Nitrogen Metabolism and Lipid Peroxidation during Hyperthermic Perfusion of Human Livers with Cancer1

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ABSTRACT

Isolation-perfusion was used as a means of heating human livers with cancer. Perfusion was at 42–42.5°C for 4 h. Perfusate constituents were analyzed in an attempt to identify factors contributing to the hepatotoxic effects of hyperthermia. During perfusion the perfusate constituents analyzed were: urea; total amino acids; uric acid; malonaldehyde; and lysosomal enzymes. Hepatic ammonia for urea synthesis is derived from degradation of amino acids, amines, and nucleic acids. An increase in proteolysis was reflected in the increase in urea from 0.6 ± 0.2 μM to 1.9 ± 8 μM and total amino acids from 1.0 ± 0.6 μM to 4.4 ± 1.7 μM during the 4 h of perfusion at 42–42.5°C. An increase in purine catabolism occurred as evidenced by an increase in uric acid from 1.7 ± 1.0 mg/100 ml to 6.1 ± 2.7 mg/100 ml. Free oxygen radicals, which can lead to lipid peroxidation, are generated by the action of xanthine oxidase on xanthine. Lipid peroxidation occurring during perfusion was assessed by an increase in malonaldehyde from 2.3 ± 1.3 μM to 10.4 ± 10.0 μM. An increase in acid phosphatase in the perfusate from 38 ± 15 units/liter to 78 ± 45 units/liter occurred, suggesting labilization of lysosomes, perhaps through lipid peroxidation. Proteolysis and lipid peroxidation are suggested to be two interrelated factors contributing to heat toxicity in the perfused human liver with cancer.

INTRODUCTION

Hyperthermia, 42–43°C, was shown to be an effective tumoricidal agent (1–5). Hyperthermia, 42°C, can be induced in the liver by isolation-perfusion (6, 7). This therapy was used to treat patients with cancer metastatic to the liver (8). Results indicate that hyperthermia was tumoricidal for metastatic colon cancer, but there remained an identifiable rim of tumor cells at the periphery of metastatic tumor nodules. However, there was marked variation in the hepatotoxic effects of heat on the liver. This varied from mild elevations in serum enzymes to hepatic necrosis in one patient which appeared directly attributable to the thermotherapy.

Heat-induced hepatotoxicity varies with the method(s) or condition(s) under which the liver is heated and is manifest through changes in hepatic functional integrity (9). The functional integrity of the isolated perfused liver can be assessed by analysis of perfusate constituents. Several investigators studied the effects of heat on liver tissue using the isolated perfused liver and described changes with temperature and time on bile flow and glucose, lactate, and fatty acid metabolism (6, 7, 9–16). The liver remained functional in a range of 42–43°C.

Perfusate constituents were analyzed during hyperthermic perfusion of human livers with cancer in an attempt to identify factors contributing to the hepatotoxic effects of heat. Data from these studies suggested that nitrogen metabolism and lipid peroxidation appeared to be two such factors. The potential contribution of these factors to heat-induced hepatotoxicity is the subject of this paper.

RESULTS

The levels of the perfusate constituents urea, total amino acids, uric acid, acid phosphatase, and malonaldehyde were analyzed and identified as factors related to the hepatotoxic effects of heat on the human liver. There was an increase in perfusate urea, phosphate, creatinine, total amino acids, uric acid, acid phosphatase, and malonaldehyde. Analysis of variance indicated that these changes were significant. Calcium decreased significantly during perfusion.

Urea concentrations increased with time throughout the perfusion. The changes were significant (P < 0.05) after 30 min in comparison to the concentration at the start of perfusion. After 60 min, the further increases in urea concentrations were not significantly different from the 60-min value. Total amino acids increased significantly in the perfusate during perfusion. At 30 min, the changes were significant (P < 0.05)
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Fig. 1. Time course of changes in perfusate levels of urea and total amino acids (\(\text{\(\alpha\)}-\text{AA}\)) during hyperthermic liver perfusion. Points, pooled data from six perfusions; bars, SE.

Fig. 2. Time course of changes in perfusate uric acid and MDA during hyperthermic liver perfusion. Points, pooled data from six perfusions; bars, SE.

Fig. 3. Time course of changes in perfusate lysosomal enzymes during hyperthermic liver perfusion. Points, pooled data from six perfusions; bars, SE.

Table 1 Effect of hyperthermic perfusion on perfusate levels of creatinine, calcium, and phosphorus

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Creatinine (mg/100 ml)</th>
<th>Calcium (meq/liter)</th>
<th>Phosphorus (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.47 ± 0.11*</td>
<td>3.91 ± 0.86</td>
<td>5.69 ± 1.58</td>
</tr>
<tr>
<td>5</td>
<td>0.58 ± 0.09</td>
<td>3.56 ± 0.68</td>
<td>5.74 ± 1.24</td>
</tr>
<tr>
<td>30</td>
<td>0.91 ± 0.25</td>
<td>3.27 ± 0.53</td>
<td>6.64 ± 2.45</td>
</tr>
<tr>
<td>60</td>
<td>1.18 ± 0.27</td>
<td>3.38 ± 0.44</td>
<td>6.69 ± 1.42</td>
</tr>
<tr>
<td>120</td>
<td>1.31 ± 0.28</td>
<td>3.41 ± 0.30</td>
<td>6.82 ± 1.67</td>
</tr>
<tr>
<td>180</td>
<td>1.41 ± 0.34</td>
<td>3.35 ± 0.32</td>
<td>5.81 ± 2.48</td>
</tr>
<tr>
<td>240</td>
<td>1.45 ± 0.38</td>
<td>3.35 ± 0.34</td>
<td>5.67 ± 2.97</td>
</tr>
</tbody>
</table>

* Mean ± SD from perfusion of six human livers with cancer at 42–42.5°C.

DISCUSSION

Hyperthermia caused hepatic injury similar to that seen after exposure to hepatotoxic chemicals (9). Hepatocellular injury was manifested by elevations in serum enzymes, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, and lactate dehydrogenase. Pathological changes consisted of a centrolobular lesion varying from fine granular degeneration to necrosis, dilatation of portal veins, and congestion of centrolobular sinusoids (9). The underlying mechanism(s) for heat-induced hepatotoxicity remains speculative. Lipid peroxidation and proteolysis occurred during hyperthermic perfusion of human livers with cancer. The results cannot be compared to perfusion at 37°C since no normothermic, nontherapeutic perfusions were carried out. Some comparisons can be made to data obtained from rat and dog liver perfusion experiments.

Perfusate urea increased with time during these perfusions (Fig. 1). Hepatic ammonia is derived for urea synthesis from degradation of amino acids, amines, and nucleic acids. Urea increased with time during perfusion of rat and dog livers, but it failed to show a significant difference between 37, 42, and 43°C (7, 9, 16). Rates of ureogenesis from endogenous substrates were unchanged or increased with temperatures up to 42°C in the perfused rat liver (11, 12, 23).

The increase in total amino acids in the perfusate during perfusion at 42.5°C indicated that there was ongoing proteolysis during perfusion at that temperature (Fig. 1). Amino acids were released into the perfusate during perfusion of rat livers until a constant level was established in the perfusion medium (24,
The accumulation of amino nitrogen or endogenous protein degradation was inhibited by amino acids with time of perfusion of the rat or dog liver at 37°C when a mixture of amino acids was added to the perfusate (6, 7, 9, 16). There was a significant (P < 0.05) increase in total amino acids with time and temperature during perfusion of the dog liver at 42 and 43°C (7, 16). Thus, proteolysis appeared to be increased during hyperthermic perfusion.

Another source of urea nitrogen could have been from the degradation of nucleic acids and purine nucleotides. Evidence for this degradation was the increase in uric acid during perfusion (Fig. 2). One origin of the purines for uric acid synthesis could come from the tumoricidal effects occurring during hyperthermic perfusion. A second source would be from adenine nucleotides which were not resynthesized to ATP. The synthesis of 1 mol of urea requires 4 ATPs. Heat treatment of isolated mouse liver mitochondria caused uncoupling of oxidative phosphorylation in a temperature-dependent fashion which becomes significant between 41 and 45°C (26). Futile cycling occurred during hyperthermic perfusion of rat livers with cancer (27). During these perfusions total phosphorus increased in the perfusate (Table 1). This also may be related to consumption of high energy intermediates.

An increased uric acid synthesis resulted from the increased purines. Uric acid synthesis required xanthine oxidase. XO acting on xanthine was the presumed source of superoxide which led to the appearance of the lipid peroxidation product MDA. MDA increased during the perfusion (Fig. 2). The xanthine oxidase activity of rat and human liver was reported to be predominantly a dehydrogenase (type D) which could be readily converted into an oxidase (type O) by proteolytic enzymes (28, 29). In order for XO to be the source of superoxide, it must be converted to the type O form at hyperthermic temperatures. In vitro, rat liver XO increased to greater than 50% O form at cytotoxic temperatures (30).

One lysosomal enzyme, p-nitrophenyl phosphatase, increased during the perfusions (Fig. 3). This could indicate labilization of the lysosomes which would be a source of proteolytic enzymes for conversion of xanthine oxidase type D to type O and enhanced proteolysis. There was no increase in β-glucuronidase activity in the perfusate. This enzyme is a glycoprotein which is cleared by the liver (31, 32). Since the enzyme level in the perfusate appeared to remain at a constant level, it could be inferred that there was a continued release of the enzyme into the perfusate during perfusion (Fig. 3). Prior studies showed that there was no inhibition of receptor-mediated endocytosis of glycoprotein by the perfused rat liver at hyperthermic temperatures (33).

However, lysosomal labilization could be the result of lipid peroxidation (34–37). Exposure of lysosomes to systems which generate superoxide, including xanthine oxidase, caused an increase in free nonsedimentable lysosomal enzymes (35–37).

Another effect which would be related to lipid peroxidation was the decrease in perfusate calcium which occurred during perfusion (Table 1). This could be referred to as calcium influx, a final event in cytotoxicity (38).

In summary, these data suggest a hypothesis to explain the hepatotoxic effects of hyperthermia. Hyperthermia causes tumor cell death along with ATP catabolism. This produces an increase in hypoxanthine and production of uric acid through the action of xanthine oxidase. Superoxide production, from the action of xanthine oxidase, leads to lipid peroxidation of cellular membranes. Labilization of the lysosomes and calcium influx follow. Further proof of this hypothesis is needed.
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