Excretion of Metabolites in Bile Following the Administration of Primaquine to Rats

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Abstract: Following the administration of primaquine diphosphate (20 mg/kg IP), six metabolites of primaquine were found in the bile of rats. Quantitative HPLC of the metabolites revealed that the sum of the six metabolites excreted in the bile represented a quantitative recovery of the dose of primaquine.

When C-14 labeled primaquine was administered to rats by intravenous or intraperitoneal routes, it has been shown that 14 % to 18 % of the label was excreted into the feces (24 h), while only 26 % to 37 % of the label was excreted into the urine (1). In the same study it was found that the C-14 label also appeared in the lumen of the small intestine, indicating that primaquine or its metabolites were being excreted into the small intestine through the bile. The study also found other indications of enterohepatic recirculation and first-pass metabolism.

The only metabolite of primaquine that has been identified in the plasma of test subjects (2) is the carboxylic acid metabolite, carboxyprimaquine (8-(3-carboxy-l-methylpropylamino)-6-methoxyquinoline) which results from the oxidative deamination of the substrate. The structure of carboxyprimaquine was first elucidated as a metabolite derived from the biotransformation of primaquine by fungi (3), and it was also identified as the major metabolite of mammals (2). In rats (1), 22 % of the primaquine was converted to the carboxylic acid metabolite, and in monkey (3) 35 % to 83 % of the dose was converted to this metabolite. High levels of the metabolite have also been reported in human plasma (4–7).

The objective of the present study was to determine the chemical nature of C-14 labeled material excreted in rat bile following the administration of primaquine.

Materials and Methods

Four male Wistar rats (400–430 g) were administered 20 mg/kg primaquine diphosphate by intraperitoneal injection, and four control rats were given saline. Using the procedure of Mulder et al. (8), the bile ducts of the rats were cannulated 24 h before administration of primaquine, so that the rats were fully recovered from the anesthesia. Bile samples of the test rats and the control rats were collected at 3, 6, 12, and 24 h after administration of the drug. The average bile flow rate was 0.51 ml/h.

High performance liquid chromatographic analysis of the bile samples were conducted using a 4.6 mm × 25 cm Whatman Partisil ODS-3 (5 μ) column with a mobile phase (1.0 ml/min) prepared using 6.6 g K_2HPO_4, 8.8 g KH_2PO_4, 4.0 g *N,N*-dimethyloctylamine, 1.8 l CH_3OH, and 2.2 l water. For the analysis, 10 μl of the bile sample was injected directly into the instrument, and the chromatograms were obtained with a dual wavelength (254 mm and 280 mm) Waters Associates Model 440 detector. Quantitations were based on peak areas, and the instrument was calibrated with a pure reference standard (3) of 8-(3-carboxy-l-methylpropylamino)-6-methoxyquinoline (carboxyprimaquine). For the quantitations of the unknown metabolites, it was assumed that their molar absorptivities were the same as that of carboxyprimaquine.

Results and Discussion

The chromatograms of the bile samples (Fig. 1) showed the presence of extremely high concentrations of primaquine metabolites. The concentration of the carboxyprimaquine (M6 in Fig. 1) reached concentrations in the 300–400 μg/ml range during the 3–6 h collection period. No significant concentration of the parent drug was detected at any time period. This was in marked contrast to the levels of carboxyprimaquine (5–10 μg/ml) that have been measured in the plasma of the rats (1). The concentrations of some of the other metabolites (Table I) reached even higher levels.

The cumulative amount of the metabolites excreted into the bile (Fig. 2) was found to rise fairly rapidly (half-life: 3–4 h) with no appreciable lag time. It was also found that the sum of the six metabolites excreted in 24 h represented a quantitative recovery of the dose of primaquine diphosphate that had been administered (Table II). From these findings and the previously reported C-14 tracer studies (1), it would appear that intraperitoneally administered primaquine was selectively trapped by the liver, and the majority of the drug was metabolized in the first pass through the liver. High levels of these metabolites (but no primaquine) were then excreted into the bile, reached the small intestines 3–6 h later, then underwent enterohepatic recirculation.

When a pure sample of carboxyprimaquine was administered in a molar amount equivalent to 20 mg/kg primaquine diphosphate, chromatograms of the bile were essentially the same as in Figure 1, except that only M3, M4, and M6 (carboxyprimaquine) were present in significant concentrations and only traces of M2 were detected. Metabolites M1 and M5 were not detected in any of the samples. A synthetic reference standard of the glycine conjugate of carboxyprimaquine (Fig. 3) was found to have a retention time between M2 and M3, and the A_{254}/A_{280} detector response ratio of the three compounds were very similar. These findings suggested that the quinoline ring of metabolites M2, M3, and M4 were not altered while their side chain functionalities were derived from the carboxylic acid. Possible structures would be the glucuronide conjugate of

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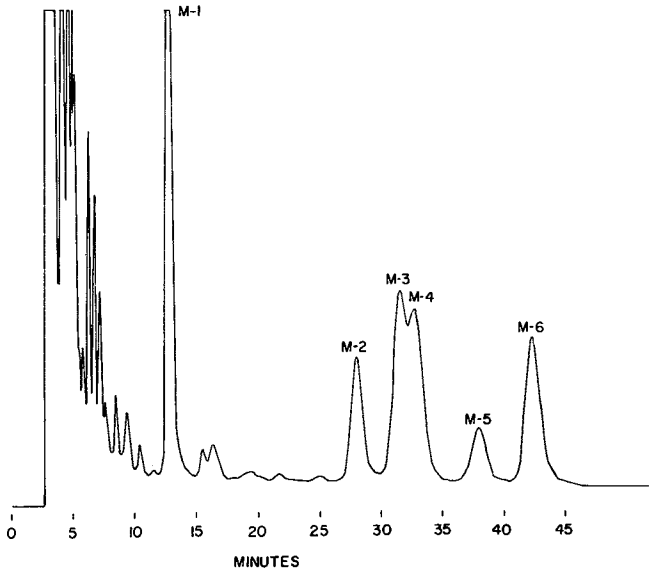

Fig. 1 High performance liquid chromatogram of the bile of rats administered 20 mg/kg primaquine diphosphate. The sample was collected over the 6–12 h time period. Only the response of the 254 nm detector is shown.

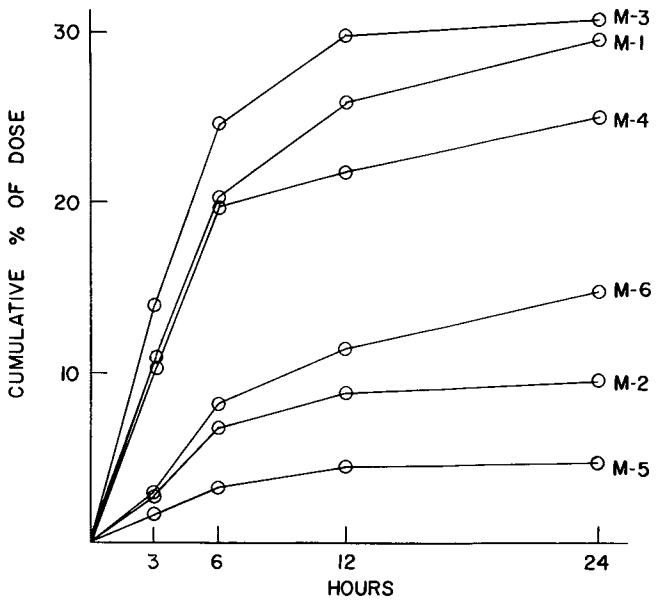

Fig. 2 Cumulative excretion of primaquine metabolites in bile. The data represents the average of two test animals.

Table I. Metabolites Excreted in Bile Following Administration of 20 mg/kg Primaquine Diphosphate.

	Metabolite Concentration in μg/ml					
Time	M1	M2	M3	M4	M5	M6[a]
0– 3 h	640	303	730	584	106	247[b]
3– 6 h	553	348	749	600	95	365
6–12 h	328	179	449	359	59	264
12–24 h	67	22	67	54	6	98

[a]carboxylic acid metabolite of primaquine (1, 2).
[b]average concentration for two test animals.

Table II. Per Cent of the Dose Excreted in the Bile in 24 Hours.

Metabolite	% of Dose
M1	29[a]
M2	9
M3	31
M4	25
M5	5
M6 (carboxyprimaquine)	15
Total[b]	114

[a]average for two animals
[b]in a non-cannulated animal, some of the metabolites could be recycled to the central compartment.

carboxyprimaquine, a conjugate of carboxyprimaquine with an amino acid other than glycine, or the metabolite of carboxyprimaquine where an additional two carbon atoms have been lost from the side chain. Of these possibilities, the amino acid conjugates of carboxyprimaquine would appear to be the most promising for future studies.

A number of other synthetic reference standards were also compared to the metabolites in bile and were found to have different retention times and A_{254}/A_{280} detector response ratios. These include: primaquine, primaquine acetate, the lactam of M6, 5-hydroxy-6-desmethylprimaquine, 6-desmethylprimaquine, and 8-amino-6-methoxyquinoline. Recent studies (9) have shown that fungi can metabolically convert primaquine to a highly lipophilic 5,5-diphenyl dimer and a 5,5-methylene linked dimer. However, these dimers were found to have retention times considerably longer than M6.

In summary, intraperitoneally administered primaquine was rapidly concentrated in the liver where the majority of the dose was transformed to the carboxyprimaquine (M6) or to other metabolites that have a common pathway (M2, M3, and M4). In the rats with cannulated bile ducts, the dose was quantitatively recovered in the bile, while in non-cannulated rats (1) approximately equal quantities of the metabolites were excreted in feces and urine.

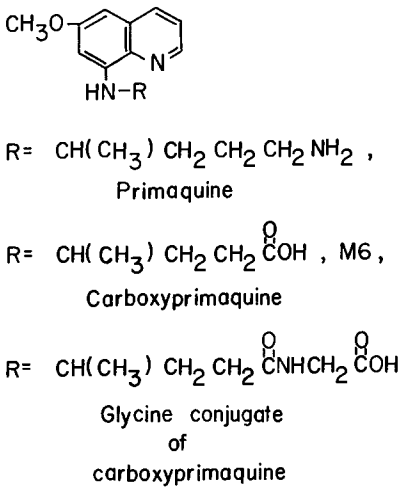

Fig. 3

Acknowledgements

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The Hypolipidemic Activity of Furoic Acid and Furylacrylic Acid Derivatives in Rodents

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Abstract: 2-Furoic acid, 3-furoic acid, 3,4-furan dicarboxylic acid and furyl-acrylic acid were evaluated for hypolipidemic activity in mice and rats. 2-Furoic acid was the most potent agent of the four tested, lowering serum cholesterol levels 41 % and serum triglyceride levels 56 % at 20 mg/kg/day in mice and serum cholesterol 50 % and serum triglyceride levels 42 % in rats. 2-Furoic acid effectively suppressed liver mitochondrial citrate exchange, ATP dependent citrate lyase, acetyl CoA synthetase, acyl CoA cholesterol acyl transferase, *sn*-glycerol-3-phosphate acyl transferase, phosphatidate phosphohydrolase and hepatic lipoprotein lipase enzymatic activities. Lipid levels after 16 days in mice were reduced in the liver. In the rat cholesterol content of the HDL fraction was elevated and lowered in the chylomicron fraction. 2-Furoic acid administration for 14 days resulted in a large portion of ^{3}H-cholesterol being excreted by the biliary route. The furoic acid derivatives appear to have promise as hypolipidemic agents and further studies on their ability to lower lipids are warranted.

Recently we have shown that a number of five-member cyclic ring structures possess potent hypolipidemic activity in rodents, e. g. succinimide (1), pyrrolidine (2) and 2-pyrrolidinones (3) have proven to lower both serum cholesterol and triglycerides in mice and rats at the low dose level of 20 mg/kg/day. These agents as well as the cyclic imides, that contain also one aromatic cyclic ring, were more potent than most commercially available agents (4), e. g. colfibrate, tiadenol, cholestyramine, nicotinic acid. All of these agents lowered serum cholesterol and triglycerides levels in CF_1 male mice approximately 40 %. Since furoic acid derivatives are available commercially, we decided to examine their capability to lower serum cholesterol and triglyceride levels in rodents. Those results are reported herein.

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Materials and Methods

Source of Compounds

2-Furoic acid, 3-furoic acid, 3,4-furandicarboxylic acid and furyl-acrylic acid were purchased from Aldrich Chemical Company, Inc. Radioisotopes were purchased from New England Nuclear. Substrates and cofactors for the enzyme reaction medium were obtained from Sigma Chemical Company.

Antihyperlipidemic Screens in Normal Rodents

Test compounds were suspended in an aqueous 1 % carboxymethylcellulose solution, homogenized, and administered to CF_1 male mice (~ 25 g) intraperitoneally for 16 days or Sprague Dawley male rats (~ 350 g) orally by an intubation needle for 14 days. On days 9 and 14 or 16, blood was obtained by tail vein bleeding and the serum separated by centrifugation for 3 min. The serum cholesterol levels were determined by a modification of the Liebermann-Burchard reaction (5). Serum was also collected on day 14 or 16 and the triglyceride content was determined by a commercial kit (Fisher, Hycel Triglyceride Test Kit).

Testing in Hyperlipidemic Mice

CF_1 male mice (~ 25 g) were placed on a commercial diet (U.S. Biochemical Corporation Basal Atherogenic Test Diet) that produced a "hyperlipidemic" state (4). After the serum cholesterol and triglyceride levels were observed to be elevated, the mice were administered test drugs at 20 mg/kg/day, intraperitoneally for an additional 14-day period. Serum cholesterol and triglyceride levels were measured at that time.

Animal Weights and Food Intake

Periodic animal weights were obtained during the experiments and expressed as a percentage of the animal's weight on day 0. After dosing for 14 days with test drugs, selected organs were excised, trimmed of fat and weighed. Food consumption was determined daily.

Toxicity Studies

The acute toxicity (LD_{50} values) (6) was determined in CF_1 male mice (~ 25 g) by administering test drugs intraperitoneally from 100 mg to 2 g/kg as a single dose. The number of deaths was recorded over a 7-day period for each group.

Enzymatic Studies

In vitro enzymatic studies were determined using 10 % homogenates of CF_1 male mouse liver with 50–200 μM of test drugs. *In vivo* enzymatic studies were determined using 10 % liver homogenates [prepared in 0.25 M sucrose + 0.001 M (ethylenedinitrilo)-tetraacetic acid, pH 7.2] from CF_1 male mice