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Enhancing Effect of High Fat Diet on 4-Nitroquinoline 1-Oxide-induced Pulmonary Tumorigenesis in ICR Male Mice

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The effects of dietary high fat on 4-nitroquinoline 1-oxide (4NQO)-induced lung tumorigenesis were investigated in male ICR mice. Two groups of mice were initially given a single subcutaneous injection of 4NQO at a dose of 15 mg/kg and, thereafter, fed either 20% corn oil-supplemented diet or a standard basal diet. Two further groups were maintained on the high fat diet or standard diet without administration of 4NQO. Mice were killed at weeks 15, 18 and 25 and the incidence of lung tumors at each time point was found to be significantly increased in the 4NQO/high fat diet group as compared to the 4NQO/standard diet group in terms of both incidence of tumor-bearing mice and the number of lesions per mouse. The results thus indicate that dietary high fat can enhance 4NQO-induced lung tumorigenesis in mice.

Key words: Lung tumorigenesis — High fat — Enhancing effect — 4-Nitroquinoline 1-oxide — Mouse

Many dietary factors have been reported to modify carcinogenesis in different organs of man and rodents.[1] For example, high fat levels have been demonstrated to exert promotion potential in mammary,[2] colon[3] and pancreas[4] carcinogenesis in animal models and, in man, a case-control study also showed that high daily food intake (fat, protein and carbohydrate) is associated with elevated relative risk of colon cancer development.[5]

With regard to lung tumorigenesis, only a few promoters have thus far been identified, significant enhancements having been found with bleomycin,[6] butylated hydroxytoluene[7] or glycerol administration.[8] 4NQO induces high incidences of pulmonary adenomas and adenocarcinomas in mice[9] and rats,[10] and this carcinogen has therefore been used in experimental models of lung tumor development. The present investigation was aimed at elucidating the influence of dietary high fat on lung tumorigenesis in the mouse 4NQO model.

4NQO (Wako Pure Chemical Industries, Osaka) was dissolved in a mixture composed of olive oil and cholesterol (50 mg of cholesterol/ml of olive oil) at a concentration of 2 mg/ml as applied previously.[9] A total of 160 6-week-old male ICR mice (Charles River Japan Inc., Atsugi) were used in the experiment. The animals were divided into 4 equal groups: groups 1 and 2 were given a single injection of 4NQO subcutaneously at a dose of 15 mg/kg body weight whereas groups 3 and 4 received a single injection of 10 ml/kg body weight of the oil mixture without 4NQO. One week thereafter, groups 1 and 3 were placed on diet (CRF-1, Oriental Yeast Co. Ltd., Tokyo; 3.5 kcal/g) containing 20% corn oil (high fat diet; 4.7 kcal/g), the main components of CRF-1 diet and the corn oil being as follows: oleic acid (22.4%: 32.8%), linolic acid (50.2%: 51.9%), linoleic acid (4.6%:1.8%), respectively. Groups 2 and 4 were maintained on basal diet without supplement. Ten mice from each group were killed at weeks 15 and 18, and all surviving animals (19 to 20 mice per group) were killed at week 25. At each time point, the lungs were excised, weighed and fixed with 10% phosphate-buffered formalin by inflation through the main bronchus. All lobes of each lung were examined using a stereoscopic microscope (SZH-111, Olympus Ltd., Tokyo), and whitish nodules and any other lesions found were counted and recorded. Lung tissue slices were taken from each lobe, including those where lesions were visible under the stereomicroscope, embedded in paraffin, cut and stained with hematoxylin and eosin, so that all tumors were confirmed histopathologically. The numbers of lung tumors were counted under the microscope.

Statistical analyses were carried out using Student's t test, Fisher's exact probability test and Peto's trend test.[11]

Body and lung weight and total calorific intake data are summarized in Table I. The body weights in groups treated with the high fat diet (groups 1 and 3) were significantly higher than the respective control values (groups 2 and 4), at each time point. However, treatment with 4NQO did not influence the body weight. Lung weights were not significantly different between groups at

Table I. Body and Lung Weights and Total Calorific Intake of Mice Initiated with 4NQO and Administered Basal or 20% High Fat Diet

Group	Treatment	15 weeks				18 weeks				25 weeks			
		No. of mice	Weights (g)		Total calorific intake	No. of mice	Weights (g)		Total calorific intake	No. of mice	Weights (g)		Total calorific intake
			Body	Lung			Body	Lung			Body	Lung	
1	4NQO →High fat	10	50.7±6.0[a, b]	0.24±0.04	1767.9	10	51.9±6.5[b]	0.28±0.04	2113.6	20	55.4±7.7[b]	0.25±0.03	2913.5
2	4NQO	10	43.6±3.7	0.26±0.08	1533.7	10	44.1±4.3	0.29±0.04	1849.4	19	46.8±6.8	0.25±0.04	2635.3
3	Vehicle →High fat	10	52.8±5.8[c]	0.26±0.03	1807.1	10	55.5±6.0[c]	0.25±0.04	2158.7	20	59.6±7.1[c]	0.25±0.02	2995.1
4	Vehicle	10	44.7±4.0	0.25±0.03	1455.5	10	46.2±4.5	0.27±0.05	1747.2	20	48.2±4.1	0.25±0.04	2462.9

a) Mean±SD.
b) Statistically different from group 2 at $P<0.01$ by Student's *t* test.
c) Statistically different from group 4 at $P<0.01$ by Student's *t* test.

Table II. Lung Tumor Development in ICR Male Mice Initiated with 4NQO and Administered Basal or 20% High Fat Diet

Group	Treatment	15 weeks		18 weeks		25 weeks	
		Incidence (%)	No. of tumors /mouse	Incidence (%)	No. of tumors /mouse	Incidence (%)	No. of tumors /mouse
1	4NQO→High fat	5/10 (50.0)	0.80±0.98[a, b]	6/10 (60.0)	1.60±1.85	16/20 (80.0)[c]	2.40±1.96[d]
2	4NQO	1/10 (10.0)	0.10±0.30	4/10 (40.0)	0.50±0.67	11/19 (57.9)	1.21±1.54
3	Vehicle→High fat	0/10 (0)	0 (0)	1/10 (10.0)	0.10±0.30	0/20 (0)	0
4	Vehicle	0/10 (0)	0 (0)	1/10 (10.0)	0.10±0.30	2/20 (10.0)	0.10±0.30

a) Mean±SD.
b) Statistically different from group 2 at $P<0.01$ by Student's *t* test.
c) Statistically different from group 2 at $P<0.01$ by Peto's trend test.
d) Statistically different from group 2 at $P<0.05$ by Student's *t* test.

any time point. Total calorific intake of mice treated with the high fat diet (groups 1 and 3) was increased relative to the basal diet control value (groups 2 and 4).

Macroscopically, lung tumors were round or oval in shape, and were mostly localized in the peripheral areas of the lungs, being clearly demarcated from the surrounding lung tissue. Tumor cells were cuboidal or columnar in shape, sometimes demonstrating vacuolated cytoplasm and exhibiting papillary arrangement. Mitotic figures were occasionally observed, but there was no evidence of local invasion or distant metastasis. On the basis of histological appearance, all of the tumors observed were therefore diagnosed as adenomas. The incidences and the numbers of lung tumors/animal at weeks 15, 18 and 25 are summarized in Table II. The incidences of lung tumors in group 1 (4NQO — high fat) were always higher than in group 2 (4NQO), and although the differences were not significant at each time point, Peto's trend test revealed a significant difference between the two groups overall ($P<0.01$). Furthermore, the mean numbers of lung tumors per mouse were significantly different between groups 1 and 2 killed at weeks 15 and 25 ($P<0.01$ and $P<0.05$, respectively).

The present experiment thus demonstrated that dietary high fat clearly enhances the development of 4NQO-initiated mouse lung tumors. This finding is in agreement with previous data which showed that the incidences of lung adenocarcinomas and adenomas induced by benzo[*a*]pyrene or N-nitrosobis(2-oxopropyl)amine are elevated in hamsters fed high fat diet.[12, 13] Epidemiological studies also showed that there was a small direct association between fat intake and the risk of human lung cancer.[14]

The target sites of 4NQO carcinogenicity in the lung are believed to be the non-ciliated bronchiolar (Clara) or type 2 alveolar cells. 4NQO is known to cause morphologic alteration in mouse Clara cells, which are capable of metabolizing carcinogens. Type 2 alveolar cells also have the same capability.[15, 16] It is reasonable to assume that factors which modify the function of these cells might

thereby modify lung tumorigenesis. For example, glycerol, which affects Clara cell function, promotes the development of mouse lung tumors initiated with 4NQO.[8] Isoproterenol, an α,β-adrenergic stimulant, which can affect secretory activity of Clara cells[17] is also known to enhance pulmonary tumorigenesis in mice treated with 4NQO or urethane.[18]

The mechanism(s) underlying the enhancing effect of high fat diet remains unclear. One possibility is that the high calorific value itself might be responsible and another is that the different composition of fatty acids could be directly involved. Beems and van Beek[12] reported that administration of unsaturated fatty acids was associated with more lung tumors than saturated fatty acid treatment, even under isocaloric conditions. Corn oil contains ω-6 polyunsaturated fatty acids, and it was reported that tumor development is enhanced by diet high in ω-6 polyunsaturated fatty acids, but not in ω-3 fatty acids, such as menhaden oil.[19] These results suggest that fatty acids themselves modify lung tumorigenesis in addition to any effect of high calorific intake. Furthermore, it has been shown for 7,12-dimethylbenz[*a*]anthracene-induced rat mammary carcinogenesis that feeding diets high in polyunsaturated fatty acids also produces more tumors than feeding diets high in saturated fatty acids.[20] Similar findings were gained for azoxymethane-induced colon carcinogenesis.[3] The corn oil used in the present experiment contained high concentrations of many unsaturated fatty acids as mentioned in "Materials and Methods." It has been reported that high dietary linoleic acid, one important unsaturated fatty acid, can inhibit phospholipid synthesis and also alter membrane fatty acid composition.[21] The possibility that these changes could occur in Clara and/or type 2 alveolar cells in the lung, and consequently modify lung tumorigenesis, requires future investigation.

Feeding of high fat diet also causes changes in lipid peroxidation and prostaglandin and/or leukotriene synthesis, which are believed to influence tumorigenesis in different target organs.[22, 23] The effects of a prostaglandin synthesis inhibitor on 4NQO-initiated mouse lung tumorigenesis are presently under investigation in our laboratory.

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