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Modifying Effects of Soybean Trypsin Inhibitor on Development of Eosinophilic Nodules and Basophilic Foci in the Exocrine Pancreas of Male Sprague-Dawley Rats Treated with 4-Hydroxyaminoquinoline 1-Oxide

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Administration of 4-hydroxyaminoquinoline 1-oxide (HAQO) to rats results in development of 2 types of pancreatic acinar lesions, namely eosinophilic nodules and basophilic foci. To cast light on the biological character of these lesions, 5-week-old male Sprague-Dawley rats were given a single intravenous injection of HAQO at a dose of 7 mg/kg and, thereafter, fed soybean trypsin inhibitor (SBTI) at dose levels of 10% and 5%. At week 57, all rats were killed for pathological examination of pancreatic tissue. The incidence of eosinophilic nodules was significantly higher in the HAQO/SBTI group than in the HAQO-alone group, whereas the basophilic acinar foci were observed to occur less frequently and to be smaller in the HAQO/SBTI-treated animals. Administration of SBTI is known to increase the blood level of cholecystokinin, a trophic factor for pancreatic acinar cells. Thus, the present findings suggest that long-term elevation of this endocrine factor can affect the two types of pancreatic acinar lesions in essentially different ways, namely enhancing development of eosinophilic nodules, while suppressing the occurrence of basophilic foci.

Key words: Soybean trypsin inhibitor — Rat — Carcinogenesis modification — Pancreatic acinar cell focus — HAQO

Long-term feeding of soybean trypsin inhibitor (SBTI)[2] to rats is known to stimulate the growth and physiological function of exocrine pancreas, resulting in hypertrophy, hyperplasia and eventually development of acinar cell tumors.[1-3] We confirmed that feeding of an SBTI diet to Sprague-Dawley rats for 4 weeks results in a significant increase of pancreatic volume in association with enlargement of acini, hypertrophy of acinar cells and an increase in intracellular zymogen granules.[4] The trophic influence of SBTI in the pancreas appears to be species-specific, since such effects were not clearly observed in hamsters, mice, dogs, calves, pigs or monkeys.[3-5] Administration of SBTI is also known to increase serum levels of cholecystokinin (CCK) in rats,[6,7] and thus, the observed enhancement of pancreatic growth after SBTI has been considered to be due to increased levels of this hormonal factor.[7]

Neoplastic lesions of the exocrine pancreas can be experimentally induced in rats by various carcinogens, such as 4-hydroxyaminoquinoline 1-oxide (HAQO),[8] azaserine,[9] 3,2′-dimethyl-4-aminobiphenyl (DMAB)[10] and N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine.[11] Histopathological studies have revealed that two types of acinar pancreatic lesions occur in these animal models, namely, eosinophilic nodules, which are believed to be precursor lesions for malignant tumors, and basophilic foci, which mimic an age-associated degenerative change in the exocrine pancreas.[12]

The present study was performed to assess whether SBTI differentially affects these 2 different types of pancreatic acinar lesions in rats treated with HAQO.

MATERIALS AND METHODS

Animals Five-week-old male Sprague-Dawley rats (Japan SLC, Inc., Shizuoka), initially weighing about 150 g, were used. The animals were housed, five per wire cage, in an air-conditioned room at 23±2°C and 60±5% humidity under a daily cycle of alternating 12-h periods of light and darkness.

Chemicals and diets 4-Hydroxyaminoquinoline 1-oxide HCl was obtained from Iwai Chemicals (Tokyo). SBTI, containing about 400 units/g as determined by the benzoyl-arginine-*p*-nitroanilide-hydrocloride (BAPA) method, was kindly provided by Fuji Oil Co., Ltd. (Osaka).

Oriental MF (Oriental Yeast Co., Ltd, Tokyo) was used as the basal powder diet.

Experimental protocol Ninety rats were divided into six groups as shown in Fig. 1. In groups 1 and 2, animals

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[2] Abbreviations: SBTI, soybean trypsin inhibitor; HAQO, 4-hydroxyaminoquinoline 1-oxide; CCK, cholecystokinin; DMAB, 3,2′-dimethyl-4-aminobiphenyl.

were given a single intravenous injection of HAQO dissolved in 0.005 *N* HCl into the saphenous vein at a dose of 7 mg/kg body weight in 0.3 ml of solution and thereafter fed either basal diet or the same diet supplemented with SBTI at a concentration of 10% (group 1; HAQO/10% SBTI) or 5% (group 2; HAQO/5% SBTI). In group 3 (HAQO group), animals only received the i.v. HAQO injection. In groups 4 and 5, animals were given an equivalent amount of the acidic aqueous solution without carcinogen and fed 10% and 5% SBTI in the diet. Group 6 (control) animals received the acidic aqueous solution and then the basal diet.

At week 52 all surviving rats were killed under ether anesthesia and the entire pancreas tissues were removed, fixed in 10% buffered formalin, trimmed free of fat, weighed, and then divided into two portions, the head portion/duodenal lobe and the splenic lobe. These two portions were routinely embedded in paraffin, and cut sections were stained with hematoxylin and eosin for histological study.

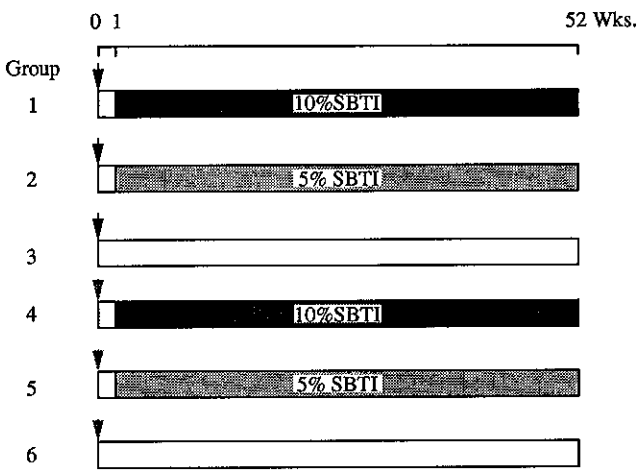

Fig. 1. Experimental design. Animals: male SD rats. ▼: 7 mg/kg HAQO (i.v.). ▼: vehicle.

Acinar cell foci of the pancreas were histologically classified as eosinophilic nodules and basophilic foci according to the criteria of Longnecker *et al.*[9] and Rao *et al.*[13] The mean numbers of nodules and foci per cm^2 of histological sections were measured with the aid of an image processor (TAS plus, Leitz, Germany).

The body and pancreatic weights and numbers of the two types of pancreatic lesions were compared among the groups using the paired Student's *t* test.

RESULTS

The data for body and pancreatic weights are summarized in Table I. Final body weights in the SBTI-treated groups were smaller than the HAQO and control group values ($P<0.01$). In contrast, the pancreas tissues of the SBTI-treated groups were increased in weight ($P<0.01$). At autopsy, multiple grayish pancreatic nodules, measuring from 1 to 3 mm in diameter were noted in the HAQO/10%SBTI and HAQO/5%SBTI group animals. These nodules appeared to involve all lobes of the organ. Such macroscopic nodular lesions were not apparent in the pancreas tissues of the HAQO, 10%SBTI, 5%SBTI or control group (see Fig. 2 for diagrammatic summary of quantitative data).

Microscopically, these nodules were shown to have histological features corresponding to the eosinophilic nodules described earlier,[9, 13] the component cells being arranged in acinar patterns. The nuclei were round to oval in shape, larger in size than those of the adjacent normal acinar cells and localized in the basal areas of the cells. Mitotic figures were occasionally noted. Poikilonucleosis with distinct nuclear outlines was frequently

Table I. Body and Pancreatic Weights and Numbers of Eosinophilic Nodules and Basophilic Foci of Male SD Rats Treated with 4-Hydroxyaminoquinoline 1-Oxide (HAQO) Followed by Soybean Trypsin Inhibitor (SBTI)

Treatment	Effective no. of rats	Final body weight (g)	Pancreatic weight (g)	Eosinophilic nodules (No./cm^2)	Basophilic foci (No./cm^2)
HAQO/10%SBTI	27	$504\pm61^{a)}$	$5.37\pm1.54^{a)}$	$5.40\pm3.22^{a,b)}$	$0.12\pm0.59^{a)}$
HAQO/5%SBTI	26	$548\pm69^{a)}$	$3.94\pm0.57^{a)}$	$4.68\pm3.47^{a,b)}$	$0.78\pm2.09^{a)}$
HAQO	26	666 ± 84	2.69 ± 0.46	$0.57\pm0.82^{b)}$	$20.15\pm18.83^{b)}$
10%SBTI	18	$502\pm62^{b)}$	$3.82\pm0.67^{b)}$	$0.13\pm0.18^{b)}$	0
5%SBTI	14	$585\pm52^{b)}$	$3.34\pm0.38^{b)}$	0.09 ± 0.24	0
Control	19	721 ± 78	2.50 ± 0.36	0.01 ± 0.02	0.14 ± 0.17

Values are represented as means $\pm$ SD.
a) Significantly different from the HAQO group at $P<0.01$.
b) Significantly different from the control group at $P<0.01$.

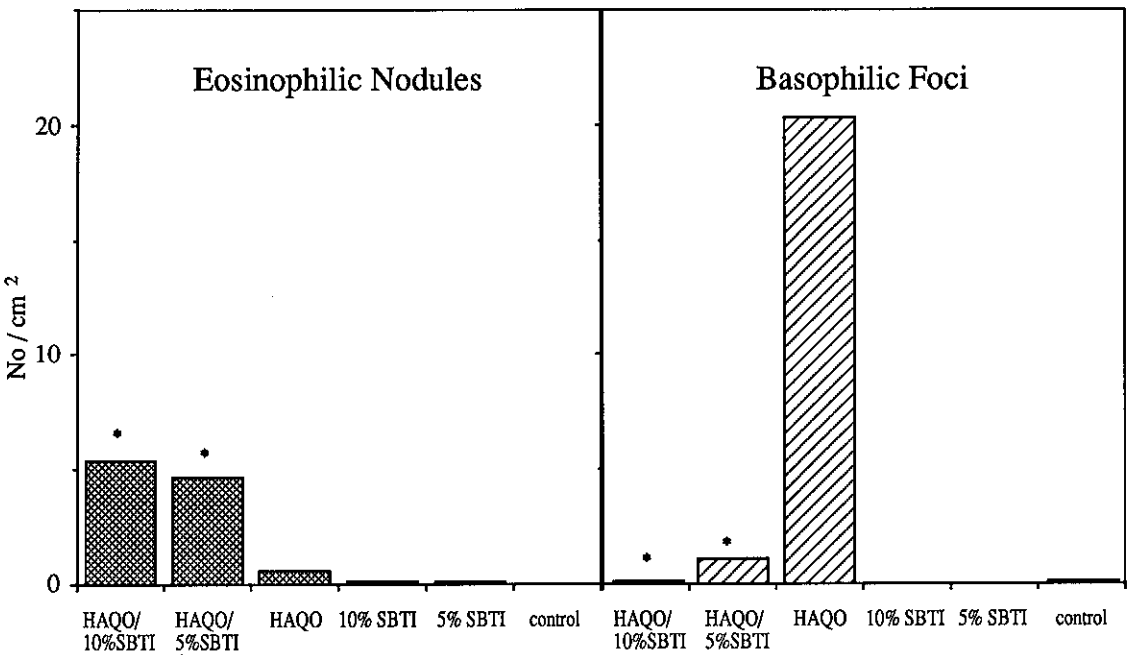

Fig. 2. Numbers of eosinophilic nodules and basophilic foci in rats fed SBTI for 51 weeks after initiation with HAQO. *: $P<0.001$ when compared with HAQO group.

seen in the larger nodules. The cytoplasm was filled with zymogen granules which were strongly stained with eosin. These nodules compressed the surrounding pancreatic tissues, appearing to form pseudocapsules, but fibrous capsules were not seen.

In the HAQO group, a large number of acinar cell foci with histological features corresponding to those of basophilic foci were apparent.[9, 13] These foci were a few to several acini in size. The component cells were larger than the adjacent normal acinar cells and arranged to exhibit acinar patterns. The cytoplasm was strongly basophilic and contained a small number of zymogen granules only faintly stained with eosin. The nuclei were irregular in size and shape, parabasally located, distinctly contoured with marginal chromatin and contained prominent nucleoli. Mitotic figures were not apparent.

Data for numbers of eosinophilic nodules and basophilic foci per cm^2 of histological sections are summarized in Fig. 2 and Table I. The frequency of eosinophilic nodules was significantly increased in the HAQO/10%SBTI and HAQO/5%SBTI groups as compared to the HAQO group value ($P<0.01$). In contrast, the number (per cm^2) of basophilic foci in rats of the HAQO/10%SBTI and HAQO/5%SBTI groups was significantly decreased as compared to the HAQO group value ($P<0.01$). A few basophilic foci were also observed in the control group rats.

DISCUSSION

The present experiments confirmed that dietary administration of SBTI dose-dependently increases pancreatic weight in both HAQO-treated and -untreated rats. It was further shown that the development of eosinophilic nodules was more pronounced in the HAQO/SBTI groups than in the HAQO-alone group, whereas the basophilic acinar foci occurred less frequently in the SBTI-treated rats. Camostat, a synthetic trypsin inhibitor, was also reported to modify pancreatic acinar lesions of rats treated with azaserine in a similar way. Thus, the numbers of basophilic foci were lower and those of acidophilic atypical acinar cell nodules were higher in the camostat-fed rats than in the controls.[14]

HAQO, a proximate carcinogenic intermediate of 4-nitroquinoline-1-oxide is both toxic and tumorigenic for rat pancreas, and a single administration of this compound is known to induce high yields of acinar cell tumors in rats after a period of 400 days.[8, 12] Therefore, the earlier appearance and higher frequency of acinar cell tumors after sequential administration of HAQO and SBTI indicate that feeding of SBTI can stimulate the development of HAQO-induced neoplastic acinar cell lesions. It has been proposed that SBTI acts on the pancreas indirectly by inhibiting trypsin within the lumen of the small intestine.[2, 6] Intraluminal trypsin is consid-

ered to inhibit pancreatic secretion by blocking the release of hormones which stimulate the pancreatic acinar cells, such as CCK, by a negative feedback mechanism in the rat.[2, 6] It has therefore been hypothesized that CCK mediates the enhancing effects of SBTI on the development of acinar cell tumors in rats. Since raw soy flour failed to modify the carcinogenic effects of azaserine in the mouse pancreas,[15] significant species differences also exist with regard to this dietary influence. In human cases, there are contrary reports concerning this feedback mechanism in patients with pancreatic disorder. For example, Ihse *et al.* reported luminal feedback regulation in pancreatic cancer patients,[16] whereas Slaff *et al.* reported that such regulation was not observed in patients with chronic pancreatitis.[17] These results indicate that further investigation is required to establish whether luminal feedback regulation exists in humans or not.

It is clear that two types of acinar lesions, eosinophilic nodules and basophilic foci, can be induced in rats by HAQO or azaserine. A general consensus from earlier studies is that basophilic foci usually stop growing, whereas some eosinophilic nodules continue to expand and eventually become neoplastic.[13, 18] Focal basophilic cellular changes are therefore apparently not part of the same spectrum of proliferative lesions as eosinophilic hyperplasias and adenomas.[19] DMAB also induces pancreatic acinar foci, which could similarly be divided into two phenotypically different populations, one eosinophilic and the other basophilic. Although the basophilic foci were found more frequently than the eosinophilic types, all of the acinar nodules and adenomas were found to be composed of eosinophilic acinar cells.[10] Furthermore, the spontaneous basophilic focus described in the aged F344 male rat is cytologically similar to the "basophilic focus" developing in the pancreas of HAQO-treated male rats.[12] Although the number of eosinophilic nodules was elevated by feeding of SBTI, that of basophilic foci was decreased. This is interesting in view of Lhoste *et al.* indication of basophilic foci 'regression' concomitant with stimulation of growth of eosinophilic nodules in camostat-fed rats.[14]

While the underlying mechanisms remain unclear, the present findings, together with other data, suggest that a long-term elevation of CCK can affect the two types of acinar cell lesion in essentially different ways, namely enhancing development of eosinophilic nodules, the precursor lesion of adenomas, but suppressing the occurrence of focal basophilic foci, which mimic age-associated degenerative disorders.

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