Clastogenicity of Quinoline Derivatives in the Liver Micronucleus Assay Using Rats and Mice

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Induction of micronucleated liver cells (MN-liver cells) was examined with the hepatocarcinogenic quinoline and its fluorinated derivatives, 3-fluoroquinoline (3-FQ) and 5-fluoroquinoline (5-FQ), using non-hepatectomized rats and mice. Male F344 rats or ICR mice were given each test chemical at a daily dose of 0.5 mmol/kg for three consecutive days by *i.p.* injection, and sacrificed at six or eleven days after the final treatment. The data may suggest that the induction frequencies of MN-liver cells by the quinoline derivatives correlate with the magnitudes of both their medium-term carcinogenicity and bacterial mutagenicity. Thus, the potently hepatocarcinogenic/mutagenic 5-FQ caused significantly higher levels of induction of MN-liver cells than the vehicle in both rats and mice. The non-hepatocarcinogenic/non-mutagenic 3-FQ showed no appreciable differences in MN-liver cell induction from the control group in rats and mice. Quinoline showed a slight and statistically insignificant increase of MN-liver cells in mice, but there was not such increase in rats. These findings may suggest the utility of the micronucleus test using hepatocytes from non-hepatectomized animals, although its sensitivity may be low as compared with hepatectomized animals.

Key words —— micronucleus test, quinolines, fluoroquinolines, non-hepatectomized

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INTRODUCTION

Quinoline was predicted to be hepatocarcinogenic by a rat medium-term bioassay system,[1] and, in fact, it is a hepatocarcinogen in rats and mice.[2–4] It is also a mutagen in bacterial tester strains in the presence of the rat liver microsomal enzyme system.[5] We have proposed a possible metabolic activation pathway of quinoline toward genotoxicity/carcinogenicity; oxidative activation might have taken place in the pyridine moiety of quinoline at positions 2 and 3.[1,6–9] When the aromatic nucleus is substituted with a fluorine (F) atom, enzymatic oxidation is known to be generally inhibited at the site of F-substitution.[10–13] Our study with 3-fluoroquinoline (3-FQ) and 5-fluoroquinoline (5-FQ) demonstrated that quinoline was deprived of mutagenicity in *Salmonella typhimurium* TA100 and of hepatocarcinogenicity in the rat medium-term bioassay system by F-substitution at position 3, but it became more mutagenic and hepatocarcinogenic by F-substitution at position 5.[1,7]

Micronucleus tests using bone marrow or peripheral blood cells have been widely used for evaluating the genotoxicity of chemicals *in vivo*. Its sensitivity, however, is not high enough for some chemicals that require metabolic activation by hepatic enzymes. In order to evaluate the clastogenic effect of chemicals in the liver, three micronucleus tests using partial hepatectomy, concurrent treatment with other mitogens, or younger rats[14] have been developed. The former two methods have disadvantages of being time-consuming due to the involvement of surgery and potential interaction of the mitogens with test chemicals, respectively. The last method using young rats is likely to be more useful in a liver micronucleus test. In fact, the usefulness of this method has been supported by the collaborative study by Japanese Environmental Mutagen Society (JEMS)/Mammalian Mutagenicity Study Group (MMS).[15]

In the present study, quinoline, 3-FQ, and 5-FQ were investigated for clastogenicity in a micronucleus test using hepatocytes from non-hepatectomized rats and mice to examine the ability of fluorine-substituted quinolines to induce micronucleated hepatocytes and to discuss it in connection with the mutagenicity in bacteria and the hepatocarcinogenicity in the medium-term bioassay system. The usefulness of this assay system is also discussed particularly in comparison with a micronucleus test using hepatocytes from hepatec-

tomized animals.

MATERIALS AND METHODS

Animals and Chemicals —— Male F344 rats (5 week old) were obtained from Charles River Japan Inc. (Atsugi, Japan). Male ICR mice (6 weeks old) were obtained from Japan SLC, Inc. (Hamamatsu, Japan). Rats and mice were randomly divided into groups and housed in cages (3 rats per cage and 5 mice per cage) with wood-chip bedding in an air-conditioned animal room at 23 ± 2°C and 50 ± 10% humidity. Food (Oriental M, Oriental Yeast Co., Tokyo, Japan) and water were available *ad libitum* throughout the experiment. 3-FQ and 5-FQ were synthesized as previously reported[7] and purified by distillation *in vacuo*. Quinoline and carboxymethylcellulose (CMC) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Quinoline was used after purification by distillation *in vacuo*. Phosphate-buffered saline (PBS) was Dulbecco's PBS and used without addition of divalent ions.

Micronucleus Tests in Non-hepatectomized Rats and Mice —— After a 1-week initial observation period, the rats (5 animals in each group) or mice (5 animals in each group) were intraperitoneally administered a test chemical suspended (0.05 mmol/ml) in olive oil at a daily dose of 0.5 mmol/kg body weight for three consecutive days. In the control group, olive oil (10 ml/kg body weight) was intraperitoneally injected. All rats were sacrificed 6 days or 11 days (rats only) after the final administration. Immediately after sacrifice, the livers were excised and perfused through the portal vein with 0.5 mM EDTA in PBS and then with 0.025% collagenase in 1/15 M phosphate buffer (pH 7.4) according to the reported procedure.[16] The livers were then cut into slices and shaken in a medium bottle with 10 ml of 10% fetal bovine serum in PBS. Undigested tissues were filtered off with a cotton mesh. Hepatocytes were collected by centrifugation at 50 × *g* for 1 min, smeared on glass slides, and dried in air overnight. After the slides were immobilized with MeOH, the cells were stained with 0.00625% acridine orange in 1/15 M phosphate buffer (pH 6.8). For each treatment, the number of micronucleated liver cells (MN-liver cells) per 2000 liver cells was counted using a fluorescent microscope, and the frequency of these cells was determined.

Quantitative Analysis of MN-liver Cells —— A cell was regarded as micronucleated when it contained a micronucleus which was spherical in shape, smaller in diameter than one-quarter of the diameter of the cell nucleus, and was seen lying in the same plane as the nucleus. Differences in the mean of frequency of micronucleated liver cells between the treated and control groups were analyzed using Dunnett's *t*-test in combination with the *F*-test.

RESULTS

Figs. 1 and 2 show the frequencies of MN-liver cells induced 6 days after *i.p.* injection of quinoline, 3-FQ, or 5-FQ to rats and mice for three consecutive days at a dose of 0.5 mmol/kg body weight. 5-FQ induced significantly higher levels of the frequency of MN-liver cells than in the control group both 6 and 11 days after the final treatment, but the induction frequency decreased on the 11th day. Quinoline showed a slight and statistically insignificant increase in MN-liver cells 6 days after the treatment in mice, but no such increase was found in quinoline-treated rats. 3-FQ showed no appreciable differences in MN-liver cell induction compared to the control groups in both mice and rats.

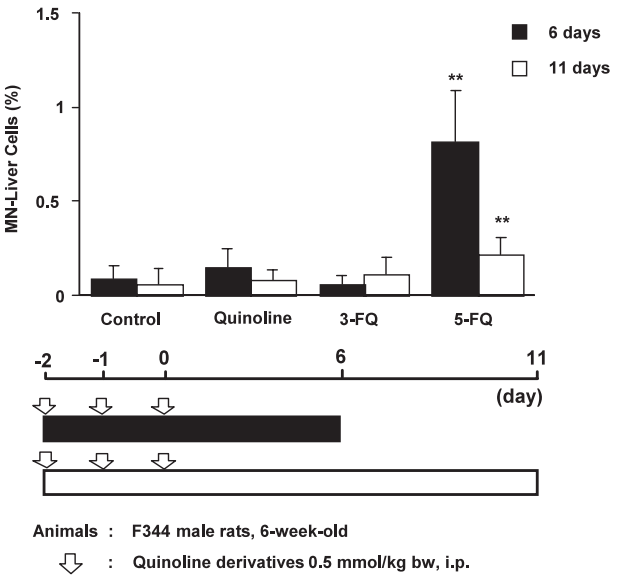

Fig. 1. Micronucleus Induction by Quinoline, 3-FQ, and 5-FQ in Hepatocytes of Rats

Each bar represents the mean ± S.D. of five individual rats. Significantly different from the control group, $^{**}p < 0.01$.

DISCUSSION

Quinoline is a hepatocarcinogen in rats and mice[2–4] and a mutagen in bacterial tester strains in the presence of the rat liver microsomal enzyme system.[5] The major metabolic intermediate of quinoline in the rat liver microsomal enzyme system is found to be 5,6-dihydroquinoline 5,6-epoxide.[17] Based on the finding that 3-hydroxyquinoline was obtained by hydrolysis of quinoline-DNA adducts under acidic or alkaline conditions, we have proposed that metabolic activation toward genotoxicity/carcinogenicity might have taken place in the pyridine moiety of quinoline at positions 2 and 3 (the enamine epoxide hypothesis) as shown in Fig. 3.[1,6–9] When the aromatic nucleus is substituted with an F atom, enzymatic oxidation is known to be generally inhibited at the site of F-substitution.[10–13] In fact, our study with 3-FQ and 5-FQ revealed that quinoline was deprived of mutagenicity in *S. typhimurium* TA100 and of hepatocarcinogenicity in the rat medium-term bioassay system by F-substitution at position 3 whereas it became more mutagenic and hepatocarcinogenic by F-substitution at position 5.[1,7] Probable reasons for the increase in mutagenicity and hepatocarcinogenicity are as follows: 5,6-epoxidation, one detoxification pathway of quinoline, is inhibited by the F atom due to its electron-withdrawing property and thereby the metabolic activation in the pyridine moiety proceeds relatively more efficiently, and there is stabilization of the enamine intermediate due to an intramolecular hydrogen bond between the F atom at position 5 and the hydroxyl group at position 4.

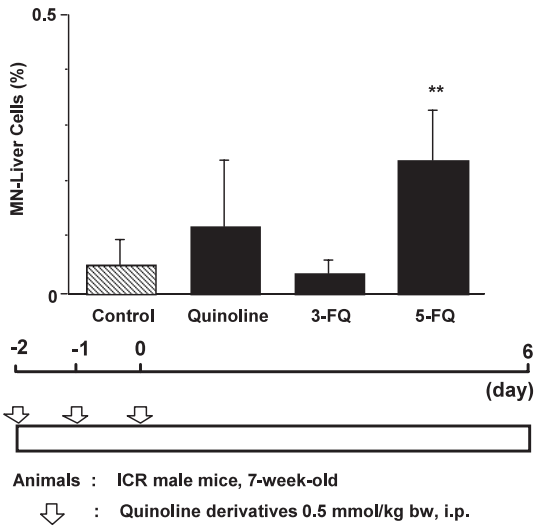

Fig. 2. Micronucleus Induction by Quinoline, 3-FQ, and 5-FQ in Hepatocytes of Mice

Each bar represents the mean ± S.D. of five individual mice. Significantly different from the control group, $^{**}p < 0.01$.

In the present study, we examined the clastogenicity of quinoline, 3-FQ, and 5-FQ in hepatocytes from non-hepatectomized mice and rats (Figs. 1 and 2). Treatment conditions were almost the same as those employed by the previous study using hepatectomized mice[16] (except for hepatectomy) for comparison. Because 5-FQ induced significantly higher levels of MN-liver cells than the vehicle, it was shown to be a clastogen in both mice and rats. On the contrary, 3-FQ showed no appreciable differences in MN-liver cell induction from the control groups, and hence was judged to be non-clastogenic in mice and rats. Since quinoline showed a considerably elevated mean of the number of MN-liver cells in mice, it was implied to be a clastogen, although it did not show any such effect in rats under the experimental conditions employed. These data of the potencies of the clastogenicity of the quinolines are consistent with our previous findings in the Ames test and rat liver medium-term bioassay in terms of similarity of the relative magnitudes of mutagenicity and carcinogenicity:[1,7] 5-FQ > quinoline > 3-FQ (negative), thus supporting the enamine epoxide hypothesis. Therefore, the clastogenictiy of quinoline might also partially contribute to its hepatocarcinogenesis in addition to its ability to induce gene mutations, as verified in a transgenic mouse model (Muta^{TM}Mouse) system.[8]

The clastogenicity of quinoline was only an equivocally positive level in the hepatocytes from

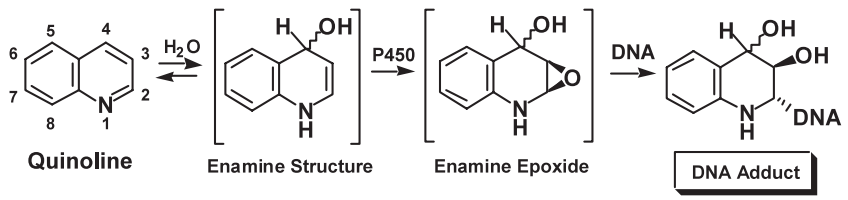

Fig. 3. Proposed Metabolic Activation Pathway of Quinoline (Enamine Epoxide Theory)

non-hepatectomized mice and was weaker than that estimated in the hepatocytes from partially hepatectomized mice under the same treatment conditions. In addition, 5-FQ was more potently clastogenic than quinoline in the present study, while the clastogenicities of quinoline and 5-FQ were of equivalent levels, *i.e.*, three times higher induction frequencies than in the control, in our previous study with hepatocytes from partially hepatectomized mice.[16] These discrepancies are, therefore, probably due to the difference of the experimental methods with and without hepatectomy. An increase in the numbers of hepatic cells in mitosis after partial hepatectomy may affect the results or yield an artificial effect: the liver micronucleus method using partial hepatectomy may be more sensitive but also may yield false positive results. The frequencies of MN-liver cells on the 11th day after the last treatment were reduced as compared with those 6 days after the treatment. Reasons for this observation might be due to inhibition of hepatocyte proliferation, suggested by a collaborative study by JEMS/MMS,[15] or due to apoptosis of cells having chromosomal damage.

In conclusion, the liver micronucleus test using hepatocytes from non-hepatectomized animals suggests that quinolines exhibit clastogenicity through the enamine epoxide pathway. The liver micronucleus method employed in this study may be useful in terms of detection of micronucleated cells in the target liver without producing false positive results, although its sensitivity may be low as compared with the method using partial hepatectomy.

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