Depression of the Hepatic Cytochrome P-450 Monoxygenase System by Treatment of Mice with the Antineoplastic Agent 5-Azacytidine

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ABSTRACT

The effects of 5-azacytidine (5-AC) administration on the hepatic cytochrome P-450 systems of mice were studied. A single i.p. dose of 5-AC (25 mg/kg) to male Swiss-Webster mice caused about a 50% depression of microsomal cytochromes P-450 and \( \Delta_5 \) and of ethymophosphine N-demethylase and ethoxyxoumarin O-deethylase activities. Depression was greatest 24 h after treatment; by 48 to 72 h, cytochromes and drug metabolism had returned to near control values. Reduced nicotinamide adenine dinucleotide-phosphate-cytochrome c reductase activity was also depressed by 5-AC, whereas reduced nicotinamide adenine dinucleotide-cytochrome c reductase was not. Incubation of 5-AC with microsomes produced no effect on drug metabolism. The prolongation of hexobarbital sleeping time by 5-AC showed that drug metabolism is also impaired by 5-AC in vivo. These studies may have important clinical implications when certain drugs are coadministered with 5-AC.

INTRODUCTION

5-AC\(^3\) (4-amino-1-\( \beta \)-o-ribofuranosyl-1,3,5-triazin-2-one), an analogue of cytidine in which nitrogen replaces carbon at position 5 of the pyrimidine ring, was prepared in the early 1960s by Czechoslovakian workers during their search for new antitumor drugs (28). 5-AC is effective in the treatment of mouse (9, 33, 34, 39) and human (1, 4, 14, 18, 31, 41-43) leukemias. Toxic side effects of 5-AC include immunosuppression, teratogenicity, abortifacient activity, inhibition of liver regeneration, and disturbance of protein synthesis and digestive functions (6,40). Clinical studies may have important clinical implications when certain drugs are coadministered with 5-AC.

5-AC depress hepatic P-450 systems. Preliminary studies showed that the acute administration of 5-AC to mice depressed the hepatic P-450 system markedly without inducing interferon. This paper describes the effects of 5-AC on the levels of P-450 and cytochrome \( \Delta_5 \) and the activities of ethymorphine N-demethylase, 7-ethoxyxoumarin O-deethylase, and NADPH- and NADH-cytochrome c reductases in murine hepatic microsomes.

MATERIALS AND METHODS

Chemicals. 5-AC, glucose 6-phosphate, NADP, NADPH, and NADH were purchased from Sigma Chemical Co. (St. Louis, MO). Ethymophosphine-HCl, sodium hexobarbital, aniline-HCl, and glucose-6-phosphate dehydrogenase were obtained from Merck and Co. (Rahway, NJ), Winthrop Laboratories (New York, NY), Eastman Kodak Co. (Rochester, NY), and Boehringer Mannheim (Indianapolis, IN), respectively. 7-Ethoxyxoumarin and 7-hydroxycoumarin were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Animals. Male Swiss-Webster mice (22 to 27 g), obtained from Taconic Farms, Inc. (Germantown, NY), were used throughout the study unless otherwise indicated. They were fed a laboratory chow supplied by Ralston Purina Co. (St. Louis, MO) and given water ad libitum. Rats (180 to 200 g) of the Sprague Dawley strain were purchased from King Co. (Madison, WI) and maintained on rat chow (Ralston Purina Co.) and water ad libitum. Animals were maintained in a controlled diurnal lighting cycle (12 h on, 12 h off).

Saline (0.9% NaCl) solutions of 5-AC (2.5 mg/ml) were prepared just prior to i.p. injection.

Tissue Preparation. Animals were killed by exsanguination, and their livers were removed immediately. When rats were used, livers were perfused in situ with cold KCl (1.15%) prior to removal. Microsomes and other subcellular fractions were prepared as described previously (8) in KCl (1.15%) and used fresh. Micromolecular protein was determined by the method of Lowry et al. (20).

Spectral Analysis. P-450 and cytochrome \( \Delta_5 \) content of tissues were determined spectrophotometrically by the methods of Matsubara et al. (23) and Omura and Sato (26), respectively. All spectral measurements were made with an Amino DW2 spectrophotometer.

Enzyme Activities. The N-demethylation of ethymorphine (2 mw), O-demethylation of 7-ethoxyxoumarin (0.25 mw), and 4-hydroxylation of aniline (0.25 mw) were measured as described by Sladek and Mannering (32), Greenlee and Poland (12), and Imai et al. (15), respectively. NADPH-cytochrome c reductase was measured using the procedure of Williams and Kamin (44), but in the absence of KCN. NADH-cytochrome c reductase was measured in the same manner but with NADH (0.8 mw) substituted for NADPH (0.8 mw).

Measurement of Hexobarbital Sleeping Time. Mice were given sodium hexobarbital i.p. (100 mg/kg in 0.9% saline solution), and sleeping time was determined as described previously (35).

Measurement of Serum Interferon. Serum interferon activity was measured by Lee Biomolecular Research Laboratories, Inc. (San Diego, CA), using L-cells challenged with encephalomyocarditis virus (5).

Analysis of Data. Student's \( t \) test was used to determine significant differences from the null hypothesis (2).

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\(^2\) To whom requests for reprints should be addressed.

\(^3\) The abbreviations used are: 5-AC, 5-azacytidine; P-450, cytochrome P-450.

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RESULTS

Effects of 5-AC on Body Weight, Liver Weight, and Hepatic Microsomal Protein. Doses of 5-AC ranging from 5 to 50 mg/kg were injected i.p. into mice, and body weight, liver weight, and hepatic microsomal protein were determined 24 h later. Chart 1 shows that 5-AC depressed liver weight in a dose-related manner, whereas body weight was unaffected. This shifted the ratio of liver weight to body weight from 6.4 to 5.4% at both 25- and 50-mg/kg doses of 5-AC. Depression of hepatic microsomal protein (both per g liver and per total liver) was also observed at the 2 highest doses of 5-AC.

Effect of 5-AC on Cytochrome b5, P-450, and Drug Metabolism. A single dose of 5-AC (25 or 50 mg/kg) depressed cytochrome b5 and P-450, ethylmorphine N-demethylase, and 7-ethoxycoumarin O-deethylase 24 h after its administration (Chart 1). The finding that ethylmorphine N-demethylase was depressed more than P-450 suggests that certain P-450 isozymes may be affected more than others by 5-AC. When the effects of 5-AC on cytochrome b5, P-450, and ethylmorphine N-demethylation are calculated per g of liver, these depressions are more marked. On this basis, a dose of 25 mg 5-AC per kg depressed cytochrome b5, and P-450, and ethylmorphine N-demethylase 59, 60, and 64%, respectively. Since 25 and 50 mg 5-AC per kg depressed P-450 and drug metabolism almost equally, the smaller dose was selected for further studies.

Time Dependence of 5-AC-mediated Effects. Mice were given injections i.p. of 25 mg 5-AC per kg, killed, and examined at various intervals thereafter for alterations in cytochrome b5, P-450, and drug metabolism (Chart 2). By 9 h after treatment, all of these components were depressed; depression was maximal 24 h after 5-AC administration, with cytochrome b5, P-450, ethylmorphine N-demethylase, and 7-ethoxycoumarin O-deethylase 66, 56, 48, and 53% of control values, respectively. Values returned to near control levels within 48 to 72 h.

Effects of 5-AC on Microsomal Electron-Transfer Components. The microsomal P-450-mediated monooxygenase system is comprised of multiple P-450 isozymes and NADPH-P-450 (c) reductase. The oxidation of certain xenobiotics may also involve NADH-cytochrome b5 (c) reductase and cytochrome b5 (21). 5-AC depressed cytochrome b5, P-450, and NADPH-P-450 (c) reductase activity, but not that of NADH-cytochrome b5 (c) reductase, thus supporting the view that the effect of 5-AC on enzyme systems is selective (Table 1). It is of interest that, although both reductases are flavoproteins, only one is depressed by 5-AC.

Chart 1. Effect of various doses of 5-AC on mice. Control values: body weight, 27.4 ± 1.1 g; liver weight, 1.8 ± 0.1 g; microsomal protein, 15.5 ± 0.5 mg/g liver; cytochrome b5, 0.29 ± 0.05 nmol/mg microsomal protein; P-450, 0.89 ± 0.17 nmol/mg microsomal protein; ethylmorphine N-demethylase, 10.97 ± 2.08 nmol HCHO formed per mg microsomal protein per min. Animals were given single doses of 5-AC (i.p.) and killed 24 h later. Points, mean; bars, SE (n = 3). * Significantly different from control values (P < 0.05).

Chart 2. Loss and recovery of microsomal cytochrome b5 and P-450 and drug-metabolizing activities in mice after a single i.p. dose of 5-AC (25 mg/kg). Mice were killed at various times after the administration of 5-AC. Control values (0 time): cytochrome b5, 0.29 ± 0.02 nmol/mg microsomal protein; P-450, 0.77 ± 0.05 nmol/mg microsomal protein; ethylmorphine N-demethylase, 10.95 ± 1.31 nmol HCHO formed per mg microsomal protein per min; 7-ethoxycoumarin O-deethylase, 1.40 ± 0.14 nmol 7-hydroxycoumarin formed per mg microsomal protein per min. Points, mean; bars, SE (n = 4). * Significantly different from control values (P < 0.05).

Table 1

<table>
<thead>
<tr>
<th>Component</th>
<th>Control</th>
<th>5-AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome b5 (nmol/mg protein)</td>
<td>0.27 ± 0.02a</td>
<td>0.22 ± 0.01b</td>
</tr>
<tr>
<td>P-450 (nmol/mg protein)</td>
<td>0.65 ± 0.04</td>
<td>0.65 ± 0.05b</td>
</tr>
<tr>
<td>NADPH-cytochrome c reductase (nmol cytochrome c reduced/min/mg protein)</td>
<td>201.7 ± 18.7</td>
<td>133.8 ± 7.7b</td>
</tr>
<tr>
<td>NADH-cytochrome c reductase (nmol cytochrome c reduced/min/mg protein)</td>
<td>588.9 ± 28.4</td>
<td>566.8 ± 82.1</td>
</tr>
</tbody>
</table>

a Mean ± SE (n = 6).

b Significantly different from control (P < 0.05).
Effect of 5-AC on in Vitro Drug Metabolism. The possibility was considered that 5-AC may be exerting its depressive effects through its activation by the P-450 system or some other microsomal system. To test this possibility, microsomal suspensions were preincubated with 5-AC in the presence of an NADPH-regenerating system before the addition of the drug substrates. Preincubation with 5-AC for 3 or 30 min did not lower the rate of ethylmorphine N-demethylation or the hydroxylation of aniline (data not shown).

Effect of 5-AC on the Subcellular Distribution of P-450, Cytochrome b₅, and Drug Metabolism. The possibility was considered that 5-AC may cause changes in hepatocyte membrane composition and alter sedimentation characteristics, thereby altering the recovery of microsomal components. Chart 3 shows that treatment of mice with 5-AC reduced cytochrome b₅, P-450, and ethylmorphine N-demethylase activity in total hepatic homogenate and the subcellular fractions to approximately the same extents. Thus, the depression of microsomal components by 5-AC is not a result of altered sedimentation characteristics.

Effects of 5-AC in Rats. Cytochrome b₅, P-450, ethylmorphine N-demethylase, and 7-ethoxycoumarin O-deethylase were depressed in hepatic microsomes 24 h after the i.p. administration of 25 mg of 5-AC per kg to rats (Table 2). The magnitudes of these depressions were similar to those observed in mice.

Effect of 5-AC on Hexobarbital Sleeping Time of Mice. The duration of sleep induced by hexobarbital is an indirect measure of the in vivo metabolism of hexobarbital by the hepatic P-450 system (35). Groups of 8 mice were given injections i.p. of saline or 25 mg of 5-AC per kg. Sodium hexobarbital (100 mg/kg) was administered 12 or 24 h later. Mice that received saline and hexobarbital 24 h later slept 36 ± 3 (SE) min. Mice that received 5-AC and hexobarbital 12 or 24 h later slept 78 ± 16 and 90 ± 14, respectively. The sleeping times of the 2 groups of mice that received 5-AC are significantly different (P < 0.05) than the group that received saline.

Effect of 5-AC on the Induction of Serum Interferon. Mice were given injections i.p. of saline or 25 mg of 5-AC per kg. Three and 12 h later, serum was collected and pooled from 3 animals/treatment/time point. None of the samples had detectable interferon activity. These results suggest that the depression of hepatic P-450 systems is not due to the induction of interferon. Some caution must be exercised in making this conclusion, because the interferon may accumulate in some tissues without appearing in the serum (13).

**DISCUSSION**

A single dose of 5-AC caused depression of microsomal drug metabolism which became maximal about 24 h after treatment. This altered metabolic activity was shown to be due to decreased levels of microsomal monooxygenase components, notably cytochromes P-450 and b₅ and the flavoprotein, NADPH-cytochrome c (P-450) reductase. However, not all components associated with the monooxygenase system are affected; e.g., NADH-cytochrome c (P-450) reductase activity was not depressed. The depression of drug metabolism was greater than the depression of P-450; this might suggest that certain P-450 isozymes are affected by 5-AC more than others, although the possibility should be considered that the loss of NADPH-P-450 reductase activity may have contributed as much to the depression of monooxygenase activity as the loss of P-450.

Most drugs are biotransformed by hepatic P-450 systems to

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**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Microsomal protein (mg/g liver)</th>
<th>Cytochrome b₅ (nmol/mg microsomal protein)</th>
<th>Ethylmorphine N-demethylase (nmol/HCHO formed/mg microsomal protein/min)</th>
<th>7-Ethoxycoumarin O-deethylase (nmol/mg microsomal protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10.6 ± 0.5²</td>
<td>0.80 ± 0.02</td>
<td>1.40 ± 0.04</td>
<td>12.14 ± 0.37</td>
</tr>
<tr>
<td>5-AC</td>
<td>8.2 ± 0.5²</td>
<td>0.51 ± 0.03³</td>
<td>0.98 ± 0.05</td>
<td>7.43 ± 0.74³</td>
</tr>
</tbody>
</table>

² Mean ± SE (n = 3).
³ Significantly different from saline (P < 0.05).
products that are less potent and more excretable than their antecedents; in fact, the duration of activity of most drugs is determined by the activity of the P-450 system. In the current studies, 5-AC depressed drug metabolism in mice by about 50%, a level that doubled the sleeping time induced by hexobarbital. 5-AC has been used therapeutically in doses ranging from 50 to 750 mg/kg/day (equivalent to approximately 1 to 15 mg/kg/day) (16, 43); thus, the doses used in the current animal studies compare favorably with those used in humans. Should a similar depressant effect of 5-AC occur in humans, the depression of drug metabolism could be an important consideration in clinical situations where this drug is used as an antineoplastic agent (1, 4, 14, 18, 31, 41-43), particularly in patients with impaired hepatic function (3, 43). Treatment of neoplastic disease frequently involves the coadministration of 2 or more drugs. Since it is not likely that the metabolism of all of these drugs will be affected equally by 5-AC, it may be necessary to know to what extent the metabolism of each is depressed by 5-AC if increased drug toxicity is to be avoided.

REFERENCES


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