to protein(s) in the molecular weight range of the cytochromes P-450 (i.e., M, 50,000–60,000; Fig. 3). However, we questioned whether direct binding of a cyclophosphamide metabolite in this manner would be responsible for the major loss of P-450 2c indicated in Fig. 2, since this same P-450 form can be suppressed by treatment with several other drugs and foreign compounds, several of which probably act by perturbing hormonal factors that regulate constitutive expression of this enzyme (12, 21). Accordingly, hepatic P-450 2c mRNA levels were examined in cyclophosphamide-treated rats to assess the mechanism by which cyclophosphamide exerts its suppressive effect. P-450 2c mRNA levels were thus found to be markedly reduced in cyclophosphamide-treated rats (Fig. 4), in accord with the major loss of P-450 2c protein and activity (Fig. 2). In contrast, PB-1 mRNA levels were unaffected (Fig. 4). These findings indicate that the suppression of P-450 2c by cyclophosphamide does not result
from a denaturation of the P-450 by acrolein, but rather, is primarily due to a decrease in P-450 2c mRNA levels and hence its rate of synthesis.

Loss of Other Male-specific P-450s in Cyclophosphamide-treated Rats and Relationship to Serum Testosterone Depletion.

In order to obtain further insight into the mechanistic basis for the decrease in P-450 2c expression in cyclophosphamide-treated rats, the response of 2 other male-specific P-450s to the same drug treatment was examined. As with P-450 2c, protein levels and activities of P-450 2a (testosterone 6β-hydroxylase, IIIA2) and P-450 RLM2 (testosterone 15α-hydroxylase, IIA2) were suppressed following cyclophosphamide treatment (Fig. 5). Previous studies with the anti-cancer drug cisplatin have established that cisplatin reduces the expression of both these P-450 proteins (as well as P-450 2c) primarily through its depletion of serum testosterone, which contributes to the maintenance of all 3 male-specific P-450s (12). Serum testosterone levels in cyclophosphamide-treated rats were therefore examined and found to be depleted in response to drug treatment (Table 1, Experiment 2). Cyclophosphamide-treated rats were therefore administered hCG5 in an effort to elevate testosterone levels and restore P-450 protein expression. Although testosterone levels were significantly elevated in cyclophosphamide-treated rats given hCG by daily injection (Table 1), there was no discernable restoration of P-450 2c mRNA (Fig. 4), protein (Fig. 6), or activity levels (data not shown). There was also no restoration of the protein or activity levels of the other male-specific P-450s, forms 2a (Fig. 6) and RLM2 (data not shown). Therefore, although the cyclophosphamide-mediated loss of serum testosterone may contribute to the observed decrease in the male-specific P-450s, this testosterone loss alone is not responsible for the reduced expression of these P-450 enzymes.

Differential Responses of the Female-predominant Hepatic Enzymes P-450 3, P-450j, and Steroid 5α-Reductase to Cyclophosphamide Treatment. Levels of the constitutive P-450 forms 3 (testosterone 7α-hydroxylase, IIA1) and j (aniline hydroxylase, IIE1) were elevated 3 days after cyclophosphamide administration (Fig. 7). An even more striking elevation of the P-450-independent enzyme steroid 5α-reductase was observed in the drug-treated rats (Table 1). This latter effect, but not the others, was reversed by hCG stimulation of testosterone production (Table 1). While P-450 3 and its associated testosterone 7α-hydroxylase activity, as well as steroid 5α-reductase activity, remained elevated over a 9-day period following drug treatment (Fig. 7 and Table 1), P-450j levels returned to near normal by 6–9 days. Moreover, aniline hydroxylase activity, which is at least 60% catalyzed by P-450j in rat hepatic microsomes (22), was not elevated in parallel to the increase in P-450j protein. Since P-450j is capable of binding a number of low molecular weight aliphatic molecules (23), we considered the possibility that P-450j might also bind the cyclophosphamide metabolite acrolein, leading to P-450j inactivation. Incubation of isolated liver microsomes with varying concentrations of acrolein revealed that P-450j-dependent aniline hydroxylase activity was inhibited by approximately 70% at 0.8 mM acrolein, whereas P-450 3-mediated testosterone 7α-hydroxylase activity was relatively insensitive to acrolein inhibition at this concentration (Fig. 8). Accordingly, the transient induction by cyclophosphamide of P-450j protein but not its associated microsomal aniline hydroxylase activity (Fig. 7) may reflect a susceptibility of this

P-450 to inactivation by the cyclophosphamide metabolite protein.

DISCUSSION

Administration of cyclophosphamide to rats results in a partial loss of hepatic microsomal P-450 hemoprotein and activity (7–9). The present study establishes that cyclophosphamide exerts a suppressive action on P-450 expression that is specific towards certain P-450 forms, including one (P-450 2c) that is an important contributor to microsomal cyclophosphamide 4-hydroxylation, a critical step in the activation of this anti-cancer drug. The effects of cyclophosphamide on the constitutively expressed P-450 proteins examined in this study can be summarized as follows.

Cyclophosphamide Suppresses Male-specific Hepatic P-450 Forms. These enzymes (P-450s 2c, 2a, and RLM2) constitute about 50% of the total P-450 content in adult male rat liver (18, 24), and their decrease following cyclophosphamide treatment is sufficient to account for most, if not all, of the decrease in total P-450 protein observed by earlier investigators. The depletion of P-450 protein by cyclophosphamide has been previously ascribed to denaturation of the P-450 by binding of the cyclophosphamide metabolite acrolein (20). However, the decline in P-450 2c mRNA levels demonstrated in the present study indicates that cyclophosphamide acts at a pretranslational level to suppress the expression of this protein. Moreover, the slow onset in the decline in individual P-450 levels, and the persistence of low enzyme levels for more than a week, imply that cyclophosphamide and its metabolites do not simply alkylate P-450 protein molecules present in the liver at the time of drug treatment.

Loss of male-specific P-450s has been reported following administration of other drugs and xenobiotics (e.g., 12, 21, 25). In an earlier study from this laboratory, the anti-cancer drug cisplatin was shown to suppress these P-450 proteins by depletion of testosterone (12), which is required for full expression of the male-specific P-450s (24, 26). The effect of cisplatin on these P-450s could be reversed by the administration of androgen, or by hCG stimulation of endogenous testosterone production in the drug-treated rats (12). In contrast, the case of the cyclophosphamide-treated rats, gonadotropin elevated serum testosterone levels but did not restore the male-specific P-450s (Fig. 6). Rather, further suppression of P-450 2c mRNA, as well as some decrease in P-450 PB1 mRNA, occurred in response to gonadotropin (Fig. 4). Thus, cyclophosphamide must perturb one or more regulatory factors, in addition to serum testosterone, that are necessary for the full expression of these proteins. A similar conclusion was reached with respect to the observed decrease in P-450 2c protein and mRNA levels following treatment of rats with 3,4,5,3',4',5'-hexachlorobiphenyl (27). Possible regulatory factors that might mediate the effect of cyclophosphamide on these P-450s include hormone secretory patterns, which contribute to the regulation of these cytochromes (18, 28, 29), alterations in the levels or activities of proteins that mediate testosterone action, or perhaps the stimulation of P-450 mRNA or protein degradation.

Cyclophosphamide Does Not Decrease, but Rather Can Elevate P-450 Forms that are Expressed Constitutively in Rats of Both Sexes. Cyclophosphamide did not decrease hepatic levels of the gender-independent P-450 PB-1 during the initial 6 days following drug administration, but, rather, elevated this P-450

5 The abbreviation used is: hCG, human chorionic gonadotropin.
to a small extent 9 days after treatment. P-450 PB-1 and P-450 2c are the major contributors to cyclophosphamide 4-hydroxylase activity in untreated adult male rat liver (6). Since P-450 2c is severely suppressed by cyclophosphamide administration, P-450 PB-1 is probably the primary cyclophosphamide 4-hydroxylase in cyclophosphamide-treated rats.

P-450 3 and steroid 5α-reductase, which are expressed at higher levels in adult female as compared with adult male liver microsomes, were both elevated severalfold 9 days after cyclophosphamide administration. The elevation of either one (30) or both these enzymes (12) by agents that suppress male-specific P-450 proteins has been observed previously. In the case of steroid 5α-reductase, the increase was reversed by hCG, indicating that the elevation of this enzyme in cyclophosphamide-treated rats is probably due to the commensurate loss of serum testosterone, which is suppressive toward expression of this enzyme (31).

P-450j protein levels were elevated 3 days following cyclophosphamide treatment, but unlike P-450 3, P-450j subsequently declined to normal or below-normal levels. In male rats, P-450j can be elevated by mechanisms involving testosterone derepression in a manner similar to that of P-450 3 (31). Thus, the initial elevation of P-450j protein may be mechanistically similar to the accompanying elevation of P-450 3. Although P-450j protein levels were initially elevated, aniline hydroxylase activity, to which P-450j is a major contributor (22), did not increase in response to drug treatment. The possibility that the apparent discrepancy between P-450j protein and P-450j activity levels 3 days after drug treatment might result from binding of the cyclophosphamide metabolite acrolein is supported by the in vitro demonstration that acrolein can significantly inhibit P-450j-mediated microsomal aniline hydroxylase activity. In contrast, P-450 3-mediated microsomal testosterone 7α-hydroxylase activity was much less sensitive to acrolein, highlighting the selectivity of the acrolein inactivation for P-450j. In carrying out these in vitro analyses, it was necessary to add exogenous NADPH cytochrome P-450 reductase, since the endogenous P-450 reductase, which is obligatory for all P-450-dependent microsomal hydroxylation reactions, was also inactivated during the initial incubation with acrolein (data not shown), in agreement with earlier reports (32). These results suggest that acrolein may exhibit some selective binding to P-450j resulting in its activation.

Hepatic microsomal P-450 reductase was partially decreased following cyclophosphamide treatment, in agreement with an earlier study (32). However, this decrease did not contribute significantly to the loss of P-450 2c-dependent microsomal activities, as judged by the inability of exogenous P-450 reductase to reverse the cyclophosphamide-induced decrease in microsomal P-450 2c activity. Under the same conditions of in vitro supplementation, exogenous P-450 reductase effectively reverses the large decrease in the activity of P-450 2c and other microsomal P-450s that occurs following depletion of hepatic P-450 reductase in hypophysectomized rats (33). The mechanism for cyclophosphamide-induced loss of P-450 reductase activity is not known with certainty, but has been proposed to involve acrolein denaturation of this flavoenzyme (34). This would indicate that in vivo treatment with cyclophosphamide can generate acrolein at a cellular concentration that is sufficiently high to inactivate hepatic enzymes, perhaps including P-450j, as discussed above.

In conclusion, cyclophosphamide administration can elicit multiple effects on hepatic P-450 enzymes resulting in their suppression, induction, or inactivation. In the case of some of these enzymes (e.g., P-450j), the response to cyclophosphamide may involve a direct interaction with the cyclophosphamide-derived metabolite acrolein. In the case of several other hepatic enzymes (e.g., P-450 2c, P-450 3, steroid 5α-reductase), however, the overall pattern of responses is similar to that observed following treatment with cisplatin (12). This suggests a common mode of effect of these 2 anti-cancer drugs on hepatic P-450 expression, namely, drug-induced perturbation in the hormonal regulation of these hepatic enzymes. Administration of acrolein to rats is known to elevate hepatic alkaline phosphatase and tyrosine-α-ketoglutarate transaminase activity, apparently by modulating pituitary-adrenal factors important for the regulation of these enzymes (34). Thus, although acrolein might indeed be the metabolite responsible for the observed effects of cyclophosphamide on P-450 profiles, a primary site for its action is likely to be the hypothalamic-pituitary-gonadal axis rather than direct alkylation of the hepatic P-450 proteins. Finally, the loss of P-450 2c following cyclophosphamide treatment suggests that cyclophosphamide may inhibit its own rate of activation in male rats. The significance of this inhibition with regard to drug efficacy is not clear insofar as P-450-mediated metabolism of this drug is generally not rate-limiting with regard to drug activation (35). However, if P-450-mediated metabolism is sufficiently suppressed, reduced drug efficacy can result (13). These results also suggest mechanisms by which cyclophosphamide may function synergistically with other anti-cancer drugs that are subject to P-450-mediated activation or inactivation. Further studies to elucidate the roles played by individual P-450 enzymes in the metabolism of other anticancer drugs, and the effects of these drugs on P-450 enzyme activity, may provide a rational basis for improvements in drug combinations designed to maximize therapeutic effect.

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