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Effect of 1,2-Dimethylhydrazine and Hydrogen Peroxide for the Duodenal Tumorigenesis in Relation to Blood Catalase Activity in Mice

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ABSTRACT

Three different mouse strains, C3H/C_s^b, C3H/HeN and B_6C_3 (C57BL x C3H) F_1, having low, high and moderate catalase activities, were studied for duodenal tumorigenesis by the combined treatment with 1,2-dimethylhydrazine (DMH)* and hydrogen peroxide (HPO). DMH alone rarely induced duodenal tumors. Administration of HPO into 3 different mouse strains induced different frequencies of duodenal tumors ; 91.7% in C3H/C_s^b, 9.5% in C3H/HeN and 31.8% in $B_6C_3F_1$ mice. The incidence of duodenal tumors was significantly increased to 52.6% and 93.8% both in C3H/HeN and $B_6C_3F_1$ mice by the combined administration of DMH and HPO. These increases in duodenal tumor were inversely correlated with the finding that administration of DMH or HPO alone or combined treatment of DMH and HPO significantly decreased mean blood catalase activities both in C3H and $B_6C_3F_1$ mice.

Oral administration of hydrogen peroxide (HPO) almost always induced duodenal tumors in mice[5] and in rats[4]. It may be effective to induce duodenal tumors by its direct action on duodenal mucosa, though the tumor yield was small[5]. The location of tumor sites is restricted to the proximal part of the duodenum between the pyloric ring and Vater's papilla. The incidence of duodenal tumors induced by HPO was inversely correlated with catalase activity in mice[6].

Small intestinal tumorigenesis has been reported by multiple s.c. injections of DMH in mice[1-3] and in rats[9,13]. A single injection of DMH at a dose of 60 mg/kg of body weight has been elucidated on the increased DNA synthesis in both small intestine and colonic glands, and occurrence of colonic tumors by multiple injection of DMH[10]. Reports on the induction of duodenal tumors are rare[12,14].

In the present experiment, we have examined a combined effect of DMH and HPO on the duodenal tumorigenesis in 3 genetically different strains of mice, and catalase activities in these mice were measured.

MATERIALS AND METHODS

Animals: Female inbred C3H and $B_6C_3F_1$ mice were obtained from Charles River Japan, Inc., Kanazawa and hypocatalasemic mice C_s^b, a mutant of C3H/C_s^a, were propagated by sibling matings in our laboratory.

*Abbreviations: HPO, hydrogen peroxide; DMH, 1,2-dimethylhydrazine; C3H, C3H/HeN; $B_6C_3F_1$, (C57BLxC3H)F_1; C_s^b, C3H/C_s^b.

Chemicals: Mice were given 0.4% HPO dissolved in distilled water, starting at 6 weeks of age, throughout the experimental period. The control mice received distilled water. 1,2-dimethylhydrazine dihydrochloride (DMH, Nakarai Chemicals, Ltd., Kyoto) adjusted to pH 7.5 with 7% w/v $NaCO_3$ solution was injected s.c. on the back at dose for a total of 1.2 mg per mouse by splitting 0.4 mg for 3 times in 2 weeks interval at starting 6 weeks of age. Mice were terminated 6 month after the start of the experiment.

Pathology: At autopsy, the intestinal tract were cut open, extended on the cardboard, fixed in 10% neutral formalin diluted in 0.05 M phosphate buffered saline. The fixed specimens were stained with alkaline phosphatase to identify the individual lesion on the mucosal surface of the intestine. After the lesions were recorded, the proximal duodenum was cut off between the pyloric ring and Vater's papilla, weighed and made for pathological studies.

Blood catalase activity: The heparinized blood samples were obtained from the axillary artery at the time of sacrificing the animals. Whole blood of 0.1 ml was mixed with 4.9 ml distilled water, and hemolyzed blood samples were immediately frozen by immersing in dry-ice alcohol and kept at −80C for up to a month prior to enzyme assay. Catalase activity was determined by the method as described previously[3]. Briefly, the reaction mixtures containing 0.5 ml of sample and 24.5 ml of a substrate solution composed of 5.4 ml of 3% H_2O_2, 7.48 g of $Na_2HPO_4{:}2H_2O$ and 3.55 g of KH_2PO_4 in 1 liter of distilled water. One ml of this mixture was mixed with 4 ml of 4% titanium sulfate solution and incubated for 24 hr at room temperature. The absorbance was measured at 415 nm and catalase activity was expressed in terms of the reaction constant (k)[11] and protein content was determined according to the method of Lowry et al[8].

RESULTS

Incidence of duodenal tumors and duodenal weights: All mice were terminated at 6 months after initial treatment. In the present experiment either control of DMH alone did not induce duodenal tumor in any strain of mice except a single tumor in C_s^b with DMH (Table 1).

Table 1. Incidence of duodenal tumors, duodenal weights and blood catalase levels in mice treated with DMH and HPO

Strain	Treatment	No. of mice examined	Duodenal tumor		Duodenal weight[b] (mg)	Blood catalase level ($x10^{-4}$, mg ± S.E.)
			No. of mice (%)	Mean No. of tumor per mouse[a]		
C3H/HeN	Control	11	0 (0)	0	17.0 ± 0.1	9.0 ± 0.2[o]
	HPO	21	2 (9.5)[c]	0.1 ± 0.07[g]	36.1 ± 1.2[k]	5.5 ± 0.3[p]
	DMH	22	0 (0)	0	20.3 ± 1.0	5.9 ± 0.5[q]
	DMH+HPO	19	10 (52.6)[d]	0.7 ± 0.3[h]	41.3 ± 1.9[l]	3.7 ± 0.6[r]
$B_6C_3F_1$	Control	12	0 (0)	0	25.4 ± 1.4	7.6 ± 0.1[s]
	HPO	22	0 (31.8)[e]	0.4 ± 0.1[i]	29.9 ± 0.9[m]	ND
	DMH	21	0 (0)	0	21.0 ± 0.2	5.3 ± 0.1[t]
	DMH+HPO	16	15 (93.8)[f]	2.0 ± 0.3[j]	39.3 ± 1.7[n]	3.1 ± 0.5[u]
C3H/C_s^b	Control	28	0 (0)	0	26.0 ± 0.1	2.5 ± 0.3
	HPO	24	2 (91.7)	2.6 ± 0.4	45.8 ± 1.6	2.7 ± 0.2
	DMH	20	1 (5.0)	0.1 ± 0.1	27.3 ± 1.5	2.0 ± 0.3
	DMH+HPO	18	18 (100.0)	4.0 ± 0.5	51.0 ± 2.9	2.5 ± 0.2

[a]Mean ± S.E. of duodenal tumor per mouse was calculated on the basis of total number of mice examined.
[b]Mean ± S.E. of duodenal wet weight was expressed as mg per 10g body weight.
c vs d; $p<0.01$ (π^2-test). e vs f; $p<0.001$.
k vs l; $p<0.05$ (t-test). g vs h; q vs r; $p<0.01$.
i vs j, m vs n, o vs p, o vs q, s vs t, s vs u; $p<0.001$.
ND: not determined.

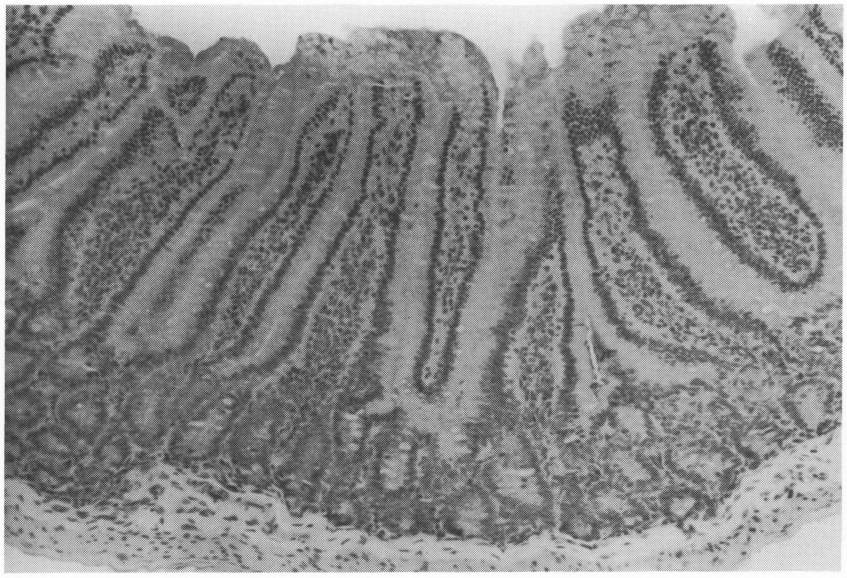

Fig. 1. Diffuse hyperplasia at the duodenum in a female C_s^b mouse who received 0.4% hydrogen peroxide for 12 weeks. H. E. ×100

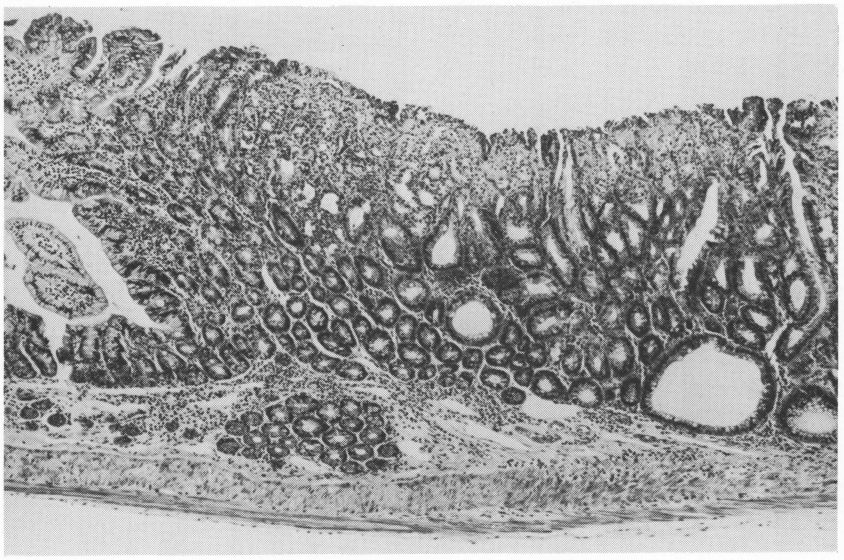

Fig. 2. Duodenal tumor at the duodenum in a female $B_6C_3F_1$ mouse who received 1.2 mg DMH and 0.4% HPO for 6 month. H. E. ×40

Incidence of duodenal tumors by HPO alone was 91.7% in C_s^b, but it was 9.5 and 31.8% in C3H and $B_6C_3F_1$, respectively. By a combined treatment of DMH and HPO, however, it increased to 5.5 times in C3H and 2.6 times in $B_6C_3F_1$ mice. Accordingly, the mean number of tumors per mouse significantly increased from 0.1 to 0.7 in C3H and 0.4 to 2.0 in $B_6C_3F_1$ mice. Duodenal weights were also significantly increased in mice treated with a combination of DMH and HPO compared to that of either HPO or DMH alone. Increase in duodenal weights was reflected by the localized or diffuse hyperplasias of duodenal villi. These hyperplastic changes

were composed of proliferation of elongated villi with some fusions of those villi (Figs. 1, 2). No invasive nor metastasizing carcinoma were found in the present experiment.

Catalase activity: Catalase activity in blood was measured at the time of termination of the experiment. In C3H mice given either DMH or HPO alone catalase activity was decreased significantly from those of control mice ($p<0.001$). Furthermore, a combined treatment of DMH and HPO significantly decreased catalase activities compared to those of DMH alone.

DISCUSSION

Oral administration of hydrogen peroxide into 3 genetically different strains of mice with various catalase activities induced different frequencies of duodenal tumors; highest in C^b_s, moderate in $B_6C_3F_1$ and lowest in C3H. These results confirmed the previous report that there is an inverse relationship between the occurrence of duodenal tumor induced by HPO and catalase activities[6].

The administered dose of DMH in the present study induced only 5% of duodenal tumor in C^b_s mice and nothing in the other two mouse strains. In combination with HPO, however, incidence of duodenal tumors increased significantly 5.5 times in C3H and 2.6 times in $B_6C_3F_1$ compared to those of HPO alone in the respective mouse strains. These results indicate that administered dose of DMH was synergistically tumorigenic with HPO in the duodenum. Furthermore, there were corresponding decrease of blood catalase levels in C3H and $B_6C_3F_1$ mice given DMH alone or DMH plus HPO even after 5 month of DMH administration. The decrease of blood catalase activities may be caused by the inhibiting action of DMH in the liver enzymes[12].

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