Hepatic Function Assessed (in Rats) during Chemotherapy with Some Anti-Cancer Drugs

I. D. Capel, M. Jenner, H. M. Dorrell, and D. C. Williams

Using rats, we studied how best to assess hepatic damage after administering therapeutic doses of each of five anti-cancer drugs or of the hepatotoxin, carbon tetrachloride. As indexes, we compared measurement of the concentration of administered antipyrine in plasma with measurement in serum of α-fetoprotein or of the activities of five enzymes that reportedly best reflect hepatic damage. The biological half-life of antipyrine in the plasma was increased more than threefold on pretreating the rats with any of the five cytotoxic drugs or with carbon tetrachloride. In contrast, the concentrations of α-fetoprotein, alkaline phosphatase, γ-glutamyltransferase, or glutamate dehydrogenase were not consistently increased. Of the enzymes tested in serum, aspartate aminotransferase and ornithine carbamoyltransferase best indicated hepatic impairment resulting from the treatment with anti-cancer drugs. Our results imply that determination of the pharmacokinetics of marker drugs such as antipyrine better indicates hepatic dysfunction induced by cytotoxic agents than does measurement of the enzymes liberated into serum as a result of damage to liver mitochondria.

Additional Keyphrases: liver disease • hepatic function assessment • cancer • enzymes as indicators of liver damage • hepatic damage induced by CC14

The hepatotoxic effects of various drugs, including anticancer agents, have been reviewed recently (1). Optimum prescribing of cytotoxic and other drugs to patients undergoing anti-cancer therapy requires the assessment of decrease in hepatic function (2). Measurement of certain enzymes in serum has been recommended in specific hepatic disorders; e.g., a mitochondrial enzyme, glutamate dehydrogenase (EC 1.4.1.3) is said to be a more specific indicator of alcohol-induced liver damage than is the widely applicable γ-glutamyltransferase (EC 2.3.2.2) (3). It has recently been recommended that a battery of tests—comprising alkaline phosphatase (EC 3.1.3.1), γ-glutamyltransferase, and aspartate aminotransferase (EC 2.6.1.1)—should always be used whenever hepatic dysfunction is suspected (4).

In a previous experiment (5) we demonstrated that the concentration in the plasma of the drug antipyrine, which demonstrably depends on the rate of hepatic hydroxylation (6), is increased by pretreatment with anti-cancer drugs. In the present paper we compare the rate at which antipyrine is cleared from plasma with serum enzyme determination as indexes to cytotoxic-drug-induced hepatic impairment. Carbon tetrachloride, which provides a good experimental model of hepatic cirrhosis (7), was administered, to compare its hepatotoxicity with the toxicities of therapeutic doses of some cytotoxic drugs used in the treatment of cancer.

Materials and Methods

Reagents

Kits for radioimmunoassay of α-fetoprotein were obtained from the Radiochemical Centre, Amersham, Bucks., U.K. Antipyrine, actinomycin D, 5-fluorouracil, and kits for assay of the serum enzymes (see below) were all supplied by Sigma Chemical Co., Poole, Dorset, U.K. Vincristine sulfate (Onconvin) was obtained from Eli Lilly & Co., Basingstoke, Hants., U.K.; cyclophosphamide (monohydrate) from Koch-Light, Colnbrook, Bucks., U.K.; and sodium methotrexate from American Cyanamid Corp., Pearl River, NY. All other chemicals and reagents were of the purest grade available and were purchased from Fisons, Loughborough, Leics., U.K.

Animals and Treatment

Male Sprague-Dawley rats weighing 300–400 g were given either an oral dose of methotrexate (0.5 mg/animal) or cyclophosphamide (4 mg/kg body wt.) in physiological saline (2 mL/kg) once daily for five successive days, or an intravenous injection of vincristine (0.03 mg/kg) or actinomycin D (0.1 mg/kg) in saline (1 mL/kg) once weekly for three successive weeks, or a single intramuscular injection of 5-fluorouracil (12.5 mg/kg) in saline (2 mL/kg) once daily for five successive days.

Positive-control animals received an intraperitoneal injection of carbon tetrachloride (2 mL/kg) dissolved in arachis oil. Two days after the end of these treatments, the animals received an oral dose of antipyrine (18 mg/kg) in isotonic saline (2 mL/kg). Blood, a total of 10 mL, was sampled by cardiac puncture from groups of three rats before and 0.50, 1, 2, 4, and 8 h after dosing. The plasma antipyrine concentration was determined by the technique of Mendelsohn and Levin (8). The biological half-life (t1/2) of the drug in the plasma was determined by linear regression analysis.

Other animals, representative of the various treatment groups but which had not received antipyrine, were killed by exsanguination via the heart, and some enzymes that reportedly reflect hepatic damage were determined in the serum.

Enzyme Assays

α-Fetoprotein in serum was determined by radioimmunoassay (9). Glutamate dehydrogenase was determined as described by Ellis and Goldberg (10) and γ-glutamyltransferase, alkaline phosphatase, ornithine carbamoyltransferase, and aspartate aminotransferase by use of the kits and methods provided by Sigma Chemical Co. (11).

Radiochemical Analyses

Duplicate radiochemical assays were performed by counting

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Table 1. Antipyrene Concentrations in Plasma of Pretreated and Control Rats at Intervals after Its Oral Administration (18 mg/kg)

<table>
<thead>
<tr>
<th>Pre-treatment with</th>
<th>Antipyrene concentration * at time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>8.6 ± 0.8</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>10.5 ± 0.5</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>14.7 ± 1.0</td>
</tr>
<tr>
<td>Vincristine</td>
<td>10.1 ± 0.7</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>21.7 ± 0.5</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>13.0 ± 0.3</td>
</tr>
<tr>
<td>Saline (control)</td>
<td>15.5 ± 0.6</td>
</tr>
</tbody>
</table>

* Milligrams of apparent antipyrene per litre of plasma, values quoted are the mean ± SEM for duplicate analyses of plasma of three animals at each time interval.

Table 2. Effect on Hepatic Enzyme Markers in Serum of Pretreatment with Cytotoxic Drugs

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Antipyrene t1/2 (h)</th>
<th>FP</th>
<th>GT</th>
<th>AP</th>
<th>OCT</th>
<th>AST</th>
<th>GD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>10.5</td>
<td>1.0 ± 1.0</td>
<td>2.2 ± 0.3</td>
<td>56.0 ± 8.0</td>
<td>250 ± 73</td>
<td>83.3 ± 15.2</td>
<td>9.7 ± 4.3</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>&gt;30</td>
<td>7.4 ± 3.0</td>
<td>2.2 ± 0.8</td>
<td>26.7 ± 7.3</td>
<td>127 ± 45</td>
<td>51.6 ± 12.5</td>
<td>7.7 ± 1.4</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>14.8</td>
<td>4.9 ± 4.3</td>
<td>1.7 ± 0.1</td>
<td>48.3 ± 17.6</td>
<td>893 ± 150</td>
<td>65.8 ± 11.2</td>
<td>9.9 ± 5.5</td>
</tr>
<tr>
<td>Vincristine</td>
<td>14.2</td>
<td>8.3 ± 2.5</td>
<td>2.0 ± 0.4</td>
<td>29.2 ± 4.4</td>
<td>350 ± 52</td>
<td>78.3 ± 1.4</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>16.3</td>
<td>9.2 ± 5.0</td>
<td>1.6 ± 0.1</td>
<td>21.2 ± 5.7</td>
<td>260 ± 42</td>
<td>111.6 ± 49.1</td>
<td>11.8 ± 2.7</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>&gt;30</td>
<td>n.d.</td>
<td>3.6 ± 0.1</td>
<td>49.2 ± 6.3</td>
<td>10,725 ± 2,085</td>
<td>208.3 ± 115.4</td>
<td>257.3 ± 153.4</td>
</tr>
<tr>
<td>Saline (control)</td>
<td>3.1</td>
<td>3.8 ± 2.7</td>
<td>1.7 ± 0.1</td>
<td>39.8 ± 11.2</td>
<td>70 ± 14</td>
<td>49.1 ± 7.2</td>
<td>9.0 ± 4.2</td>
</tr>
</tbody>
</table>

Serum enzyme values are mean ± SD for duplicate assays on three animals per group. Abbreviations: FP, α-fetoprotein, in µg/L of serum; GT, γ-glutamyltransferase, in Sigma units/mL of serum; AP, alkaline phosphatase, in n mole product formed/min per mL of serum; OCT, ornithine carbamoyltransferase, in Sigma units/mL of serum; AST, aspartate aminotransferase, in Sigma–Frankel units/mL of serum; GD, glutamate dehydrogenase, in IU/L of serum; and n.d., not detected.

Measurement of α-fetoprotein is a valuable indicator of hepatocarcinoma and of tissue regrowth in patients after partial hepatectomy (13). It also is increased after chemical damage of the type induced by carbon tetrachloride (14). Our results, however, confirm other reports (13) that after severe liver injury induced by high doses of carbon tetrachloride there is no increase in α-fetoprotein concentration. 5-Fluorouracil administration after partial hepatectomy completely inhibits cell division, and hence regeneration (15). Similarly, inhibition of protein synthesis by the cytostatic agents probably accounts for our observation of a lack of an appreciable increase in serum α-fetoprotein during their use.

All the serum enzymes we investigated have been recommended as indicators of various forms of hepatic dysfunction; indeed, γ-glutamyltransferase, reportedly more sensitive than aspartate aminotransferase, is widely estimated for the detection of minor alterations in liver function induced by drugs or disease (16). It has recently been suggested (13) that in cases of alcoholic liver disease the enzymes of the mitochondrial membranes are not affected, whereas glutamate dehydrogenase, characteristic of the inner-mitochondrial matrix, is increased in serum. Presumably the anti-cancer drugs administered in this experiment do not impair liver function by an effect on the mitochondria, because most of the mitochon- dria-derived serum enzymes were increased after carbon tetrachloride treatment only, but not by the cytotoxic drugs. Of the serum enzymes comprising the recommended “battery of hepatic function tests” (4)–γ-glutamyltransferase, aspartate aminotransferase, and alkaline phosphatase—none was sensitive enough to detect the damage resulting from treatment with anticancer drugs.

Measurement of ornithine carbamoyltransferase has recently been claimed to be as sensitive as histology for the detection of chemically induced liver damage (17). Of the enzymes we studied, it was the most responsive to the effects of for two cycles in a Model 2650 liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, IL 60515).

**Results**

Table 1 gives the concentration of antipyrene in plasma. Table 2 compares the plasma t1/2 for the drug with values for the enzymes in serum in animals treated with cytotoxic drugs or chemically damaged. The elimination of antipyrene from the plasma was delayed by pretreatment with cytotoxic drugs, resulting in increased t1/2 values. This increase in t1/2 was most marked in the rats treated with 5-fluorouracil, being similar (increased 10-fold) to that of the carbon tetrachloride-treated animals.

The activities of ornithine carbamoyltransferase and aspartate aminotransferase in serum were extremely sensitive indicators of the chemical damage induced by carbon tetrachloride; these two enzymes were also the most increased by most (though not all) of the cytotoxic drugs.

We could see no consistent differences in the relative toxicities of the various anti-cancer drugs as judged from a comparison of the values for enzymes released into the serum in response to their administration.

**Discussion**

In a recent review, Bond (2) emphasizes the dangers of prescribing anti-cancer drugs to patients with impaired liver function but overlooks the hepatotoxicity of these drugs themselves. Although in some reports methotrexate is demonstrably hepatotoxic only when administered in the dosage used for treating psoriasis (12), Zimmerman (1) has recommended periodic liver biopsy during on-going therapy of cancer with methotrexate. Clearly, detection of the potential hepatotoxic effects of the anti-cancer drugs, although crucial to the therapy, is being neglected largely owing to a lack of a sufficiently sensitive test of liver function.
the cytotoxic drugs and carbon tetrachloride-induced damage.

Hepatic clearance tests involving the use of dyes or drugs as marker compounds are becoming more popular for estimating hepatic function (18). The hitherto popularly used sulfobromophthalein is potentially dangerous, and results with both this compound and indocyanine green depend greatly on the rate of hepatic blood flow (19). The concentration of antipyrine in plasma provides a reliable indication of remaining functional cell mass in other forms of hepatic impairment (20). In our comparison, antipyrine $t_{1/2}$ values not only most sensitively indicate toxicity but could provide an estimate of the relative impairment resulting from different treatments. If these results are verified in man, this, combined with the fact that highly sensitive assays permitting salivary analysis are available, strongly recommends determination of antipyrine clearance as the best means of evaluating hepatic impairment during treatments with cytotoxic drugs.

References