Altered Cobalamin Distribution in Rat Hepatomas and in the Livers of Rats Treated with Diethylnitrosamine

John C. Linnell, Edward V. Quadros, David M. Matthews, Harold P. Morris, and Lionel A. Poirier

Department of Experimental Chemical Pathology, The Vincent Square Laboratories of Westminster Hospital, London, SW1V, 2RH, United Kingdom [J. C. L., E. V. Q., D. M. M.]; Department of Biochemistry, Howard University, Washington, D. C. 20059 [H. P. M.]; and Nutrition and Metabolism Section, National Cancer Institute, Bethesda, Maryland 20014 [L. A. P.]

SUMMARY

The distribution of cobalamin cofactors was investigated in the livers and tumors of rats bearing transplanted Morris 7777 or 7800 hepatomas, in the livers of rats treated with the hepatocarcinogen diethylnitrosamine, and in normal rats. There was a significant increase in the proportion of methylcobalamin both in livers and tumors from rats bearing the hepatomas 7777 and 7800 compared to the proportion of methylcobalamin in the livers of normal rats. The total cobalamin content of the hepatomas was significantly lower than that of host or control livers. Similarly, the total cobalamin content of the livers from the tumor-bearing rats was less than that in control animals.

The administration to rats of an acute dose of diethylnitrosamine led to an 84% increase in the hepatic concentration of methylcobalamin. Chronic administration of diethylnitrosamine slightly increased the hepatic methylcobalamin concentration, but this was not statistically significant. Liver weight was reduced, and the hepatic content of total cobalamin fell to 55% of that in control animals.

INTRODUCTION

Cobalamin has been implicated in the formation and growth of tumors (30). The activities of several chemical carcinogens have been reported to be increased in animals fed high dietary levels of cobalamin (5, 9, 22, 25, 28, 29). Furthermore, Rous sarcomas grow faster in chicks supplemented with cobalamin than in animals not so treated (26). Abnormalities in the metabolism of cobalamin have been seen during tumor formation and development. High levels of total cobalamin have been reported in the plasma of rats bearing a transplantable leukemia (2) and in the urine of leukemic patients (27). In patients with chronic myeloid leukemia the serum total cobalamin level is abnormally high and is associated with increases in the serum transcobalamin (21, 37, 38). The total cobalamin content of rat liver tumors is low (23, 39). Similarly, the chronic administration of the hepatocarcinogens, N,N-dimethyl-4-aminoazobenzene and of 3''-methyl-N,N-dimethyl-4-aminoazobenzene to rats led to decreased plasma and liver levels of total cobalamin (6, 24). Indirect evidence indicates that Me-Cbl* is a cofactor whose metabolism may be deranged in developing tumor systems. The high dependence upon exogenous methionine in certain tumor lines (1, 12, 14) and the methyl-reversible folate deficiency seen in rats bearing the Novikoff hepatoma (32) point to a metabolic abnormality in the transfer of methyl groups from the folate pool to form methionine in tumors. Similarly, the methyl-reversible folate deficiency and the decreased levels of S-adenosylmethionine, of the enzyme N5-methyltetrahydrofolate:homocysteine methyltransferase, and of folate polyglutamates in the livers of rats treated with DENA (3, 33, 36) point to a metabolic abnormality in the activity of methyltransferase enzyme in the livers of carcinogen-treated rats. Me-Cbl appears to be the cofactor essential to this reaction (7). We have therefore investigated the distribution of cobalamin cofactors in transplanted hepatomas and in the livers of rats treated with hepatocarcinogen DENA.

MATERIALS AND METHODS

Animals, Tumors, and Diets. Morris 7777 and 7800 hepatomas were inoculated i.m. into female Buffalo rats at Howard University and were sent to the National Cancer Institute 11 days later. The animals were then housed 5 to 6 together in hanging stainless steel wire mesh cages. Food (Wayne Lab-Blox) and water were available ad libitum. When the tumors had reached 2 to 3 cm in diameter (32 to 39 days after transplantation), the rats were weighed and killed by decapitation. Uninoculated tumor-free female Buffalo rats of the same age and treated the same as the tumor-bearing animals were also killed at the same time to be used as controls. After exsanguination livers and tumors were immediately excised. The livers were weighed and immediately frozen on a dry-ice bath. The tumors were placed on ice, and the grossly viable neoplastic tissue of each tumor was isolated from the necrotic material. The collected tumor samples were then also placed on the dry-ice bath. When the individual frozen livers and tumor samples were then wrapped in aluminum foil and shipped in dry ice to Westminster Hospital. Animals were sacrificed, and the tissues were prepared under red light to minimize possible photo-decomposition of the cobalamin cofactors.

1 Supported by a grant from the Wellcome Trust and by USPHS Grant CA 10729.
2 To whom requests for reprints should be addressed.
3 Recipient of a Commonwealth Tropical Medicine Award from the Ministry of Overseas Development.
4 The abbreviations used are: Me-Cbl, methylcobalamin; DENA, diethylnitrosamine.
DENAF Administration. The effects of acute and chronic administration of DENA on cobalamin distribution in the livers of rats were also investigated. Male NIH Sprague-Dawley rats (175 to 225 g) were used in the experiments. They were housed 5 to 6 together in hanging stainless steel wire mesh cages. In both experiments food (Wayne Lab-Blox) and water were available ad libitum. In the chronic studies DENA (100 ppm in the drinking water) was administered to rats for 3 weeks; similar control animals received no carcinogen.

Fifty-eight hr before sacrifice, the DENA-treated animals were given drinking water without carcinogen. The animals were weighed and killed by decapitation, and their livers were prepared as described above. In the acute studies the rats were fed Wayne Lab-Blox for 2 weeks before treatment with DENA. Each rat then received an i.p. injection of 25 mg of DENA in 2 ml of corn oil. Rats similarly treated with 2 ml of corn oil containing no carcinogen were used as controls. Previous studies have shown that signs of folate deficiency after the administration of a single dose of DENA are at a maximum 3 days after injection (31). Thus, 72 hr after injection with DENA, the animals were sacrificed, and their livers were removed as described above and stored at or below -20°C until analyzed for cobalamins.

Estimation of Total and Individual Cobalamins. Samples (0.5 to 1.0 g) were cut from the center of each piece of tissue by the light of a red photographic lamp, weighed, wrapped in a tared square of aluminum foil, and homogenized in distilled water (10 ml) with a Potter-Elvehjem homogenizer. Total cobalamin was estimated by a radioisotopic method (20) extracting the liver homogenates with a cyanide-containing acetate buffer (16). Further aliquots were extracted with hot ethanol and then were desalted and concentrated. Cobalamins were separated by 1- or 2-dimensional chromatography and located bioautographically by over-layering the chromatogram with an agar medium containing Escherichia coli mutant and a tetrazolium growth indicator. The zones were quantitated by photometric scanning and comparison with standards (17-19). Reference compounds were kindly supplied by Glaxo Laboratories, Greenford, Middlesex, England.

RESULTS

Cobalamins in Rats Bearing Transplantable Hepatomas. In rats implanted with Morris 7777 or 7800 hepatomas, terminal body and liver weights were significantly lower than in control animals (Table 1). In each group of tumor-bearing rats total cobalamin concentrations were significantly higher in the livers than in tumors from the same animals (p < 0.02). In rats bearing the Morris 7777 hepatoma, liver total cobalamin concentrations were significantly lower than those in the control animals (Table 2). The

Table 1

Mean liver and body weights of rats bearing hepatomas or treated with DENA

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Duration of experiment (days)</th>
<th>Body wt (g)</th>
<th>Liver wt (g)</th>
<th>Liver wt Body wt x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>23*</td>
<td>211 ± 4*</td>
<td>7.0 ± 0.2</td>
<td>3.3 ± 0.1</td>
</tr>
<tr>
<td>Morris 7800</td>
<td>4</td>
<td>23</td>
<td>171 ± 12*</td>
<td>5.4 ± 0.6*</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Morris 7777</td>
<td>5</td>
<td>30</td>
<td>177 ± 6°</td>
<td>5.7 ± 0.2°</td>
<td>3.2 ± 0.2°</td>
</tr>
<tr>
<td>Control acute</td>
<td>5</td>
<td>3</td>
<td>361 ± 8</td>
<td>12.7 ± 1.1</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>DENA acute</td>
<td>5</td>
<td>3</td>
<td>305 ± 10°</td>
<td>10.8 ± 0.5</td>
<td>3.6 ± 0.1°</td>
</tr>
<tr>
<td>Control chronic</td>
<td>10</td>
<td>21</td>
<td>342 ± 13</td>
<td>13.5 ± 0.5</td>
<td>3.9 ± 0.1°</td>
</tr>
<tr>
<td>DENA chronic</td>
<td>9</td>
<td>21</td>
<td>350 ± 17</td>
<td>8.9 ± 0.4°</td>
<td>2.6 ± 0.1°</td>
</tr>
</tbody>
</table>

J. C. Linnell et al.
proportions of Me-Cbl in livers and tumors from both transplanted groups were each significantly higher than the proportions of Me-Cbl in the livers of control animals. Highest proportions of Me-Cbl were found in tumors from the Morris 7777-implanted animals, where there was an almost 15-fold difference from the controls. As a concentration, Me-Cbl was likewise significantly increased in both liver and tumor tissue from animals bearing the Morris 7777 hepatoma, but in the Morris 7800-implanted group only the livers contained significantly higher concentrations of Me-Cbl than the livers of the control animals.

Effects of DENA Administration. Acute administration of DENA had no apparent effect on liver weight of the animals, but their mean body weight was significantly lower than that of the controls (Table 1). Chronic administration of much smaller amounts of DENA for 21 days reduced the liver weight but had no effect on the body weight.

Acute administration of DENA led to a mean increase of 84% in the liver concentration of Me-Cbl by comparison with that in control animals, without significantly affecting adenosylcobalamin, hydroxycobalamin, or total cobalamin concentrations (Table 3). In the chronic experiment, the proportion of Me-Cbl in the liver was slightly but not significantly increased above that in the control animals. The whole-liver content of total cobalamin in the DENA-treated rats was reduced to almost half that in control animals (p < 0.001) (Table 4), probably due to the reduction in liver weight in the DENA-treated rats (Table 1).

DISCUSSION

The raised levels of Me-Cbl found in the livers of rats treated with the hepatocarcinogen DENA and the increased proportion of Me-Cbl in hepatomas 7777 and 7800 constitute the main findings of the present investigation. Tissue cobalamin concentrations vary considerably in rats of different age, sex, and strain, as the present and previous results (35) show. In any comparative study it is thus important to use appropriate control animals. Contamination of tissue samples with blood is unlikely to have contributed significantly to the tissue Me-Cbl since concentrations of this and other cobalamins are much higher in most organs than in blood. In healthy male Wistar rats for example, liver Me-Cbl concentrations were some 20 times higher than in erythrocytes and more than 200 times higher than in plasma (35).

Me-Cbl has a coenzyme function in the transfer of methyl groups from the folate pool to form methionine; this coenzyme may play a key role in the regulation of folate metabolism and in biological methylations. Previous indirect evidence suggests that the transfer of methyl groups from the folate pool is deficient in tumor cells in vitro (1, 13, 14), in rats bearing the Novikoff hepatoma (32), and in rats treated with DENA (33, 36). However, Grossman et al. (11) have shown that the apoenzyme levels of the methyltransferase responsible for this transfer are relatively normal in the livers and tumors of hepatoma-bearing rats. The present studies show that the levels of the coenzyme Me-Cbl are ample in both tumors and host livers. The 2 sets of observations are difficult to reconcile.

Although a role for Me-Cbl in the regulation of growth has not been established, relatively high levels of Me-Cbl have previously been reported in human fetal plasma (4) and in fetal tissues (15), which are consistent with the increased methyltransferase activity observed in fetal liver and brain (8). The increased Me-Cbl recently found in a case of pri-

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Liver cobalamins 72 hr after acute DENA administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cyanocobalamin was detected in any sample.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total cobalmins</td>
</tr>
<tr>
<td></td>
<td>Whole liver</td>
</tr>
<tr>
<td></td>
<td>(ng)</td>
</tr>
<tr>
<td>Control</td>
<td>1198 ± 95</td>
</tr>
<tr>
<td>DENA</td>
<td>1207 ± 151</td>
</tr>
</tbody>
</table>

• Mean ± S.E.
• Significantly higher than control (p < 0.001).

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Liver cobalamins after chronic DENA administration for 3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cyanocobalamin was detected in any sample.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total cobalmins</td>
</tr>
<tr>
<td></td>
<td>Whole liver</td>
</tr>
<tr>
<td></td>
<td>(ng)</td>
</tr>
<tr>
<td>Control</td>
<td>1023 ± 160</td>
</tr>
<tr>
<td>DENA</td>
<td>563 ± 64</td>
</tr>
</tbody>
</table>

• Mean ± S.E.
• Significantly lower than control (p < 0.02).
CANCER RESEARCH VOL. 37

12. Halpern, B. C., Clark, B. R., Hardy, D. N., Halpern, R. M., and Smith, R. A. N5-Methyltetrahydrofolate (34). Together with our present findings, these observations suggest that the folate cycle is involved in the regulation of Me-Cbl synthesis, and apart from acting as a cofactor in the methylenetetrahydrofolate reductase system, Me-Cbl may either directly or indirectly be involved in nuclear acid synthesis and cell proliferation.

REFERENCES


Altered Cobalamin Distribution in Rat Hepatomas and in the Livers of Rats Treated with Diethylnitrosamine


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/37/9/2975