Comparative Carcinogenicity of 4-Nitroquinoline 1-oxide and 4-Hydroxyaminoquinoline 1-oxide in 3 Strains of Mice. (29979)

YASUHIKO SHIRASU* (Introduced by Richard S. Yamamoto)
National Cancer Institute, National Institutes of Health, Bethesda, Md.

4-Nitroquinoline 1-oxide (4NQO), first synthesized by Ochiai *et al*(1), was found to have carcinogenicity by Nakahara *et al*(2). Since the discovery of the carcinogenic action, many reports have described its powerful effect. This compound was shown to cause skin tumors by skin-painting or sarcomas by subcutaneous injection(2,3). Lung cancer also could be induced in mice and rats by subcutaneous injections when a mixture of olive oil and cholesterol was used as a vehicle(4,5). Recently, 4-hydroxyaminoquinoline 1-oxide (4HAQO), a reduction product of 4NQO(6), was proven by us to be a carcinogen(7). Subcutaneous injection of 4HAQO produced sarcomas and epithelial tumors in mice at the injection site, whereas skin-painting failed to yield tumors(7,8). Local sarcomas were induced in rats by subcutaneous injection of 4HAQO(8,9).

It seemed important to compare the carcinogenicity of these 2 related compounds, because 4NQO might exert its carcinogenic action through the reduced form as suggested by the microbial metabolism studies(10). To compare the effect of these 2 compounds and also to explore possible strain differences, a study was carried out using 3 strains of mice. A relatively small dose of these carcinogens was employed because larger amounts might obscure the comparison of the carcinogenic effects of these compounds.

Materials and methods. With the aid of Tween 80 (0.5%), homogeneous suspensions of 4NQO and 4HAQO·HCl were made in physiological saline for the subcutaneous injections at the interscapular area of mice. The 3 strains of mice used in this experiment were C3H/HeN, C57BL/6JN and noninbred albino (General Purpose White Swiss). All mice were obtained from the NIH Animal Production Section. They were 8 weeks old at start of the experiment and were maintained on a diet of Purina Chow and water *ad libitum*. The mice of each strain were divided into 3 groups, each consisting of 20 mice, 10 females and 10 males, except the 4HAQO group of non-inbred albino mice in which 11 females and 9 males were used. The first and second groups were treated with 4HAQO and 4NQO, respectively. Ten injections of 0.1 ml of 0.1% suspension of the carcinogens, *i.e.*, 0.1 mg per mouse per time were administered once a week. The dose was almost equimolar because of a slight difference in the molecular weight of these carcinogens. The third group of control mice was treated with 0.1 ml of the vehicle. A careful autopsy was performed on the animals when they died or were sacrificed in moribund condition. The animals were kept under observation for 32 weeks, at which time all surviving animals were sacrificed and autopsied. The tumors and other organs were fixed in 10% formalin, sectioned and stained with hematoxylin and eosin for histological study.

Results and discussion. As shown in Table I, it is evident that 4HAQO was more effective than 4NQO in producing tumors in all 3 strains. Although the tumor incidence was relatively low because of the small dose of the carcinogen employed, sufficient numbers of the tumors were obtained to compare the relative carcinogenicity of the two compounds.

The local tissue injury provoked by injections of these carcinogens was more remarkable in the mice of the 4HAQO groups than in those of the 4NQO groups in all 3 strains. The C57BL mice developed more pronounced tissue reaction than C3H mice, with noninbred albino mice showing the least reaction.

The main tumors obtained were sarcomas and papillomas at the injection site, and also leukemia. There were some strain differences in production of the tumors. More sarcomas developed in the C3H mice than in the other 2 strains. Leukemia was mostly observed in the non-inbred albino mice. The C57BL mice showed the lowest incidence of tumors. In this experiment no remarkable sex difference

* Visiting Associate on leave of absence from Inst. of Physical and Chemical Research, Tokyo, Japan.

TABLE I. Comparative Carcinogenicity of 4HAQO and 4NQO in 3 Strains of Mice.*

Strain and sex		Control		4HAQO				4NQO			
		Effective No.†	No. of mice with tumor	Effective No.	No. of mice with tumor			Effective No.	No. of mice with tumor		
					Sarcoma	Papilloma	Leukemia		Sarcoma	Papilloma	Leukemia
C3H/HeN	♀	10	0	10	2(21,21)‡	1(15)	0	10	0	0	0
	♂	10	0	10	4(21,22, 22,26)	0	0	10	1(24)	0	0
Total		20	0	20	6	1	0	20	1	0	0
C57BL/6JN	♀	10	0	10	1(26)	0	0	10	0	0	0
	♂	10	0	10	1(26)	2§(15,17)	1(26)	10	0	0	0
Total		20	0	20	2	2	1	20	0	0	0
Non-inbred albino	♀	10	0	11	1(26)	1(13)	3(9,12, 22)	10	0	0	1(13)
	♂	7	0	9	1(22)	0	2(19,28)	6	0	0	1(27)
Total		17	0	20	2	1	5	16	0	0	2
Grand total		57	0	60	10	4	6	56	1	0	2

* Dosage of the carcinogens: Subcutaneous injections with 0.1 ml of 0.1% suspension (0.1 mg), once weekly, 10 times. Observation period: 32 weeks.
† Effective number means the number of animals surviving after the appearance of the first tumor in each group.
‡ Numbers in parentheses are weeks on which the tumors were first noticed.
§ One was found in a leukemia case.

was seen in the tumor yield of these two compounds. The first cases of sarcomas and leukemia appeared earlier in the 4HAQO than in the 4NQO group (Table 1). Sarcomas appeared from 132 days up to 185 days after the start of the experiment and grew rapidly to kill the hosts or to make them moribund within 2 to 6 weeks. Histologically, the majority of the sarcomas were fibrosarcomas except for one case of rhabdomyosarcoma in a male C3H mouse of the 4HAQO group.

The papillomas began to appear after about 100 days. Although 2 of 4 papillomas regressed during the term of the experiment, one persisted until its host died of leukemia, the other one to the end of the study. There was no skin cancer as described for the ddOM mice in the previous report(8). No papilloma developed in 4NQO groups in this experiment probably because of the low dose of 4NQO employed. Comparing the present results with previous ones(8) the 3 strains used in this study seem to be less susceptible than ddOM mice to the local tumor induction of these carcinogens.

Most of the leukemia cases were observed in the non-inbred albino mice except for one case in the C57BL mice of the 4HAQO group. There was no case of leukemia in the C3H mice. The first leukemia appeared as early as 63 days and successively thereafter up to 197 days. The course of the disease was so rapid that the animals died or were killed in moribund condition from a few days to 2 weeks after the swelling of the superficial lymph nodes was noticed. A generalized enlargement of the lymph nodes was found at autopsy. The spleen was moderately increased in size. The liver remained normal in size or was slightly enlarged. The total white blood cell counts examined in 6 of 8 leukemic animals ranged from 19,200-335,000/mm^3 with the mean value of 108,000/mm^3. Microscopically, the lymph nodes and spleen were overrun with leukemic cells resulting in obliteration of the normal architectural makings. As a rule, there was leukemic infiltration of the liver, kidney, lung, bone marrow and other organs. All these cases were diagnosed as lymphocytic leukemia. Generalized leukemia might have developed from the local lymphoma near the injection site, because in some

cases the enlargement of the brachial lymph nodes of one or both sides was first observed and thereafter the enlargement of the lymph nodes throughout the body ensued.

Recently, 4NQO was shown to cause leukemia in ICR mice(11). Two cases of leukemia were observed earlier in mice of the ddOM strain given 4HAQO(8). The present study showed that non-inbred albino mice were susceptible to the leukemogenic action of these 2 compounds. Accordingly, these 2 compounds seem to be leukemogenic in susceptible strains of mice.

Besides the above mentioned tumors, one to several very tiny pulmonary tumor-like nodules were observed in several non-inbred albino mice including a control mouse, when they were sacrificed at the end of experiment. Because of the small size of the nodules a complete examination could not be carried out, although some of them were shown to be adenomas by histological examination. No pulmonary tumor, however, could be found in the C3H and C57BL mice. A mammary adenocarcinoma, which might be spontaneous, was found in a C3H female mouse of 4HAQO group.

The more active carcinogenic action of 4HAQO compared with 4NQO might suggest that 4NQO acts through the reduced form, although the exact nature of the mechanism of action of these compounds would depend on future metabolism studies. Recent metabolic studies on the carcinogenic aromatic amines or amides have indicated the possibility that their hydroxylamines might be closer to the proximal carcinogens because of the more active carcinogenicity of the latter compared with the parent compounds(12-15). The results of the present study are of interest in this respect.

Summary. A comparative study of the carcinogenicity of 4-nitroquinoline 1-oxide and 4-hydroxyaminoquinoline 1-oxide was carried out using C3H/HeN, C57BL/6JN and non-inbred albino strains of mice. Ten subcutaneous injections of these carcinogens at a same and relatively low dose (0.1 mg per time) were made once a week into the same site on the back of the mice. More sarcomas developed in the C3H mice than in the other 2 strains, whereas leukemia was mostly observed in non-inbred albino mice. The C57BL mice showed the least tumor incidence. The tumor yields obtained within the observation period of 32 weeks clearly showed that 4-hydroxyaminoquinoline 1-oxide was more active than 4-nitroquinoline 1-oxide. The result of this study would support the hypothesis that 4-nitroquinoline 1-oxide acts through its reduced form.

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