Induction of Hepatic Neoplastic Lesions in Mice with a Single Dose of Hycanthone Methanesulfonate after Partial Hepatectomy

Hiroyuki Tsuda, D. S. R. Sarma, S. Rajalakshmi, J. Zubroff, Emmanuel Farber, Robert P. Batzinger, Young-Nam Cha, and Ernest Bueding

Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania 19140 (D. S. R. S., S. P., J. Z., E. F.); and Department of Pathobiology, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore, Maryland 21205 [R. P. B., Y-N. C., E. B.]

ABSTRACT

Experiments were designed to determine whether hycanthone methanesulfonate (1-[(2-diethylamino)ethyl]amino)-4-(hydroxymethyl)thioxanthen-9-one monomethanesulfonate), an antischistosomal drug, and its analog, IA-4-N-oxide (8-chloro-2-[2-diethylamino)ethyl]-2H[1]benzothiopyran[4,3,2-cd]indazole 5-methanol monomethanesulfonate), will induce neoplastic lesions in the livers of mice not infected with Schistosoma mansoni. All the mice received a single i.m. injection of hycanthone methanesulfonate (76 mg/kg), IA-4-N-oxide (80 mg/kg), or an equivalent volume of the solvent, 0.9% NaCl solution, 42 hr after partial hepatectomy. Of the mice receiving hycanthone methanesulfonate and living 200 days or longer, hepatocellular carcinoma was seen in 11.5% and liver sarcoma was seen in 4.2%. This type of malignant neoplasm was not seen in the animals receiving either IA-4-N-oxide or 0.9% NaCl solution. In addition, mice receiving hycanthone methanesulfonate showed a significantly higher incidence of both type 1 (43% compared to 21% in controls) and type 2 (21% compared to 12% in controls) hepatocyte neoplasms. Mice receiving IA-4-N-oxide showed no increased incidence of neoplasms.

INTRODUCTION

Administration to mice infected with Schistosoma mansoni of a single dose of HMS1 (Chart 1), a widely used antischistosomal drug (18, 20, 23, 24), induced a significant increase in hepatic neoplasms (13, 14). No hepatomas were observed when the same dose of HMS was given to mice not infected with the parasite (13, 14). Since several known carcinogens have been shown to induce liver neoplasms in mice or rats when administered as a single dose after PH but not after a sham operation (see Ref. 10), it became of interest to observe whether HMS might be hepatocarcinogenic if given to mice during liver regeneration.

The results presented in this communication indicate that a single dose of HMS given 42 hr after PH induces a significant increase in hepatic neoplasms when compared to control mice or to mice receiving a single dose of a HMS analog, IA-4-N-oxide.

MATERIALS AND METHODS

Five- to 6-week-old, randomly bred male mice (Swiss-Webster CF-1, from Charles River Laboratories, Inc., Wilmington, Mass.) were used in this study. They had free access to food and water and were used after 1 week of acclimatization. For most of the duration of the experiment, the mice were housed singly. HMS was a gift from Dr. A. E. Farah, Sterling-Winthrop. IA-4-N-oxide was prepared by the method described by Hulbert et al. (18) from IA-4, kindly provided by Dr. Frederick De Serres of the National Institute of Environmental Health Services. [methyl-3H]Thymidine (specific activity, 20 Ci/mmol) was obtained from New England Nuclear, Boston, Mass.

Mice were anesthetized using pentobarbital sodium, and 67% PH was performed by a procedure similar to that described by Higgins and Anderson (17) for rats. After surgery, the animals were given 5% glucose in water instead of plain water for 2 days. To determine the peak of the S-phase, mice were given i.p. injections of [methyl-3H]thymidine (5 Ci/g body weight) at different time periods between 5 and 54 hr after PH. One hr after the injection, the animals were killed, liver DNA was isolated (22), and the specific activity was determined. DNA was measured by the procedure described by Burton (5). For the carcinogenic studies, all the mice were subjected to PH and 42 hr later were divided into 3 groups. One group received i.m. HMS (76 mg per kg) equivalent to 60 mg of hycanthone per kg body weight, a second group received an equimolar i.m. dose of IA-4-N-oxide (80 mg per kg), and a third group received an equivalent volume of 0.9% NaCl solution.

The mice were weighed periodically and observed carefully. They were either sacrificed when moribund or allowed to die naturally. Approximately 2- to 3-mm-thick sections were taken from all liver lobes, in addition to any grossly abnormal areas, and fixed in 10% (0.075 M phosphate buffer, pH 7.4) formalin, then dehydrated in a graded series of ethanol, embedded in paraffin, and stained with hematoxylin-eosin. Other organs were also checked carefully for any gross abnormalities.

RESULTS

Initial experiments were carried out to determine the peak of S phase of the liver cell following PH. Under our experimental conditions, the peak of DNA synthesis occurred 42 hr after PH.

Since the first neoplasm (type 1) was seen in an animal that died at 205 days after the start of the experiment, only animals that survived longer than 200 days were used to compile the data. The results presented in Table 1 indicate that the mean survival time of mice in the HMS group is not significantly different from that of the control group.
Classification of the Lesions. The neoplastic changes observed in the present experiment were classified histopathologically into 3 types: (a) hepatic nodules; (b) hepatocellular carcinomas; and (c) sarcomas. Hepatic nodules were further divided into 2 types, type 1 and type 2.

Type 1 nodules (Figs. 1 and 2), occurring either as single or multiple lesions, were composed of proliferating parenchymal cells, ranging in size from microscopic to a massive growth involving a complete liver lobe. The lesions consisted mainly of closely packed single liver cell plates with little difference from normal lobular structure. Usually, there was a slight compression of surrounding nonnodular areas. Occasionally, some of the type 1 nodules showed a slightly increased basophilia in both cytoplasm and nucleus. Mitotic activity was rare. Type 2 nodules (Figs. 3 and 4) usually arose within type 1; the size varied from microscopic to 1 to 2 cm in diameter. Histologically, convoluted cell plates were 2 or more cells thick, and the normal lobular pattern was lost with an absence of the obvious central vein (terminal hepatic vein) and portal triads. In some of the type 2 nodules, the vascular spaces were wide and distinct.

Type 2 nodules had a tendency to show increased basophilia in both cytoplasm and nucleus. Mitotic activity was slightly elevated. These lesions showed expansive growth and compressed the surrounding areas, but there was no clear-cut evidence of invasion. Borders between type 1 and 2 nodules were not always well defined, and in some regions an apparent transition between the two was seen.

Hepatocellular carcinomas (Figs. 5 and 6), trabecular or papillary in structure, were well defined and irregular in arrangement. The trabeculae were multiple-cell-thick structures, with wide blood spaces. Some of these lesions exhibited direct invasion into the surrounding liver tissue or remote metastases to the lung (Fig. 7). Usually, the tumor cells showed cytological atypia.

Lesions were classified as sarcomas when they consisted of packed proliferation of spindle-shaped mesenchymal cells (Fig. 8).

When the specimens exhibited mixed types of lesions, the most advanced lesion was used for scoring purposes, e.g., "type 2" was designated when the specimen showed both types 1 and 2 hepatic nodules; "hepatocellular carcinoma" was designated when the specimen showed types 1 and 2 nodules and hepatocellular carcinoma.

Incidence of Preneoplastic and Neoplastic Lesions. The results presented in Table 1 show that hepatocellular carcinomas (11.5% incidence) were seen exclusively in the mice given HMS. Furthermore, the mice in this group developed significantly increased numbers of both type 1 (42.7% compared to 21.2% in the control group) and 2 (20.8% compared to 12.1% in the control group) hepatic nodules. However, mice in the IA-4-N-oxide group showed no significant difference from controls in the incidence of nodules. A few mice (4.2%) in the HMS group developed sarcomas. In one animal, a part of the sarcoma showed angiosarcomatous growth with proliferation of endothelial-like cells, which tended to form irregular channels similar to vascular spaces. This sarcoma closely resembled that observed in a mouse infected with schistosomes and treated with a single dose of HMS (14). Glandular arrangement of the tumor tissue, suggesting bile duct origin, was not observed.

The results presented in Table 2 indicate that the incidence of tumors in organs other than liver (lung adenoma, kidney fibrosarcoma, leukemia, and testicular interstitial cell tumor) was not significantly different in the 3 groups.

In assessing the effects of HMS on the liver, the expression of results in Table 1 is conservative, in that only one lesion was used in any single mouse. It should be pointed out that HMS administration was consistently associated with an increase in all 3 types of hepatocellular proliferative lesions. For example, the mice in the HMS group had 72 type 1 and 31 type 2 nodules, as compared to 22 type 1 and 8 type 2 nodules in the 0.9% NaCl solution control group and 23 type 1 and 10 type 2 nodules in the IA-4-N-oxide group. Thus, regardless of how

---

**Table 1**

Survival time and neoplastic lesions in the livers of mice treated with a single dose of HMS or IA-4-N-oxide 42 hr after PH

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean survival time (days)</th>
<th>No. of animals</th>
<th>Hepatic nodules Type 1</th>
<th>Hepatocellular carcinoma</th>
<th>Sarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HMS, 76 mg/kg i.m.</td>
<td>546 ± 142</td>
<td>96</td>
<td>41(42.7)</td>
<td>11(11.5)</td>
<td>4(4.2)</td>
</tr>
<tr>
<td>2</td>
<td>IA-4-N-oxide, 80 mg/kg i.m.</td>
<td>482 ± 114</td>
<td>61</td>
<td>13(16.1)</td>
<td>10(12.4)</td>
<td>0(0)</td>
</tr>
<tr>
<td>3</td>
<td>0.9% NaCl solution i.m.</td>
<td>553 ± 127</td>
<td>66</td>
<td>14(21.2)</td>
<td>8(12.1)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

*a* Animals surviving less than 200 days were excluded from the effective number.

*b* Mean ± S.D.

*c* Significantly different from Group 2 at p < 0.001 and Group 3 at p < 0.005 (χ² test).

*d* Numbers in parentheses, percentage of mice with the neoplastic lesions.

*e* Significantly different from Group 3 at p < 0.001 (χ² test).

*f* Significantly different from Groups 2 and 3 at p < 0.005 (χ² test). Two mice had metastases to the lung; one had a hemangiosarcoma-like lesion.
the results are expressed, the administration of HMS is associated with quite a large increase in types 1 and 2 neoplasms, as well as in carcinomas.

DISCUSSION

The results of this study clearly show that a single dose of the antischistosomal agent, HMS, given i.m. 42 hr after PH significantly increased the incidence of liver cell neoplasms of 3 types, types 1 and 2 nodules and hepatocellular carcinoma and sarcoma in normal mice in the absence of a known infection with S. mansoni. While this manuscript was in preparation, Bulay et al. (4) reported that multiple doses of HMS administered to noninfected mice increased the incidence of liver cell neoplasms. However, in their studies, the average survival of both the HMS-treated and control mice was shorter than the latency of HMS-induced hepatocarcinomas (4). Therefore, quantitative evaluation of their data is not possible.

HMS, a commonly used drug in schistosomiasis (18, 20, 23, 24), has been shown to be mutagenic in a variety of mammalian and nonmammalian test systems (1, 3, 9, 15, 16) and teratogenic in mice and to induce malignant cell transformation in Rauscher virus-infected rat embryo cells (see Ref. 3 for a review). In addition, HMS induces damage to liver DNA in rats (26), a property shared with several liver carcinogens (25). Thus, the carcinogenic action of HMS is not unexpected.

An analog of HMS, IA-4-N-oxide, also an antischistosomal agent (15), did not induce any significant increase in liver neoplasms when given in an equivalent single dose under conditions in which HMS was effective. It is interesting that IA-4-N-oxide has been reported to be much less mutagenic than HMS (2).

Since infection with schistosomes or other trematodes is associated with liver cell necrosis and compensatory liver cell proliferation (7, 8, 13, 14, 21) and since HMS has now been found to be carcinogenic when given to mice during liver cell regeneration, it is possible that the efficacy of HMS in increasing liver neoplasms in mice infected with S. mansoni might be related to the liver cell injury and regeneration found under these conditions. It is noteworthy in this context that an otherwise noncarcinogenic dose of dimethylaminosamine-induced cholangiocarcinoma in Syrian golden hamsters infected with Opsihtorhchis viverrini (27) and that low doses of 2-amino-5-azotoluene produced a much higher incidence of hepatomas in mice infected with S. mansoni than in noninfected mice (11).

The classification of mouse hepatic neoplasms (6, 28) merits some comment. Walker et al. (29) subdivided the parenchymal cell neoplasms into 2 types, A and B, depending on their histological characteristics, with type B showing more malignant behavior. Jones and Butler (19) are in agreement with this classification. Gellaty (12) further subdivided type A lesions into 2 groups according to their morphological characteristics and their behavior on transplantation. During this study, it was found that type A lesions could be subdivided into 2 types, which we designate as type 1 and 2 nodules, depending on whether the liver cell plates are arranged as single (type 1) or multiple (type 2). Since type 2 lesions were not infrequently found inside type 1 nodules, it is reasonable to suggest that the type 2 nodule might arise from the type 1. The lesions exhibiting histological characteristics of cancer, such as well-defined trabecular patterns, showed invasion of surroundings and metastasis and therefore are classified as carcinomas. These correspond to Walker’s type B nodules. Recently, Williams et al. (30) observed that both type A and B nodules of Walker can be transplanted to the mammary fat pad and that the percentage of takes was greater for the type B nodules.

These results are generally consistent with the formulation as used in this study.

ACKNOWLEDGMENTS

We wish to thank Dr. A. Medline of the Department of Pathology, Toronto Western Hospital, Toronto, Ontario, Canada, for his review of the slide material, Patrick M. Dolan for his highly competent and effective technical assistance, and Helen Alston for her excellent secretarial help.

REFERENCES

H. Tsuda et al.


Fig. 1. Type 1 nodule compressing normal liver tissue. The nodule is composed of cells closely resembling the normal hepatocytes. HMS group. H & E, x 40.

Fig. 2. Type 1 nodule. Hepatocytes are arranged in single-cell plates. HMS group. H & E, x 100.

Fig. 3. Type 2 nodule (upper right) and surrounding type 1 nodule (lower left). Convoluted liver cell plates are 2 or more cells thick and tend to show trabecular structure in the center of type 2 nodule. Nuclei are prominent. HMS group. H & E, x 100.

Fig. 4. Type 2 nodule compressing surrounding normal hepatocytes. IA-4-W-oxide group. H & E, x 100.
Fig. 5. Hepatocellular carcinoma composed of multiple-cell-thick plates. Blood space is wide and distinct. HMS group. H & E, × 100.

Fig. 6. Hepatocellular carcinoma showing papillary and trabecular structures. Acinar structure is also observed; cells and nuclei vary in size. HMS group. H & E, × 100.

Fig. 7. Fibrosarcoma composed of dense proliferation of spindle-shaped atypical cells. HMS group. H & E, × 100.

Fig. 8. Pulmonary metastasis from the trabecular carcinoma shown in Fig. 5. H & E, × 40.
Induction of Hepatic Neoplastic Lesions in Mice with a Single Dose of Hycanthone Methanesulfonate after Partial Hepatectomy
