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Experimental Study of the Influence of *Schistosoma japonicum* Infection on Carcinogenesis of Mice Liver Treated with N-2-fluorenylacetamide (2-FAA)

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Introduction

Although a relationship between urinary bladder cancer and schistosomiasis haematobia is generally accepted, it has been debated whether or not *Schistosoma mansoni* or *Schistosoma japonicum* infection play a role in the pathogenesis of hepatocellular carcinoma (Cheever, 1978; Mott, 1978; Edington, 1979; Bueding *et al.*, 1980).

Experimentally, it has been reported that in comparison with control animals, some chemical compounds can produce a significantly higher incidence of hepatocellular carcinoma in animals infected with *S. mansoni* (Domingo *et al.*, 1967; Liu *et al.*, 1969; Haese *et al.*, 1973; Haese and Bueding, 1976) or *S. japonicum* (Shigefuku, 1943).

The present study was carried out investigating the influence of *S. japonicum* infection on the development of the liver tumor initiated by N-2-fluorenylacetamide.

Materials and Methods

A total number of 173 4-week-old female ddY mice were randomly divided into three groups; (1) schistosomiasis-carcinogen group, 77 mice infected by tail immersion with *S. japonicum* (40 Japanese strain cercariae per each mouse) and orally administered with 2-FAA 4 weeks after infection; (2) carcinogen-only control group, 86 mice administered with 2-FAA; (3) carcinogen-only long-term group, 10 mice were administered with 2-FAA for 60 weeks in order to examine the possibility of tumor formation. Each group was fed on normal basal diet for the first four weeks, and then on a diet containing 0.03% of 2-FAA. Mice were anesthetized with diethylether and sacrificed during administration periods of 2-FAA from 9 to 40 weeks when they were moribund and had masses or ascites on palpation. The dying mice during the administration periods were autopsied as soon as possible. Macroscopically, the liver was meticulously examined for evidence of tumor. The other organs, especially the lungs, were examined for distant metastasis.

Each tissue was fixed in buffered 10% formalin and embedded in paraffin. Specimens were cut into serial sections with 5μ in thickness and stained with hematoxylin-eosin and periodic acid Shiff.

Results

The incidence of liver tumor in each group and the recovery worm pair number are summarized in Table 1. In relation to the recovery worm pair number, the mice harbored approximately an average of 5 worm pairs per mouse by sixteen weeks of study (range

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Table 1 Incidence of the liver tumors in various administration periods and recovery worm number

Administration period (week)	Schistosomiasis-carcinogen group			Carcinogen-only group	
	No. of mice sacrificed	Ave. No. of worm pair recovered	No. of mice with liver tumor	No. of mice sacrificed	No. of mice with liver tumor
9	12	5(3-13)*	0	15	0
12	12	4(2- 5)	0	15	0
16	11	5(1-10)	2	15	0
20	10	3(1- 6)	3	10	0
28	6	2(1- 3)	2		
30	6	2(1- 3)	5		
36	5	2(1- 3)	5	5	0
37	5	2(2)	3	5	2
38	5	2(1- 4)	3	11	2
40	5	1(1)	1	10	2
Total	77		24(31.17%)†	86	6(6.98%)

* Range of worm pair.

† Significantly different from carcinogen-only group at $p<0.005$ (χ^2 test).

Table 2 Distribution of the liver tumor in liver lobes of mice in schistosomiasis-carcinogen group

Total number of mice	No. of mice with the liver tumors in			
	Right lobe	Median lobe	Left lobe	Caudate lobe
24	18(75.0%)	22(91.7%)	21(83.3%)	4(16.7%)*

* Significantly different from the other lobes at $p<0.005$ (χ^2 test).

1-13). The worm pair was reduced by degrees with elongation of study, but not eliminated.

In the schistosomiasis-carcinogen group, the first liver tumor was observed 16 weeks after the administration of 2-FAA. Thereafter, the liver tumors were continuously observed and were noted in a total of 24 of the 77 mice (31.17%) by 40 weeks. In the carcinogen-only group, the liver tumor was first observed at the 37 th week and was noted in 6 of the 86 mice (6.98%) by 40 weeks. The incidence rate of liver tumors in the total period of 40 weeks was significantly greater in schistosomiasis-carcinogen group than in carcinogen-only group using a chi-square test ($p<0.005$).

Macroscopically, the liver tumors in the schistosomiasis-carcinogen group protruded from the liver surface as solitary or multiple nodules which often occupied most of one or several liver lobes (Fig. 1). On a cut surface, the tumors were well demarcated against the adjacent liver tissues as greyish-yellow nodules of varying diameter in size (Fig. 2). The characteristics of the liver tumors in carcinogen-only group were similar to those in schistosomiasis-carcinogen group except for being smaller in size and less in number (Fig. 3).

The distribution of the liver tumors in the various liver lobes in the schistosomiasis-carcinogen group is described in Table 2. Of 24 mice with the liver tumor, 18 mice (75.0%) developed the liver tumor in right lobe, 22 (91.7%) in median lobe, 21 (83.3%) in left lobe and 4 (16.7%) in caudate lobe. The in-

Table 3 Incidence and observation periods of the liver lesions in each group by 40 weeks

	No. of mice with		
	hyperplastic nodules		hepatocellular carcinoma
	Type 1	Type 2	
Schistosomiasis-carcinogen group (total 77 mice)	6(7.8%)	10(13.0%)*	8(10.3%)†
Observation periods (week)	16-30	16-37	28-40
Carcinogen-only group (total 86 mice)	4(4.7%)	2(2.3%)	0(0 %)
Observation periods (week)	37-38	40	

* Significantly different from carcinogen-only group at $p<0.01$ (χ^2 test).
† Significantly different from carcinogen-only group at $p<0.005$ (χ^2 test).

cidence rate of the liver tumor was the lowest in caudate lobe and was significantly different from those in the other lobes ($p<0.005$). No significant differences, however, were found amongst the other lobes.

Microscopic examination of the liver tumors both in schistosomiasis-carcinogen group and carcinogen-only control group disclosed hepatocellular carcinoma or hyperplastic nodules. The hyperplastic nodules could be divided into two types according to Tsuda's Classification (1979). Type 1 nodule (Fig. 4) consisted mainly of closely packed basophilic liver cells. There was the slight compression of the non-nodular areas caused by these lesions. Mitotic figure was uncommon. Type 2 nodule (Fig. 5), showing more advanced behavior than Type 1, was composed of a large amount of proliferating hepatocytes. Liver cell plates consisted of two or more cells in thickness. The Type 2 nodules were slightly or moderately compressing the non-nodular areas. Moderate degrees of anisocytosis and pleomorphism were often observed. Mitotic figure was occasionally obsered. Type 1 and 2 nodules were sometimes observed individually or side by side in the same liver (Fig. 6).

Hepatocellular carcinoma, trabecular or papillary in structure, was composed of eosinophilic or basophilic tumor cells with severe degrees of anisocytosis and pleomorphism (Fig. 7, 8). The trabeculae were multiple-cell-thick strurtures. Blood space was wide and distinct.

In schistosomiasis-carcinogen group, hyperplastic nodules and hepatocellular carcinoma were usually found to be devoid of the schistosome eggs and granulomas. These were noted in normal liver tissues or near the tumor margin.

The incidence and observation periods of the liver lesions in each group are shown in Table 3 (when the specimens exhibited mixed types of lesions, the most advanced lesion was adopted for tabulation purposes). Type 1 nodules were noted in 6 out of the 77 mice (7.8%) in the schistosomiasis-carcinogen group and in 4 of the 86 mice (4.7%) in the carcinogen-only group. There was no significant difference between the two groups. The mice in the schistosomiasis-carcinogen group, however, displayed a significantly higher incidence rate of both the Type 2 nodule (13% compared to 2.3% in carcinogen-only group) and hepatocellular carcinoma (10.3% compared to 0% in carcinogen-only group).

The Type 1 nodule was observed in the liver lesions of the schistosomiasis-carcinogen group during the period from 16 to 30 weeks. Type 2 nodule was first noted simultaneously with the appearance of Type 1 nodule, but was observed up to 37 weeks. There was a tendency to decrease occurrence of Type 1

nodule with the appearance of Type 2 nodule. The incidence of Type 2 nodule also decreased with the appearance of hepatocellular-carcinoma which was observed from 28 to 40 weeks. The carcinogen-only group showed a delayed appearance of both Type 1 (37 weeks) and Type 2 (40 weeks) nodules. The Type 2 nodule developed 3 weeks after the first appearance of Type 1 nodule.

Of the 10 mice maintained until 60 weeks, 6 mice developed Type 1 or 2 nodules. No hepatocellular carcinoma was observed in any of them.

No distant metastasis was observed in any other organs in all groups.

Discussion

Some observations of the influence of schistosoma infection on carcinogenesis of the mice liver initiated by hepatocarcinogens have been reported by other laboratories which indicated that carcinogen plus schistosomiasis resulted in an early and marked production of hepatocellular carcinoma (Domingo *et al.*, 1967 ; Haese *et al.*, 1973 ; Haese and Bueding, 1976). However, these pay little attention to the effects of the schistosoma infection on the development of the liver tumors in the course of time. In this paper, author reported the time course development of the liver tumors in order to elucidate the pathogenesis of the higher occurrence of the liver tumor in the schistosomal liver.

The results indicated that the liver tumors including hyperplastic nodules which were divided into two types, Type 1 and 2, depending on their histological characteristics and hepatocellular carcinoma had a much higher incidence and earlier development in schistosomiasis-carcinogen group in comparison with carcinogen-only control group at 40 weeks after administration of 2-FAA.

In schistosomiasis-carcinogen group, the Type 1 nodule was first observed 16 weeks after administration of 2-FAA and the incidence diminished by degrees with increasing incidence of Type 2 nodule. The Type 1 nodule might therefore be a precursor of Type 2 nodule. This was supported by the dual occurrence of both Type 1 and Type 2 nodules in the same liver. Type 2 nodule has been found to arise within the Type 1 (Tsuda *et al.*, 1979). Here the Type 2 nodule was noted simultaneously with the appearance of Type 1 nodule in the schistosomiasis-carcinogen group, but in the carcinogen-only group Type 1 nodule was first observed at 37 weeks and the Type 2 nodule developed 3 weeks later. It was suggested that *S. japonicum* infection brought about an early onset of Type 1 nodule and furthermore promoted to arise the Type 2 nodule from the Type 1 nodule.

Hepatocellular carcinomas in schistosomiasis-carcinogen group were found at 28 weeks and increased with a corresponding disappearance of Type 2 nodules. Since hyperplastic hepatic lesions have been defined as pre-neoplastic lesions (Farber, 1976), it was considered that hepatocellular carcinoma may arise from Type 2 nodules. On the other hand, hepatocellular carcinoma did not develop until 60 weeks in the carcinogen-only group, it was evident that *S. japonicum* infection also promoted to arise the hepatocellular carcinoma possibly from Type 2 nodule. The higher incidence of liver tumors in schistosomal liver might be attributed to accelerating effects of schistosome infection both on the induction of the Type 1 nodule and additionally on the subsequent progressive development of the tumor.

Experimental methods for the rapid induction of a hyperplastic nodule in the liver of animals have been developed by many investigators. Partial hepatectomy (Cradock, 1973), various metabolizing enzyme inducers (Peraino *et al.*, 1977 ; Guethner and Mannering, 1977 ; Kitagawa *et al.*, 1978 ; Ito *et al.*, 1978, 1980 ; Hasegawa *et al.*, 1982) and other carcinogenic agents (Solt *et al.* 1977) enhance the production of hyperplastic nodules or hepatocellular carcinoma when administered after or before hepatocarcinogens.

The mechanism of rapid induction of the hyperplastic nodules in the schistosomal liver is not clearly established yet and further experiments should be needed. There are some reports on this problem. A single dose of the antischistosomal drug, hycanthone at 42 hours after hepatectomy, significantly increas-

ed the incidence of liver cell neoplasia in normal mice and it was suggested that the efficacy of hycanthone in promoting the liver neoplasms in mice infected with *S. mansoni* may be related to liver injury and regeneration induced by the schistosomes infection (Tsuda *et al.*, 1979). It was further reported that the mouse with schistosomiasis persisting for an extended period could produce hepatocellular hyperplasia. The hyperplastic response in schistosomiasis rendered the liver cell susceptible to carcinogenic attack by a carcinogen, and schistosoma infection might additionally act as a promoter (Bueding *et al.*, 1980).

The incidence of distant metastasis of hepatocellular carcinoma was generally considered low for mice. However, some studies examined the lungs in more detail showed the incidence to be much greater. Metastasized behavior of the liver neoplasms in mice has been accepted to be defferent according to several conditions such as strain, sex and age of the animals (Kyriazis *et al.*, 1974 ; Vesselinovich *et al.*, 1978 ; Frith *et al.*, 1981). On the histological examination of the organs, especially the lungs, no distant metastasis was observed in the present study. It was considered that hepatocellular carcinomas in ddY mice might rarely metastasize to the other organs by 40 weeks although they could be rapidly induced by *Schistosoma japonicum* infection.

The incidence of the liver tumors in schistosomiasis-carcinogen group was the highest in the median lobe, intermediate in the left lobe and the right lobe and the lowest in the caudate lobe. The low incidence of the liver tumors in the caudate lobe has been reported in normal animals (Takayama and Inui, 1967 ; Lawson and Paund, 1974 ; Frith *et al.*, 1979). The *S. japonicum* infection may have perhaps no influence on the location in the liver of the tumors induced by a carcinogen.

Summary

The influence of *Schistosoma japonicum* infection on the development of the liver tumors initiated by 2-FAA was studied in 173 ddY mice. Animals were divided into three groups ; schistosomiasis-carcinogen group, carcinogen-only group and carcinogen-only long-term group.

After the administration of 2-FAA, the first liver tumor was observed at 16 weeks in schistosomiasis-carcinogen group and at 37 weeks in carcinogen-only group. Overall, the liver tumors were noted in 24 of 77 mice (31.17%) in schistosomiasis-carcinogen group and in 6 of 86 mice (6.98%) in carcinogen-only group. There was significant difference between the two groups ($p<0.005$).

Microscopically, the tumors revealed hyperplastic hepatic nodules divided into Type 1 and Type 2 and hepatocellular carcinoma. The mice of the schistosomiasis-carcinogen group developed significantly increased numbers of both Type 2 nodule ($p<0.01$) and hepatocellular carcinoma ($p<0.005$). In carcinogen-only long-term group, no hepatocellular carcinoma was observed.

The liver tumors had much higher incidence, earlier development and more advanced nature in the schistosomiasis-carcinogen group compared with the carcinogen-only control group.

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寄生虫の発癌性に関する実験的研究：発癌剤を投与した日本住血吸虫感染マウスの肝腫瘍発生について

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日本住血吸虫症と肝腫瘍発生の関係について検索する目的で，正常及び日本住血吸虫感染マウスに発癌剤を投与し，両者における肝腫瘍発生について病理学的検討を行つた．

実験には正常な4週令 ddY 雌マウスと日本住血吸虫セルカリア40隻を感染させた同種マウスを用い，N-2-fluorenylacetamide (2-FAA) を経口投与し，投与後9週から40週までの肝腫瘍発生を比較した．

肉眼的に，感染群は16週より腫瘍発生がみられ，40週までに77例中24例（31.17%）に認めた．一方，非感染群は37週より腫瘍発生がみられ，40週までに86例中6例（6.98%）であつた．40週までの肝腫瘍発生頻度において，両群間に有意の差を認めた。

組織学的に，これらの肝腫瘍は肝細胞癌，Type 1 結節性増殖巣及び Type 1 と肝細胞癌の中間型を示す Type 2 結節性増殖巣に分類することができた．頻度は感染群で肝細胞癌10.3%，Type 1 結節性増殖巣7.8%，Type 2 結節性増殖巣13%，非感染群では各々，0%，4.7%及び2.3%であり，Type 2 結節性増殖巣及び肝細胞癌の発生頻度は両群間において有意の差を認めた．

以上の結果から，日本住血吸虫感染群は非感染群に比較して肝腫瘍発生の時期が早く，発生頻度が高く，組織学的により進んだ腫瘍の性状を呈していた．

Explanation of Figures

Fig. 1 Gross appearace of the liver tumors in the schistosomiasis-carcinogen group sacrificed at 28 weeks. Multiple nodules are seen in the various liver lobes.

Fig. 2 Gross appeprance of the liver tumors on cut surface in the schistosomiasis-carcinogen group sacrificed at 28 weeks. The liver tumors are well demarcated against the adjacent liver tissues.

Fig. 3 Gross appearance of the liver tumors in carcinogen-only group sacrificed at 40 weeks. Multiple-small-round nodules are seen in all over the liver.

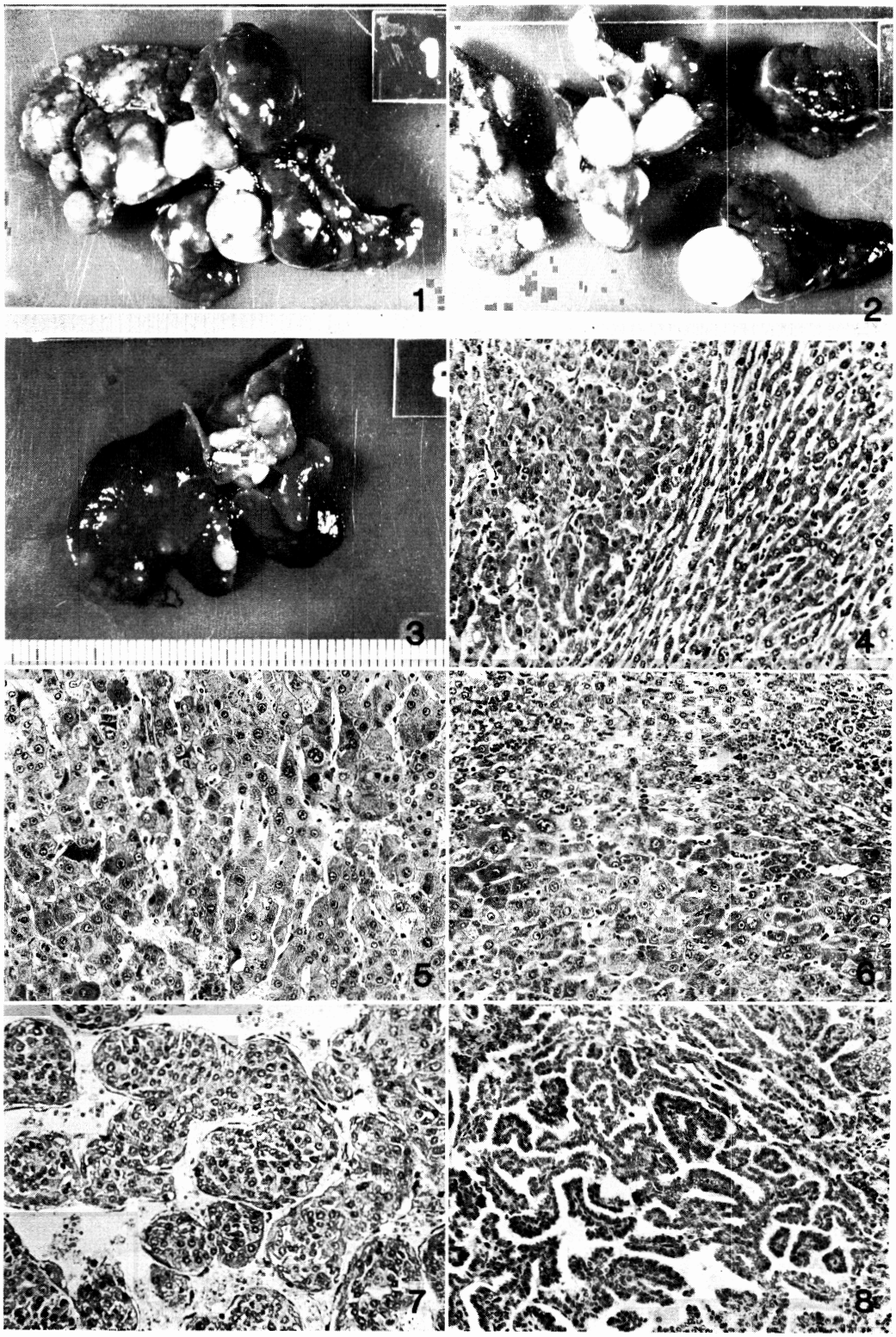
Fig. 4 Microscopic picture of Type 1 hepatic nodule. The nodule consists of closely-packed hepatocytes. Slight compression of non-nodular area is seen. H & E stain (×100)

Fig. 5 Microscopic picture of Type 2 hepatic nodule. Liver cell plates are two or more cells in thickness and a moderate degree of anisocytosis and of pleomorphism is seen. H & E stain (×100)

Fig. 6 Coexistence of Type 2 nodule (lower side) and Type 1 nodule (upper side). H & E stain (×100)

Fig. 7 Microscopic picture of trabecular hepatocellular carcinoma. The trabeculae are multiple-cell-thick. Blood space is wide and distinct. H & E stain (×100)

Fig. 8 Microscopic picture of papillary hepatocellular carcinoma. Papillomatous proliferation of basophilic tumor cells is seen. H & E stain (×50)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