The Influence of Schistosoma mansoni Infection on Carcinogenesis of Mouse Livers Initiated by N-2-fluorenylacacetamide

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Summary: The influence of S. mansoni infections on the occurrence of liver tumors initiated by N-2-fluorenylacacetamide was investigated. Four-week-old female ddY mice were treated as follows; mice exposed to 20 cercariae of S. mansoni (schistosomiasis group); mice given perorally a carcinogen (carcinogen group); and mice receiving both a carcinogen and S. mansoni (schistosomiasis carcinogen group). The first observation of liver tumors in one of the schistosomiasis carcinogen animals was made during the 21st week and in two of the carcinogen animals during the 40th week. The incidence of liver tumors by 40 weeks was 21 of 45 mice (46.7 %) in the schistosomiasis carcinogen group and 2 of 32 mice (6.3 %) in the carcinogen group. This difference was significant (p<0.005). None of the animals in the schistosomiasis group developed a liver tumor. Microscopic examination of the liver tumors disclosed hyperplastic nodules Type 1 and 2, and hepatocellular carcinoma. The mice in the schistosomiasis carcinogen group had a significantly higher incidence of both Type 1 nodules (13.3 % compared to 0 % in the carcinogen group) and hepatocellular carcinomas (26.7 % compared to 0 % in the carcinogen group). The schistosomiasis carcinogen treatment resulted in early and marked production of liver tumors.

Key words: Schistosoma mansoni — N-2-fluorenylacacetamide — hyperplastic nodule — hepatocellular carcinoma — carcinogenicity

Introduction

The relationship between hepatocellular carcinoma and schistosomiasis mansoni has been debated etiologically in human cases (Edington et al. 1965; Martinez et al. 1965; Hinz, 1966; Cheever et al. 1967; Mott, 1978; Edington, 1979). A high incidence of hepatocellular carcinoma in mice exposed to Schistosoma mansoni infections and carcinogens has been reported by many investigators (Domingo et al. 1967; Liu et al. 1969; Haese et al. 1973; Haese and Bueding, 1976). Several investigators have studied the influence of Schistosoma japonicum infections on carcinogenesis in mouse livers (Shigefuku, 1943; Miyasato, 1984). The present study was undertaken using a similar experimental system to Miyasato (1984) with S. mansoni infection and 2-FAA (N-2-fluorenylacacetamide), to confirm the influence of schistosomiasis on initiated mouse liver.

Materials and Methods

109 four-week-old female ddY mice were divided into three groups; 1) schistosomiasis carcinogen group; 45 mice were exposed intraperitoneally to 20 Puerto Rican strain cercariae of S. mansoni per mouse...
and provided with normal rodent food for the first four weeks, and thereafter with an oral carcinogenic diet containing 0.03% 2-FAA; 2) schistosomiasis group; 32 mice were exposed intraperitoneally to 20 Puerto Rican strain cercariae of S. mansoni per mouse and provided with normal rodent food throughout the study period; 3) carcinogen group; 32 mice were provided with rodent food for four weeks and then were placed on an oral carcinogenic diet containing 0.03% 2-FAA.

Animals were sacrificed every 10 weeks with diethyl-ether; however, they were autopsied as soon as possible when they were moribund or had ascites on palpation. The worms were recovered by a portal perfusion method. All visceral organs, especially the liver, were removed and meticulously examined for evidence of tumors. Investigations of distant metastases were carried out in those animals with liver tumors.

Each tissue sample was fixed in buffered 10% formalin and embedded in paraffin. Specimens were cut into serial sections, 5μ in thickness, and stained with haematoxylin and eosin, and periodic acid Schiff.

**Results**

The incidence of liver tumors and the number of recovered worm pairs are tabulated in Table 1. The average number of recovered worm pairs ranged from 1.5 to 4.5 in the schistosomiasis carcinogen group and from 1.7 to 3.8 in the schistosomiasis group. No significant differences in the recovered worm pair numbers were found between the two groups at each administration period of 2-FAA. Similar results were obtained for the total average number of worm pairs.

No liver tumors were detected in the schistosomiasis group. In the schistosomiasis carcinogen group, liver tumors were

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<tr>
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<td>Incidence of mice with tumors*</td>
<td>Worm pair number</td>
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<td>0 - 10</td>
<td>0/9</td>
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% of mice with tumor 46.7***

* : Number of mice with tumors/number of mice sacrificed.
** : Numbers in parentheses, range of worm pairs.
*** : Significantly different from carcinogen group at p<0.005 and schistosomiasis group at p<0.005 (normal difference test).
found after 21 weeks and were totally developed in 21 of 45 mice (46.7%) by 40 weeks. Liver tumors were found in only 2 of 32 mice (6.3%) after 31 to 40 weeks in the carcinogen group.

After 40 weeks, the incidence of liver tumors in the schistosomiasis carcinogen group was significantly higher (by the normal difference test) than the frequencies in the carcinogen group ($p<0.005$) and the schistosomiasis group ($p<0.005$).

Gross examination of liver tumors from animals in the schistosomiasis carcinogen group revealed protruded-multiple nodules, which were large in size, yellowish-white in color, slightly hard in consistency and well-circumscribed (Fig. 2). The characteristics of the liver tumors in the carcinogen group resembled those in the schistosomiasis carcinogen group except they were smaller in size and number (Fig. 3).

Microscopically, the liver tumors were classified into three types, hyperplastic nodules Type 1 and Type 2, and hepatocellular carcinoma, according to the Classification of Tsuda et al. (1979). The Type 1 nodule (Fig. 4) was composed of proliferating parenchymal cells which consisted mainly of closely packed single liver cell plates similar to normal lobular structure. There was usually a slight compression of the surrounding nonnodular areas. Mitotic activity was rare. Type 2 nodules (Fig. 5), composed of convoluted cell plates, were two or more cells thick, and the normal lobular pattern was lost with the absence of the central vein and portal triads. Mitotic activity was slightly elevated. These lesions showed expansive growth and compressed the surrounding areas, but there was no clear-cut evidence of invasion. Type 1 and 2 nodules were frequently found side by side (Fig. 6), and Type 2 nodules were occasionally inside Type 1 nodules. Hepatocellular carcinoma was trabecular in structure. The trabecular type had multiple-cell-thick structures with various sized blood spaces (Fig. 7). Some of these trabecular lesions invaded surrounding liver tissue and metastasized to the lung. If two or three different types were found microscopically in the liver lesions (Type 1, Type 2, and/or hepatocellular carcinoma), then the classification was

| TABLE 2 | Incidence of the liver lesions in each group after 40 weeks |
|-----------------|-----------------|-----------------|-----------------|
|                | No. of mice     | No. of mice with |                 |
|                |                 | hyperplastic nodules | hepatocellular carcinoma |
| Schistosomiasis carcinogen group | 45 | 6 (13.3%)* | 3 (6.7%) | 12 (26.7%)** |
| Period (week)  |                 | 21 - 40          | 26 - 28         | 29 - 40         |
| Carcinogen group | 32 | 0 | 2 (6.3%) | 0 |
| Period (week)  |                 | 40 |                 |                 |
| Schistosomiasis group | 32 | 0 | 0 | 0 |

*: Significantly different from the carcinogen group and schistosomiasis group at $p<0.005$ (normal difference test).

**: Significantly different from the carcinogen group and schistosomiasis group at $p<0.005$ (normal difference test). 4 cases metastasized to the lungs. Coexistence of Type 1 (10/12, 83.3%), of Type 2 (9/12, 75.0%).
made according to the predominant type present.

The incidence of the microscopic liver lesions is described in Table 2. In the schistosomiasis carcinogen group, histological examination revealed the presence of Type 1 nodules in 6 mice (13.3%), Type 2 nodules in 3 mice (6.3%) and hepatocellular carcinomas in 12 mice (26.7%). In the 12 mice with the hepatocellular carcinomas, coexistence with Type 1 nodules was found in 10 mice (83.3%), and with Type 2 nodules in 9 mice (75.0%). On the other hand, Type 2 nodules were noted in only 2 of the 32 mice in the carcinogen group. The incidence of Type 1 nodules with hepatocellular carcinomas in the schistosomiasis carcinogen group were significantly higher than in the other two groups at p<0.005 (normal difference test). To further establish a correlation between the occurrence of the microscopic liver lesions and the experimental periods, a histogram was constructed, as shown in Fig. 1. In the schistosomiasis carcinogen group, Type 1 nodules were first found after 21 weeks, Type 2 nodules after 26 weeks, and hepatocellular carcinoma finally appeared after 29 weeks with a subsequent increase in the number of lesions. Moreover, the Type 2 nodules in the schistosomiasis carcinogen group were detected 14 weeks earlier than those in the carcinogen group.

Table 3 shows the distribution of the liver tumors in the liver lobes of the schistosomiasis carcinogen group. The frequencies were as follows: right lobe, 13/21 (61.9%); median lobe, 16/21 (76.2%); left lobe, 18/21 (85.7%); and caudate lobe, 12/21 (57.1%). The only significant difference was between the left lobe (with the highest incidence) and the caudate

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**Fig. 1.** Incidence of microscopic liver lesions with different observation periods.
liver neoplasm with Schistosoma mansoni

Discussion

It has been reported that S. mansoni infection in animals treated with a certain hepatocarcinogen induces an early and marked production of hepatocellular carcinoma (Domingo et al. 1967; Haese et al. 1973; Haese and Bueding, 1976). A role of S. mansoni in hepatocarcinogenicity was suggested by several investigators; that is, the parasitic infection may act as a promotor (Bueding et al. 1980) or may cause specific liver conditions similar to those induced by partial hepatectomy (Tsuda et al. 1979). The process of carcinogenicity initiated in hepatocytes by carcinogens has been morphologically established since hyperplastic nodules in the liver were demonstrated to be a precursor of the hepatocellular carcinoma (Farber, 1976). However there are few reports on the time course for development of the liver neoplasm in schistosomal animals. Miyasato (1984) indicated that S. japonicum infection combined with 2-FAA rapidly induced hyperplastic nodules, and accelerated the transformation from hyperplastic nodules to the appearance of hepatocellular carcinomas. The present study describes in detail the influence of S. mansoni infections on the time course for development of liver tumors initiated by 2-FAA.

As shown in Tables 1 and 2, the liver tumor was initially observed at 21 weeks and 21 of 45 (46.7%) mice developed the liver tumors in the schistosomiasis carcinogen group by 40 weeks. These 21 tumors included 6 with Type 1 nodules, 3 with Type 2 nodules, and 12 with hepatocellular carcinomas. In contrast to this, only 2 of 32 (6.3%) mice developed Type 2 nodules in the carcinogen group by 40 weeks.

The results indicate an earlier development and a significantly higher incidence of liver tumors in the schistosomiasis carcinogen group than in the carcinogen group or schistosomiasis group.

Of 12 mice with hepatocellular carcinomas, coexistence with Type 1 nodules was found in 10 (83.3%), and with Type 2 nodules in 9 mice (75.0%). This high incidence of coexistence firmly supports the theory (Farber, 1976) that the hyperplastic nodule is a precursor of the hepatocellular carcinoma.

With regard to the distribution of the liver tumors in the liver lobes, a signifi-
cant difference was found only between the left lobe (the highest incidence) and the caudate lobe (the lowest incidence). There was insufficient data and evidence to elucidate a mechanism for this difference.

In 4 of 12 animals with hepatocellular carcinomas, metastases to the lungs were observed. Tumors in the schistosomiasis carcinogen group which had metastasized to the lungs were all of the trabecular type in the liver. This type of liver tumor is considered to be much more likely to metastasize to the lungs (Takayama, 1968; Vesselinovitch et al. 1978; Frith et al. 1981).

The time course for development of the liver tumor, as shown in Fig. 1, clearly illustrates the tendency for an early occurrence and accelerated growth of the liver tumor. Type 1 hyperplastic nodules appeared at an early stage, then Type 2 hyperplastic nodules occurred, and finally hepatocellular carcinoma was detected. This observation was supported by the fact that Type 2 nodules were frequently found inside Type 1 nodules (Tsuda et al. 1979), or Type 1 and 2 nodules were often side by side (Fig. 6) and the hepatocellular carcinoma increased with a decrease in the Type 2 nodules (Miyasato, 1984). Since the time course for development of the liver tumor is very similar to that in S. japonicum (Miyasato, 1984), there is a possibility that the mechanism of the early induction and high incidence of liver tumors in S. mansoni is the same as in S. japonicum, although the species are different.

The mechanism of the early induction of liver tumors has been studied by many investigators. It was considered that a partial hepatectomy was closely related to the early induction of liver tumors (Craddock, 1973; Craddock and Frei, 1974). The combination of partial hepatectomy and 2-FAA had a synergic effect on the occurrence of liver tumors (Kitagawa, 1971; Solt et al. 1977; Ito et al. 1978; Hasegawa et al. 1982). This enhancing effect may be due to the liver cell proliferation during a regenerative stage after the partial hepatectomy.

Generally, the regeneration of hepatocytes occurs with liver injury. In human cases, Bueding et al. (1980) suggested that the increased incidence of liver carcinoma was due to the stimulation of cell proliferation by the infection which leads to a greater susceptibility of the hepatic tissue to environmental or medical carcinogens. In the present experiment, regeneration of parenchyma, after liver injury by the S. mansoni infection, occurred during the period from 16 to 20 weeks after administration of 2-FAA (Figs. 10, 11, and 12). Furthermore, the liver tumor was initially detected just after the beginning of the regeneration of the hepatic parenchyma.

These results strongly support the concept that cell proliferation during regenerative stages induced by a S. mansoni infection plays a significant role in the higher incidence and earlier development of liver tumors.

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References


Craddock, V.M. (1973). Induction of liver tumors in rats by a single treatment with nitroso compounds given after partial hepap-


Fig. 2. Gross appearance of a liver tumor from the schistosomiasis carcinogen group at 40 weeks. Various sized white nodules are seen in the liver.

Fig. 3. Gross appearance of a liver tumor from the carcinogen group at 40 weeks. A few small nodules are seen.

Fig. 4. Microscopic features of a Type 1 hyperplastic nodule. The Type 1 nodule is on the left side. The structure is normal, but it is compressing the surrounding nonnodular area. (H and E ×200)

Fig. 5. Microscopic features of a Type 2 hyperplastic nodule. The Type 2 nodule is in the upper half of this figure. The liver cell plates are thicker and the vascular space is wider than in the Type 1 nodule. (H and E ×200)

Fig. 6. Nodules Type 1 and 2 are side by side. On the left side there is a Type 1 nodule, and on the right side a Type 2 nodule. The demarcation is not clear. (H and E ×200)

Fig. 7. Hepatocellular carcinoma of a trabecular type. Multiple-cell-thick liver plates are seen with vascular spaces of various sizes. (H and E ×100)
Fig. 8. Microscopic features of a metastasis in the lung in the schistosomiasis carcinogen group at 40 weeks. (H and E ×50)

Fig. 9. High-power view of Fig. 8. The metastasized tumor cells have glandular structures. (H and E ×200)

Fig. 10. Microscopic features of liver tissue in the schistosomiasis carcinogen group at 16 weeks. Granulomas, tightly located, are numerous with expansive growth and the hepatic parenchyma is severely injured. The central veins are few in number and the portal triads are destroyed. (H and E ×50)
Fig. 11. Microscopic features of liver tissue in the schistosomiasis carcinogen group at 20 weeks. Parenchymal regeneration becomes dominant, and the granulomas are decreased in size and number, compared with Fig. 10. (H and E ×50)

Fig. 12. Microscopic features of liver tissue in the schistosomiasis carcinogen group at 25 weeks. Parenchymal regeneration becomes more dominant with a marked decrease in both the size and number of granulomas. (H and E ×50)