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METABOLITES OF QUINOLINE, A HEPATOCARCINOGEN, IN A SUBCELLULAR MICROSOMAL SYSTEM

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It was evidenced that quinoline was metabolized to quinoline 1-oxide catalyzed by cytochrome P-450-linked mixed function monooxygenase system and to 5,6-trans-dihydroxy-5,6-dihydroquinoline by cytochrome P-448-linked one. The third metabolite, 3-hydroxyquinoline, may be produced in another course of metabolism, which might be connected with genotoxicity of quinoline, as previously discussed.

KEYWORDS —— quinoline; metabolism; microsome; cytochrome; dihydrodiol; epoxide

Quinoline, a hepatocarcinogen,[1] is known to require enzymic activation for its genotoxicities. Chemically, quinoline binds covalently to nucleic acid[2] and biologically, it mutates bacterial cells only in the presence of rat liver microsomal fractions.[3] The mixed function monooxygenase system is assumed to take part in its metabolic activation. Here we report the metabolites of quinoline in a subcellular microsomal enzyme system.

Materials and Methods

The ^{3}H-quinoline preparation used has a specific activity of 29.8 Ci/mol.[2] 2,8-Dideuterioquinoline was prepared by the catalytic dehalogenation of 8-chloroquinoline with D_2 gas, followed by its treatment with alkaline D_2O. Hepatic microsomal fractions were prepared from the rat pretreated with the enzyme inducers.[2] The inducers used were phenobarbital (PB), 3-methylcholanthrene (3MC) and polychlorinated biphenyl (PCB).

The reaction mixture for quantitation of the metabolites of quinoline was prepared in HEPES buffer (0.05 M, pH 7.45) containing 1 mg/ml of microsomal protein, 3 mM NADPH and 0.5 mM quinoline. Incubation was carried out for 15 min at 37°C. The metabolites of ^{3}H-quinoline in the reaction mixture were chromatographed on a thin-layer silica gel plate developed with the following solvent systems: (A) chloroform/ethanol (9:1 V/V), (B) hexane/acetone (1:1 V/V), (C) ethyl acetate/acetic acid/methanol (6:1:3 V/V). The chromatograms were cut into pieces (0.5 x 1.0 cm) and the radioactivity was counted in 5 ml of liquid scintillation solution.

Table I. Metabolites of Quinoline

Fraction[a]	Rf values Solvent systems[b]			Ratio of metabolites (%)[c] (enzyme inducer used)			
	A	B	C	none	PB	3MC	PCB
I	0.00	0.00	0.00	0.0	3.2	7.1	8.9
II	0.20	0.18	0.57	9.4	4.2	58.4	83.2
III	0.44	0.13	0.50	4.1	36.9	3.3	4.8
IV	0.51	0.60	0.83	2.1	0.0	2.2	0.0
V	0.66	0.73	0.74	84.4	55.6	29.0	3.1

a) These fractions were assigned to the following metabolites, respectively: I, unidentified; II, 5,6-trans-dihydroxy-5,6-dihydroquinoline; III, quinoline 1-oxide; IV, 3-hydroxyquinoline; V, quinoline and other unidentified metabolites.
b) See the text.
c) Enzyme inducer used for the pretreatment of rats.

Separation and Structure Determination of the Metabolites

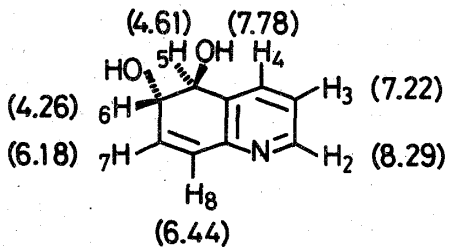
Five fractions were found on the radiochromatogram and denoted as fractions I - V, respectively, in each incubation mixture. Their Rf values are shown in Table I. The relative amounts of the fractions I to V are shown in the same table in percentages of the total radioactivity found on each chromatogram. On the basis of Rf values and UV spectrum, fraction III was assigned to quinoline 1-oxide and fraction IV was assigned to 3-hydroxyquinoline. Fraction V seems to contain the starting material quinoline and other unknown metabolites. The metabolites at the origin on the chromatogram were not identified. The chemical structure of the fraction II was studied by ^{1}H-NMR with the help of the metabolic data from 2,8-dideuterioquinoline used as the starting material, as follows.

Structure of the Metabolite from the Fraction of II

Analysis of the ^{1}H-NMR spectrum of this metabolite enabled us to formulate its structure as shown in Fig. 1. The chemical shifts of each proton measured in dimethyl sulfoxide-d_6 are shown in δ-unit in the same figure and the spin-spin coupling constants (J values) are also shown. It appears that the spectrum consists of three groups of signals: 3 protons in the aromatic region, 2 protons in the ethenic region, and 2 protons in the aliphatic -CH-O- region. The pattern of the signals in the field below 7.0 may be assigned to the signals due to the protons at 3- and 4-, and 2-positions, respectively, of the pyridine moiety of the quinoline ring; a quartet for 3-proton, a broad doublet (slightly spin-spin coupled probably with 2- and 8-protons) for 4-proton and a slightly split doublet at the lowest field for 2-proton. Such signal assignments can be supported by the spin-spin coupling parameters; $J_{2,3}$ = 5.0 Hz and $J_{3,4}$ = 7.6 Hz, which are characteristic of the couplings of pyridine ring protons. The signals between 6.0 and 7.0 may be assigned to vicinal ethenic protons, each of which is slightly coupled with another proton. This consists of a typical AB type of the signal pattern, the coupling constant ($J_{A,B}$) being 9.9 Hz. The signal between 4.0 and 5.0 may be assigned to vicinal protons adjacent to an oxygen function. To be noteworthy, all the resonance lines between d 4.0 and 5.0 are broadened, whereas they become sharpened upon addition of D_2O to the solution measured for NMR spectrum. This is a strong indication of the fact that the vicinal CH groups giving broadened signals are bound to the OH group. It turns out that the

signals in the highest region are assigned to the protons in the structure of OH-CH-CH-OH. All the NMR information described above leads to a tentative conclusion that this metabolite is either 5,6-dihydroxy-5,6-dihydroquinoline or 7,8-dihydroxy-7,8-dihydroquinoline. Usage of deuterium-labeled quinoline enabled us to derive the final conclusion on the structure. Thus, 2,8-dideuterioquinoline took the place of non-labeled quinoline in the metabolic study. In the spectrum of the deuterated metabolite, the signal of 2-proton disappeared and that of 3-proton deformed from a quartet to a doublet. This is unequivocal evidence for the signal assignments of the aromatic ring protons described. Deuterium labeling also produced a complete reduction of signal-intensity of one of the ethenic protons at the lower field (6.44), accompanied by deformation of the signal pattern of the other at the higher field (6.18). In addition to these changes in the signal pattern in the ethenic region, a slight change was produced in the aliphatic proton region; a triplet of the higher field of signals (4.26) became two doublets, whereas the lower field of signals (4.61) remained intact. It is therefore safely concluded that the signal at 6.44 is due to the 8-proton and that the siganl at 4.26 is due to the 6-proton, the spin-spin coupling with which becomes negligible by deuterium replacement of 8-hydrogen. Thus, all the signal-assignments propose the unequivocal structure of 5,6-dihydroxy-5,6-dihydroquinoline for this metabolite, as shown in Fig. 1.

Further information on the conformational structure is derived from the spin-spin coupling data shown in Fig. 1. Thus, a large $J_{5,6}$ value of 9.9 Hz must come from the anti-conformation of 5- and 6-protons, hence the two hydroxyl groups are situated in a trans configuration and both are in an equatorial conformation. This is supported by the fact that the rather smaller value (2.4 Hz) found for $J_{6,7}$ fits nearly perpendicular dihedral angle of H_7-C_7-C_6-H_6.[4] This fact enables us to formulate the steric structure as shown in Fig. 2. The absolute configuration is open to further investigation.



$J_{2,3}$ = 5.0 Hz
$J_{3,4}$ = 7.6 Hz
$J_{5,6}$ = 9.9 Hz
$J_{7,8}$ = 10.0 Hz
$J_{6,7}$ = 2.4 Hz
$J_{6,8}$ = 1.6 Hz

Fig. 1

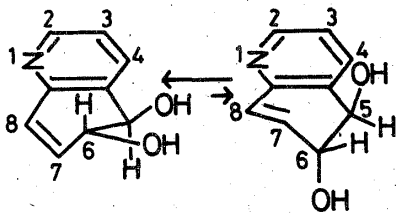


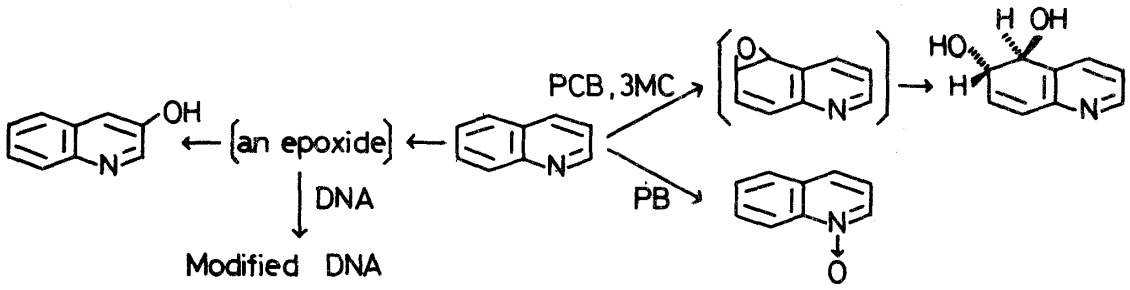
Fig. 2

Metabolic Pathway of Quinoline in Microsomal System

As shown in Table I, the relative amounts of the metabolites are strongly dependent upon the enzyme inducer used for the pretreatment of the animal. The principle metabolite was quinoline 1-oxide (the fraction III) with the PB-pretreatment, whereas it was the 5,6-dihydro-5,6-diol (the fraction II) with either the 3MC- or PCB-pretreatment. It is, therefore, strongly suggested that quinoline 1-oxide is formed by cytochrome P-450-linked monooxygenases and that the

dihydro-diol is produced by the cytochrome P-448 type. These suggestions were supported by the effects of selective enzyme inhibitors on quinoline metabolism. Thus, 7.8-benzoflavone, an inhibitor for the cytochrome P-448, inhibited the formation of the dihydro-diol completely. On the other hand, SKF-525A, an inhibitor for the cytochrome P-450, suppressed the formation of quinoline 1-oxide to a remarkable extent. In addition, in the presence of 1 mM 3,3,3-trichloropropene oxide (TCPO), an inhibitor for the epoxide hydrase, the formation of the dihydro-diol was inhibited. In a lapse of time, the fraction V gradually decreased and the fraction II increased stoichiometrically. These results suggest that the 5,6-dihydro-diol (the fraction II) was produced by the epoxide hydrase from the 5,6-epoxide (the fraction V), which was not separated from the starting material quinoline on the chromatogram under the experimental conditions employed.

All the data described here propose the following major metabolic pathways of quinoline in a subcellular microsomal enzyme system, as shown in following chart. It is noteworthy that our previous work on the DNA modification indicates that another metabolic epoxidation process might be taking place in the pyridine moiety, probably in a minor course of the metabolism, to form an ultimate metabolite which might modify DNA bases leading to the genotoxicity of quinoline. 3-Hydroxyquinoline (the fraction IV) might have been produced in the latter minor course of metabolism. The structure of the ultimate carcinogen is therefore assumed to be the 3,4-dihydro-3,4-epoxide or the 1,2,3,4-tetrahydro-4-hydroxy-2,3-epoxy-1,4-hydrate, which will be fully discussed in a forthcoming paper.



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