Modification of the Carcinogenic Potency of Quinoline, a Hepatocarcinogen, by Fluorine Atom Substitution: Evaluation of Carcinogenicity by a Medium-Term Assay

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The potent mutagen, 5-fluoroquinoline (5-FQ), and non-mutagenic 3-fluoroquinoline (3-FQ) were tested for hepatocarcinogenicity using a medium-term assay system employing quinoline, a moderately mutagenic hepatocarcinogen, as a reference. F344 male rats were given a single i.p. injection of a submanifestational dose of diethylnitrosamine (DEN, 200 mg/kg). Then, quinoline, 3-FQ, or 5-FQ at two doses (0.1% and 0.05%) was added to their diet for a period of 6 weeks, starting from 2 weeks after the DEN injection. Control groups were administered DEN alone. All rats were subjected to a partial (two-thirds) hepatectomy at the end of week 3 and sacrificed at the end of week 8. The number and areas of GST-P (placental glutathione *S*-transferase)-positive foci induced in the liver increased significantly as a result of treatment with 0.1% quinoline, and this increase was dramatic with 5-FQ at both doses, whereas no increases were noted with 3-FQ at either dose. Thus, the results of the medium-term carcinogenicity assay predicted that quinoline, a hepatocarcinogen, would be deprived of carcinogenicity by fluorine atom substitution at position 3, and would conversely be endowed with a higher carcinogenic capacity by substitution at position 5. A semi-quantitative relationship was demonstrated between carcinogenic and mutagenic potencies.

Key words quinoline; hepatocarcinogen; carcinogenicity; medium-term carcinogenesis; fluorine substituent effect

Quinoline is a hepatocarcinogen in rats¹⁾ and is also a potent mutagen in bacterial tester strains in the presence of rat liver microsomal enzymes.²⁾ As we previously reported, quinoline is metabolized to 5,6-dihydroquinoline-5,6-diol, quinoline *N*-oxide, and 3-hydroxyquinoline in the rat liver microsomal enzyme system, and the major metabolic intermediate is 5,6-dihydroquinoline-5,6-diol produced by the enzymatic hydrolysis of 5,6-dihydroquinoline 5,6-epoxide.³⁾ We confirmed that 5,6-dihydroquinoline 5,6-epoxide is much less mutagenic than quinoline in *S. typhimurium* TA100, regardless of the presence or absence of the rat liver S-9 mix.⁴⁾ Furthermore, Willems *et al.* also reported the same finding.⁵⁾ We demonstrated that ³H-labeled quinoline was covalently bound to nucleic acid in the presence of a microsomal enzyme fraction isolated from 3-methylcholanthrene-treated rat liver, and that the quinoline-DNA adduct, once produced in DNA, released ³H-label quantitatively as 3-hydroxyquinoline under either acidic or alkaline conditions.⁶⁾ This suggested that metabolic activation might have taken place in the pyridine moiety of quinoline, as illustrated in Chart 1.⁶⁾ Since oxidative metabolism is effectively inhibited by halogen-substitution at the metabolic site,⁷⁾ 3-fluoroquinoline (3-FQ) and 5-fluoroquinoline (5-FQ) were prepared and tested for mutagenicity in *S. typhimurium*

TA100. Our results revealed that quinoline was deprived of mutagenicity by fluorine (F)-substitution at position 3 but became more mutagenic by F-substitution at position 5, as had been expected from our biochemical predictions.^{8,9)} In the present study, the *in vivo* genotoxicity of quinoline, 3-FQ, and 5-FQ were investigated in a medium-term bioassay system for hepatocarcinogenesis which was established by the 1st Department of Pathology of Nagoya City University Medical School and is referred to as the DEN-PH model.¹⁰⁾ According to this system, a single i.p. injection of a submanifestational dose of diethylnitrosamine (DEN) is given at the beginning of week 1, followed by six weeks of *p.o.* administration of quinoline derivatives to be tested, and a partial hepatectomy (PH) is performed at the end of week 3. Then, GST-P (placental glutathione *S*-transferase)-positive foci induced in the liver are quantified at the end of week 8 as preneoplastic end-point marker lesions.

MATERIALS AND METHODS

Animals and Chemicals A total of 112 male F344 rats (5-weeks-old) were obtained from Charles River Japan, Inc., Atsugi. They were randomly divided into groups and housed at 3 or 4 rats per cage, each of which contained

Chart 1. Proposed Metabolic Activation Pathways of Quinoline (Enamine Epoxide Theory)

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wood-chip bedding. The animal room was air-conditioned at $24 \pm 2^\circ\text{C}$ and $55 \pm 5\%$ humidity. Food (Oriental M, Oriental Yeast Co., Tokyo) and water were available *ad libitum* throughout the experimental period. 3-FQ and 5-FQ were synthesized from the corresponding aminoquinolines *via* the Schiemann reaction¹¹⁾ and purified by distillation *in vacuo*. Quinoline, obtained from Wako Pure Chemical Industries, Osaka, was used after purification by distillation *in vacuo*. DEN was obtained from Tokyo Chemical Industry Co., Tokyo. The test compounds were thoroughly mixed with powdered basal diet (Oriental M) and stored at room temperature until use.

Treatment of Animals The experimental protocol is shown in Fig. 1. After a one week initial observation period, the rats (16 animals for each chemically treated or control group) were intraperitoneally injected with DEN dissolved in 0.9% NaCl at a dose of 200 mg/kg body weight. Starting from two weeks after this, the animals were maintained on a powdered basal diet supplemented with quinoline, 3-FQ, or 5-FQ, or unsupplemented in the case of the control. Two doses (0.1 and 0.05%) of each quinoline were tested. All rats were subjected to a two-thirds PH at the end of week 3 and sacrificed at the end of week 8. Immediately after sacrifice, the livers were excised and cut into slices 2–3 mm thick. Three of these, one from the caudate lobe and two from the right anterior lobe, were fixed in ice-cold acetone for subsequent immunohistochemical examination of GST-P expression, as previously described.¹²⁾

GST-P Immunohistochemical Examination The avidin-biotin-peroxidase complex (ABC) method estab-

lished by Hue *et al.*¹³⁾ was employed to identify GST-P-positive liver foci. After deparaffinization, liver sections were treated sequentially with rabbit anti-rat GST-P (1:5000), biotin-labeled goat anti-rabbit IgG (1:400), and ABC (Vectastain ABC kit, PK4001, Vector Laboratories, Inc., Burlingame, CA). Sites of peroxidase binding were demonstrated by the diaminobenzidine method. The sections were counterstained with hematoxylin for microscopic examination.

Quantitative Analysis of GST-P-Positive Foci The number and areas of GST-P-positive foci which were greater than 0.2 mm in diameter and the total areas of foci observed in liver sections were estimated using a color video image processor (VIP-21C, Olympus-Ikegami Tsushin Co., Tokyo). The significance of differences between the means of the treated and control groups with respect to body weight, between the weights of liver and kidney relative to body weight, and in the number and areas of GST-P-positive foci were analyzed using Student's *t*-test and Welch's *t*-test in combination with the *F*-test.

RESULTS

Body, liver, and kidney weights at the end of week 8 are summarized in Table 1. Body weights were found to decrease remarkably in the 0.1% 5-FQ group and slightly in the 0.1% quinoline group. A severe decrease in food consumption (FC) appeared temporarily right after the partial hepatectomy in all groups. The 0.1% 5-FQ group showed less FC throughout the experimental period (Fig. 2). In contrast, liver weight and liver weight relative to body weight increased remarkably in groups treated with 5-FQ and quinoline at both doses. It is known that an increase in liver weight usually reflects an increase in genotoxicity toward the liver. No appreciable changes were observed in kidney weights relative to body weight in any groups, although a slight increase was found in the 0.1% quinoline group. The numbers and areas of GST-P-positive liver cell foci are summarized in Table 2 and illustrated in Fig. 3. The greatest increases in numbers of foci were in the 5-FQ groups, followed by the quinoline groups. Those of the 3-FQ groups were at the control level. The areas of foci in the 5-FQ groups and in the 0.1% quinoline group were also greatly increased. The order of suspected carcinogenic potencies of these sub-

Fig. 1. Experimental Protocol of the Medium-Term Bioassay for Hepatocarcinogenesis

Table 1. Body, Liver, and Kidney Weights at the End of Week 8

Group/Treatment	No. of rats	Body weight (g)	Liver weight		Kidney weight	
			Weight (g)	Relative to body weight (%)	Weight (g)	Relative to body weight (%)
1. Quinoline 0.1%	16	245.4 $\pm$ 16.2**	11.76 $\pm$ 1.27	4.78 $\pm$ 0.34***	1.80 $\pm$ 0.19	0.74 $\pm$ 0.08**
2. Quinoline 0.05%	16	255.9 $\pm$ 14.6	10.24 $\pm$ 0.87	4.00 $\pm$ 0.20***	1.79 $\pm$ 0.13	0.70 $\pm$ 0.04*
3. 3-FQ 0.1%	16	259.6 $\pm$ 8.7	8.83 $\pm$ 0.40	3.40 $\pm$ 0.11***	1.76 $\pm$ 0.09	0.68 $\pm$ 0.03
4. 3-FQ 0.05%	16	264.1 $\pm$ 9.9	8.59 $\pm$ 0.72	3.25 $\pm$ 0.20	1.72 $\pm$ 0.07	0.65 $\pm$ 0.03
5. 5-FQ 0.1%	13	216.1 $\pm$ 15.5***	11.95 $\pm$ 0.90	5.54 $\pm$ 0.21***	1.46 $\pm$ 0.11	0.68 $\pm$ 0.03
6. 5-FQ 0.05%	14	251.8 $\pm$ 14.5*	11.99 $\pm$ 1.07	4.76 $\pm$ 0.25***	1.70 $\pm$ 0.11	0.67 $\pm$ 0.02
7. None	14	266.1 $\pm$ 16.1	8.47 $\pm$ 0.60	3.18 $\pm$ 0.14	1.78 $\pm$ 0.13	0.67 $\pm$ 0.03

Data are means $\pm$ S.D. values. Significantly different from the control (group 7), * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Fig. 2. Variation of Body Weight (B.W.) and Food Consumption (F.C.)

Fig. 3. Numbers and Areas of GST-P-Positive Foci Induced in the Liver

Significantly different from basal diet group; * $p < 0.01$; ** $p < 0.001$.

Table 2. Numbers and Areas of GST-P-Positive Liver Cell Foci in Rats

Group/Treatment	No. of rats	Number (No./cm ²)	Area (mm ² /cm ²)
1. Quinoline 0.1%	16	9.23 ± 3.43**	0.687 ± 0.352*
2. Quinoline 0.05%	16	6.57 ± 1.37**	0.432 ± 0.124
3. 3-FQ 0.1%	16	4.44 ± 1.67	0.302 ± 0.148
4. 3-FQ 0.05%	16	4.18 ± 2.62	0.304 ± 0.272
5. 5-FQ 0.1%	13	21.95 ± 5.23**	2.494 ± 0.688**
6. 5-FQ 0.05%	14	17.23 ± 5.53**	1.474 ± 0.578**
7. None	14	4.28 ± 1.61	0.332 ± 0.189

Data are means ± S.D. values. Significantly different from the control (group 7), * $p < 0.01$; ** $p < 0.001$.

stances was found to be parallel to that of their known mutagenicities in *S. typhimurium* TA100.^{8,9)}

DISCUSSION

The medium-term assay system (DEN-PH model) employed in the present study has been widely used to test over 170 chemicals to date.^{10,14)} The end-point marker, GST-P-positive liver foci expression, is regarded as being highly useful for predicting carcinogenicity.^{12,15)} Results reported so far allow us to conclude that this assay system is highly reliable for the prediction of the potency of various chemicals to induce carcinogenesis in the liver.¹⁶⁾ On this basis, it is worth emphasizing that fluorine atom

substitution at an appropriate position of the aromatic ring structure might be effective in depriving these molecules of genotoxicity. As illustrated in Chart 2, an F-atom at position 5 might stabilize the key-intermediate 1,4-hydrated structure through hydrogen bonding between the F-atom and OH group at position 4, resulting in a more ready formation of its 2,3-epoxide, the ultimate genotoxic form. In contrast, an F-atom at position 3 might inhibit epoxidation of the 1,4-hydrated enamine structure because of the electron-withdrawing effect of an F-atom against the one-electron-step oxidation of the 2,3-double bond.

It may be worth mentioning briefly that these three chemicals examined in this study were subjected to a micronucleus induction assay, recently proposed by Shimada and Igarashi,¹⁷⁾ which employs partially hepatectomized mice. This method is expected to be useful as a short-term *in vivo* assay to detect genotoxicity, including carcinogenicity, in the liver. As a preliminary study, a part of each liver excised after the 6-week-treatment of partially hepatectomized rats with these chemicals in the present study was subjected to the micronuclei induction test. The results revealed that 5-FQ induced micronuclei to an appreciably greater extent than quinoline and 3-FQ, as was revealed by the present medium-term carcinogenicity assay. However, no appreciable differences were observed when micronuclei were counted 5 or 10 d after the treatment of partially hepatectomized

Chart 2. Proposed Fluorine Effects on Metabolic Activations

mice with these chemicals. Details, including the discrepancy found in both the experiments, will be discussed elsewhere.¹⁸⁾

REFERENCES

- Hirao K., Shinohara Y., Tsuda H., Fukushima S., Takahashi M., Ito N., *Cancer Res.*, **36**, 329—335 (1976).
- Nagao M., Yahagi T., Seino Y., Sugimura T., Ito N., *Muta. Res.*, **42**, 335—342 (1977).
- Tada M., Takahashi K., Kawazoe Y., *Chem. Pharm. Bull.*, **30**, 3834—3837 (1982).
- Saeki K., Takahashi K., Kawazoe Y., *Biol. Pharm. Bull.*, **16**, 232—234 (1993).
- Willems M. I., Dubois G., Boyd D. R., Davies R. J. H., Hamilton L., McCullough J. J., van Bladeren P. J., *Mutat. Res.*, **278**, 227—236 (1992).
- Tada M., Takahashi K., Kawazoe Y., *Chem.-Biol. Interactions*, **29**, 257—266 (1980).
- LaVoie E. J., Tulley-Freiler L., Bedenko V., Hoffmann D., *Mutat. Res.*, **116**, 91—102 (1983); Hecht S. S., LaVoie E. J., Bedenko V., Pingaro L., Katayama S., Hoffmann D., Sardella D. J., Boger E., Lehr R. E., *Cancer Res.*, **41**, 4341—4345 (1981); Hubenman E., Slaga T. J., *ibid.*, **39**, 411—414 (1979); Diamond L., Cherian K., Harvey R. G., DiGiovanni J., *Mutat. Res.*, **136**, 65—72 (1984); Miller E. C., Miller J. A., *Cancer Res.*, **20**, 133—137 (1960); Miller J. A., Miller E. C., Finger G. C., *ibid.*, **13**, 93—97 (1953); *idem*, *ibid.*, **17**, 387—398 (1957).
- Takahashi K., Kamiya M., Sengoku Y., Kohda K., Kawazoe Y., *Chem. Pharm. Bull.*, **36**, 4630—4633 (1988).
- Kamiya M., Sengoku Y., Takahashi K., Kohda K., Kawazoe Y., "Antimutagenesis and Anticarcinogenesis, Vol. II," ed. by Kuroda Y., Shankel D. M., Waters M. D., Plenum Pub. Corp., New York, 1990, pp. 441—446.
- Ito N., Imaida K., Hasegawa R., Tsuda H., *C.R.C. Crit. Rev. Toxicol.*, **19**, 385—415 (1989); Ito N., Tsuda H., Tatematsu M., Inoue T., Tagawa Y., Aoki T., Uwagawa S., Kagawa M., Ogiso T., Masui T., Imaida K., Fukushima S., Asamoto M., *Carcinogenesis*, **9**, 387—394 (1988).
- Araki M., Saneyoshi M., Harada H., Kawazoe Y., *Chem. Pharm. Bull.*, **16**, 1742—1746 (1968).
- Tatematsu N., Mera Y., Ito N., Satoh K., Sato K., *Carcinogenesis*, **6**, 1621—1626 (1985).
- Hue S.-M., Raine L., Fanger H., *J. Histochem. Cytochem.*, **29**, 577—580 (1981).
- Ito N., Tatematsu M., Hasegawa R., Tsuda H., *Toxicol. Pathol.*, **17**, 630—641 (1989).
- Sato K., Kitahara A., Satoh K., Ishikawa T., Tatematsu M., Ito N., *Jpn. J. Cancer Res. (Gann)*, **75**, 199—202 (1984).
- Ogiso T., Tatematsu M., Tamano S., Tsuda H., Ito N., *Toxicol. Pathol.*, **13**, 257—265 (1985); Ogiso T., Tatematsu M., Tamano S., Hasegawa R., Ito N., *Carcinogenesis*, **11**, 561—566 (1990); Imaida K., Tatematsu M., Kato T., Tsuda H., Ito N., *Jpn. J. Cancer Res. (Gann)*, **80**, 326—330 (1989).
- Igarashi M., Shimada H., *Hen-igensei Shiken*, **3**, 21—25 (1994).
- Saeki K., Kadoi M., Kawazoe Y., Igarashi M., Shimada H., in preparation.