Acta Medica et Biologica
Vol. 42, No. 2, 71-78, 1994

A High Fat Diet Containing Large Amounts of Saturated Fatty Acids Induced in Hamsters by Oral Administration of N-nitroso-bis (2-oxopropyl) Amine Suppresses Hepatic and Biliary Tract Carcinogenesis

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Received February 22, 1994

Summary. **Tumorigenesis of the hepatic and biliary tract with N-nitroso-bis (2-oxopropyl) amine (BOP) was examined following the application of a high fat diet to hamsters. The animals were classified into four groups according to diet and BOP application: first, a standard diet; second, a high fat diet; third, BOP with a standard diet; fourth, BOP with a high fat diet. The high fat diet was rich in saturated fatty acids. No neoplasm was detected in any of the animals treated for 10 weeks, nor in the first and second groups of animals treated for 20 weeks. In the third group of animals, carcinogenic reaction appeared in the intra- and extrahepatic bile ducts and in the gallbladder. In the fourth group of animals, carcinogenic reaction was induced in the intrahepatic and upper extrahepatic bile ducts. However, the incidence of carcinogenic reaction which developed in the lower extrahepatic bile duct and gallbladder was greater in the third group of animals than in the fourth group.**

These results suggest that a high fat diet containing large amounts of saturated fatty acids suppresses carcinogenesis in the lower extrahepatic bile tract.

INTRODUCTION

It has been shown that both the volume and composition of diet affect the incidence and development of neoplasms in several tissues; for example, the restriction of food and calorie intake results in the inhibition of tumorigenesis.[1,2] Moreover, it was demonstrated that a high fat diet increased carcinogenesis of the hepatic and biliary tract and shortened the life span of the experimental animal.[3-5] It has also been recently observed that carcinogenesis can be determined by the essential fatty acids in the fat, the degree of their unsaturation and the location of the unsaturation.[6-8] Hepatic and biliary tract neoplasms have been able to be induced experimentally by the administration of N-nitroso-bis [2-oxopropyl] amine (BOP) in hamsters and guinea pigs.[9-11] However, the effects of a high fat diet containing large amounts of saturated fatty acids on tumorigenesis of the hepatic and biliary tract tissues with BOP have not previously been examined.

The experiments in this series were designed to investigate in hamsters whether a high fat diet influences tumorigenesis of the hepatic and biliary tract tissues with BOP.

MATERIALS AND METHODS

Experiment I

Animals

Seventy-five male Syrian Golden hamsters aged 6 weeks (Seiwa Experimental Animals, Ltd., Fukuoka) were used. They were allowed free access to a standard diet (MF diet, Oriental Yeast Co., Osaka) and tap water, and were adapted to the breeding room for a week before use. Room conditions were maintained as follows: temperature, 22±2℃; humidity, 55±15%; light: dark cycle, 12 h (lighting from 08 : 00 to 20 : 00 h). The experiment was started when the animals reached 7 weeks of age.[12] The individual body weight, food and water intake were recorded between 10 : 00 and 12 : 00 h daily.

Study design

At first the hamsters were divided into two groups according to the experimental periods: 39 animals for 10 weeks and 36 animals for 20 weeks. Then they were further classified into four groups according to diet and BOP application: first, a standard diet (Table 1); second, a high fat diet (Table 1, butter was used as fat)[13]; third, a standard diet with BOP; fourth, a high fat diet with BOP.

Dietary schedule

Animals in the first and third groups received a standard diet for either 10 or 20 weeks. Animals in the second and fourth groups received a high fat diet during the first 5 weeks of the experiment; this was switched to the standard diet during the remaining scheduled periods. The third and fourth groups of animals received N-nitroso-bis (2-oxopropyl) amine (BOP, Nacalai Tesque Inc., Kyoto) in drinking tap water at a concentration of 20 ppm. It was noted that BOP did not affect the amount of water intake during the entire experiment (Table 2).

Table 1. Composition of experimental diets.

Ingredients	Standard diet (%)	High fat diet (%)
Carbohydrate	54.5	50.0
Protein	24.0	20.0
Fat	5.1	20.0
Vitamins	a little	1.5
Minerals	6.2	5.0
Fiber	3.2	3.5
Water	7.0	a little
Calorie density (kcal/100 g)	360	450

Blood chemistry

A blood sample was taken from each animal when sacrificed. The collected blood was immediately cooled with ice water and centrifuged at 2000 rpm for 20 min. The plasma was separated and preserved at −20℃ until measurement. The following parameters showing nutrition and hepatic function were estimated with an autoanalyzer (Hitachi-736, Hitachi Co., Tokyo): total protein (TP, Biuret method); total cholesterol (Tch, cholesterol oxidase colorimetric method) and glutamic-pyruvic transaminase (GPT, ultraviolet method).[14,15]

Histopathological examination

Under ether anesthesia, the liver, bile duct, gallbladder, pancreas and duodenum of each animal were removed together from the body on the day scheduled for the examination. The specimens obtained were fixed with 10% formalin, and 5 μm thick slices were cut off at 1.0 mm intervals. The bile duct was sectioned, the proximal half of the duct referred to as the upper bile duct, and the distal half duct as the lower bile duct. The specimens were then judged according to the histopathological criteria after being prepared with hematoxylin and eosin.[9,10,16]

Analysis of data

Pathological observations were compared with Fisher's exact test, and the statistical significance of differences among values was evaluated by ANOVA and Duncan's multiple range test: $p<0.05$ was regarded as a significant difference.

Table 2. Water and BOP intake during the experimental periods.

		I	II	III	IV
	10W	(n=10)	(n=9)	(n=10)	(n=10)
	20W	(n=9)	(n=10)	(n=8)	(n=9)
Water	10W	867±41	711±17[a]	957±35	868±35
(ml)	20W	2124±91	1869±74[b]	1829±78	1749±58
BOP	10W			19.1±0.7	17.4±0.7
(g)	20W			41.1±1.2	36.2±2.1

The animals received a standard diet (I), high fat diet (II), standard diet with BOP (III) or a high fat diet with BOP (IV) for 10 or 20 weeks (W).
Numbers in parentheses are the numbers of specimens. Values are the means ± SE.
[a]$p<0.05$ vs I, III and IV. [b]$p<0.05$ vs I.

Experiment II

On the basis of the results obtained in Experiment I, the experiment was repeated in hamsters under the same conditions. Twenty-three animals were divided into two groups: those on a standard diet with BOP, and those on a high fat diet with BOP. Twenty weeks later, histopathological examination was made of the carcinogenic reaction in the extrahepatic lower bile duct and gallbladder.

		No. of animals examined	No. of animals presenting factor	Percent of incidence
1. INTRAHEPATIC BILE DUCT				
Cholangiocellular carcinoma	I	8	6	75
	II	9	6	67
Carcinoma *in situ*	I	8	7	88
	II	9	8	89
Adenoma	I	8	8	100
	II	9	9	100
Proliferation of bile duct	I	8	8	100
	II	9	9	100
2. EXTRAHEPATIC BILE DUCT				
a. UPPER BILE DUCT				
Invasive carcinoma	I	8	0	0
	II	9	0	0
Carcinoma *in situ*	I	8	4	50
	II	9	5	56
Atypical epithelium	I	8	6	75
	II	9	7	78
Proliferation of goblet cell	I	8	4	50
	II	9	4	44
b. LOWER BILE DUCT				
Invasive carcinoma	I	8	2	25
	II	9	0	0
Carcinoma *in situ*	I	8	5	63
	II	9	4	44
Atypical epithelium	I	8	5	63
	II	9	5	56
Proliferation of goblet cell	I	8	5	63
	II	9	7	78
3. GALLBLADDER				
Carcinoma *in situ*	I	8	1	$13^{a}_{b,c}$
	II	9	0	0
Atypical epithelium	I	8	1	13
	II	9	0	0
Proliferation of goblet cell	I	8	1	13
	II	9	1	11

Fig. 1. Incidence of hepatic and biliary tract neoplasms in animals on a standard diet and BOP (I), and in animals on a high fat diet and BOP (II). [a]$p<0.005$ vs 1 (I, carcinoma *in situ*). [b]$p<0.0002$ vs 1 (II, carcinoma *in situ*). [c]$p<0.01$ vs 2a (II, carcinoma *in situ*).

RESULTS

Experiment I

Subjects of analysis

Histopathologically, no neoplasm was detected in the visceral organs collected from any of the animals treated for 10 weeks. Histopathological changes in the hepatic and biliary tract tissues were noted in the animals treated for 20 weeks.

Distribution and incidence of neoplasm

In the first and second groups of animals, no neoplasm developed in the intra- or extra- hepatic bile ducts, gallbladder, pancreas or duodenum.

As shown in Fig. 1, a series of histopathological findings were obtained in the third group of animals: the intrahepatic bile duct tended to show a high incidence of neoplasms, and the areas affected in order of incidence were the upper bile duct, the lower bile duct and the pancreas or the gallbladder. In the intrahepatic bile duct, cholangiocellular carcinoma, carcinoma *in situ*, adenoma and bile duct proliferation were seen. There were carcinoma *in situ*, atypical epithelium, and goblet cell proliferation in the upper extrahepatic bile duct. Invasive carcinoma, carcinoma *in situ*, atypical epithelium, and goblet cell proliferation were seen in the lower extrahepatic bile duct. In the gallbladder carcinoma *in situ*, atypical epithelium and goblet cell proliferation were seen. When compared to the incidence of carcinoma *in situ*, a significantly lower incidence was seen in the lower extrahepatic bile duct.

In the fourth group of animals, almost the same occurrence and distribution of neoplasms were obtained in the intrahepatic and upper extrahepatic bile ducts as in the third group of animals. The incidence of carcinogenic reaction in the lower extrahepatic bile duct tended to be low, and in the gallbladder the incidence was significantly lower than in the intrahepatic bile duct (Fig. 1).

As to the pancreas and duodenum, in the third group of animals carcinoma *in situ*, adenoma, atypical epithelium, proliferation of the pancreatic ducts and goblet cells were seen in the pancreas, but no meaningful finding appeared in the duodenum. In

		No. of animals examined	No. of animals presenting factor	Percent of incidence
PANCREAS				
Invasive carcinoma	I	8	0	0
	II	9	0	0
Carcinoma *in situ*	I	8	1	13
	II	9	1	11
Adenoma	I	8	1	13
	II	9	0	0
Atypical epithelium	I	8	2	25
	II	9	0	0
Proliferation of pancreatic duct	I	8	7	88
	II	9	4	44
Proliferation of goblet cell	I	8	1	13
	II	9	5	56
DUODENUM				
Invasive carcinoma	I	8	0	0
	II	9	0	0
Carcinoma *in situ*	I	8	0	0
	II	9	1	11
Atypical epithelium	I	8	0	0
	II	9	0	0

Fig. 2. Incidence of neoplasms in the pancreas and duodenum in animals on a standard diet and BOP (I), and in animals on a high fat diet and BOP (II).

the pancreas of the fourth group of animals, carcinoma *in situ* and proliferation of the pancreatic duct were seen, though no adenoma or atypical epithelium was detected. No significant difference between the two groups of animals was seen in the incidence of the carcinogenic reaction (Fig. 2).

Diet and calorie intake

Diet intake during the experimental periods was unaffected by BOP administration in animals on either the high fat diet or the standard diet. Calorie intake in the animals on the high fat diet was greater than that in animals on the standard diet (Table 3).

Body weight

The body weight after 20 weeks of treatment was similar in all four groups of animals (Table 3).

Blood substances showing nutrition and hepatic function

Total protein in the blood was unaffected by BOP. Total cholesterol in animals with and without BOP was similar, though BOP increased the level of cholesterol. An increase in GPT was seen in animals on BOP (Table 4).

Experiment II

Incidence of carcinogenic reaction

Carcinogenic reaction was reproduced in the lower extrahepatic bile duct and gallbladder. However, the incidence of carcinogenic reaction at these sites was higher in animals on the standard diet than in animals on the high fat diet (Fig. 3).

DISCUSSION

Oral administration of BOP in drinking water at a concentration of 20 ppm for 20 weeks resulted in a high incidence of hepatic and biliary tract neoplasms in hamsters (Fig. 1). This is in keeping with the previous view that orally administered BOP can effectively induce hepatic and bile tract carcinoma in hamsters.[9-11]

The areas affected in order of the incidence of carcinoma were: the intrahepatic bile duct; the upper bile duct; the lower bile duct; and the gallbladder

Table 3. Food and calorie intake, and body weight.

	I (n=9)	II (n=10)	III (n=8)	IV (n=9)
Standard diet (g)	906±24	825±43	986±23	772±34
High fat diet (g)		308±20		269±8
Calorie (kcal)	3262±86	4116±148[a]	3550±83	3989±145[b]
Body weight (g)	129±4	133±3	133±5	133±5

The animals received a standard diet (I), high fat diet (II), standard diet with BOP (III) or a high fat diet with BOP (IV) for 20 weeks.
Numbers in parentheses are the numbers of specimens. Values are the means ±SE.
[a]$p<0.05$ vs I and III. [b]$p<0.05$ vs I and III.

Table 4. Blood substances showing nutrition and hepatic function.

	I (n=9)	II (n=10)	III (n=8)	IV (n=9)
TP (g/dl)	5.8±0.1	6.1±0.1	5.4±0.8	6.4±0.1
Tch (mg/dl)	198±12	148±17[a]	320±38[b]	285±19
GPT (U)	328±70	162±38[c]	479±52[d]	384±29

The animals received a standard diet (I), high fat diet (II), standard diet with BOP (III) or a high fat diet with BOP (IV) for 20 weeks.
Numbers in parentheses are the numbers of specimens. Values are the means ±SE.
[a,c]$p<0.01$ vs III and IV. [b]$p<0.01$ vs I. [d]$p<0.05$ vs I.

or the pancreas (Figs. 1 and 2). This occurrence and distribution of hepatic and biliary tract neoplasms partially corresponds to the results of Pour and his coworkers.[10]

The incidences of intrahepatic and upper extrahepatic bile duct carcinogenic reaction observed in animals on the high fat diet and BOP administration were almost the same as those in animals on the standard diet and BOP administration (Fig. 1), in spite of reports that a high fat diet increases carcinogenesis of the hepatic and biliary tract.[3-5] It is not easy to explain this finding, but several research works have revealed that carcinogenesis can be determined by the essential fatty acids in the fat, the degree of their unsaturation, and the location of the unsaturation.[6-8] Considering these reports together with the fact that the high fat diet used in this study was rich in saturated fatty acid,[13] it is possible that saturated fatty acids in the diet may play an important role in the phenomena observed. Likewise, this may confirm the finding that the incidence of carcinogenic reaction in the lower extrahepatic bile duct and gallbladder was lower in animals on a high fat diet than in animals on a standard diet (Fig. 3). In other words, the high fat diet is active in suppressing carcinogenesis in the bile tract.

In higher animals, excessive calorie intake has been considered to be the main factor in producing a tumorigenetic effect.[17,18] However, it was difficult in this study to attribute the high incidence of neoplasms in hamsters to an excessive intake of calories alone.[12] The high incidence could also be explained by the decrease in carbohydrate intake or the increase in the protein/carbohydrate ratio.[19,20] In this examination, calorie intake in animals on the high fat diet and BOP was greater than in animals on the standard diet and BOP (Table 3), and the protein/carbohydrate ratio in the high fat diet was lower than that in diets previously reported.[3,4] Diet components seem to be unrelated to regional differences observed in the incidence of carcinoma in the hepatic and biliary tract.

The nutritional condition of the animals affects the development of neoplasms.[6] In this study, diet and water intake for the experimental periods were similar in the groups of animals on BOP (Tables 2 and 3). Total protein and body weight also were similar in all four groups of animals (Tables 3 and 4). These results are in keeping with the finding that hamsters on BOP maintained body weight gain until 20 weeks of age.[12] The possibility that the regionally observed tumorigenetic difference is derived from the nutritional condition of the animals is excluded.

BOP induces pancreatic carcinogenetic reaction to a higher degree in hamsters.[21] Although it has been presumed that the carcinogenetic effect of BOP on the hepatic and biliary tract is not dependent on the density of BOP in bile juice,[10] carcinogenesis has been reproduced by BOP administration even when animals had the common bile duct resected.[22] In this study, BOP provoked a carcinogenic reaction in the pancreas, but no difference was seen in the incidence

			No. of animals examined	No. of animals presenting factor	Percent of incidence
LOWER BILE DUCT					
	IC	I	14	4	
	CIS	I	14	2	38
	AE	I	14	10	
	IC	II	9	0	
	CIS	II	9	0	15[a]
	AE	II	9	4	
GALLBLADDER					
	CIS	I	14	3	54
	AE	I	14	12	
	CIS	II	9	0	22[b]
	AE	II	9	4	

Fig. 3. Incidence of carcinogenic reaction in the extrahepatic lower bile duct and gallbladder in animals on a standard diet and BOP (I), and in animals on a high fat diet and BOP (II).
[a,b]$p<0.05$ vs I. IC: invasive carinoma; CIS: carcinoma *in situ*; AE: atypical epithelium.

of the reaction in animals which received standard and high fat diets (Fig. 2). It appears that the fat metabolism has no influence on carcinogenesis of the pancreas with BOP.

As mentioned above, recent studies have emphasized that the location of the unsaturation of essential fatty acids is all important in determining the influence of a dietary fat source on carcinogenesis.[6-8] However, because cholesterol has been shown to change mucosal permeability in response to several substances and to modulate BOP absorption,[23] and as some bile acids promote carcinogenesis,[24,25] these bile components should also be remembered in relation to the carcinogenesis regionally observed. Further study of these aspects is necessary.

In summary, a high fat diet containing large amounts of saturated fatty acids with BOP changed the incidence of carcinogenic reaction in the hepatic and biliary tracts. Although this diet did not change the incidence of carcinogenic reaction in the intrahepatic and upper extrahepatic bile ducts, the incidence of carcinogenic reaction in the lower extrahepatic bile duct and gallbladder was decreased. A high fat diet may thus suppress carcinogenesis in specific sites in the hepatic and biliary tracts.

Acknowledgements. The author is greatly indebted to Prof. Dr. K. Hatakeyama (First Dept. of Surg., Niigata Univ. Sch. of Med., Niigata) and to Dr. T. Sakaguchi (First Dept. of Physiol., Niigata Univ. Sch. of Med., Niigata) for their critical advice.

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