Comparative Carcinogenicity of 4-Hydroxyaminoquinoline-1-oxide and Its Diacetyl Derivative in Mice and Rats[1] (35459)

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4-Hydroxyaminoquinoline-1-oxide (HAQO) is a potent carcinogen for rats and mice (1–3). Although HAQO or its metabolite(s) react with DNA and RNA *in vivo* (4, 5), HAQO has shown little or no *in vitro* reactivity with nucleophiles at neutrality in the absence of oxygen (6, 7). On the other hand, the *O,O'*-diacetyl derivative of HAQO reacts with DNA and RNA nonenzymatically at neutrality to yield products with similar fluorescent properties to those of the nucleic acid adducts formed from HAQO *in vivo* (6). In view of these observations, the greater carcinogenicity of lipid-soluble esters of *N*-hydroxy-2-acetylaminofluorene and several other hydroxamic acids compared to the hydroxamic acids (8), and the data implicating the sulfuric acid ester of *N*-hydroxy-2-acetylaminofluorene as an ultimate carcinogenic form of this hydroxamic acid in the rat liver (9–11), carcinogenicity assays were carried out with HAQO and DiAcHAQO. The results of comparative assays in which these compounds were injected subcutaneously into mice or rats or applied cutaneously to mice are reported here.

Materials and Methods. HAQO, HAQO·HCl, and 4-nitroquinoline-1-oxide (NQO) were synthesized and kindly supplied by Dr. Kei Sato, Faculty of Education, Hirosaki University, Hirosaki, Japan. DiAcHAQO (mp 110° dec. in a Fisher-Johns apparatus or 125° according to the rapid time–temperature curve obtained with the Accumelt instrument, American Instrument Co.) was prepared by the method of Kawazoe and Araki (12). The DiAcHAQO was prepared at biweekly intervals to minimize loss due to its reactivity; all of the quinoline derivatives were stored at —20°. 7-Methylbenz[a] anthracene (7-Me-BA) was obtained from Eastman Organic Chemicals.

Female mice (CD-1), initial weights of 20–25 g, and male rats (CD-random-bred), initial weights of 160–190 g, were obtained from the Charles River Breeding Laboratories, Wilmington, Mass. and given Wayne Breeder Blox (Allied Mills, Chicago, Ill.) and water *ad libitum*. The rats were caged individually and the mice were in groups of 5. For studies on skin carcinogenesis the compounds were dissolved in dimethylsulfoxide–acetone (1:3) or 100% ethanol as indicated in footnote *a* to Table I, and 0.15 ml was applied to the shaved dorsal skin of each mouse. Beginning 2 weeks after the last application of the test compounds and continuing until the termination of the experiments all of the mice were treated in the same area twice weekly with 0.15 ml of a 0.33% solution of croton oil (S. B. Penick and Co., New York, N.Y.) in acetone. The backs of the mice were shaved at intervals as needed. The papillomas were counted at 2–3-week intervals; representative papillomas and all of the carcinomas were confirmed by histological study. For the injections the compounds were dissolved or suspended in tricaprylin without heat with the aid of a magnetic stirrer and injected sc in the right hind leg. Details as to doses and numbers of injections are

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TABLE I. Skin Tumor Induction in Groups of 35 Mice with Cutaneously Administered HAQO, DiAcHAQO, or NQO.[a]

Expt. no.	Compound	No. of applications	No. of mice with papillomas/no. of mice alive and (total no. of papillomas) at: (months): 4	6	7	10	No. of mice with carcinomas by: 12
I	7-Me-BA	6	15/34 (59)[b]	18/34 (86)	20/33 (87)	17/26 (61)	13
	HAQO	12	6/35 (9)	10/35 (20)	13/33 (39)	13/27 (24)	9
	DiAcHAQO	12	4/33 (6)	7/33 (11)	11/32 (21)	15/29 (24)	3
	Solvent control	12	0/35 (0)	0/35 (0)	0/35 (0)	3/32 (4)	0
II	7-Me-BA	6	20/35 (112)	25/34 (122)	26/34 (159)	18/27 (92)	13
	HAQO·HCl	12	2/35 (2)	6/35 (16)	10/34 (23)	8/32 (18)	1
	DiAcHAQO	12	4/35 (4)	14/34 (28)	15/34 (38)	17/33 (39)	3
	NQO	12	6/35 (12)	10/34 (34)	11/33 (34)	14/29 (25)	4
	Solvent control	12	1/35 (1)	1/35 (1)	1/35 (1)	1/35 (1)	0

[a] Female mice received 0.3 ml of solvent containing 230 μg of 7-Me-BA, 233 μg of DiAcHAQO, or an equimolar amount of HAQO, HAQO·HCl, or NQO 3 times/wk for the indicated number of applications. Dimethylsulfoxide–acetone (1:3 by vol) was used as solvent in Expt. I; absolute ethanol was used as solvent in Expt. II, except that (because of poor solubility in ethanol) in Expt. II the 7-Me-BA was dissolved in acetone. Two weeks after the last application of the test compounds twice weekly applications of 0.15 ml of croton oil (0.33% in acetone) were initiated and continued to the end of the experiments.

[b] No. of mice with papillomas/no. of mice alive, with the total no. of papillomas in parentheses.

TABLE II. Sarcoma Induction in Groups of 35 Mice Injected se with HAQO and DiAcHAQO.[a]

Compound	No. of injections	No. of mice with sarcomas at injection site by (months):				No. of mice with:				No. of mice killed tumor-free at 17 months
		6	8	10	17	Lymphoid tumors	Mammary carcinomas	Lung adenomas	Other tumors	
HAQO	15	3	11	16	19	6	1	0	1[b]	2
DiAcHAQO	15	5	9	15	20	1	1	1	0	5
HAQO	10	5	13	15	18	3	1	1	0	5
DiAcHAQO	10	1	4	8	11	2	0	2	0	13
HAQO	5	0	1	4	9	6	0	0	1[c]	14
DiAcHAQO	5	0	0	2	9	0	2	2	2[d]	15
Solvent control	15	0	0	0	0	1	1	1	0	26

[a] Female mice were injected se for the number of times indicated with 0.1 ml of tricaprylin containing 0.1 mg of HAQO or 0.15 mg of DiAcHAQO. Injections were at weekly intervals.

[b] One squamous cell carcinoma at the injection site.

[c] One keratoacanthoma at injection site.

[d] One bronchiogenic carcinoma and 1 hepatocellular carcinoma.

given in Tables II and III. All of the tumors were confirmed by histological examination. In all of the experiments the solutions were prepared immediately before use.

Results and Discussion. In mice there were no significant differences in the activities of HAQO and DiAcHAQO whether they were compared as initiators for the induction of skin tumors or were injected subcutaneously for the induction of sarcomas. Thus, application of a total of 12 μmoles of HAQO, HAQO·HCl, DiAcHAQO, or NQO to the skin of mice and subsequent applications of croton oil caused papillomas to develop in up to 50% of the mice with averages of 2–3 papillomas/tumor-bearing mouse (Table I). The first papillomas developed 3–4 months after the first application of the test compounds, and the number of papillomas increased up to about the seventh month. A few squamous cell carcinomas of the skin developed 9–12 months after the first application of each of the quinoline derivatives. These data on DiAcHAQO are consistent with the statement, unsupported by data, of Kawazoe *et al.* (13) that total doses of 1.5 or 10.5 mg of DiAcHAQO induced malignant skin tumors in mice. Our data and those of Kawazoe *et al.* (13) contrast with an early report of Shirasu (1) in which he failed to induce tumors of the skin or subcutaneous tissue of mice with an acetyl derivative of HAQO.

HAQO and DiAcHAQO each induced sarcomas in 25% of the mice which received 5 injections (3.2 μmoles), while 31–57% of those which received 10 or 15 injections developed sarcomas (Table II). The first sarcomas were observed 4–5 months after the first injection when 10 or 15 injections were administered and at 6–8 months when only 5 injections were given. All of the sarcomas were diagnosed as fibro- and myosarcomas. Some lymphoid tumors and lymphatic leukemias occurred in the mice injected with HAQO and DiAcHAQO; further studies would be necessary to ascertain whether the incidences were significantly higher than those of the control mice. HAQO and DiAcHAQO also had equal activity for lung tumor induction when single doses of 53 mμmoles were injected into newborn mice of

TABLE III. Comparative Carcinogenicity in Rats of Subcutaneously Injected HAQO and DiAcHAQO.[a]

Expt. no.	Compound	No. of injections	No. of rats/group	No. of rats with sarcomas at injection site by (months): 6	8	12	16	No. of rats killed tumor-free at end of expt.[b]
I	HAQO	1	16	2	5	9	11	4[c]
		2	16	5	8	13	14	1
		3	16	11	13	15	15	1
	DiAcHAQO	1	16	0	0	0	0	15
		2	16	0	0	0	0	11
		3	16	0	0	1	3	9[c]
	Solvent control	3	16	0	0	0	0	13
II	HAQO · HCl	3	20	15	20	20	20[d]	0
	DiAcHAQO	3	20	0	0	1	1[d]	16
	Solvent control	3	20	0	0	0	0[d]	18

[a] Male rats were injected sc for the number of times indicated with 0.2 ml of tricaprylin containing 1.0 mg of HAQO or an equimolar amount of DiAcHAQO or HAQO · HCl. Injections were at weekly intervals.

[b] Expts. I and II were terminated at 16 and 14 months, respectively.

[c] Each of these groups had 1 rat with lymphatic leukemia.

[d] Data obtained at 14 months.

the DDD strain (14). When either compound was injected lung adenomas were found in about 50% of the mice, while only 5% of the controls developed lung tumors.

As observed previously by others (1, 2), HAQO also induces sarcomas readily in rats (Table III). Under the same conditions, however, DiAcHAQO was very much less active. Thus, a single injection of 1 mg (6.4 μmoles) of HAQO induced fibro- and myosarcomas in 70% of the rats within 16 months, and 2 or 3 injections of 1 mg each induced 80–100% incidences within 8–12 months. In contrast, equivalent injections of DiAcHAQO induced sarcomas in only 4 of 36 rats which received 3 injections, and no tumors developed in rats which received only 1 or 2 injections.

While, like HAQO, DiAcHAQO is a potent carcinogen for mice, we have no explanation at present for its very much lower carcinogenic activity for the subcutaneous tissue of rats. Likewise, it is not possible to interpret the data presented above in terms of the nature of the ultimate carcinogenic form of HAQO. Thus, the greater reactivity of DiAcHAQO with tissue nucleophiles under nonoxidizing conditions (6) and the apparent similarity between the products formed from HAQO in ascites hepatomas (4, 5) and those formed nonenzymatically by reaction of DiAcHAQO and nucleic acids (6) support the idea the DiAcHAQO could be a prototype for the active form of HAQO *in vivo*. The carcinogenicity data provide no support for this model, since the ultimate carcinogenic form should have greater intrinsic activity. On the other hand, the carcinogenicity data do not rule out the possible importance of esters of HAQO in carcinogenesis. Thus, relative carcinogenic activities depend not only on the intrinsic activities of the compounds, but also on their availabilities at the critical sites of the target cells. Data on the absorption and metabolism of HAQO and DiAcHAQO are needed to assess these factors. Other derivatives of HAQO must also be considered as possible ultimate carcinogens. HAQO is readily oxidized to a free radical (15–17), which through reaction with cellular nucleophiles or through indirect mechanisms (7, 18–20) may be important in carcinogenesis by this compound.

Summary. 4-Hydroxyaminoquinoline-1-oxide and its *O,O'*-diacetyl derivative were compared with respect to their abilities to induce skin tumors in mice (with subsequent application of croton oil) and to induce sar-

comas at the site of sc injection in rats or mice. The two compounds had approximately equal activities in the two mouse assays; however, the diacetyl derivative was much less active than its parent compound in the induction of sarcomas in rats.

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