Initiating Activity of Diethylnitrosamine in a Rapid Production Model for Pancreatic Carcinomas in Syrian Hamsters

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The potential initiating activity of diethylnitrosamine (DEN) was studied in a rapid production model for pancreatic carcinogenesis in hamsters developed in our laboratory incorporating the principle of selection based on resistance to cytotoxicity, originally demonstrated for liver carcinogenesis in rats. Female Syrian golden hamsters were given DEN at a dose of 100 mg/kg body weight or N-nitrosobis-(2-oxopropyl)amine (BOP) at a dose of 70 mg/kg body weight as initiators followed by 3 cycles of augmentation pressure (choline-deficient diet combined with DL-ethionine, L-methionine upon return to basal diet and then administration of 20 mg/kg body weight BOP), and killed 10 weeks after the beginning of the experiment. DEN followed by the augmentation pressure induced a 65% incidence of total pancreatic lesions including 15% carcinomas, while BOP followed by the augmentation pressure induced 100% incidence of total pancreatic lesions and 84.2% for carcinomas. These yields were significantly greater than those observed for augmentation pressure alone. The results thus indicate that DEN possesses weak initiating activity for pancreatic carcinogenesis under the present experimental conditions.

Key words: Diethylnitrosamine — Pancreatic carcinogenesis — Rapid production model — Hamster

Recently, we established a rapid production model for pancreatic carcinomas[1, 2] based on the principle of selection pressure developed from liver studies.[3, 4] In this model, N-nitrosobis(2-oxopropyl)amine (BOP),[4] a well-known pancreatic carcinogen, was given as an initiator, followed by repeated augmentation pressure, the term adopted for the selection or promoting procedure, which consists of a toxic DL-ethionine supplementation on a choline-deficient (CD) diet step, switch to L-methionine to assist compensatory regeneration and low-level exposure to BOP to inhibit cell division in non-initiated populations. Pancreatic ductal lesions including carcinomas could be induced within only 10 experimental weeks after appropriate BOP initiation. The model is thus advantageous for detection of potential initiating activity of environmental substances for pancreatic carcinogenesis, since little is known about etiological factors involved in human pancreatic cancer development.

Diethylnitrosamine (DEN), a well known hepatocarcinogen which exerts strong initiation activity for liver carcinogenesis in rats[5-7] and mice,[8-10] also possesses strong carcinogenicity for the upper respiratory tract in hamsters.[11, 12] Parsa *et al.*[13] reported that dimethylnitrosamine (DMN), a related nitroso compound, can induce cancerous changes in adult human pancreas in organ-culture for 10 weeks, and that cells derived from DMN-treated explants produce multiple nodules of carcinoma when injected subcutaneously into nude mice. This suggests that N-nitroso compounds may be causal factors for human pancreatic carcinogenesis.

In the present experiment, we studied the potential initiating activity of DEN for pancreatic carcinogenesis in hamsters in our rapid production model. Since in the BOP initiation case cholangiocellular lesions were also induced, an investigation of liver tissue was also included.

MATERIALS AND METHODS

Animals A total of 101 female Syrian golden hamsters (Japan SLC Inc., Hamamatsu) aged 6–7 weeks and weighing about 100 g at the commencement were used. The hamsters were housed five per plastic cage on tips, in an air-conditioned room at 24°C and 60% humidity with a daily 12 h alternating cycle of light and dark, and given food and tap water *ad libitum*. Commercially available

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[4] Abbreviations: BOP, N-nitrosobis(2-oxopropyl)amine; DEN, diethylnitrosamine; CD diet, choline-deficient diet.

basal Oriental MF (Oriental Yeast Co. Ltd., Tokyo) and Lombardi CD (Dyet Inc., Pennsylvania, USA) diets were purchased and stored under refrigeration.

Chemicals DL-Ethionine and L-methionine were purchased from Nacalai Tesque Inc. (Kyoto) and DEN was supplied by Wako Pure Chemical Industries Ltd. (Osaka). BOP was kindly donated by Dr. Yukio Mori (Laboratory of Radiochemistry, Gifu Pharmaceutical University, Gifu). Each of the chemicals was dissolved in sterilized saline immediately before usage.

Experimental groups Hamsters were divided into 5 groups according to the treatments shown in Fig. 1. As the initiation step, either 100 mg/kg body weight DEN or 70 mg/kg body weight BOP was injected intraperitoneally (i.p.) or subcutaneously (s.c.), respectively. Eleven days after the initiation, the hamsters were given 500 mg/kg body weight daily i.p. injections of DL-ethionine while being fed CD diet *ad libitum*. Basal diet was restored and the hamsters received an i.p. injection of 800 mg/kg body weight of L-methionine on the fifth day after the first injection of DL-ethionine. Two days later, the hamsters were given a 50 mg/kg body weight injection of DEN i.p. or 20 mg/kg body weight of BOP s.c. This procedure, consisting of administration of DL-ethionine while on CD diet, L-methionine and DEN or BOP injection, is termed one augmentation pressure cycle.

Group 1 received 100 mg/kg body weight of DEN for initiation followed by 3 simple 50 mg/kg body weight DEN treatments. Group 2 received 100 mg/kg body weight of DEN for initiation followed by 3 augmentation pressure cycles with 50 mg/kg body weight of DEN. Group 3 received 3 augmentation pressure cycles with 20 mg/kg body weight of BOP, without initiation. Groups 4 and 5 received 100 mg/kg body weight of DEN or 70 mg/kg body weight of BOP, respectively, for initiation followed by 3 augmentation pressure cycles with the 20 mg/kg body weight of BOP. All surviving hamsters were killed 10 weeks after the initiation.

Histological examination The pancreas and liver tissues were removed, weighed and examined histologically. The tissues were fixed in 95% ethanol containing 1% acetic acid for 2 h followed by ethanol overnight at 4°C, embedded in paraffin and stained with hematoxylin and eosin. The pancreatic tissues were flattened on a filter paper to avoid shrinkage on fixation, then divided into the three lobes — splenic, gastric and duodenal — and horizontal slices were taken from each. From the liver, slices were taken through the 3 main lobes including the gall-bladder.

Pancreatic lesions were diagnosed according to the criteria described by Pour and Wilson[14] and Kozuka *et al.*[15] Liver lesion diagnosis was based on the criteria described by Bannasch *et al.*[16] The numbers of lesions
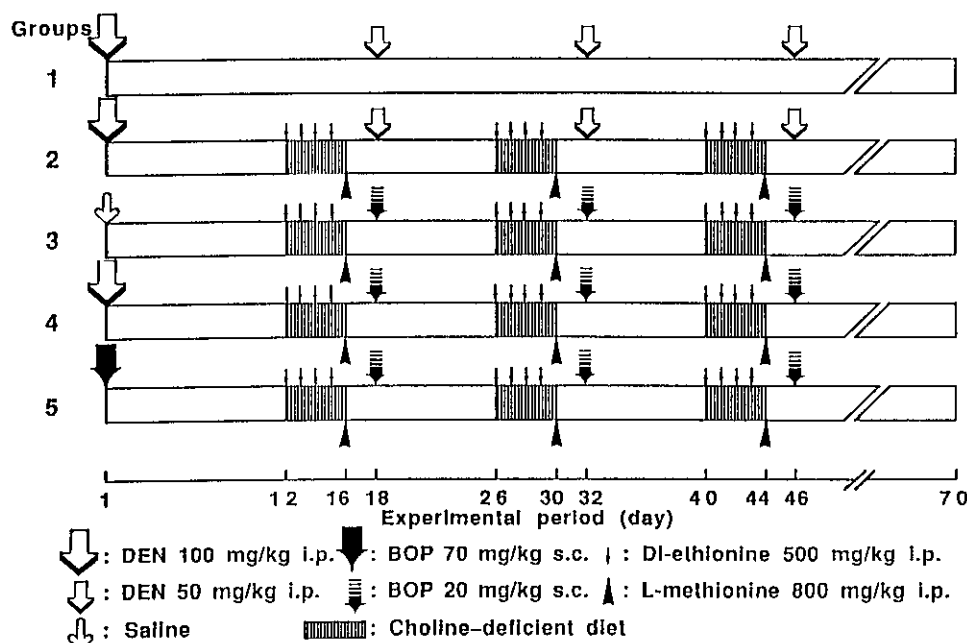

Fig. 1. Experimental design for studying initiation activity of DEN for pancreatic carcinogenesis in hamsters.

Table I. Experimental Details and Body, Pancreas and Liver Weights of Hamsters

Group	Treatment	Number of hamsters		Body weight[a] (g)		Relative organ weight[a] (g/100 g body wt.)	
		Initial	Effective	Initial	Final	Pancreas	Liver
1	DEN(100, 50×3)	20	20	96.8±7.9	162±19	0.46±0.07	4.09±0.23[c]
2	DEN(100, 50×3) +Et+Met+CD	21	20	97.1±6.4	127±14[b]	0.49±0.10	4.44±0.66[c]
3	Saline+BOP(20×3) +Et+Met+CD	19	19	97.0±7.4	133±16[b]	0.48±0.07	3.78±0.26
4	DEN(100)+BOP(20×3) +Et+Met+CD	21	20	96.9±7.1	126±13[b]	0.53±0.09	4.03±0.35[c]
5	BOP(70, 20×3) +Et+Met+CD	19	19	96.4±6.4	120±13[b, d]	0.54±0.07[c]	3.84±0.26

a) Data are shown as means±SD.
b) Significantly different from group 1, $P<0.001$.
c) Significantly different from group 3, $P<0.05$.
d) Significantly different from group 3, $P<0.01$.
e) Significantly different from group 3, $P<0.001$.

Table II. Incidences and Numbers of Pancreatic Lesions

Group	Effective number of hamsters	Incidence (%)				Mean number of pancreatic lesions/hamster[a, b]
		Total pancreatic lesions[a]	Ductal hyperplasias	Atypical ductal hyperplasias	Ductal carcinomas	
1	20	0 (0)	0 (0.0)	0 (0)	0 (0)	0.00±0.00
2	20	4 (20.0)	4 (20.0)	0 (0)	0 (0)	0.20±0.40[c]
3	19	4 (21.0)	2 (10.5)	3 (15.8)	0 (0)	0.32±0.73
4	20	13 (65.0)[d]	8 (40.0)	5 (25.0)	3 (15.0)	1.00±0.95[d]
5	19	19 (100.0)[e]	19 (100.0)[e]	16 (84.2)[e]	17 (89.5)[e]	7.16±3.76[f]

a) Pancreatic lesions include hyperplasia, atypical hyperplasia and carcinoma categories.
b) Data are shown as means±SD.
c) Significantly different from group 1 at $P<0.05$.
d) Significantly different from group 3 at $P<0.05$.
e) Significantly different from group 3 at $P<0.01$.
f) Significantly different from group 3 at $P<0.001$.

were counted under the microscope and the data were statistically analyzed by the chi-square test for incidences and Student's t test for multiplicities of lesions.

RESULTS

Experimental details Initial and effective numbers of hamsters, their body weights and relative pancreas and liver weights are summarized in Table I. One hamster each from groups 2 and 4 died, presumably due to the toxicity inherent in the augmentation pressure as found in a previous study.[1] Final body weights of hamsters from groups 2–5, which received augmentation pressure, were decreased as compared to those of hamsters from group 1. Relative pancreas weight increased due to tumor development in hamsters from group 5, and relative liver weights were slightly increased in hamsters from groups 1, 2 and 4 as compared with those of group 3.

Incidence and numbers of pancreatic and liver lesions
Incidences and numbers of pancreatic lesions are shown in Table II. All pancreatic lesions observed were of ductal origin and were histologically divided into hyperplasia (Fig. 2), atypical hyperplasia (Fig. 3) of duct cells and carcinomas (Figs. 4 and 5). No acinar cell and islet cell lesions were observed in any of the hamster groups. No pancreatic lesions developed in hamsters from group 1. Hyperplasias were evident in hamsters from groups 2–5 and the incidence reached 100% in group 5 animals.

Fig. 2. Hyperplasia in a hamster from group 4. Metaplasia of normal cuboidal duct epithelial cells to tall columnar cells and partial multilayering are evident. (×600)
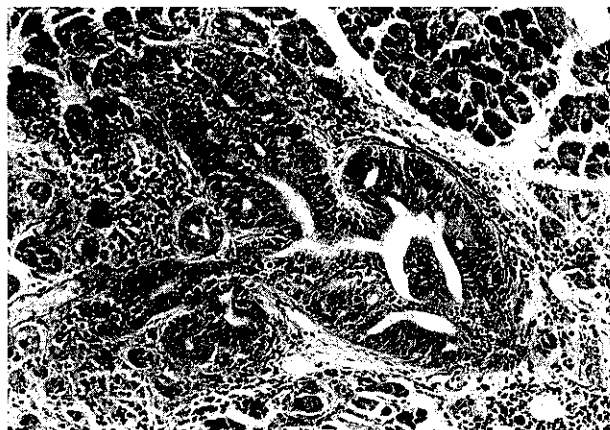

Fig. 4. Intraductal carcinoma in a hamster from group 4. Cribriform pattern and bridge formation are evident. Note atypical cells and mitotic figures. (×500)
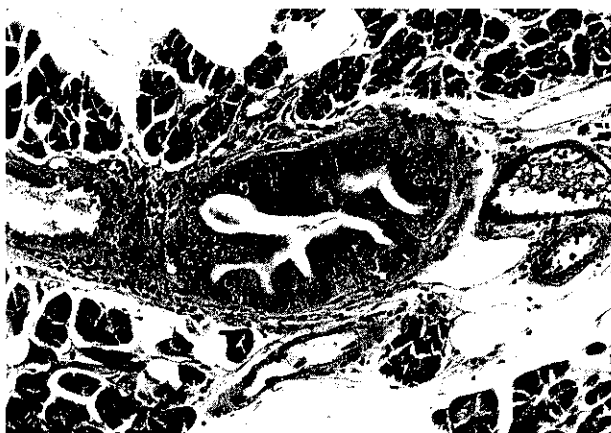

Fig. 3. Atypical hyperplasia which developed in a hamster from group 4. Epithelial cells show significant multilayering and papillary proliferation. (×600)
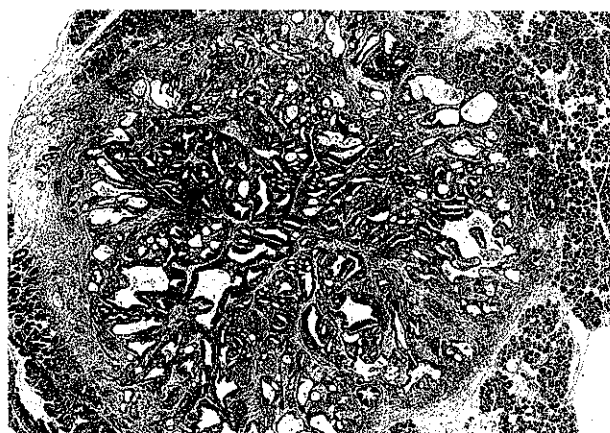

Fig. 5. Invasive carcinoma in a hamster from group 5. (×80)

Atypical hyperplasias developed in hamsters from groups 3-5, and carcinomas were found in animals from groups 4 and 5. The incidences of total pancreatic lesions in hamsters from groups 4 and 5 were significantly increased as compared to the appropriate control group 3 value. Mean numbers of pancreatic lesions increased significantly in hamsters of groups 4 and 5. Histopathologically, the carcinomas which developed in hamsters from groups 4 and 5 were all of tubular duct cell type (Figs. 4 and 5).

Observed liver lesions are summarized in Table III. Bile duct proliferation was evident in 1 out of 20 hamsters (5%) from group 2, and in 14 out of 19 hamsters (73.7%) from group 5. Two cholangiocellular adenomas and four cholangiocellular carcinomas were also found in hamsters from group 5. No hepatocellular or gall bladder lesions developed in any of the hamster groups.

DISCUSSION

It is evident from the present result that DEN possesses a weak initiation activity for pancreatic carcinogenesis in hamsters. The dose of DEN used in the present experiment was chosen on the basis of pilot study

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Table III. Incidence of Liver Lesions

Group	Effective No. of hamsters	Incidence (%)		
		Bile duct proliferation	Cholangiocellular adenomas	Cholangiocellular carcinomas
1	20	0 (0)	0 (0)	0 (0)
2	20	1 (5)	0 (0)	0 (0)
3	19	0 (0)	0 (0)	0 (0)
4	20	0 (0)	0 (0)	0 (0)
5	19	14 (73.7)[a]	2 (10.5)	4 (21.1)

a) Significantly different from group 3 at $P<0.01$.

findings which revealed more than 100 mg/kg body weight to be toxic under the present experimental conditions (unpublished data). Compared to the repeated 4, 2, 1 or 0.5 mg/kg body weight injections used for studies of upper respiratory tract carcinogenesis in hamsters,[12] the level of carcinogen exposure was relatively high. Nevertheless the initiating activity of DEN for pancreatic carcinogenesis was far weaker than that of BOP, and indeed, our previous findings showed that even 30 mg/kg body weight of BOP given as the initiation step induced a 55% incidence of pancreatic adenocarcinomas.[2]

The present initiating activity of DEN for pancreatic carcinogenesis was clearly dependent on repeated augmentation pressure for expression. Similarly, it has been reported that N-methyl-N-nitrosourea (MNU), a direct multi-target carcinogen, or 1,2-dimethylhydrazine (DMH), a colon carcinogen, when given intraperitoneally as an initiator followed by or prior to partial hepatectomy with selection procedure are capable of inducing putative preneoplastic altered liver cell foci,[17] suggesting that lack of detectable hepatocarcinogenic initiating activities for these agents under normal conditions is due to the absence of an appropriate promotion stimulus. Further studies are clearly required to determine what roles the augmentation pressure plays in the expression of initiating activity of DEN for pancreatic carcinogenesis. Ductal cell proliferation could be relatively specifically induced by the augmentation pressure when the animals were initiated with BOP, but not without initiation (unpublished data). Whether the same thing could happen under DEN initiation would be an interesting point for further study. Moreover, an evidence that the augmentation pressure consisting of BOP administration but not that of DEN was required for the expression of the initiating activity of DEN would provide another clue.

The present results thus support the findings by Parsa *et al.* that DMN, another N-nitroso compound, can produce cancerous changes in organ-cultured adult human pancreas[13] and embryonal rat pancreas[18] despite their hitherto negative carcinogenicity for this tissue *in vivo*. That DMN and DEN are considered to be distributed in the environment, being detectable in commercially available foodstuffs such as bacon or smoked meat,[19] is of clear importance in this context. Even more importantly, perhaps, many nitroso compounds can be synthesized endogenously from the precursor in the presence of nitrate under acidic conditions in the gut.[20]

In conclusion, the present results strongly suggest that exposure to exogenous or endogenous N-nitroso compounds may play a role in the development of pancreatic cancer in man. However, further assessment of interspecies variation and tissue-specific response, especially in the light of the demonstrated lack of initiating activity for carcinogenesis in the biliary system, appears warranted. Further investigation regarding the augmentation pressure mechanism is under way in our laboratory.

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