Effects of 5-Fluorouracil on Drug-metabolizing Enzymes in the Rat

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SUMMARY

The effect of pretreatment of rats with 5-fluorouracil (5-FU) on hepatic drug-metabolizing enzyme activities was examined. Male rats, weighing 165 g, were given a single i.p. injection of 5-FU, 120 mg/kg, and 7 days later the drug-metabolizing enzyme activities of a 10,000 x g supernatant fraction from liver homogenates were examined. Aniline hydroxylase, ethylmorphine N-demethylase, and 2-(methylthio)benzothiazole S-demethylase activities were depressed 41, 40, and 25%, respectively, while the protein content of the 10,000 x g supernatant fraction was unchanged. On Day 7, pentobarbital sleeping time was increased 80% compared to controls. On Day 7, control and 5-FU-treated rats weighed 212 and 165 g, respectively, while on Day 14 when they weighed 249 and 195 g, respectively, there were no longer differences in enzyme activities. Seven days after two successive daily i.p. injections of 5-FU, 60 mg/kg, aniline hydroxylase and ethylmorphine N-demethylase activities were depressed while S-(methylthio)benzothiazole S-demethylase was unchanged. Activation in vitro of cyclophosphamide to alkylating metabolites by 10,000 x g supernatant fraction of liver homogenates from rats 7 days after pretreatment with 5-FU, 120 mg/kg, was decreased 25% compared to controls. Decreases occurred in body and liver weights 7 and 14 days after 5-FU pretreatment. However, there was no apparent correlation between these decreases and the changes in enzyme activities. The depression of hepatic drug-metabolizing enzyme activities after pretreatment with 5-FU may be due to the direct action of the drug on the synthesis and/or turnover of these enzymes, delayed growth, or a combination of these effects.

INTRODUCTION

Relatively little is known of the effects of anticancer drugs on the action, metabolism, or toxicity of other drugs used concomitantly in cancer patients (20, 23). Decreased activities of hepatic microsomal drug-metabolizing enzymes have recently been reported in rats treated with 5-FU (5). Cyclophosphamide, procarbazine, mechlorethamine, and various steroids are metabolized by hepatic microsomal enzymes (1). Some of these drugs are used in combination with 5-FU (2, 5, 6, 12). This report describes decreases in several hepatic drug-metabolizing enzyme activities following pretreatment of rats with 5-FU. This study may be relevant to the clinical situation, since cancer patients are usually treated with a variety of carcinostatic and other drug combinations (4, 20). A preliminary report of our investigation has already appeared (16).

MATERIALS AND METHODS

Animals and Tissue Preparation. Male rats, weighing 165 ± 3.0 g (obtained from Sprague-Dawley Laboratories, Madison, Wis.), were given i.p. injections of 5-FU dissolved in 1% Na2CO3 (w/v); control animals received an equivalent volume of 1% Na2CO3. In all experiments animals had free access to a standard Purina laboratory chow diet and tap water. All animals were sacrificed between 9 and 10 a.m.

Animals were decapitated, and the livers were removed, rinsed in several volumes of cold 0.9% NaCl solution, weighed, placed in 4 volumes of ice-cold 154 mM KCl-10 mM phosphate buffer, pH 7.4, and homogenized at 4° with a Potter homogenizer fitted with a motor-driven Teflon pestle. The homogenates were centrifuged at 10,000 x g for 20 min at 4° in a Sorvall refrigerated centrifuge. A microsomal pellet was obtained by centrifuging an aliquot of the 10,000 x g supernatant fraction in a Spinco Model L ultracentrifuge (rotor No. 50) at 105,000 x g for 60 min. Protein determinations were performed according to Lowry et al. (17) with bovine serum albumin as standard.

Incubation Mixtures. All incubations were carried out in duplicate, in open 25-ml Erlenmeyer flasks. The flasks were equilibrated for 10 min at 37° on a Dubnoff shaking water bath, and reactions were initiated by the addition of an aliquot (0.5 or 1.0 ml, as indicated) of 10,000 x g supernatant fraction of liver homogenates. Blank determinations lacking substrates were run with the assay mixtures and results were calculated after subtraction of the blanks. Under the conditions of each assay, enzyme activities were zero-order with respect to concentration of substrate, and product formation was directly proportional to incubation time and protein concentration.

Incubation Mixture for Ethylmorphine N-Demethylation and 2-(Methylthio)benzothiazole S-Demethylation. The reaction mixture contained NADP (1 μmole), glucose 6-phosphate (12 μmoles), magnesium chloride (12.5 μmoles),
semicarbazide hydrochloride (30 μmoles), Na+/K+ phosphate buffer, pH 7.4 (1 mmole), 0.5 ml of the 10,000 × g supernatant fraction (equivalent to 200 mg, wet weight, of liver), and the substrate ethylmorphine hydrochloride (10 μmoles) or 2-(methylthio)benzothiazole (20 μmoles) to a final volume of 3.0 ml. The 2-(methylthio)benzothiazole was added after it had been dissolved in 0.05 ml ethanol. With ethylmorphine and 2-(methylthio)benzothiazole as substrates, the incubations proceeded for 15 and 30 min, respectively. The reactions were terminated by the addition of 1.0 ml 20% ZnSO₄·7H₂O (w/v) followed by 1.0 ml saturated Ba(OH)₂·8H₂O. After chilling and centrifugation, an aliquot of the supernatant was assayed for formaldehyde formed, with the use of the Nash reagent, as previously described (19), with formaldehyde as standard.

Incubation Mixture for Aniline Hydroxylase. The reaction mixture contained NADP (0.5 μmole), glucose 6-phosphate (10 μmoles), MgCl₂ (25 μmoles), Na+/K+ phosphate buffer, pH 7.4 (200 μmoles), 1.0 ml of the 10,000 × g supernatant fraction (equivalent to 400 mg, wet weight, of liver), and the substrate aniline hydrochloride (10 μmoles) to a final volume of 4.0 ml. The reaction proceeded for 20 min and was terminated by the addition of 2.0 ml 20% trichloroacetic acid (w/v). After chilling and centrifugation, an aliquot was assayed spectrophotometrically for p-aminophenol formation, as previously described (19), with p-aminophenol as standard.

Incubation Mixture for Cyclophosphamide Activation. The reaction mixture contained NADP (0.5 μmole), glucose 6-phosphate (10 μmoles), MgCl₂ (25 μmoles), Na+/K+ phosphate buffer, pH 7.4 (200 μmoles), 1.0 ml of the 10,000 × g supernatant fraction (equivalent to 400 mg, wet weight, of liver), and the substrate cyclophosphamide (72 μmoles) to a final volume of 4.0 ml. The reaction proceeded for 10 min and was terminated by the addition of 2.0 ml 5.5% ZnSO₄·7H₂O (w/v) followed by 2.0 ml 4.5% Ba(OH)₂ (w/v). The mixture was centrifuged, an aliquot of the supernatant was extracted twice with methylene chloride to remove unchanged cyclophosphamide, and alkylating metabolites of cyclophosphamide were determined colorimetrically by reaction with 4-(p-nitrobenzyl)pyridine as previously described (21). Cyclophosphamide activation was expressed as equivalents of acid-hydrolyzed cyclophosphamide (1 × HCl for 30 min in boiling water) reacting with 4-(p-nitrobenzyl)pyridine.

Chemicals. Cyclophosphamide and 5-FU were gifts from Meade Johnson & Co., Evansville, Ind., and Hoffman-LaRoche, Nutley, N. J., respectively. Glucose 6-phosphate (disodium salt) and NADP (sodium salt) were obtained from Sigma Chemical Co., St. Louis, Mo. Ethylmorphine hydrochloride was obtained from Merck Chemical Division, Rahway, N. J. Aniline hydrochloride was obtained from Mallinckrodt Chemical Works, New York, N. Y., and 2-(methylthio)benzothiazole and 4-(p-nitrobenzyl)pyridine were obtained from Eastman Kodak Co., Rochester, N. Y.

RESULTS

Male Sprague-Dawley rats, weighing 165 ± 3.0 g, were given a single i.p. injection of 5-FU, 120 mg/kg, and 7 days later, drug-metabolizing enzyme activities of the 10,000 × g supernatant fraction from liver homogenates were examined. Aniline hydroxylase, ethylmorphine N-demethylase, and 2-(methylthio)benzothiazole S-demethylase activities per g liver were depressed 41, 40, and 25%, respectively, compared to control animals. After 14 days there no longer were differences in hepatic drug-metabolizing enzyme activities (Table 1).

In addition to measuring drug-metabolizing enzyme activities, other biological parameters were followed in an attempt to characterize the basis for the alterations by 5-FU on enzyme activities. Parameters that were monitored included body weight, liver weight, and protein contents of 10,000 × g supernatant fraction and the 105,000 × g microsomal pellet from liver homogenates.

Protein content of liver 10,000 × g supernatant fraction was unchanged on both Days 7 and 14 (Table 2), which indicated that the decreased enzyme activities on Day 7 were not the result of a marked decrease in total liver protein. In contrast, microsomal protein content was depressed 10% on Day 7 but had returned to normal on Day 14 (Table 2). However, the relationship of the small decrease in microsomal protein content on Day 7 to the marked decreases in enzyme activities in 5-FU-treated rats is unclear.

Following 5-FU treatment, the rats showed some anorexia for 2 to 4 days, which was accompanied by a loss in body weight averaging 30 g. Food intake then increased and growth resumed, which accounts for the observation that by Day 7 the rats had returned to their initial body weight, 160 g, while control rats weighed 212 g (Table 2). Although by Day 14 there were still differences in body weight between control (249 g) and drug-treated rats (195 g) (Table 2), enzyme activities were similar (Table 1). Therefore, differences in enzyme activities were not due solely to differences in body weights of the animals.

Decreases in liver weights were observed 7 days after either a single i.p. injection of 5-FU, 120 mg/kg, or 2 successive daily injections of 5-FU, 60 mg/kg. These changes could not account for the decreased enzyme activities, since these activities had returned to normal 14 days after a single i.p. injection of 5-FU, 120 mg/kg, although differences in liver weights still persisted. Upon gross examination, whole livers and liver slices from 5-FU-treated rats appeared normal with no indication of necrosis.

Because the activities of hepatic drug-metabolizing enzymes were depressed 7 days after the administration of a single i.p. injection of 5-FU, 120 mg/kg, pentobarbital sleeping time was measured on Day 7. As shown in Table 3, on Day 7 after 5-FU there was an 80% increase in pentobarbital sleeping time.

Since 7 days after a single i.p. injection of 5-FU, 120 mg/kg, the activities of hepatic drug-metabolizing enzymes were depressed, while pentobarbital sleeping time was increased, the effect of 2 successive daily i.p. injections of 5-FU at 60 mg/kg on enzyme activities in vitro was examined. As can be seen (Table 1), 7 days after 2 successive daily i.p. injections of 5-FU, 60 mg/kg, the activities of ethylmorphine N-demethylase and aniline hydroxylase were...
Table 1

Ethylmorphine N-demethylase, aniline hydroxylase, and 2-(methylthio)benzothiazole S-demethylase activities of rat 10,000 × g supernatant fraction from liver homogenates 7 and 14 days after a single i.p. injection of 5-FU (120 mg/kg) and 7 days after 2 successive daily injections of 5-FU (60 mg/kg).

Standard conditions of assay were used. Values represent the mean ± S.E. of 7 and 5 rats/group for Days 7 and 14, respectively, after a single injection of 5-FU (120 mg/kg) and of 5 rats/group 7 days after 2 successive daily injections of 5-FU (60 mg/kg). When results are statistically different, the numbers in parentheses represent percentage of control, which is taken as 100.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Day 7 Control</th>
<th>5-FU (120 mg/kg)</th>
<th>Day 14 Control</th>
<th>5-FU (60 mg/kg)</th>
<th>Day 7 5-FU (60 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylmorphine N-demethylase (μmoles formaldehyde/g liver/15 min)</td>
<td>5.6 ± 0.25 (100)</td>
<td>3.4 ± 0.50* (60)</td>
<td>6.4 ± 0.50</td>
<td>5.2 ± 0.72</td>
<td>6.4 ± 0.46 (100)</td>
</tr>
<tr>
<td>Aniline hydroxylase (μmoles p-amino phenol/g liver/20 min)</td>
<td>0.25 ± 0.023 (100)</td>
<td>0.15 ± 0.022* (59)</td>
<td>0.34 ± 0.024</td>
<td>0.32 ± 0.024</td>
<td>0.32 ± 0.014 (100)</td>
</tr>
<tr>
<td>2-(methylthio)benzothiazole S-demethylase (μmoles formaldehyde/g liver/30 min)</td>
<td>2.6 ± 0.09 (100)</td>
<td>1.9 ± 0.09* (75)</td>
<td>2.6 ± 0.13</td>
<td>2.4 ± 0.14</td>
<td>2.9 ± 0.25</td>
</tr>
</tbody>
</table>

*p < 0.005 compared to control value.
**p < 0.01 compared to control value.
***p < 0.001 compared to control value.

Table 2

Body weight, liver weight, and protein contents of 10,000 × g supernatant fraction and 105,000 × g microsomal pellet from liver homogenates of rats 7 and 14 days after a single i.p. injection of 5-FU (120 mg/kg) and 7 days after 2 successive daily injections of 5-FU (60 mg/kg).

Values represent the mean ± S.E. of 7 and 5 rats/group for Days 7 and 14, respectively, after a single injection of 5-FU (120 mg/kg) and 5 rats/group 7 days after 2 successive daily injections of 5-FU (60 mg/kg). When results are statistically different, the numbers in parentheses represent percentage of controls, which is taken as 100.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Day 7 Control</th>
<th>5-FU (120 mg/kg)</th>
<th>Day 14 Control</th>
<th>5-FU (60 mg/kg)</th>
<th>Day 7 5-FU (60 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>212 ± 4</td>
<td>160 ± 4*</td>
<td>249 ± 9</td>
<td>195 ± 10*</td>
<td>214 ± 1</td>
</tr>
<tr>
<td>Liver wt (g)</td>
<td>9.3 ± 0.23 (100)</td>
<td>8.2 ± 0.11* (88)</td>
<td>9.5 ± 0.21 (100)</td>
<td>8.0 ± 0.34* (84)</td>
<td>9.0 ± 0.37 (100)</td>
</tr>
<tr>
<td>10,000 × g supernatant fraction protein (mg/g liver)</td>
<td>170 ± 4</td>
<td>160 ± 3</td>
<td>180 ± 5</td>
<td>180 ± 6</td>
<td>160 ± 3</td>
</tr>
<tr>
<td>10,000 × g supernatant fraction protein (mg/g liver)</td>
<td>48 ± 0.9 (100)</td>
<td>43 ± 1.1* (90)</td>
<td>48 ± 1.6</td>
<td>48 ± 2.4</td>
<td>44 ± 0.88</td>
</tr>
<tr>
<td>10,000 × g supernatant fraction protein (mg/g liver)</td>
<td>48 ± 0.9 (100)</td>
<td>43 ± 1.1* (90)</td>
<td>48 ± 1.6</td>
<td>48 ± 2.4</td>
<td>44 ± 0.88</td>
</tr>
</tbody>
</table>

*p < 0.001 compared to control value.
**p < 0.005 compared to control value.
***p < 0.01 compared to control value.
****p < 0.02 compared to control value.
*****p < 0.05 compared to control value.

almost one-half that of controls. Differences in 2-(methylthio)benzothiazole S-demethylase activities were not statistically significant although after 5-FU treatment this activity appeared to be depressed.

After 2 successive daily injections of 5-FU, 60 mg/kg, the animals showed some anorexia for 2 to 4 days which was accompanied by a loss in body weight. Food intake then increased and growth resumed so that by Day 7 drug-
The small decrease in liver microsomal protein 7 days after a single i.p. injection of 5-FU, 120 mg/kg, conversion in vitro was examined. As can be seen (Table 4), 7 days after a single i.p. injection of 5-FU, 120 mg/kg (Table 2). Therefore, the small decrease in liver microsomal protein 7 days after a single injection of 5-FU, 120 mg/kg (Table 2). Therefore, the small decrease in liver microsomal protein content after a single injection of 5-FU, 120 mg/kg, conversion of cyclophosphamide to active alkylating metabolites by the 10,000 x g supernatant fraction from liver homogenates was depressed 25%.

**DISCUSSION**

We have demonstrated that pretreatment of rats with 5-FU caused a reversible decrease in the activities of aniline hydroxylase, ethylmorphine N-demethylase, and S-(methylthio)benzothiazole S-demethylase as measured in the 10,000 x g supernatant fraction from liver homogenates. These results agree with those of Donnelli et al. (10), who measured aniline hydroxylase, aminopyrine N-demethylase, and p-nitroanisole O-demethylase activities after pretreatment of rats with 5-FU. In addition we found that the protein content of 10,000 x g supernatant fraction from liver homogenates was unchanged, which indicated that the decrease in enzyme activities was a selective effect rather than the result of a marked depression in total protein synthesis. In this regard it is of interest that among the biochemical consequences of 5-FU is an inhibition in the synthesis of specific proteins without apparent effects on total protein synthesis (18).

Decreases were noted in body and liver weights 7 and 14 days after 5-FU pretreatment, and liver microsomal protein content was slightly depressed 7 days after a single i.p. injection of 5-FU, 120 mg/kg. However, there was no apparent correlation between these parameters and changes in enzyme activities.

Pentobarbital sleeping time was increased when measured on Day 7 after pretreatment with 5-FU, 120 mg/kg. Our results are consistent with the report that pretreatment of rats with cyclophosphamide, which depressed the activities of hepatic drug-metabolizing enzymes in vitro (10), increased sleeping time, and plasma and brain half-lives of pentobarbital (9).

Our study showed that after 5-FU pretreatment the activation of cyclophosphamide in vitro to alkylating products was depressed. Various agents, including SKF-525A, phenobarbital, morphine, 3-methylcholanthrene, thioacetamide, and cobalt chloride, which alter rat and mouse hepatic microsomal enzyme activities, have been examined as to their effect on the therapeutic efficacy and toxicity of cyclophosphamide (13, 21). However, to our knowledge, comparable studies have not been done following pretreatment with anticancer drugs that are known to inhibit the activities of hepatic microsomal drug-metabolizing enzymes.

The therapeutic efficacy of cyclophosphamide appears to be unchanged following pretreatment with phenobarbital or SKF-525A (13, 21). However, SKF-525A, 2,4-dichloro-6-phenylphenoxyethyl-diethylamine, and chloramphenicol, all inhibitors of microsomal drug-metabolizing enzymes, decreased the lethality of cyclophosphamide to mice and rats (7, 13). As many as 8 metabolites of cyclophosphamide have been found (3). It is possible that pretreatment with agents such as 5-FU, which decrease the rate of activation of cyclophosphamide, may change the overall composition of active and inactive metabolites occurring in tumor and normal tissues. Since 5-FU and cyclophosphamide are used clinically as part of multiple drug regimens (2, 5, 6, 12), the implications of a possible depression by 5-FU in the rate of cyclophosphamide activation in regard to antitumor efficacy and toxicity of cyclophosphamide remain to be explored.

Decreased food intake can depress hepatic drug-metabolizing enzymes (8, 14, 15). Animals pretreated with 5-FU became anorexic and lost weight for 2 to 4 days, but then food intake increased and growth resumed. Therefore it is unclear whether the decrease in enzyme activities after 5-FU

### Table 3

**Pentobarbital sleeping time 7 days after a single i.p. injection of 5-FU (120 mg/kg)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sleeping time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>69 ± 2.9 (100)</td>
</tr>
<tr>
<td>5-FU</td>
<td>124 ± 12.3* (180)</td>
</tr>
</tbody>
</table>

*p < 0.001 compared to control value.

### Table 4

**Cyclophosphamide activation by 10,000 x g supernatant fraction from liver homogenates of rats 7 days after a single i.p. injection 5-FU (120 mg/kg)**

<table>
<thead>
<tr>
<th>Assay, cyclophosphamide activation (nmol alkylating product/g liver/10 min)</th>
<th>7-day control rats</th>
<th>5-FU-treated rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-day control rats</td>
<td>787 ± 7.5 (100)</td>
<td>592 ± 14.2* (75)</td>
</tr>
</tbody>
</table>

*p < 0.001 compared to control value.
is due to the action of 5-FU on synthesis and/or turnover of drug-metabolizing enzymes, delayed growth, a depression of these enzymes due to a drug-related anorexia, or a combination of these 3 effects. It would be of interest to attempt to dissociate the direct effect of 5-FU on enzyme activities from the possible indirect effects due to reduced food intake.

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