Communications to the Editor

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DEPRIVATION OF THE MUTAGENIC PROPERTY OF QUINOLINE: INHIBITION OF MUTAGENIC METABOLISM BY FLUORINE SUBSTITUTION

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3-Fluoro-, 2- and 3-chloro-quinolines were deficient in mutagenicity in S. typhimurium TA100, whereas all the other fluoro and chloro derivatives examined were mutagenic. Together with mutagenic property of methylquinolines, the mutagenic metabolite of quinoline is presumed to be the 2,3-dihydro-2,3-epoxide of 1,4-hydrate form of quinoline. A possibility is proposed that a fluorine substitution may deprive the aromatic compounds of the genotoxicity.

KEYWORDS —— fluoroquinoline; methylquinoline; chloroquinoline; quinoline; mutagenicity; metabolism; mutagenic activation

It is a widely accepted concept that a fluorine (F) atom substituted on the aromatic ring blocks the oxidative genotoxic metabolism at the site of the F substitution in polycyclic aromatic hydrocarbons.[1-7] It is possible that introducing an F-substituent at a putative site in the molecule will deprive the molecule of any genotoxic side effects without affecting the other biological activities attributed to the molecule, since the F atom is sometimes ignored in the interactions with biomolecules, including enzymes. The steric disturbance of the F atom may be minimal because of its small van der Waals radius (1.35 Å), close to that of hydrogen (1.20 Å). It is worth noting that a methyl substituent also suppresses an enzymic oxidation at the arenic double bond involved in the methyl substitution.[1,8-10]

In the present study, we examined the substituent effect on the mutagenicity of hepatotumorigenic quinoline in Salmonella typhimurium TA100 (donated by Dr. B. Ames) in the presence of an S9 mix. The aim of this study is to determine whether quinoline is deprived of its mutagenic property through specific inhibition of the mutagenic metabolism by introducing a substituent such as fluorine. In addition, this study attempts to confirm the mutagenic activation process of quinoline that is responsible for mutagenesis and probably for carcinogenesis. The metabolic activation pathway of quinoline has previously been studied based mainly on the structure of metabolites and the property of the DNA-bound adduct.[11-14] In this study, quinoline and its 3-, 5-, 6-, and 8-fluoro derivatives were synthesized and tested for mutagenicity in TA100 in the presence of an S9 mix consisting of the

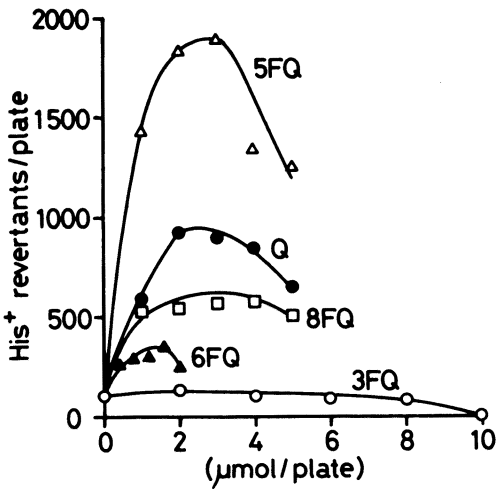


Fig. 1. Mutagenicity of Fluoroquinolines in TA100 (1.68 nmol P450/plate)

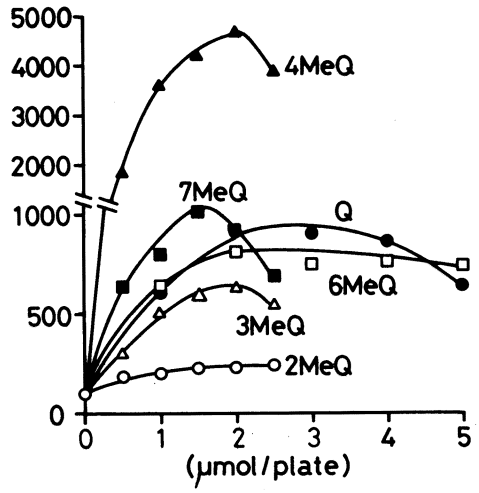


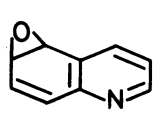
Fig. 2. Mutagenicity of Methylquinolines in TA100 (1.68 nmol P450/plate)

(**FQ**, fluoroquinoline; **MeQ**, methylquinoline; Q, quinoline)

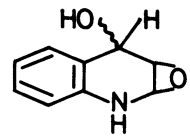
S9 obtained from rat liver (induced with phenobarbital and 5,6-naphthoflavone, Oriental Yeast Co., Tokyo), KCl, $MgCl_2$, glucose-6-phosphate (G-6-P), G-6-P dehydrogenase, NADPH, NADH, and isotonic phosphate buffer (pH 7.4). The mutagenicity assay was carried out after preincubation with the chemicals for 20 min.[15] TA100 is a tester strain sensitive to the mutagenicity of quinoline. In 3-fluoroquinoline, the F atom is located on the pyridine moiety involved in the genotoxic metabolism,[11-13] whereas that of 5-, 6-, and 8-fluoroquinolines is located on the benzene moiety which is presumed to be the site involved in the detoxication process. In addition, some methyl and chloro derivatives were also tested. It is noted here that none of the derivatives examined induced revertants without an S9 mix.

Mutagenicity of fluoroquinolines The mutagenicity of fluoroquinolines in the presence of the S9 mix, in comparison to that of quinoline, are shown in Fig. 1. 5-Fluoroquinoline was more potently mutagenic than quinoline itself, and 8-fluoroquinoline followed. The 6-Fluoro derivative was weakly mutagenic. 3-Fluoroquinoline was not mutagenic. It is, therefore, safely concluded that the 3-fluoro derivative would be much weaker than the other fluoroquinolines assayed, even if it might be mutagenic.

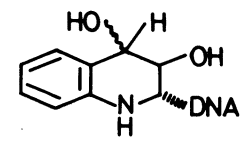
Mutagenicities of methylquinolines Mutagenicities of methylquinolines are shown in Fig. 2. These derivatives were previously tested in a similar assay system in which ATP was used as an additional cofactor.[16] There were no significant discrepancies in the relative mutagenic potencies in any of these compounds. 4-Methylquinoline was the most mutagenic, followed by 7-methylquinoline and quinoline. 3-Methyl and 6-methyl derivatives were somewhat less mutagenic, while the 2-methylquinoline was much less active than the other methylquinolines.



The major epoxide in microsomal metabolism



Presumed mutagenic metabolite



Presumed DNA-quinoline adduct

(The absolute configurations have not been examined yet.)

Mutagenicities of 2-, 3-, 4- and 6-chloroquinolines (Data not shown) 4-Chloroquinoline proved to be weakly mutagenic, whereas 2- and 3-chloroquinolines were non-mutagenic. 2-Chloroquinoline was previously reported to be non-mutagenic and non-carcinogenic.[17,18] It is to be noted that, contrary to the fluoroquinolines, all the chloroquinolines examined were cytotoxic regardless of the presence or absence of the S9 mix. The mechanism of enhanced cytotoxicity of chloro derivatives is now under investigation.

Discussion The 3-fluoro substituent completely deprived the quinoline molecule of its mutagenicity, whereas other fluorines substituted on the benzene moiety did not. These results are consistent with our earlier proposal[11-13] that the oxidation of the pyridine moiety is the mutagenic activation process of quinoline. Thus, it is possible that the 3,4-dihydro-3,4-epoxide or a species of the 2,3-epoxide are responsible for quinoline mutagenesis, if the ultimate reactant is an arene epoxide.

The fluorine at position-5 increased the mutagenicity to a remarkable extent. This potentiating effect is well understood, when the following proposals previously documented are taken into consideration: (1) The F-substituent inhibits enzyme oxidation not only of the double bond involved in the F-substitution but also of the double bond at located at the position peri to the fluorine,[3,19] although the opposite effect has been documented.[20,21] (2) An arenic double bond at a peri position to the ring nitrogen carrying a lone pair of electrons resists exzyme oxidation.[22] In fact, the 7,8-dihydro-7,8-diol or its precurser, 7,8-dihydro-7,8-epoxide, was not found in the metabolites of quinoline.[11,14] Thus, in 5-fluoroquinoline, the formation of 3,4- and 5,6-dihydro-epoxides must be suppressed by the 5-F-substituent while that of 7,8-dihydro-epoxide must be suppressed by the ring nitrogen at the peri position. Therefore, only the double bond available to an epoxidation is limited to the 2,3-double bond of 1,4-dihydro-4-hydroxyquinoline, a hydrate form of quinoline.[23] Namely, 1,2,3,4-tetrahydro-4-hydroxyquinoline 2,3-epoxide is the most probable structure of the mutagenic principle of quinolines.

Looking at the mutagenicities of methylquinolines, an extraordinary increase in the mutagenicity of 4-methyl isomer may suggest that the position-4 cannot be involved in the oxidative activation and that a bulky methyl group at the position-4 might protect the adjacent 2,3-dihydro-epoxide from an detoxifying attack of epoxide hydrolase, resulting in an enhancement of the mutagenicity. This mechanism may be related to that involved in the enhancement effect of a bay region methyl group on tumorigenicity of polycyclic aromatic hydrocarbons.[4,24,25] The 2-methyl isomer is very weakly mutagenic, suggesting that this position may be

involved in the DNA binding as shown in the chart. It is known that methyl-substitution at the site involved in the epoxide formation is known to partially reduce the mutagenicity.[8-10]

The fact that 2- and 3-chloroquinolines are non-mutagenic while the 4-chloro isomer is weakly mutagenic seems to eliminate the possibility that the 3,4-epoxide would be the mutagenic metabolite.

In conclusion, the present study, together with our previous studies,[11-13] reveal that the mutagenic metabolite is produced through a microsomal oxidation of the pyridine moiety but not the benzene moiety and that the 2,3-epoxide of 1,4-hydrate form of quinoline may be the most probable structure of the mutagenic principle among all the other possible arenic epoxides, although direct evidence is not yet available. In addition, the introduction of a halogen (F or Cl) onto position-3 deprives the quinoline molecule of its mutagenic property. It is worth noting that 3-fluoroquinoline is much less cytotoxic than the 2- or 3-chloro derivatives, although the latter two are also deprived of the mutagenicity. A comprehensive review of the substituent effects on mutagenicity and cytotoxicity of quinolines will be published in detail in a forthcoming full paper.

REFERENCES

1) E. J. LaVoie, L. Tulley-Freiler, V. Bedenko and D. Hoffmann, Mutation Res., **116**, 91 (1983).
2) S. S. Hecht, E. J. LaVoie, V. Bedenko, L. Pingaro, S. Katayama, D. Hoffmann, D. J. Sardella, E. Boger and R. E. Lehr, Cancer Res., **41**, 4341 (1981).
3) E. Hubenman and T. J. Slaga, Cancer Res., **39**, 411 (1979).
4) L. Diamond, K. Cherian, R. G. Harvey and J. DiGiovanni, Mutation Res., **136**, 65 (1984).
5) E. C. Miller and J. A. Miller, Cancer Res., **20**, 133 (1960).
6) J. A. Miller, E. C. Miller and G. C. Finger, Cancer Res., **13**, 93 (1953).
7) J. A. Miller, E. C. Miller and G. C. Finger, Cancer Res., **17**, 387 (1957).
8) S. K. Yang, Drug Metab. Dispos., **10**, 205 (1982).
9) S. K. Yang, M. W. Chou, H. B. Weems and P. D. Fu, Biochem. Biophys. Res. Commun., **90**, 1136 (1979).
10) T. Kinoshita, M. Konieczny, R. Santella and A. M. Jeffrey, Cancer Res., **42**, 4032 (1982).
11) M. Tada, K. Takahashi and Y. Kawazoe, Chem. Pharm. Bull., **30**, 3834 (1982).
12) M. Tada, K. Takahashi, Y. Kawazoe and N. Ito, Chem.-Biol. Interact., **29**, 257 (1980).
13) M. Tada, K. Takahashi and Y. Kawazoe, "Microsome, Drug Oxidation and Drug Toxicity" ed. by R. Sato and R. Kato, Japan Scientific Societies Press, Tokyo, 1982, p.517.
14) E. J. LaVoie, E. A. Adams, A. Shigematsu and D. Hoffmann, Carcinogenesis, **4**, 1169 (1983).
15) K. Takahashi, T. Kaiya and Y. Kawazoe, Mutation Res., **187**, 191 (1987).
16) M. Dong, I. Schmertz, E. LaVoie and D. Hoffmann, "Polynuclear Aromatic Hydrocarbons" ed. Jones and Freudenthal, vol. 3, pp. 97-108, Raven Press, New York (1978).
17) M. Nagao, T. Yahagi, Y. Seino, T. Sugimura and N. Ito, Mutation Res., **42**, 335 (1977).
18) K. Hirao, Y. Shinohara, H. Tsuda, S. Fukushima, M. Takahashi and N. Ito, Cancer Res., **36**, 329 (1976).
19) S. Amin, J. Camanzo and S. S. Hecht, Cancer Res., **44**, 3772 (1984).
20) G. K. B. Prasad, S. Mirsadeghi, C. Biehlert, R. A. Byrd and D. R. Thakker, J. Biol. Chem., **263**, 3676 (1988).
21) D. R. Buhler, F. Ünlü, D. R. Thakker, T. J. Slaga, A. H. Conney, A. W. Wood, R. L. Chan, W. Levin and D. M. Jerina, Cancer Res., **43**, 1541 (1983).
22) E. J. LaVoie, E. A. Adams and D. Hoffmann, Carcinogenesis, **4**, 1133 (1983).
23) D. L. Tullis and S. Banerjee, Cancer Lett., 241 (1984).
24) S. S. Hecht, S. Amin, K. Huie, A. A. Melikian and R. G. Harvey, Cancer Res., **47**, 5310 (1987).
25) A. A. Milikian, S. Amin, K. Huie, S. S. Hecht and R. G. Harvey, Cancer Res., **48**, 1781 (1988).

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