4-ニトロキノリン1-オキサイドによってマウスに発生した肥満症の特性

誌名	日本獣醫學雑誌 = The Japanese journal of veterinary science
ISSN	00215295
著者名	水谷,武夫
発行元	日本獣医学会
巻/号	39巻2号
掲載ページ	p. 141-147
発行年月	1977年4月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

Jap. J. vet. Sci., **39**, 141～147 (1977)

Characterization of Obesity in Mice Induced with 4-Nitroquinoline 1-Oxide

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(Received for publication July 30, 1976)

Abstract. Massive and persistent obesity was induced in mice by a single intracerebral injection with 4-nitroquinoline 1-oxide (NQO). It was characterized by determining the effect of strains of mice or vehicles of NQO on the development of obesity, and by comparing the body composition, organ weight, and glucose tolerance between NQO-obese and control mice. Treatment with NQO resulted in a higher incidence of obesity in C57BL/6 mice than in ddOM, C3Hf and DBA/2 mice. A much higher obesity-inducing rate was also given by treatment with NQO prepared in 10% lecithin than in 0.5% Tween 80 or 30% dimethyl sulfoxide. The weights of the liver and heart, as well as water, protein and fat contents of the body, were significantly larger in NQO-obese mice than in controls. NQO-obese C57BL/6 mice showed to fall transiently in a state of chemical diabetes 12 weeks after treatment with NQO.

Obesity has been produced in rodents by giving injury to the hypothalamus with selective electrolytic lesioning [3, 8] or chemical agents, such as goldthioglucose (GTG) [1] and bipiperidyl mustard [10]. As a relatively simple technic for producing experimental obesity in mice [11], a single intracerebral injection with the carcinogen, 4-nitroquinoline 1-oxide (NQO) [9], has been reported. The present paper deals with the characterization of NQO obesity by determining the effect of strains of mice or vehicles of NQO on the development of obesity. In it, comparison was also made on body composition, organ weight, and glucose tolerance between NQO-obese animals and controls.

Materials and Methods

Animals: All the mice used in the present experiments were produced in this laboratory. Inbred mice of the ddOM, C57BL/6, C3Hf and DBA/2 strains and F_1 hybrid mice of BDF_1 (C57BL/6×DBA/2) and DBF_1 (DBA/2×C57BL/6) were used at 6 to 8 weeks of age. ICR mice of a closed colony were obtained from a commercial breeder (Nihon Clea Co., Tokyo). They were allowed to have water and commercial pellets (CMF; Oriental Co., Tokyo) ad libitum. They were housed in a room under controlled lighting (8:00 AM to 8:00 PM), temperature (24±1°C), and relative humidity (55±5%).

Administration with NQO: NQO was suspended to a concentration of 2 mg/m*l* in physiologic saline containing 0.5% Tween 80. Each mouse of the different strains was injected intracerebrally with 0.02 m*l* of the resulting suspension in the same manner as described previously [11]. In order to determine the effect of vehicles of NQO on the development of obesity, such amount of NQO was mixed with 10% lecithin or 30% dimethyl sulfoxide (DMSO) as for the resulting mixture to contain 0.5, 1.0 or 2.0 mg/m*l* of NQO. Then ddOM mice were given intracerebrally with 0.02 m*l* of the resulting mixture. Control animals were given with 0.02 m*l* of the vehicle used by the same route.

Organ weight and body composition: At autopsy the liver, kidney, heart and adrenals were removed and weighed rapidly. The carcass was homogenized in a Waring blender. For determination of water the homogenate was dried at 70°C until a constant

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weight was obtained. Fat was then determined by using a Soxlet reflux apparatus. The dried defatted residue was used for determination of protein by the micro-Kjeldahl method.

Glucose tolerance test: NQO-obese mice of the C57BL/6 strain was starved for 18 to 20 hours after a collection of blood sample for determination of the blood glucose level in the fed state. Then they were injected intraperitoneally with the aqueous solution of 10% (w/v) glucose in a dose of 1 mg/g body weight after the collection of blood sample for determination of the basal level of blood glucose. Blood samples were then collected 30, 60 and 120 minutes after the injection for the determination of blood glucose. The blood glucose was determined by the glucose-oxidase method (reagents; Boehringer) using 20 μl samples collected from the coccygeal vein.

Results

As shown in Table 1, treatment with NQO resulted in a higher incidence of obesity and a lower mortality in C57BL/6 mice than in mice of any other strain. In contrast, ICR mice showed a low incidence of obesity and a relatively high mortality. In the F_1 hybrid mice the obesity-inducing rate was 49% in BDF_1 and 22% in DBF_1.

Tables 2, 3 and 4 present the effect of different vehicles on the development of obesity 5 to 10 weeks after treatment with NQO. Obesity was induced in 11, 56 and 100% of the surviving mice at the concentration of 0.05, 0.1 and 0.2% of NQO, respectively, 10 weeks after administration with NQO prepared in 10% lecithin. In 0.5% Tween 80 the incidence of obesity was 26 and 45% at the concentration of 0.1 and 0.2% of NQO, respectively. Obesity was induced in 63% of the mice treated with 0.2% of NQO in 30% DMSO, but in none of the mice treated with 0.05 or 0.1% of NQO.

The organ weights of the NQO-obese and control mice of the ddOM, BDF_1 and C57BL/6 strains are given in Tables 5, 6 and 7, respectively. The liver and heart

Table 1. Incidence of obesity in different strains of mice 5 and 10 weeks after treatment with NQO

Strain of mice	Sex[1]	Initial No. of mice	5-Week			10-Week		
			No. of mice surviving	No. of obese mice	%	No. of mice surviving	No. of obese mice	%
ddOM	F	60	47	20	43	47	21	45
	M	60	41	7	17	41	7	17[2]
C57BL/6	F	50	49	32	65	49	34	69
	M	20	20	9	45	20	14	70
C3Hf	F	24	24	7	29	20	6	30
DBA/2	F	22	7	1	14	6	1	17
BDF_1[3]	F	60	46	7	15	41	9	22
DBF_1[4]	F	42	38	14	37	35	17	49
ICR	F	91	75	0	0	61	2	3
	M	40	26	1	4	24	3	13

Remarks.

NQO=4-nitroquinoline 1-oxide. NQO was suspended in physiologic saline containing 0.5% Tween 80 to a concentration of 2 mg/ml, and 0.02 ml of the resulting suspension was injected intracerebrally.

[1]: F=Female. M=Male.

[2]: 8 weeks after treatment with NQO.

[3]: F_1 hybrid (C57BL/6♀×DBA/2♂).

[4]: F_1 hybrid (DBA/2♀×C57BL/6♂).

Table 2. Incidence of obesity by NQO suspended in 10% lecithin to different concentrations

NQO conc. (%)	Dose (mg)	Initial No. of mice	5-Week			10-Week		
			No. of mice surviving	No. of obese mice	%	No. of mice surviving	No. of obese mice	%
0	0	20	19	0	0	19	0	0
0.05	0.01	20	20	2	10	19	2	11
0.1	0.02	20	16	9	56	16	9	56
0.2	0.04	20	13	9	69	12	12	100

Remarks.

NQO=4-nitroquinoline 1-oxide. NQO was suspended in sterilized water containing 10% lecithin to different concentrations, and 0.02 m*l* of the resulting suspension was injected intracerebrally to each mouse of the ddOM strain.

Table 3. Incidence of obesity by NQO suspended in 0.5% Tween 80 to different concentrations

NQO conc. (%)	Dose (mg)	Initial No. of mice	5-Week			10-Week		
			No. of mice surviving	No. of obese mice	%	No. of mice surviving	No. of obese mice	%
0	0	40	40	0	0	40	0	0
0.05	0.01	40	40	0	0	40	0	0
0.1	0.02	26	25	8	32	23	6	26
0.2	0.04	60	47	20	43	47	21	45

Remarks.

NQO=4-nitroquinoline 1-oxide. NQO was suspended in physiologic saline containing 0.5% Tween 80 to different concentrations, and 0.02 m*l* of the resulting suspension was injected intracerebrally to each mouse of the ddOM strain.

Table 4. Incidence of obesity by NQO dissolved in 30% DMSO to different concentrations

NQO conc. (%)	Dose (mg)	Initial No. of mice	5-Week			10-Week		
			No. of mice surviving	No. of obese mice	%	No. of mice surviving	No. of obese mice	%
0	0	20	19	0	0	17	0	0
0.05	0.01	20	17	0	0	17	0	0
0.1	0.02	20	19	0	0	19	0	0
0.2	0.04	19	8	5	63	8	5	63

Remarks.

NQO=4-nitroquinoline 1-oxide. NQO was dissolved in sterilized water containing 30% dimethyl sulfoxide (DMSO) to different concentrations, and 0.02 m*l* of the resulting solution was injected intracerebrally to each mouse of the ddOM strain.

were significantly larger in the NQO-obese mice than in the controls. The weight of the liver was about twice as large in the NQO-obese animals as in the controls.

Table 8 shows the body composition of the NQO-obese and control mice of the

Table 5. Organ weights in NQO-obese and control mice of ddOM strain 10 weeks after treatment with NQO

		Obese mice	Control mice	
No. of mice		6	6	
Body weight	(g)	48.1 ± 4.4	25.8 ± 2.4	
Liver	(g)	2.59± 0.66[1)]	1.23± 0.17	$p<0.001$
Kidney	(mg)	435.50±66.29	302.83±34.82	$p<0.001$
Heart	(mg)	164.58±22.16	126.50±14.08	$p<0.01$
Adrenals	(mg)	21.00± 3.76	11.75± 1.34	$p<0.001$

Remarks.

Organ weight is wet weight. NQO=4-nitroquinoline 1-oxide.

1): Mean value ± standard deviation.

Table 6. Organ weights in NQO-obese and control BDF_1 hybrid mice 14 weeks after treatment with NQO

		Obese mice	Control mice	
No. of mice		5	5	
Body weight	(g)	53.0 ± 3.6	27.4 ± 5.2	
Liver	(g)	1.84± 0.17[1)]	1.10± 0.19	$p<0.001$
Kidney	(mg)	346.60±28.36	293.40±27.91	$p<0.05$
Heart	(mg)	148.00±12.47	121.40± 5.60	$p<0.001$
Adrenals	(mg)	10.80± 1.10	6.20± 0.84	$p<0.001$

Remarks.

Organ weight is wet weight. NQO=4-nitroquinoline 1-oxide.

1): Mean value ± standard deviation.

Table 7. Organ weights in NQO-obese and control mice of C57BL/6 strain 14 weeks after treatment with NQO

		Obese mice	Control mice	
No. of mice		5	5	
Body weight	(g)	55.8 ± 1.3	23.8 ± 0.8	
Liver	(g)	2.95± 0.38[1)]	1.19± 0.06	$p<0.001$
Kidney	(mg)	343.20±11.26	258.80±19.54	NS
Heart	(mg)	142.20±16.81	103.60± 3.58	$p<0.001$
Adrenals	(mg)	8.50± 1.23	6.40± 0.89	NS

Remarks.

Organ weight is wet weight. NQO=4-nitroquinoline 1-oxide.

1): Mean value ± standard deviation. NS=Not significant ($p>0.05$).

ddOM strain 5 and 10 weeks after treatment with NQO. Water protein and fat content of the body were significantly higher in the NQO-obese animals than in the controls. The fat content was approximately three times as large in the NQO-obese animals as in the controls. It attained 40% of the body weight 10 weeks after treatment with NQO.

The results of the glucose tolerance test on the NQO-obese animals in different stages are summarized in Table 9. Blood glucose levels in the fed and basal state were much higher at 12 weeks than at 4, 8, 20 and 30 weeks after treatment with NQO. As shown in Fig. 1, the incidence

Table 8. Body composition in NQO-obese and control mice 5 and 10 weeks after treatment with NQO

		(A) Control (5)[1]	(B) Obese (5)	(C) Obese (10)	(A) vs (B)	(B) vs (C)
No. of mice[3]		5	4	4		
Body weight	(g)	22.7 ±1.8	35.5 ±3.9	46.5 ±3.7		
Water	(g)	14.56±1.306[2]	18.87±1.821	20.46±1.164	$p<0.01$	NS
	(%)	64.01±2.07	53.17±1.00	44.05±1.38		
Protein	(g)	4.43±0.320	5.28±0.443	5.71±0.370	$p<0.05$	NS
	(%)	19.52±0.75	14.89±0.35	12.29±0.35		
Fat	(g)	2.43±0.706	10.14±1.467	18.73±1.980	$p<0.001$	$p<0.001$
	(%)	10.71±2.87	28.49±2.08	40.21±1.08		

Remarks.

[1]: In parentheses is shown time in weeks after treatment with NQO.

[2]: Mean value ± standard deviation.

NQO=4-nitroquinoline 1-oxide. NS=Not significant ($p>0.05$).

[3]: ddOM strain.

Table 9. Glucose tolerance in NQO-obese C57BL/6 mice in different stages after treatment with NQO

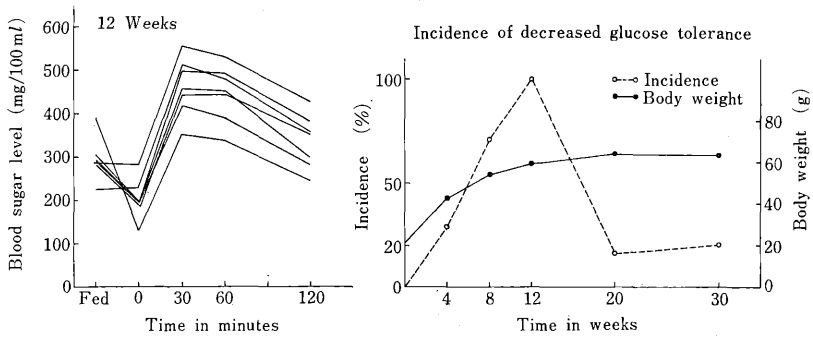
Time in weeks	No. of mice	Body weight (g)	Time in minutes				
			Fed	0	30	60	120
4	7	42.9±2.8	183±12[1]	147±13	286± 43	290± 60	217±60
8	7	54.3±3.2	209±54	156±30	308± 93	347±124	275±89
12	7	59.5±2.9	296±50	201±47	463± 67	448± 65	334±63
20	6	60.2±1.9	183±57	131±32	210±104	213± 96	165±46
30	5	64.2±5.9	130±22	151±32	216± 67	242± 76	188±78

Remarks.

[1]: *Mean value ± standard deviation (blood glucose level in mg/100 ml).*

NQO=4-nitroquinoline 1-oxide.

Fig. 1. Glucose tolerance curves in each NQO-obese mouse of C57BL/6 strain 12 weeks after treatment with NQO, and relationship between incidence of decreased glucose tolerance and body weight gain



of glucose intolerance was 100% at 12 weeks after treatment with NQO.

Discussion

Obesity-inducing rate and mortality in mice after treatment with NQO varied

from one strain of mice to another. It is also of interest to note that there was a difference in the rate of obesity between BDF_1 and DBF_1 mice.

In order to confirm whether goldthioglucose (GTG) induced obesity in ICR mice or not, a dose of 1.0 mg/g of the agent was injected intraperitoneally to ICR mice. Obesity was induced in 75% of the mice 10 weeks after injection. This result indicates that obesity could not readily be induced in ICR mice by intracerebral injection with NQO.

In the present study lecithin was the most effective vehicle for the induction of NQO obesity. This suggests that lecithin may be related to the constitution of the nervous tissue. In contrast, the 0.05 and 0.1% levels of NQO in DMSO failed to induce obesity in mice. It is considered that the obesity-inducing activity of NQO may have been lowered by the chemical and physical properties of DMSO [4].

The increase in the liver and heart weights of NQO-obese animals in the present study is consistent with the finding of Marshall [6] that the enlargement of the liver, heart and kidney had likely been the nonspecific effect of prolonged hyperphagia and seen in "regulatory", as well as "metabolic", obesity. Drachman [2] also suggested that splanchnomegaly might be one of the adaptations to an increased food intake in GTG-obese mice.

The results of the present study on carcass analysis of NQO-obese mice are in agreement with the findings of GTG-obese animals reported by Margaret [5]. They indicate that the increase in body weight could be ascribed almost entirely to that in fat content in NQO-obese mice.

In the present study the incidence of glucose intolerance at 12 weeks of the experimental period and intermittent glucosuria in NQO-obese C57BL/6 mice demonstrated that the NQO-obese animals had fallen transiently in a state of chemical diabetes. To lend support to this result, it has been reported that GTG treatment caused obesity and induced profound and intense diabetic changes in KK, ICR and C57BL mice [7].

NQO-obese mice might be expected to be excellent experimental animals for the study of obesity or diabetes mellitus, particularly the diabetes in the "prediabetic" phase.

Acknowledgments: The author thanks Mr. T. Yamamoto, Mr. K. Ohno and Mr. A. Ozaki for their technical assistance. He is indebted to Dr. A. Ohta, professor at the Tokyo College of Pharmacy, for his generous supply of the carcinogen. He is grateful to Dr. S. Yamamoto, professor emeritus at the University of Tokyo, Drs. T. Mitsuoka and C. Kaneuchi, of his institute, and Dr. Y. Shirasu, of the Institute of Environmental Toxicology, for their encouragement and helpful criticism.

This work was supported in part by research grants from the Ministry of Education, Science and Culture of the Japanese Government.

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