

Inhibitory Effect of Fumaric Acid on Hepatocarcinogenesis by Thioacetamide in Mice

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The inhibitory effect of fumaric acid (FA) on hepatocarcinogenesis was examined in mice fed thioacetamide (TAA). A group of male ICR mice was fed TAA at a level of 0.035% in the diet for 40 weeks and then fed a basal diet for 48 weeks. Hepatic tumors developed in 11 of the 24 animals of this group and they were diagnosed as hepatocellular carcinomas. However, cirrhotic lesions and the enlargement of hepatocyte nucleoli were not as marked in mice as in previous findings in rats fed TAA. The effect of FA on the carcinogenesis was examined in a group of mice fed this compound at a level of 1% in a basal diet after ingestion of TAA. The inhibitory effect of FA on TAA carcinogenesis was so marked that no hepatic carcinomas were found in any of the 15 animals fed FA in combination with TAA.

Keywords fumaric acid; thioacetamide; mouse liver; hepatocellular carcinoma; anticarcinogenesis

Introduction

In our pharmacological studies on the extract of *Capsella bursa-pastoris* (Cruciferae), fumaric acid (FA) was isolated and identified as the component of the herb responsible for inhibiting the growth of subcutaneously transplanted Ehrlich tumors in mice and gastric ulceration in rats.^{1,2)} Thereafter, we found that FA reduced the toxic symptoms in mice and rats injected with mitomycin C or aflatoxin B₁³⁻⁵⁾ and that FA suppressed hepatocarcinogenesis in rats fed 3'-methyl-4-(dimethylamino)azobenzene or thioacetamide (TAA).^{6,7)}

TAA is a potent hepatotoxin of which the toxic and carcinogenic activities have been well studied.⁸⁻¹⁰⁾ It is an interesting agent in its mechanism of carcinogenesis, for it has not demonstrated any direct interaction with cellular deoxyribonucleic acid (DNA): it does not form DNA adducts,¹¹⁾ it fails to induce repair DNA synthesis,¹²⁾ and it is negative in the Ames assay.¹³⁾ Most of these studies were done on rats⁸⁾ but a limited number of studies on mice reported a higher incidence of hepatic carcinomas in the TAA-treated animals.^{14,15)} The present study was undertaken to examine whether FA would inhibit hepatocarcinogenesis in mice fed TAA, comparing the histopathological findings of livers in mice with the previous ones in rats.⁷⁾

Materials and Methods

Animals and Their Diet A total of 54 male ICR mice (CLEA Japan Inc., Tokyo), 4 weeks of age and weighing 20–23 g, were used. Commercial diet CE-2 (CLEA Japan Inc.) was used as the basal diet, and an experimental diet containing 0.035% TAA or 1% FA was also prepared by the same manufacturer. TAA was purchased from Wako Pure Chemical Co., Tokyo, and FA was purchased from Nakarai Chemicals, Ltd., Tokyo. The animals were housed in an air-conditioned room kept at 23 °C and 60% relative humidity with a 12-h light and 12-h dark cycle.

Experimental Procedure Thirty-nine mice were given the diet containing 0.035% TAA for 40 weeks, after which they were divided into groups 1 and 2. Twenty-four mice of group 1 were then given the basal diet for 48 weeks, and 15 mice of group 2 were given the diet containing 1% FA for the same period. The other 15 mice formed the control group and were maintained on the basal diet throughout the feeding period. At the end of the feeding schedule, all animals were killed and their livers were examined histologically. Tissue samples for microscopic examination were fixed in a 10% formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Results and Discussion

None of the 15 animals in the untreated control group showed macroscopic or microscopic changes in the liver. Neoplastic and histologic changes in the livers of the 2

experimental groups were as follows:

Group 1 The effect of feeding with TAA was examined in the 24 animals, and hepatic carcinomas had developed in 11 of them (Table I). Histologically, all were diagnosed as hepatocellular carcinomas (Fig. 1a and 1b). Hyperplastic nodules of hepatocytes were prominent (Fig. 1c), while the proliferation of fibrous and ductal cells was slight. Liver cirrhosis was noted only in 2 animals (Fig. 2d).

Group 2 The inhibitory effect of FA on the carcinogenesis was marked in this group fed this compound after ingestion of TAA. No hepatic carcinomas had developed in any of the 15 animals (Table I). Hyperplastic nodules of hepatocytes were noted in 2 animals and liver cirrhosis was noted in 1. Other livers showed no marked distortion in the lobular architecture. Pleomorphism and anisocytosis of hepatocytes, irregularly contoured nuclei and increase of nuclear chromatin were seen in most livers, but these alterations in the cellular architecture were ameliorated by the administration of FA.

Although most carcinogenic and biochemical studies of TAA were carried out on rats,⁷⁻¹³⁾ a limited number of studies on mice suggested that mouse liver was more susceptible to the carcinogenic effect of TAA than rat liver.^{14,15)} These earlier findings were confirmed by our studies: the incidence of tumor induction in ICR mice (45.8%, Table I) was almost double that in DONRYU rats (22%) fed TAA under the same feeding schedule.⁷⁾ There were other differences in the histological findings between mice and rats. First, the hepatic tumors that had developed in mice were exclusively hepatocellular carcinomas, while those in rats were a mixture of hepatocellular carcinomas and papillary adenocarcinomas. Second, a number of lesions of cirrhosis and cholangiofibrosis were constantly found in the livers of rats, while liver cirrhosis was found in only 2 of 24 mice. Third, the enlargement of hepatocyte nucleoli by TAA was striking in rats but not in mice. The enlargement of hepatocyte nucleoli as well as liver cirrhosis in rats fed TAA are assumed to be toxic symptoms rather than precursory alterations in the hepatocarcinogenesis. Detailed studies should also be made on mice to elucidate the modes of action of TAA in the hepatocarcinogenesis.

We previously found that the administration of FA effectively suppressed hepatocarcinogenesis in rats that had been fed either a diet containing 0.06% 3'-methyl-4-(dimethylamino)-azobenzene for 50 d⁶⁾ or a diet containing 0.035% TAA for 40 weeks.⁷⁾ In the present study, such an

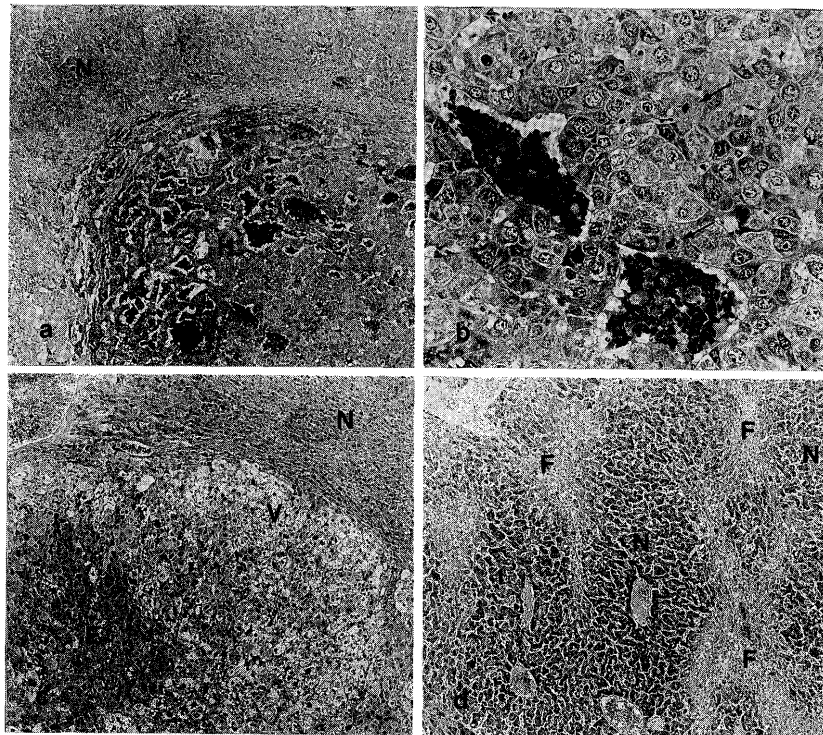


Fig. 1. Micrographs of Mouse Liver Tissues

a) Hepatocellular carcinoma in mouse of group 1. Hepatocellular carcinoma (H) compresses surrounding parenchyma (N). $\times 60$. b) Higher magnification of micrograph-a. Mitotic figures (arrows) are seen. $\times 300$. c) Hyperplastic nodule of hepatocytes in mouse of group 1. The nodule consists of vacuolated cells (V) and eosinophilic cells (E). $\times 60$. d) Liver tissue from mouse of group 1. Cirrhosis. Islands of liver cells (N) are surrounded by proliferating fibers (F). $\times 60$.

TABLE I. Inhibitory Effect of FA on Induction of Hepatocellular Carcinomas and Hyperplastic Nodules of Hepatocytes in Livers of Mice fed TAA

Group	No. of mice	Body weight (g) ^{a)}	Liver weight (g) ^{a)}	No. of mice with:		
				Hepatocellular carcinoma ^{b)}	Hyperplastic nodules of hepatocytes ^{b)}	Cirrhosis
Control	15	48.1 \pm 7.5	2.2 \pm 0.2 ^{c)}	0	0	0
1	24	39.9 \pm 4.5	5.7 \pm 2.8	11 (45.8)	13 (54.2)	2
2	15	42.0 \pm 5.8	2.5 \pm 0.7 ^{c)}	0 ^{d)}	2 (13.3) ^{e)}	1

a) Mean \pm S.D. b) Numbers in parentheses are percentages. c) Significant at $p < 0.01$ by Student's *t*-test as compared with body or liver weight in group 1. d, e) Significant at $p < 0.01$ (d) and $p < 0.05$ (e) by Fischer's exact probability test as compared with incidence of hepatocellular carcinomas or hyperplastic nodules of hepatocytes in group 1.

anticarcinogenic activity was also shown in mice whose livers were more susceptible to the carcinogenic effect of TAA than were rat livers. Since FA was given to the animals after cessation of the intake of a carcinogen, FA inhibited the development of carcinomas not as much by affecting the metabolism of the carcinogen but, rather, by affecting the progression of carcinogenesis. FA has various kinds of activities supposedly related to the present finding: it inhibits the growth of subcutaneously transplanted Ehrlich tumors in mice,¹⁾ and it reduces the toxicity of mitomycin C, aflatoxin B₁, and some carcinogens.^{3-5,16)} TAA has been reported to demonstrate no interaction with cellular DNA,^{8,11-13)} and FA has been found to exert no effect on repair DNA synthesis of hepatocytes.⁵⁾ Therefore, it is unlikely that FA inhibits TAA carcinogenesis by the repair of genomic damage. On the other hand, FA enhances *de novo* DNA synthesis of hepatocytes in the liver to counteract the toxicity of a hepatotoxin, whereas such activity of FA is not shown in the hepatic cancer cells growing in abdominal ascites.⁵⁾ One possible explanation of the mode of action

of FA is that it stimulates the proliferation of normal or intact hepatocytes in the liver thus enhancing the elimination of damaged or altered hepatocytes.

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