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Effects of Cigarette Smoke on *N*-Nitrosobis(2-oxopropyl)amine-induced Pancreatic and Respiratory Tumorigenesis in Hamsters

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Influences of cigarette smoke on *N*-nitrosobis(2-oxopropyl)amine (BOP)-induced pancreatic duct and respiratory tract tumorigenesis were investigated using a hamster two-stage carcinogenesis model. Male 5-week-old hamsters were divided into 5 groups. Group 1 was s.c. injected with BOP at a dose of 10 mg/kg once a week for 3 weeks as an initiation treatment together with cigarette smoke exposure over the same 4-week period. Group 2 was exposed to cigarette smoke for 26 weeks after the BOP-initiation. Groups 3 and 4 were respectively given the BOP-initiation alone and the 26-week cigarette smoke exposure without initiation. Group 5 served as a sham-smoked negative control. The experiment was terminated 30 weeks after the first BOP injection. The incidence of pancreatic adenocarcinomas was significantly decreased in Group 1 as compared to the Group 3 value ($P<0.01$) while the Group 2 value did not show any change. In contrast, the incidence of laryngeal and tracheal proliferative lesions (hyperplasias and papillomas) was significantly increased in Group 2 over Group 3 ($P<0.01$). The incidence of pulmonary hyperplasias was also increased in Group 2 over Group 3 ($P<0.05$), although that of pulmonary adenomas or adenocarcinomas was decreased in Group 2 as compared to the Group 3 value ($P<0.01$). Cigarette smoke exposure in the BOP-initiation phase (Group 1) did not affect the development of respiratory proliferative lesions. No animals in Groups 4 and 5 developed any tumors in the pancreas or respiratory tract. Our results thus indicate that cigarette smoke exposure inhibits pancreatic carcinogenesis when given in the initiation phase, whereas it modulates (enhances or suppresses) the development of proliferative lesions in the respiratory tract if applied during the promotion stage to hamsters pretreated with BOP.

Key words: Cigarette smoke — Pancreas — Respiratory tract — BOP — Hamster

Epidemiological studies have suggested that cigarette smoking is closely associated with increased risk of cancers in various organs such as the lung, oropharynx, pancreas and stomach.[1-3] It has been shown that even a single dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a strong tobacco-specific nitrosamine, can induce respiratory tract tumors in hamsters, but smoke inhalation does not result in an increase in respiratory tract tumor incidence in NNK-initiated animals.[4] However, we recently found that cigarette smoking exerts promoting effects on upper respiratory tract tumorigenesis in hamsters initiated with diethylnitrosamine.[5] Nevertheless, experimental influences of cigarette smoking on tumorigenesis in organs other than the respiratory tract have not been elucidated.

A carcinogenic nitrosamine, *N*-nitrosobis(2-oxopropyl)amine (BOP), preferentially induces pancreatic ductal cancers in hamsters.[6] Because of the histological and biological similarities to human tumors, the hamster-BOP pancreatic carcinogenesis model has been extensively applied to assess modifying effects of chemicals on pancreatic carcinogenicity.[7] BOP also has the ability to induce tumors of the lung, liver and kidney of hamsters,[8] and it has been shown that the Syrian golden hamster is particularly suitable for examining the effects of carcinogenic substances which may target the respiratory tract.[9] In the present study, the effects of cigarette smoke exposure, in either the initiation or promotion stage, on pancreatic as well as respiratory tract tumorigenesis were examined in hamsters treated with BOP.

MATERIALS AND METHODS

One hundred and ten male 5-week-old Syrian golden hamsters were obtained from Sankyo-Labo Service (Tokyo). They were housed, five animals per plastic cage, under standard laboratory conditions (room temperature, 23±2°C; relative humidity 60±5%; a 12 h/12 h light/dark cycle), and were given basal diet (Oriental MF, Oriental Yeast Co., Tokyo) and tap water *ad libitum*. Non-filter cigarettes were purchased from Japan Tobacco Co. Ltd. (Tokyo).

Animals in Groups 1–3, each consisting of 30 hamsters, were initiated with BOP (Nacalai Tesque Inc., Kyoto), dissolved in physiological saline just before administration and s.c. injected once a week for 3 weeks at a dose of 10 mg/kg body weight (see Fig. 1 for experimental protocol). Group 1 was simultaneously given

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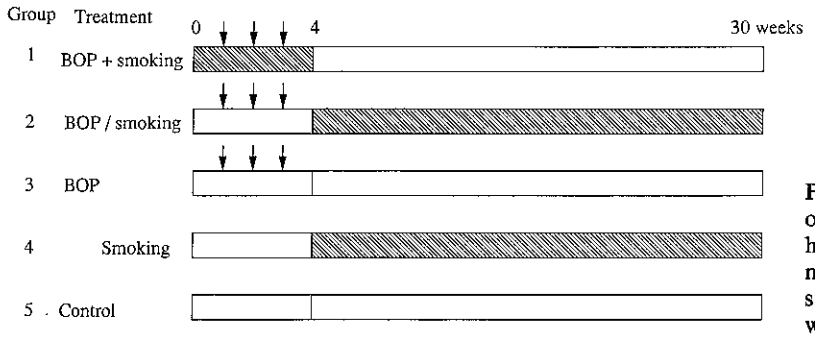

Fig. 1. Protocol for assessing effects of smoking on BOP-induced tumorigenesis in Syrian golden hamsters (5-week-old males). Cigarettes: a commercially available brand; ↓: BOP (10 mg/kg, s.c.); ▨: smoking (9 min × 2/day, 5 days/week).

cigarette smoke exposure for 4 weeks. Cigarette smoke was transnasally administered 5 days a week by using a Hamburg type II smoking machine (Borgwaldt, Germany) under the following conditions: exposure period, 9 min/time; exposure frequency, twice/day; doses, 30 cigarettes/time; inhalation volume, 35 ml; inhalation flow, 17.5 ml/s; dilution of cigarette smoke, 1/7. After BOP-initiation, Group 2 received cigarette smoke exposure for the following 26 weeks under the same conditions as Group 1. Group 3 served as a BOP-positive control. Ten animals in Group 4 were treated with cigarette smoke alone for 26 weeks, similarly to Group 2. Another 10 animals in Group 5 served as non-treatment controls. Animals killed upon becoming moribund or found dead were autopsied for histological examination. At the 30th week of the experiment, all surviving animals were killed under ether anesthesia. At autopsy, the pancreatic and respiratory tissues (larynx, trachea and lung) were carefully removed and routinely processed for histological examination.

Statistical significance of inter-group differences was evaluated using Fisher's exact probability test and Student's t test.

RESULTS

As shown in Fig. 2, long-term exposure to cigarette smoke in the promotion phase caused marked growth retardation regardless of whether with or without BOP-initiation. Short-term smoking exposure in the initiation phase also caused growth retardation, but the final body weights were comparable to those of non-treatment controls. The BOP treatments did not adversely influence the body weights of hamsters as compared to matched controls without BOP-initiation, and in fact values were a little increased. Except for 2 animals found dead in the early stage of the experiment, all others were included in the effective number; most of them were killed at the termination and a few were killed after becoming moribund several days before the termination.

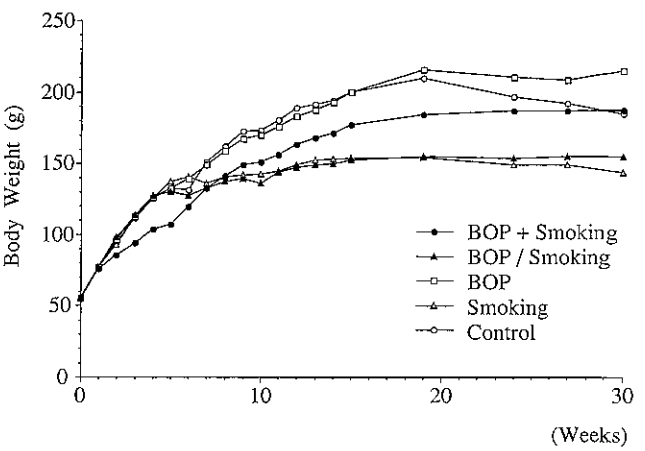

Fig. 2. Body weights of hamsters exposed to cigarette smoke during initiation or promotion phase of BOP-induced carcinogenesis.

Pancreatic precancerous and cancerous lesions induced by BOP in hamsters were judged as dysplastic lesions and carcinomas, as defined previously.[10] Table I summarizes data on the effects of cigarette smoke in terms of pancreatic proliferative lesions. Smoke exposure in the initiation stage significantly decreased the incidence and multiplicity of pancreatic carcinomas ($P<0.01$ and $P<0.05$), whereas smoking in the promotion stage did not influence the development of carcinomas or dysplastic lesions. Cigarette smoke itself did not induce such proliferative lesions in the pancreas. Table II shows the distribution of pancreatic proliferative lesions. The number of carcinomas per animal in the gastric lobe was significantly reduced by simultaneous smoking exposure in the initiation phase ($P<0.05$).

Preneoplastic and neoplastic lesions in the larynx or trachea were diagnosed as papillary hyperplasias and papillomas.[5] Pulmonary proliferative lesions were classified as hyperplasias and neoplasias (adenomas or adeno-

Table I. Effects of Cigarette Smoke on Pancreatic Proliferative Lesions in Hamsters Treated with BOP

Group	Effective no. of animals	Incidence of (%)		No. of lesions/animal	
		Carcinomas	Dysplastic lesions	Carcinomas	Dysplastic lesions
1. BOP+smoking	29	5 (17.2)**	20 (68.9)	0.24±0.64[a]*	1.38±1.57
2. BOP/smoking	29	12 (41.3)	23 (79.3)	0.77±1.22	1.53±1.31
3. BOP	30	17 (56.6)	19 (63.3)	0.80±1.03	1.40±1.50
4. Smoking	10	0	0	0	0
5. Control	10	0	0	0	0

a) Mean±SD.
*, **: Significantly different from Group 3 (* $P<0.05$, ** $P<0.01$).

Table II. Distribution of BOP-induced Pancreatic Proliferative Lesions in Hamsters

Group	Splenic lobe		Gastric lobe		Duodenal lobe		Head	
	Adc[a]	Dys	Adc	Dys	Adc	Dys	Adc	Dys
1	0.13±0.44[b]	0.27±0.64	0.03±0.18*	0.31±0.66	0	0	0.07±0.26	0.79±0.68
2	0.30±0.70	0.33±0.60	0.13±0.34	0.40±0.67	0.03±0.18	0.03±0.18	0.27±0.45	0.77±0.73
3	0.33±0.66	0.30±0.74	0.26±0.52	0.46±0.86	0.03±0.18	0.03±0.18	0.17±0.38	0.60±0.72

a) Adc: adenocarcinoma; Dys: dysplastic lesion.
b) Mean±SD.
: Significantly different from Group 3 ($P<0.05$).

Table III. Effects of Cigarette Smoke on Proliferative Lesions in the Larynx/Trachea and Lungs of Hamsters Treated with BOP

Group	Effective no. of animals	No. of hamsters with			
		Larynx/trachea lesions (%)		Lung lesions (%)	
		Hyp[a]	Pap	Hyp	Ad/Adc
1	29	2 (7)	2 (7)	6 (21)	22 (76)
2	29	11 (38)**, ##	3 (10)	13 (45)*	14 (48)**, #
3	30	2 (7)	1 (3)	5 (17)	24 (80)
4	10	0	0	0	0
5	10	0	0	0	0

a) Hyp: hyperplasia; Pap: papilloma; Ad: adenoma; Adc: adenocarcinoma.
*, ** : Significantly different from Group 3 (* $P<0.05$, ** $P<0.01$).
#, ## : Significantly different from Group 1 (# $P<0.05$, ## $P<0.01$).

carcinomas). Table III summarizes data on the effects of cigarette smoke on the development of proliferative lesions in the larynx and trachea or the lung. Smoking exposure in the promotion phase significantly increased the incidence of papillary hyperplasias in the larynx and trachea as compared to values for Groups 1 or 3 ($P<0.01$). Smoking in the promotion phase also showed a tendency to increase the incidence of papillomas in the larynx and trachea, although this effect was not statistically significant. Long-term promotion-phase exposure also significantly ($P<0.05$) increased the incidence of pulmonary hyperplasias while significantly ($P<0.01$) decreasing the incidence of pulmonary neoplasias. Simultaneous smoking exposure in the initiation phase did not modulate the development of proliferative lesions either in the larynx and trachea or in the lung. No proliferative lesions were induced by smoke inhalation alone.

DISCUSSION

The results of the present study are equivocal in demonstrating a negative influence of cigarette smoke, i.e., promotive effects were found for upper respiratory tract tumorigenesis, but a blocking influence was exerted

on initiation of pancreatic ductal carcinogenesis in hamsters with BOP. While long-term cigarette smoke exposure remarkably reduced body weight gain, this influence on growth was not considered sufficient to have essentially altered the direct effects of cigarette smoke in the promotion phase, because reduction in weight is generally associated with decreased tumor yields.

While the underlying mechanisms await elucidation, the presently observed promotive effects of cigarette smoke on the development of proliferative lesions in the upper respiratory tract are consistent with our previous results on cigarette smoke enhancement of tumorigenesis in the upper respiratory tract of hamsters initiated with diethylnitrosamine.[5] One possibility regarding the mechanism is a direct action of irritating substances such as nicotine and formaldehyde included in cigarette smoke, because of the lack of any systemic effect on pancreatic carcinogenesis when inhaled during the promotion phase in this study. Nicotine is unstable, but it has been demonstrated that topical application of this irritant enhances skin tumorigenesis in mice initiated with benzo[*a*]pyrene (B[a]P).[11] Formaldehyde has also been found to have a promoting activity on glandular stomach carcinogenesis in rats after initiation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and sodium chloride.[12] A second possibility is involvement of known complete carcinogens such as NNK, *N'*-nitrosonornicotine (NNN) and B[a]P, which exert tissue-specific promotion effects as well as initiation activities with or without metabolic activation. An additional factor worthy of mention is alveolar macrophages, which have the ability to clean inhaled cigarette smoke by endocytosis.[13] Their activation by smoking is known to yield oxygen radicals and proteolytic enzymes.[13]

In the present study, cigarette smoke clearly modulated the yield of proliferative lesions in the lung when administered in the promotion phase. However, the situation is complicated by an apparent lesion dependence of response with pancreatic carcinomas being decreased in the initiation phase, in clear contrast to the decrease of pulmonary adenomas or adenocarcinomas in the promotion phase, although an increase was evident in the incidence of pulmonary hyperplasias. It has been reported that progression to malignancy involves a decline in the frequency of hyperplasias and an increase in the frequency of adenomas during NNK treatment in A/J mice.[14] If a similar relationship holds for hamsters, the smoke exposure might in fact have exerted an inhibitory effect on pulmonary tumorigenesis in the present study.

The observation of an anti-initiation influence of cigarette smoke on BOP-induced pancreatic ductal carcinogenesis was an unexpected result. The most likely possibility is that some disturbance of metabolic enzymes, especially those of phase I, might have been caused by smoke inhalation. NNK, a tobacco-specific nitrosamine, has been found to induce pancreatic cancers in a long-term bioassay using rats.[15] Recently, it has also been reported that NNK can transplacentally induce pancreatic tumors in hamsters.[16] Both BOP and NNK need metabolic activation to exert mutagenicity or carcinogenicity[17] and their alkylating metabolites might be the direct causative agents.[18] At the same time, they would be expected to induce drug metabolic enzymes and, in fact, alterations in these by cigarette smoke have been demonstrated in the lung and liver of rats.[19] In addition, a case-control multicenter study on lung cancer patients revealed a pronounced effect of tobacco smoke on the metabolism of xenobiotics and on prooxidant state.[20] Such alterations in xenobiotic metabolism could have affected the pancreatic carcinogenicity of BOP.

In conclusion, it should be borne in mind that the modifying effects of cigarette smoke on carcinogenic action are complicated in practice. The results of the present study do not provide unequivocal evidence of enhancement but suggest that an influence in the promotion stage may be more important than in the initiation phase, when extrapolated to the human environment.

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