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THE EFFECT OF DIET AND PHENOBARBITONE ON QUININE METABOLISM IN THE RAT AND IN MAN

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Abstract—Quinine is metabolized by rat liver microsomes, the enzyme requires NADPH. The activity is increased by phenobarbitone treatment, and decreased by low protein diet. Plasma clearance of quinine in the rat is also increased by phenobarbitone treatment and decreased by feeding a purified protein free diet. Human liver metabolizes quinine in the microsomal fraction, but the plasma clearance of quinine *in vivo* was only slightly altered by phenobarbitone in the dosage of 125 milligrams (mg)/day for 3 days.

IN MAN, when quinine is taken by mouth, half of the dose can be recovered from the urine, as the 2-hydroxy compound (quinine carbostyril); a number of other products can also be found in the urine.[1, 2] Knox[3] found an enzyme in rabbit liver that oxidises quinine and related compounds. The enzyme was a water soluble flavoprotein closely resembling aldehyde oxidase. However, this enzyme was not found in the livers of other animals. Moreover, K_m for quinine seemed rather high (5×10^{-4}M) in comparison with the plasma levels found in therapeutic use, (2×10^{-5}M = 7μg/ml). We thought it worthwhile to see if the microsomal hydroxylating enzymes of rat and human liver would attack quinine, and whether the soluble enzyme found in rabbits could be found in other species.

MATERIALS AND METHODS

Quinine was measured fluorometrically in an Aminco–Bowman spectrofluorometer, at 350 and 450 mμ excitation and emission wavelengths. 0·5 μg/ml quinine base in N sulphuric acid was used as the standard.

Plasma levels of quinine and metabolites were estimated by a modification of the method of Brodie, Udenfriend and Baer.[4] An 0·5 ml sample (plasma or incubation mixture) was added to 1 ml N NaOH and 3·5 ml water, and extracted with 5 ml ethylene dichloride, by shaking for 10 min in a stoppered 15 ml test tube. The tube was then centrifuged, and an aliquot (1–4 ml) of the ethylene dichloride taken off. The quinine was then extracted into 3 ml N H_2SO_4.

An aliquot of the alkaline aqueous supernatant was then extracted with butanol to remove quinine metabolites. The butanol was extracted with sulphuric acid and the acid extract assayed fluorometrically as for quinine.

Control experiments showed that all of the quinine and up to 20% of quinine metabolites were removed by the first ethylene dichloride extraction.

Animals

Male Sprague–Dawley rats were bought from Carworth Farms, (Carworth Europe Limited, Alconbury, Huntingdon). Some were maintained on stock pellets (diet 41B), with tap water to drink, others given 1 mg/ml sodium phenobarbitone in the drinking water for at least one week before use. A third group was given a purified protein free diet for at least one week.[5] In this way, rats with normal, high, and low microsomal hydroxylating enzyme activity were prepared. The animals were fed *ad lib.* up to the time of killing. Quinine was given intraperitoneally (i.p.) or intravenously (i.v.) as a dose of 50 or 25 mg/kg.

New Zealand white rabbits fed SG1 diet and water *ad lib.*, weighing about 2 kg were used for some experiments on metabolism of quinine *in vitro*.

Human experiments

Volunteers (AMGL, WM and VS) took quinine hydrochloride by mouth in gelatine capsules in the morning. Plasma quinine levels were then measured at intervals. The experiment was repeated after taking phenobarbitone for several days.

In vitro *metabolism of quinine*

Liver homogenates were made with 2 g liver and 18 ml 0·15 M KCl using a Dounce type all glass homogeniser. (Blaessig Glass Co.) or an Ultra Turrax Blender. (Janke and Kunkel. Staufen im Br. Germany.)

The post mitochondrial supernatant (PMS) remaining after 10 min centrifugation at 9000 *g* in a refrigerated centrifuge, was used for some experiments. In others, the PMS was spun at 104,000 *g* for 60 min and the supernatant taken off (post microsomal supernatant). The microsomal pellet was resuspended in 0·15 M KCl.

Liver preparations were incubated at 37° in air by shaking in incubation mixtures which contained 300 μmoles sodium phosphate buffer pH 7·4, 25 μmoles $MgCl_2$, 10 μmoles sodium isocitrate and 1·5 μmoles NADP in a volume of 3 ml. Quinine hydrochloride 0·15 μmoles was added to give a final concentration of 5×10^{-5}M. Where microsomes were used for incubation, isocitric dehydrogenase (Sigma Chemical Co. Type IV, 0·01 ml) was added.

0·5 ml samples were taken, on addition of liver preparations, in the cold. (Zero time sample.) The reaction was started by warming the incubation mixture to 37°. Samples were then taken after 5, 10 and 20 min of incubation, and the disappearance of quinine measured.

RESULTS

Plasma levels in rats

After i.p. injection of quinine into rats, the plasma levels fell exponentially with time. Table 1 shows plasma levels of quinine and metabolites in rats with high, medium and low microsomal hydroxylation activity.

The plasma half life for quinine, after i.p. injection of 50 mg/kg, was 0·7 hr for rats given phenobarbitone, 1·5 hr for stock rats and 3·0 hr for rats fed a protein free diet. After i.v. dosage (25 mg/kg) the half lives were 0·7, 1·2 and 3·4 hr in the respective groups. The ratios of metabolite to quinine were near to one for phenobarbitone treated rats, about 0·5 for stock rats, and about 0·3 for rats fed protein free diets.

Plasma levels in man

After an oral dose of quinine HCl, the plasma levels of quinine reached a maximum at about 3 hr, and then fell exponentially with time.

TABLE 1. PLASMA LEVELS OF QUININE AND METABOLITES IN RATS FED STOCK OR PROTEIN FREE DIETS, OR WITH PHENOBARBITONE IN THE DRINKING WATER

	60 min after injection		120 min after injection	
Dietary treatment	Quinine (μg/ml)	Metabolites (μg/ml)	Quinine (μg/ml)	Metabolites (μg/ml)
Stock diet	2·4 ± 0·5	1·2	1·5 ± 0·5	0·9
Stock diet and phenobarbitone water	1·1 ± 0·2	0·8	0·4 ± 0·1	0·6
Protein free diet	4·1 ± 0·5	1·3	3·2 ± 0·2	0·5

Rats were prepared as described in the section on methods and given 50 mg/kg quinine hydrochloride i.p. Four rats were killed at each time from each of the groups. Results are expressed as mean ± standard deviation.

TABLE 2. LOCATION OF QUININE METABOLISM IN LIVER FRACTIONS FROM HUMAN, FROM RAT WITH AND WITHOUT PHENOBARBITONE TREATMENT, AND FROM RABBIT

	Source			
Liver fraction	Stock rat	Rat + phenobarbitone quinine metabolized (μg)	Human	Rabbit
Homogenate	21	15	11	34
Post mitochondrial supernatant	19	15	12	37
Microsomes	7	10	10	6
Soluble fraction (post microsomal)	3	0	0	36

Tissue fractions equivalent to 250 mg liver were used for the human, 100 mg liver from stock rat and 25 mg liver for rat + phenobarbitone and rabbit liver. Changes of less than 2 μg between initial and final quantities of quinine in the incubation mixture were regarded as not significantly different from zero. Incubation time was 10 min except for rabbit liver where 20 min incubation was allowed.

A dose of 300 mg quinine HCl resulted in levels of around 1·5 μg quinine base/ml plasma 4 hr later.

The plasma half life for quinine was 5·9, 5·8 and 5·6 hr for AMCL. Twenty-four hr after dosage with phenobarbitone (125 mg/day for 4 days), the plasma half life was 5·2 hr. Three days later the half life was 5·3 hr. For WM the plasma half life before phenobarbitone was 5·5 hr and after phenobarbitone was 5·1 hr.

The mean ratio of metabolites to unaltered quinine was 0·23 before and 0·27 after phenobarbitone for AMCL, while for WM the ratios were 0·29 and 0·32.

Quinine metabolism in vitro

Rabbit liver. A few experiments were performed with rabbit liver to confirm the findings reported by Knox.[3] Rabbit liver preparations metabolized quinine at a rate

greater than 30 μmoles/g liver/hr. In the rabbit the activity was found in the soluble (post microsomal) fraction. Quinine metabolism in the rabbit did not depend on the addition of NADP.

Rat liver. Rat liver preparations metabolize quinine readily. The whole of the activity found in the homogenate was recovered in the post mitochondrial fraction, and essentially none was found in the soluble (post microsomal) fraction. (See Table 2.)

The maximum initial rate of activity was found when about 5×10^{-5}M quinine was present, both higher ($1{\cdot}5 \times 10^{-4}$M) and lower ($1{\cdot}5 \times 10^{-5}$M) concentrations of quinine gave lower activities, while at 5×10^{-4}M quinine concentration, no quinine metabolising activity could be obtained.

While quinine was lost from the incubation mixture, a number of fluorescent metabolites extractable into butanol appeared. These have not been identified.

The rate of quinine metabolism was markedly increased by pre-treatment of the rat with phenobarbitone and decreased by feeding a protein free diet (Table 3).

In liver from phenobarbitone treated rats, 65 per cent of the quinine metabolizing activities could be recovered from the microsomes. The microsomes required a NADPH generating system and their activity was totally inhibited in a gas-phase of CO.

TABLE 3. EFFECT OF DIET AND PHENOBARBITONE ON THE METABOLISM OF QUININE BY RAT LIVER POST MITOCHONDRIAL FRACTION, *IN VITRO*

Dietary treatment	Quinine metabolism μmoles/g liver/hr
Stock diet + phenobarbitone	10·8 ± 2·3
Stock diet	3·5 ± 0·9
Protein free diet	0·15 ± 0·02

Post mitochondrial supernatant (PMS) fraction of liver was prepared and incubated as described in the section on methods. For stock animals 1 ml of 10% PMS was used, for phenobarbitone treated animals 0·25 ml of 10% PMS was used, for protein depleted animals 25% homogenate were made and 1 ml of PMS used.

Results are expressed as mean ± standard deviation for at least seven animals in each group. Incubation time was 10 min.

However, some quinine metabolism took place when post mitochondrial fractions were incubated with CO, or in the absence of NADPH. (In contrast, no aniline hydroxylation or pyramidon demethylation takes place under these conditions.) The CO resistant activity was 10–20 per cent of the total in phenobarbitone treated animals and up to 50 per cent in stock animals. This activity was lost when the PMS was separated into microsomes and soluble supernatant and could not be recovered by recombination of the fractions.

Human liver. We obtained 120 g of fresh, normal liver from a 7-year-old child during the repair of a traumatic tear of the liver. Pieces of the liver were deep frozen, and thawed just before use. Microsomal cytochrome P-450 content was 12 nmoles/g wet wt., aniline hydroxylation activity was 2 μmoles/g/hr after one weeks storage and 0·9 μmoles/g/hr after 3 months storage.

At this latter time, quinine metabolism was measured and found to be 0·71 μmoles/g/hr. The quinine metabolizing activity of the post mitochondrial fraction was greater than that of the homogenate, and 75 per cent of the activity could be recovered in the microsomes. There was no activity in the soluble phase. The activity of the post mitochondrial fraction was totally inhibited by CO.

DISCUSSION

It is clear that the major site of quinine metabolism in the rat liver is in the microsomes. The rate of quinine metabolism *in vivo* and *in vitro* is radically altered by feeding low protein diets and by phenobarbitone treatment. Quinine metabolism changes in step with the changes in liver microsomal P-450 and other microsomal hydroxylation reactions.[5]

The evidence from a single human liver specimen suggests that microsomal enzymes are the site of quinine metabolism in man.

The rate of quinine metabolism *in vitro* is changed by a factor of 3·2 in going from stock to phenobarbitone pre-treated rats, and a factor of 23 on going to protein depleted rats. However, *in vivo*, the plasma half life alters only by a factor of 2 in going from the stock to either of the other groups. This suggests that the rate of quinine metabolism *in vivo* depends on many factors. Measurement of quinine metabolism *in vitro* with liver preparations, in which all permeability barriers are broken, reveals only a few of these factors.

The very low rate of quinine metabolism by the liver preparations from protein depleted rat livers could be due to loss of enzyme activity during preparation, since P-450 levels and aniline hydroxylation are less severely depressed in these livers.[5]

The finding that some quinine metabolizing activity in the post mitochondrial fraction is not dependent on NADP addition and is CO resistant, is puzzling. It suggests that P-450 may not be involved in this part of the several reactions which quinine undergoes.

Ths studies in man, *in vivo*, showed no clear cut induction of quinine metabolism; perhaps the length of exposure to phenobarbitone was insufficient to cause any decrease in plasma half life of quinine.

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