Jpn. J. Cancer Res. 82, 1211–1219, November 1991

Induction of Renal Cell Tumors in Rats and Mice, and Enhancement of Hepatocellular Tumor Development in Mice after Long-term Hydroquinone Treatment

Masa-Aki Shibata,[1] Masao Hirose, Hikaru Tanaka, Emiko Asakawa, Tomoyuki Shirai and Nobuyuki Ito

First Department of Pathology, Nagoya City University Medical School, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467

Hydroquinone (HQ) was administered to F344 rats and B6C3F$_1$ mice of both sexes at a level of 0.8% in the diet for two years. This treatment induced renal tubular hyperplasia as well as adenomas, predominantly in males of both species, and was associated with chronic nephropathy in rats. In addition, the occurrence of epithelial hyperplasia of the renal papilla was increased in male rats. Foci of cellular alteration of the liver were significantly reduced in number by HQ in rats, but in contrast, were increased in mice, where development of hepatocellular adenoma was also enhanced in males. The incidence of squamous cell hyperplasia of the forestomach epithelium was significantly higher in mice of both sexes given HQ than in the controls, but no corresponding increase in tumor development was observed. The present study strongly indicates potential renal carcinogenicity of HQ in male rats and hepatocarcinogenicity in male mice. Thus, it is possible that HQ, which is present in the human environment, may play a role in cancer development in man.

Key words: Hydroquinone — Carcinogenicity study —Rat and mouse — Kidney tumor — Liver tumor

Hydroquinone (HQ; 1,4-benzenediol),[2] a metabolite of benzene, is widely used in industry as a developer in black-and-white photography and as an antioxidant.[1,2] It also has applications in cosmetics for skin care products and treatment of hyperpigmentation.[1,2] In addition, this compound has been found in cigarette smoke (up to 80 μg/cigarette).[3] In nature, HQ is widely distributed in the leaves of many plants, fruit (Chinese anise) and in the bark and buds of pear trees as a component of the glucoside arbutin.[1]

HQ is generally reported as non-mutagenic in the Ames assay,[2,4,5] but there is extensive evidence for its clastogenicity in mammal cells both *in vitro* and *in vivo*.[6–10] Benzene has been reported to exert toxicity, mutagenicity and carcinogenicity in man and experimental animals.[11–13] These effects are likely to be due to benzene metabolites such as phenol, catechol (1,2-benzenediol) and HQ.[12,14] Although it was previously shown that treatment with HQ in the diet for two years was not associated with any carcinogenic activity in rats,[15] a recent long-term gavage study revealed this phenolic compound to have positive carcinogenicity in both rats and mice.[16] Two-stage studies on benzene derivatives in our own laboratory demonstrated that catechol but not HQ or resorcinol (1,3-benzenediol) can enhance tumor development of forestomach and glandular stomach of rats initiated with *N*-methy-*N'*-nitro-*N*-nitrosoguanidine in association with alteration of DNA synthesis.[17–19] Indeed, catechol itself induced gastric carcinomas in rats and adenomatous hyperplasia in mice in two-year carcinogenicity studies.[20] The purpose of the present long-term study was to investigate whether dietary HQ at the same dosage level as catechol, an isomer, would cause cancer development in any organs of rats and mice of either sex.

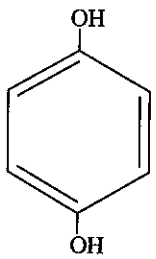
MATERIALS AND METHODS

Test chemical The HQ (1,4-benzenediol; purity >99%) used in this study was purchased from Wako Pure Chemical Industries, Ltd., Osaka. The chemical structure is shown in Fig. 1.

Animals Fischer 344 rats and B6C3F$_1$ mice of both sexes, approximately 5 weeks old, were obtained from Charles River Japan, Inc., Atsugi and held in quarantine for a 1 week acclimatization period prior to experimentation. The animals were randomly assigned to the control and experimental groups on the basis of body weights. They were housed in plastic cages (five/cage), with hardwood chips for bedding, under controlled conditions of temperature (22±2°C), humidity (60±10%) and lighting (12-h light/dark cycle). Formulated or control diets (Powdered diet MF, Oriental Yeast Co., Ltd., Tokyo) and tap-water were freely available.

[1] To whom all correspondence should be addressed.

[2] Abbreviations: HQ, hydroquinone; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; EQ, ethoxyquin.



Hydroquinone
(1,4-benzenediol)

CAS No. 123-31-9

Fig. 1. Chemical structure of hydroquinone (HQ) assayed in rats and mice.

Two-year study design Diet containing 0.8% HQ was fed to groups of 30 male and 30 female rats for 104 weeks and to groups of 30 male and 30 female mice for 96 weeks. The dose level of HQ chosen was the same as used in earlier studies of HQ and its analogs.[17–20] Since the expected concentration of HQ in the diet was confirmed 2 weeks after preparation, the diet was prepared twice a month. Control rats and mice received powdered basal diet without any chemical supplement. All animals were examined twice daily for general health and signs of toxicity. Individual body weights were measured weekly for the first 14 weeks and once every 4 weeks thereafter. Food and water intakes were measured over a 2-day period before each weighing. At the completion of the 2-year administration period, all surviving animals were deprived of food overnight and then killed by exsanguination under ether anesthesia.

Pathology All animals were given a complete gross necropsy and the liver and kidneys were weighed. The organs that were taken and fixed in 10% phosphate-buffered formalin for routine histopathological examination included those weighed, as well as the following: heart, spleen, aorta, lymph nodes, thymus, pituitary, thyroids, parathyroids (if found), adrenals, trachea, lungs, tongue, salivary glands, esophagus, stomach, small and large intestines, pancreas, gall bladder (in mice), urinary bladder, testes, prostate, epididymis, seminal vesicles, mammary gland, ovaries, uterus, vagina, skeletal muscle, skin, eyes, Harderian glands and all gross lesions. Lungs, stomach, intestinal tract, and urinary bladder were inflated with fixative. Tissues from animals that died or became moribund during the experiment were also examined histopathologically. In addition, for quantitative evaluation of lesion development in the liver, areas of liver sections were determined using a color image processor (SPICCA-II, Nippon Avionics Co., Ltd., Tokyo).

Statistical analyses The data were subjected to analyses of variance and the significance of differences between the means was tested by using Student's t test. The incidences of histopathologic lesions were analyzed by the two-sided Fisher's exact probability test and the severity of non-neoplastic lesion development by the Mann-Whitney test.

RESULTS

No treatment-related clinical signs were observed in either species throughout the study. No significant differences in survival were noted between HQ-treated and control groups for rats (Fig. 2a) or mice (Fig. 2b). Body weights of rats and mice at representative periods are presented in Figs. 3a and 3b, respectively. Statistically significant reduction in weight gain was noted in rats of both sexes and male mice given HQ. Food and water consumption values for HQ-treated rats and mice were comparable to those of respective control animals. Average amounts of HQ intake, calculated from food consumption, were 351 and 368 mg/kg/day in male and female rats, and 1046 and 1486 mg/kg/day in male and female mice, respectively.

Organ weights Average liver and kidney weights for both species are shown in Table I. Statistically significant increases were seen in both the absolute and relative liver weights in male rats given HQ. Average absolute or relative kidney weights were significantly higher in rats of both sexes given HQ than in corresponding controls. In mice, relative weights only of the liver and kidneys showed significant elevation in females treated with HQ.

Pathology Macroscopically, granular appearance or indentation of the kidneys was increased in male rats treated with HQ. In addition, small nodular lesions (<1 mm in size) were grossly visible in the forestomach lumen of many mice both sexes given HQ.

(1) Histopathology in rats Treatment-related changes were observed in the kidneys and liver of rats as summarized in Table II. Chronic nephropathy occurred in both control and treated groups of males. However, this age-related renal disease was observed to be more severe in males given HQ. This phenomenon was also seen in females given HQ, but the changes were slight. Epithelial hyperplasia of the renal papilla was increased in male rats given HQ. This change is considered to be a component of advanced chronic nephropathy. Statistically significant numbers of renal tubular hyperplasias and microscopic adenomas developed in males given HQ. Tubular hyperplasias (Fig. 4a) were composed of tubules with stratified epithelial cells which partially or fully filled the tubular lumina. Cystic forms were frequent. Tubular hyperplasias were considered synonymous with

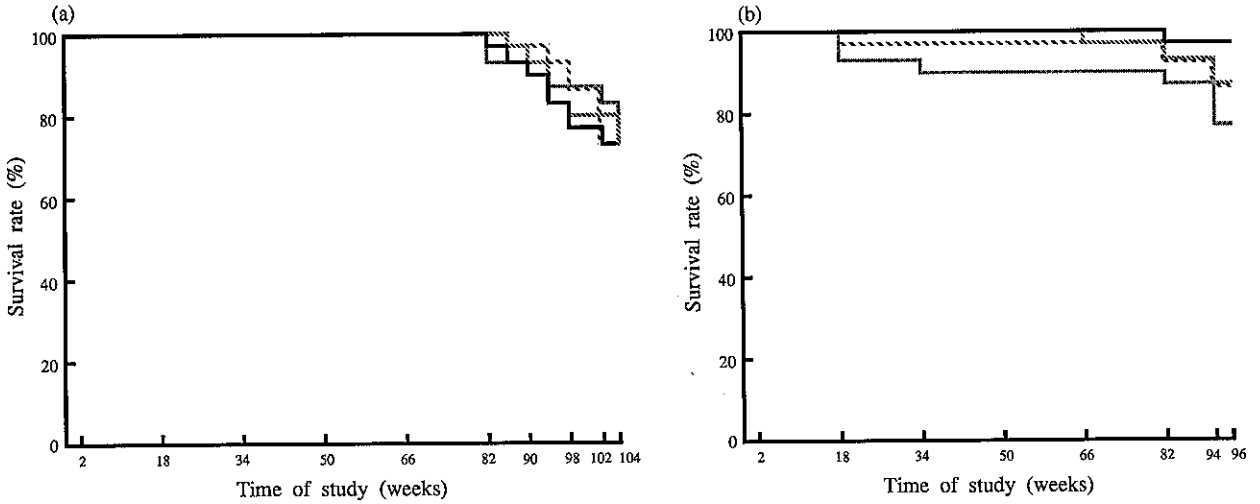


Fig. 2. Survival curves for rats (a) and mice (b): males given HQ at dietary levels of 0% (▒▒▒) or 0.8% (▬▬); females given HQ at dietary levels of 0% (▚▚▚) or 0.8% (▒▒▒).

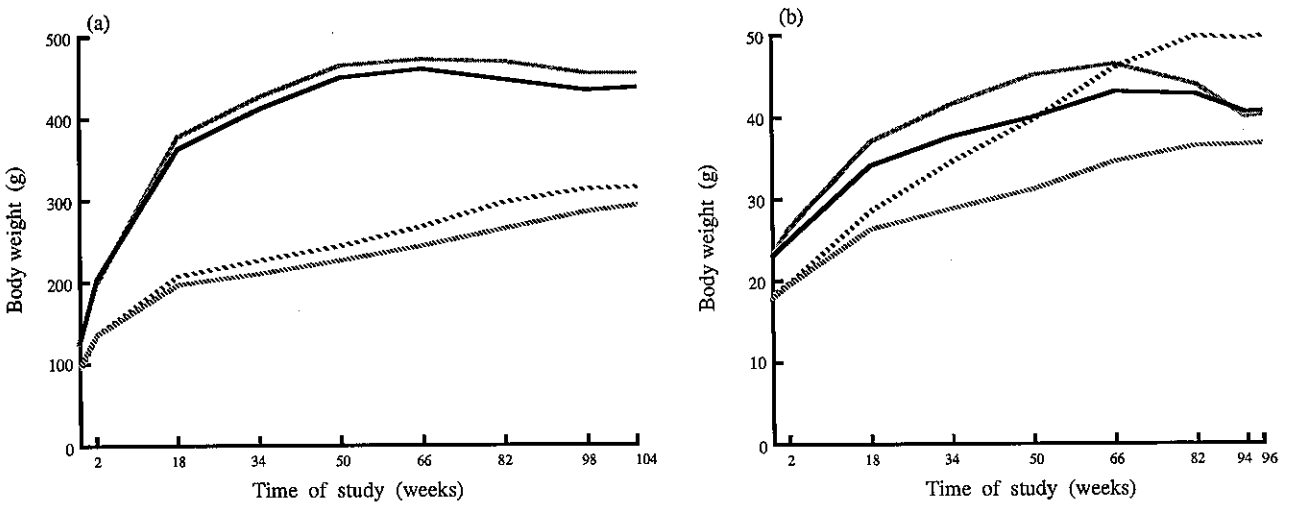


Fig. 3. Growth curves for rats (a) and mice (b): males given HQ at dietary levels of 0% (▒▒▒) or 0.8% (▬▬); females given HQ at dietary levels of 0% (▚▚▚) or 0.8% (▒▒▒).

such terms as "dysplastic foci" or "dysplastic tubular epithelium." Tubular adenomas also exhibited cystic (Fig. 4b) or solid forms (Fig. 4c), in both cases being composed of relatively uniform epithelial cells with clear or pale basophilic cytoplasm and round nuclei with prominent nucleoli. A significant reduction in bile duct hyperplasia of liver was noted in males given HQ. Since the incidence of foci of cellular alteration in the liver showed a tendency for decrease in males receiving HQ, this lesion was further assessed quantitatively (Table III). Average numbers of foci per cm^2 of liver section analyzed showed a statistically significant reduction in both sexes given HQ. Most of the liver foci were of basophilic character.

No treatment-associated proliferative or neoplastic lesions were observed in the forestomach or glandular stomach of rats. With regard to other tissues observed, similar frequencies to known spontaneous and incidental findings commonly seen in this strain of rats were noted in both control and treated rats of either sex.

Table I. Final Body, Liver and Kidney Weights of Animals Given Hydroquinone (HQ) in the Diet

Treatment	No. of surviving animals	Final body wt. (g)	Liver		Kidneys	
			Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
Rats						
Male						
None	24	438±38	8.08±4.89	1.86±1.15	2.02±1.21	0.47±0.28
HQ	22	422±44	12.07±1.13**	2.89±0.37**	3.42±0.52**	0.82±0.16**
Female						
None	22	306±42	7.24±1.46	2.38±0.38	1.86±0.10	0.62±0.09
HQ	22	283±28*	7.31±1.03	2.62±0.55	1.91±0.08	0.68±0.06*
Mice						
Male						
None	22	37±6	2.11±0.86	5.81±2.57	0.66±0.11	1.78±0.23
HQ	27	38±5	2.12±0.65	5.75±2.21	0.68±0.06	1.81±0.17
Female						
None	26	47±7	1.65±0.28	3.60±1.02	0.48±0.04	1.05±0.20
HQ	26	34±5**	1.76±0.52	5.27±1.74**	0.49±0.06	1.48±0.28**

* Significantly different from the corresponding control value, $P<0.05$.
** Significantly different from the corresponding control value, $P<0.01$.

Table II. Incidences of Lesions of the Kidneys and Liver in Rats Given Hydroquinone (HQ) in the Diet

Site/lesion	No. of rats with lesions (%)			
	Males		Females	
	None	HQ	None	HQ
No. examined	30	30	30	30
Kidneys				
Chronic nephropathy (+)	17 (57)	10 (33)	1 (3)	8 (27)
(++)	0	9 (30) **	0	0 **
(+++)	0	5 (17)	0	0
Hyperplasia, papilla	2 (6)	11 (37)*	0	0
Tubular hyperplasia	1 (3)	30 (100)**	0	2 (7)
Adenoma	0	14 (47)**	0	0
Liver				
Bile duct hyperplasia	8 (27)	1 (3)*	0	1 (3)
Foci of cellular alteration	21 (70)	14 (47)	22 (73)	19 (63)
Hyperplastic nodule	2 (7)	1 (3)	2 (7)	2 (7)
Hepatocellular carcinoma	1 (3)	1 (3)	0	1 (3)

Grading: +, slight; ++, moderate; +++, severe.
* Significantly different from the control value, $P<0.05$.
** Significantly different from the control value, $P<0.01$.

(2) Histopathology in mice Table IV summarizes treatment-related lesions observed in the kidney, liver and forestomach of mice. The increase in advanced chronic nephropathy associated with HQ treatment in rats was not evident in the case of mice. However, significant development of renal tubular hyperplasia was noted in males receiving HQ (Fig. 5a). Moreover, in this group, renal cell adenomas were also observed, although the occurrence of this lesion was not statistically significant (Fig. 5b). The features of the renal lesions in mice were similar to those induced by HQ in rats.

Centrilobular hypertrophy of the hepatocytes was observed in HQ-treated males only, this being statistically significant. In contrast to rats, the incidence of liver foci

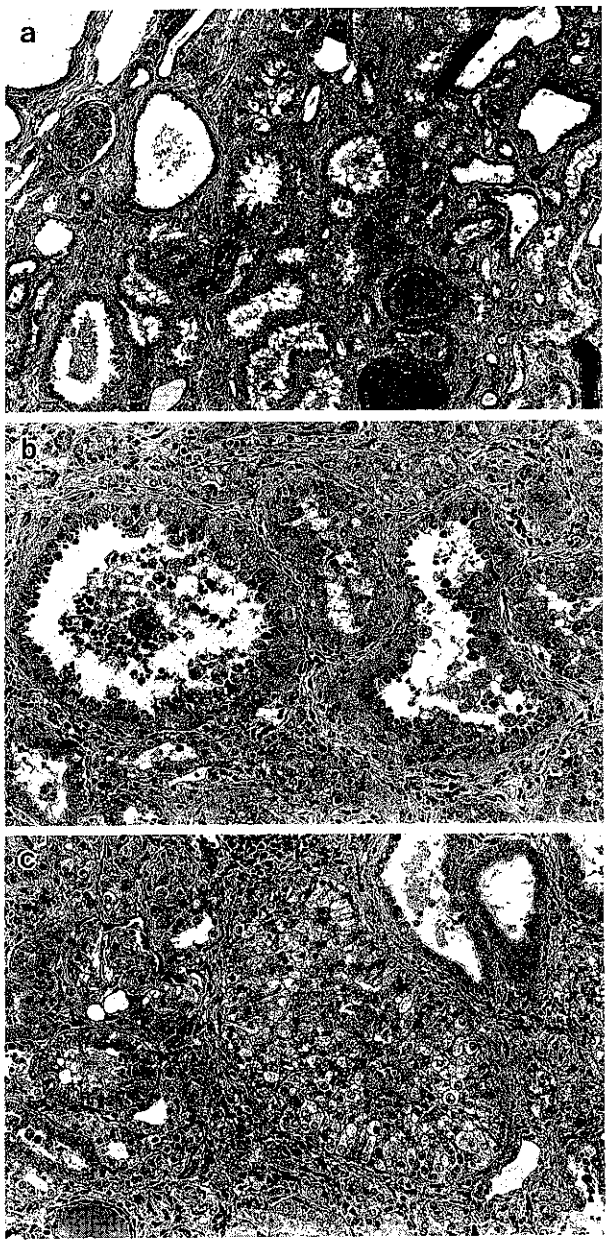


Fig. 4. Renal preneoplastic and neoplastic lesions in male rats treated with 0.8% HQ in the diet for 104 weeks. (a) Multiple tubular hyperplasias and tubular cell adenomas were seen in cases of chronic nephropathy. H & E stain. ×100. (b) Cystic and (c) solid tubular cell adenomas composed of relatively uniform epithelial cells with clear or pale basophilic cytoplasm and round nuclei with prominent nucleoli. H & E stain. ×200.

in mice was significantly increased in males given HQ. In addition, numbers of foci per cm^2 liver were significantly increased in this group (Table III). The liver foci observed in mice were of various types such as basophilic, eosinophilic and clear cell. The incidence of the hepatocellular adenoma was also elevated in HQ-treated males. However, no differences in hepatocellular carcinoma development between control and treatment groups were observed.

Table III. Quantitative Data for Foci of Cellular Alteration in the Liver of Animals Given Hydroquinone (HQ) in the Diet

Treatment	No. of animals	No. of foci of cellular alteration	
		Total	No./liver (cm^2)
Rats			
Male			
None	30	50	1.18±1.17
HQ	30	25	0.57±0.69*
Female			
None	30	171	4.38±4.12
HQ	30	47	1.38±1.37**
Mice			
Male			
None	28	6	0.37±1.10
HQ	30	23	1.16±1.54*
Female			
None	29	1	0.07±0.39
HQ	30	3	0.13±0.44

* Significantly different from the corresponding control value, $P<0.05$.
** Significantly different from the corresponding control value, $P<0.01$.

Significantly elevated incidences of squamous cell hyperplasia but not tumor development in the forestomach epithelium were observed in mice of both sexes given HQ. No significant differences with respect to tumor incidence at any other organ site were observed between control and treated mice.

DISCUSSION

While in one earlier study, no pathological changes were noted when levels of up to 1% HQ were fed to SD rats for two years,[15] a recent gavage study of similar length in F344 rats demonstrated induction of renal tubular adenomas in males and leukemias in females, along with enhanced hepatocellular adenoma development in $B6C3F_1$ mice.[16] In the present study, occurrence of both precancerous and neoplastic lesions in the kidneys was similarly noted, but was additionally found in male mice. Although only the incidence of renal tubular hyperplasia and not tubular adenoma was statistically significant, since spontaneous occurrence of renal neoplastic lesions in this mouse strain is very rare,[21,22] the finding was considered to be biologically significant.

Table IV. Incidences of Lesions of the Kidney, Liver and Forestomach in Mice Given Hydroquinone (HQ) in the Diet

Site/lesion	No. of mice with lesions (%)			
	Males		Females	
	None	HQ	None	HQ
No. examined	28	30	29	30
Kidneys				
Pyelonephritis	2 (7)	0	0	0
Interstitial nephritis	0	0	0	1 (3)
Cyst	0	2 (7)	0	1 (3)
Tubular hyperplasia	0	9 (30)**	0	0
Adenoma	0	3 (10)	0	0
Liver				
Hypertrophy	0	26 (67)**	0	3 (10)
Foci of cellular alteration	4 (14)	14 (47)*	0	2 (7)
Hepatocellular adenoma	6 (22)	14 (47)*	0	1 (3)
Hepatocellular carcinoma	7 (26)	6 (20)	1 (3)	0
Forestomach				
Hyperplasia	1 (4)	11 (37)**	3 (10)	14 (47)**
Squamous cell carcinoma	0	1 (3)	0	1 (3)

* Significantly different from the control value, $P<0.05$.
** Significantly different from the control value, $P<0.01$.

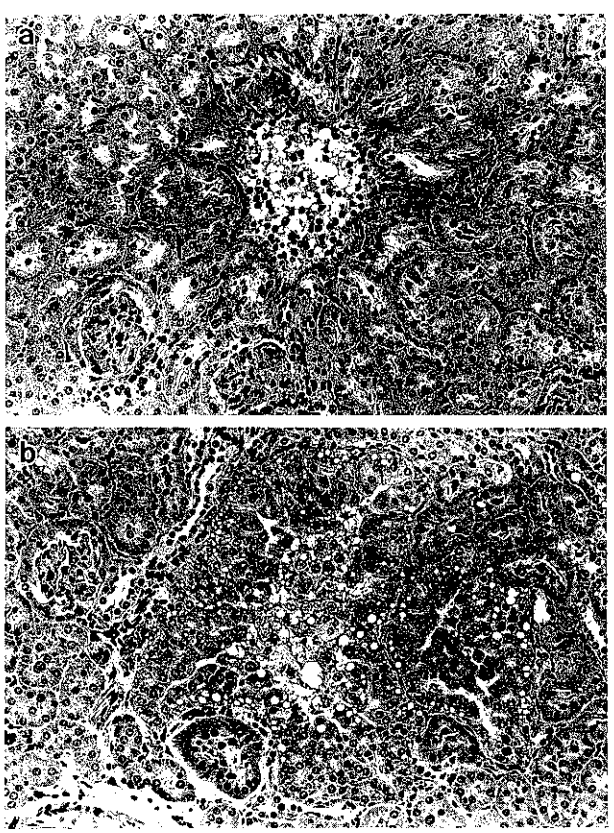


Fig. 5. Renal preneoplastic and neoplastic lesions in male mice treated with 0.8% HQ in the diet for 96 weeks. (a) Tubular cell hyperplasia composed of uniform epithelial cells with hyperchromatic round nuclei. (b) Solid tubular cell adenoma made up of uniform cells with granular cytoplasm. Note scant fibrovascular stroma at the periphery of the lesion. H & E stain. ×200.

The current study also provided evidence that HQ exerts an inhibitory influence on liver foci in rats of both sexes while, in contrast, enhancing development of not only foci but also hepatocellular adenomas in male but not female mice. Recently it was also found that HQ can exert inhibitory effects on putative preneoplastic lesions, placental glutathione S-transferase-positive foci, in rats initiated with diethylnitrosamine (DEN) (unpublished). Similarly, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are phenolic antioxidants like HQ, are known to inhibit hepatocellular tumors and their preneoplastic foci in rat two-stage hepatocarcinogenesis.[23-25] In contrast, BHA promotes development of liver foci in mice after DNA initiation[26] and BHT treatment alone is associated with enhanced mouse spontaneous liver tumors.[27-29] Fundamental differences therefore appear to exist between rats and mice regarding the effects of non-genotoxic compounds such as antioxidants on tumor development of the liver. The antioxidant ethoxyquin (EQ) also inhibits rat

hepatocarcinogenesis[24,25] and treatment with this antioxidant alone causes precancerous lesions of the renal tubules in rats.[30] In addition, EQ and BHA both exert promoting activity on rat renal carcinogenesis after suitable initiation.[25] The present findings also demonstrate that HQ inhibited bile duct hyperplasia development in male rats, as did BHA.[31] Thus, the observed effects induced by HQ in the rodent liver are essentially similar to those reported for the other phenolic antioxidants BHA and BHT.

HQ readily undergoes autoxidation to form quinone metabolites in aqueous solution and in the presence of oxygen *in vitro*.[2,16] The dominant feature of quinone is the capacity to undergo reversible oxidation-reduction reactions with concomitant formation of free radicals.[32–34] It is possible that these oxygen radicals can react directly with macromolecules such as DNA, RNA and thiol grougs of enzymes. It is also known that they are not only cytotoxic but also mutagenic, and therefore possibly carcinogenic.[35–37] In fact, the mutagenicity associated with the one-electron reduction of various quinones tested in *Salmonella typhimurium* TA 104 was attributed to generation of such oxygen radicals[38] and quinone formation was postulated to play a role in the diethylstilbestrol-induced carcinogenic process of the kidney in hamsters.[39] Species such as free radicals and quinone metabolites may play a role in HQ-associated renal carcinogenesis.

Numerous chemicals that lack genotoxicity are capable of inducing neoplasms in rodents, exposure is to high doses that cause cytotoxicity and/or chronic hyperplasia in the target organs. Prolonged exposure to nongenotoxic nephrotoxins, exemplified by unleaded gasoline and 1,4-dichlorobenzene, may lead to compensatory renal cell division which could be directly involved in the increased incidence of renal tumors in male rats.[40] Furthermore, it has been suggested that increases in renal cell replication may be directly linked to male-rat-specific protein α_{2u}-globulin.[41,42] Accumulation of this protein in proximal tubules is related to nephropathy in male rats.[40] However, the renal cell tumor development in male rats under the long-term influence of HQ was found to be not associated with α_{2u}-globulin nephropathy (unpublished data). Furthermore, since the occurrence of renal neoplastic lesions in male mice was not accompanied by renal toxicity, a different mechanism(s) from those involving α_{2u}-globulin must be considered. This area is the subject of ongoing research in our laboratory.

HQ, in contrast to catechol, as shown in previous studies,[17,18] did not cause tumor development of the forestomach and glandular epithelia in either of the species used in the present investigation, but brought about only a hyperplastic response even after prolonged treatment. This suggests that it is not a stomach carcinogen. Indeed, HQ has no promoting activity for rat two-stage forestomach or glandular stomach carcinogenesis[18] and does not alter DNA synthesis or expression of the pepsinogen isozyme 1 used to predict preneoplasia.[19]

HQ is widely distributed in our environment due to its use in industry, cosmetics and medicine, and its presence in cigarette smoke. Accordingly, humans are frequently exposed to HQ as well as other benzene metabolites. In conclusion, the present study strongly suggests that since HQ has apparent carcinogenic potential for rodents, there is a possibility that it may play a role in human cancer development.

ACKNOWLEDGMENTS

This study was supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare of Japan, as well as grants from the Experimental Pathology Research Association, Japan, and the Society for Promotion of Pathology of Nagoya, Japan.

(Received May 31, 1991/Accepted August 1, 1991)

REFERENCES

1) Raff, R. and Ettling, B. V. Hydroquinone, resorcinol and pyrocatechol. *In* "Encyclopedia of Chemical Technology," 2nd Ed., Vol. 11, pp. 462–492 (1966). John Wiley, New York.
2) Final report on the safety assessment of hydroquinone and pyrocatechol. *J. Am. Coll. Toxicol.*, **5**, 123–165 (1986).
3) Ishiguro, S., Sakuma, H., Kusama, M., Yano, S., Shimojima, N. and Sugawara, S. Glass capillary column gas chromatographic analysis of tobacco and cellulose cigarette smoke. I. Acidic fractions. *Sci. Pap. Cent. Res. Inst. Jpn. Tob. Salt Public Corp.*, **118**, 207–211 (1976).
4) Florin, I., Rutberg, L., Curvall, M. and Enzell, C. R. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology*, **18**, 219–232 (1980).
5) Sakai, M., Yoshida, D. and Mizusaki, S. Mutagenicity of polycyclic aromatic hydrocarbons and quinones on *Salmonella typhimurium* TA97. *Mutat. Res.*, **156**, 61–67 (1985).
6) Morimoto, K. and Wolff, S. Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. *Cancer Res.*,

40, 1189–1193 (1980).
7) Gocke, E., King, M-T., Eckhardt, K. and Wild, D. Mutagenicity of cosmetics ingredients licensed by the European communities. *Mutat. Res.*, **90,** 91–109 (1981).
8) Tunek, A., Hogstedt, B. and Olofsson, T. Mechanism of benzene toxicity. Effects of benzene and benzene metabolites on bone marrow cellularity, number of granulopoietic stem cells, and frequency of micronuclei in mice. *Chem.-Biol. Interact.*, **39,** 129–138 (1982).
9) Chignell, C. F. Structure-activity relationships in the free-radical metabolism of xenobiotics. *Environ. Health Perspect.*, **61,** 133–137 (1985).
10) Crebelli, R., Conti, G. and Carere, A. On the mechanism of mitotic segregation induction in *Aspergillis nidulans* by benzene hydroxy metabolites. *Mutagenesis,* **2,** 235–238 (1987).
11) Snyder, R., Dimitriadis, E., Guy, R., Hu, P., Cooper, K., Bauer, H., Witz, G. and Goldstein, B. D. Studies on the mechanism of benzene toxicity. *Environ. Health Perspect.*, **82,** 31–35 (1989).
12) Barale, R., Marrazzini, A., Betti, C., Vangelisti, V., Loprieno, N. and Barrai, I. Genotoxicity of two metabolites of benzene: phenol and hydroquinone show strong synergistic effects *in vivo. Mutat. Res.*, **244,** 15–20 (1990).
13) Aksoy, M. Hematotoxicity and carcinogenicity of benzene. *Environ. Health Perspect.*, **82,** 193–197 (1989).
14) Glatt, H. R., Padykula, R., Berchtold, G. A., Ludewig, G., Platt, K-L., Klein, J. and Oesch, F. Multiple activation pathways of benzene leading to products with varying genotoxic characteristics. *Environ. Health Perspect.*, **82,** 81–89 (1989).
15) Carlson, A. J. and Brewer, N. R. Toxicity studies on hydroquinone. *Proc. Soc. Exp. Biol.*, **84,** 684–688 (1953).
16) National Toxicology Program. Toxicology and carcinogenesis studies of hydroquinone in F344/N rats and B6C3F$_1$ mice (gavage studies). Natl. Toxicol. Program Tech. Rep. Ser., No. 366, (1989).
17) Hirose, M., Fukushima, S., Kurata, Y., Tsuda, H., Tatematsu, M. and Ito, N. Modification of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced forestomach and glandular stomach carcinogenesis by phenolic antioxidants in rats. *Cancer Res.*, **48,** 5310–5315 (1988).
18) Hirose, M., Yamaguchi, S., Fukushima, S., Hasegawa, R., Takahashi, S. and Ito, N. Promotion by dihydroxybenzene derivatives of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced F344 rat forestomach and glandular stomach carcinogenesis. *Cancer Res.*, **49,** 5143–5147 (1989).
19) Shibata, M.-A., Yamada, M., Hirose, M., Asakawa, E., Tatematsu, M. and Ito, N. Early proliferative responses of forestomach and glandular stomach of rats treated with five different phenolic antioxidants. *Carcinogenesis,* **11,** 425–429 (1990).
20) Hirose, M., Fukushima, S., Shirai, T., Hasegawa, R., Kato, T., Tanaka, H., Asakawa, E. and Ito, N. Stomach carcinogenicity of caffeic acid, sesamol and catechol in rats and mice. *Jpn. J. Cancer Res.*, **81,** 207–212 (1990).
21) Ward, J. M., Goodman, D. G., Squire, R. A., Chu, K. C. and Linhart, M. S. Neoplastic and nonneoplastic lesions in aging (C57BL/6N×C3H/HeN)F$_1$ (B6C3F$_1$) mice. *J. Natl. Cancer Inst.*, **63,** 849–854 (1979).
22) Tamano, S., Hagiwara, A., Shibata, M.-A., Kurata, Y., Fukushima, S. and Ito, N. Spontaneous tumors in aging (C57BL/6N×C3H/HeN)F$_1$ (B6C3F$_1$) mice. *Toxicol. Pathol.*, **16,** 321–326 (1988).
23) Imaida, K., Fukushima, S., Shirai, T., Ohtani, M., Nakanishi, K. and Ito, N. Promoting activities of butylated hydroxyanisole and butylated hydroxytoluene on 2-stage urinary bladder carcinogenesis and inhibition of γ-glutamyl transpeptidase-positive foci development in the liver of rats. *Carcinogenesis,* **4,** 895–899 (1983).
24) Ito. N., Tsuda, H., Sakata, T., Hasegawa, R. and Tamano, S. Inhibitory effect of butylated hydroxyanisole and ethoxyquin on the induction of neoplastic lesions in rat liver after an initial treatment with N-ethyl-N-hydroxyethylnitrosamine. *Gann,* **74,** 466–468 (1983).
25) Tsuda, H., Sakata, T., Masui, T., Imaida, K. and Ito, N. Modifying effects of butylated hydroxyanisole, ethoxyquin and acetaminophen on induction of neoplastic lesions in rat liver and kidney initiated by *N*-ethyl-*N*-hydroxyethylnitrosamine. *Carcinogenesis,* **5,** 525–531 (1984).
26) Hagiwara, A., Diwan, B. A. and Ward, J. M. Modifying effects of butylated hydroxyanisole, di(2-ethylhexyl)-phthalate or indomethacin on mouse hepatocarcinogenesis initiated by N-nitrosodiethylamine. *Jpn. J. Cancer Res.*, **77,** 1215–1221 (1986).
27) Lindenschmidt, R. C., Tryka, A. F., Goad, M. E. and Witschi, H. P. The effects of dietary butylated hydroxytoluene on liver and colon tumor development in mice. *Toxicology,* **38,** 151–160 (1986).
28) Olsen, P., Meyer, O., Bille, N. and Würtzen, G. Carcinogenicity study on butylated hydroxytoluene (BHT) in Wistar rats exposed *in utero. Food Chem. Toxicol.*, **24,** 1–12 (1986).
29) Witschi, H. P. Enhanced tumor development by butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. *Food Chem. Toxicol.*, **24,** 1127–1130 (1986).
30) Manson, M. M., Green, J. A. and Driver, H. E. Ethoxyquin alone induces preneoplastic changes in rat kidney whilst preventing induction of such lesions in liver by aflatoxin B$_1$. *Carcinogenesis,* **8,** 723–728 (1987).
31) Ito, N., Fukushima, S., Hagiwara, A., Shibata, M. and Ogiso, T. Carcinogenicity of butylated hydroxyanisole in F344 rats. *J. Natl. Cancer Inst.*, **70,** 343–352 (1983).
32) Bachur, N. R., Gordon, S. L., Gee, M. V. and Kon, H. NADPH cytochrome P-450 reductase activation of quinone anticancer agents to free radicals. *Proc. Natl. Acad. Sci. USA,* **76,** 954–957 (1979).
33) Buffinton G. D., Ollinger, K., Brunmark, A. and Cadenas, E. DT-diaphorase-catalyzed reduction of 1,4-naphthoquinone derivatives and glutathionyl-quinone conjugates. *Biochem. J.*, **257,** 561–571 (1989).

34) Thor, H., Smith, M. T., Hartzell, P., Bellomo, G., Jewell, S. A. and Orrenius, S. The metabolism of menadione (2-methyl-1,4-naphthoquinone) by isolated hepatocytes. *J. Biol. Chem.*, **257**, 12419–12425 (1982).
35) Levin, D. E., Hollstein, M., Christman, M., Schwiers, E. A. and Ames, B. N. A new *Salmonella* tester strain (TA 102) with A-T base pairs at the site of mutation detects oxidative mutagens. *Proc. Natl. Acad. Sci. USA*, **79**, 7445–7449 (1982).
36) Price, P. J., Suk, W. A., Skeen, P. C., Chirigos, M. A. and Huebner, R. J. Transforming potential of anticancer drug adriamycin. *Science*, **187**, 1200–1201 (1975).
37) Ames, B. N. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science*, **221**, 1256–1264 (1983).
38) Chesis, P. L., Levin, D. E., Smith, M. T., Ernster, L. and Ames, B. N. Mutagenicity of quinones: pathways of metabolic activation and detoxification. *Proc. Natl. Acad. Sci. USA*, **81**, 1696–1700 (1984).
39) Segura-Aguilar, J., Cortes-Vizcaino, V., Leombart-Bosch, A., Ernster, L., Monsalve, E. and Romero, F. J. The levels of quinone reductases, superoxide dismutase and glutathione-related enzymatic activities in diethylstilbestrol-induced carcinogenesis in the kidney of male Syrian golden hamsters. *Carcinogenesis*, **11**, 1727–1732 (1990).
40) Borghoff, S. J., Short, B. G. and Swenberg, J. A. Biochemical mechanisms and pathobiology of α_{2u}-globulin nephropathy. *Annu. Rev. Pharmacol. Toxicol.*, **30**, 349–367 (1990).
41) Short, B. G., Steinhagen, W. H. and Swenberg, J. A. Unleaded gasoline and 2,2,4-trimethylpentane: promoting effects on the development of a typical cell foci and renal tubular cell tumors in rats exposed to *N*-ethyl-*N*-hydroxyethylnitrosamine. *Cancer Res.*, **49**, 6369–6378 (1989).
42) Goldsworthy, T. L., Lyght, O., Burnett, V. L. and Popp, J. A. Potential role of $\alpha 2_u$-globulin, protein droplet accumulation and cell replication in the renal carcinogenicity of rats exposed to trichloroethylene, perchloroethylene, and pentachloroethane. *Toxicol. Appl. Pharmacol.*, **96**, 367–379 (1988).