REFERENCES

1. Mason JW, Amiodarone. *N Engl J Med* **316**: 455–466, 1987.
2. Gross SA, Bandyopadhyay S, Klaunig JE and Somani P, Amiodarone and desethylamiodarone toxicity in isolated hepatocytes in culture. *Proc Soc Exp Biol Med* **190**: 163–169, 1989.
3. Daniels JM, Brien JF and Massey TE, Pulmonary fibrosis induced in the hamster by amiodarone and desethylamiodarone. *Toxicol Appl Pharmacol* **100**: 350–359, 1989.
4. Fruncillo RJ, Bernhard R, Swanson BN, Vlasses PH and Ferguson RK, Effect of phenobarbitone on the pharmacokinetics and tissue levels of amiodarone in the rat. *J Pharm Pharmacol* 37: 729–731, 1985.
5. Young RA and Mehendale HM, *In vitro* metabolism of amiodarone by rabbit and rat liver and small intestine. *Drug Metab Dispos* **14**: 423–429, 1986.
6. Young RA and Mehendale HM, Effect of cytochrome P-450 and flavin-containing monooxygenase modifying factors on the *in vitro* metabolism of amiodarone by rat and rabbit. *Drug Metab Dispos* **15**: 511–517, 1987.
7. Larrey D, Tinel M, Letteron P, Geneve J, Descatoire V and Pessayre D, Formation of an inactive cytochrome P-450Fe(II)-metabolite complex after administration of amiodarone in rats, mice and hamsters. *Biochem Pharmacol* **35**: 2213–2220, 1986.
8. Halpert JR, Multiplicity of steroid-inducible cytochromes P-450 in rat liver microsomes. *Arch Biochem Biophys* **263**: 59–68, 1988.
9. Wrighton SA, Maurel P, Schuetz EG, Watkins PB, Young B and Guzelian PS, Identification of the cytochrome P-450 induced by macrolide antibiotics in the rat liver as the glucocorticoid responsive cytochrome P-450p. *Biochemistry* **24**: 2171–2178, 1985.
10. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
11. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T and Mayer RT, Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: A series of substrates to distinguish between different induced cytochromes P-450. *Biochem Pharmacol* **34**: 3337–3345, 1985.
12. Wrighton SA, Schuetz EG, Watkins PB, Maurel P, Barwick J, Bailey BS, Hartle HT, Young B and Guzelian P, Demonstration in multiple species of inducible hepatic cytochromes P-450 and their mRNAs related to the glucocorticoid-inducible cytochrome P-450 of the rat. *Mol Pharmacol* **28**: 312–321, 1985.
13. Brien JF, Jimmo S, Brennan FJ, Ford SE and Armstrong PW, Distribution of amiodarone and its metabolite, desethylamiodarone, in human tissues. *Can J Physiol Pharmacol* **65**: 360–364, 1987.
14. Camus P and Mehendale HM, Pulmonary sequestration of amiodarone and desethylamiodarone. *J Pharmacol Exp Ther* **237**: 867–873, 1986.

Biochemical Pharmacology, Vol. 39, No. 10, pp. 1629–1631, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Comparison of Mongolian gerbil and rat hepatic microsomal monooxygenase activities: high coumarin 7-hydroxylase activity in the gerbil

(*Received* 27 *October* 1989; *accepted* 1 *February* 1990)

The Mongolian gerbil (*Meriones unguiculatus*) has been widely utilized as an animal model of unilateral hemispheric global ischaemia [1–3]. Although many pharmacological investigations have been undertaken using this model, studies of drug metabolism in the gerbil have not been well documented. Extensive studies on species differences in microsomal monooxygenase (MMO*) activities [4] do not include the gerbil amongst the species investigated. However, the pharmacokinetics, including limited metabolic studies, of coumarin (2*H*-1-benzopyran-2-one) in the gerbil have been reported [5]. Man is exposed to coumarin via its addition to toiletries and tobacco products [6]. Coumarin, in combination with cimetidine, is currently undergoing clinical trials for the treatment of various malignancies [7–9], and there have also been several human trials involving coumarin preparations for the treatment of lymphoedemas [10, 11]. A suitable animal model for man with respect to coumarin metabolism and toxicity has yet to be found [6].

It is well-recognized that the biotransformation of drugs, in particular by the cytochrome P450-dependent MMO system, can profoundly affect their pharmacological, and toxicological, activities. Hence, we have investigated various P450-dependent MMO activities [aniline 4-hydroxylase; benzphetamine *N*-demethylase; 7-ethoxycoumarin *O*-deethylase (7-ECOD); and, particularly, coumarin 7-hydroxylase (COH)] of gerbil liver microsomes, and compared these with those observed in the rat, a species for which extensive information on hepatic drug metabolism is available.

Materials and methods

All substrates, enzymes and cofactors were obtained from the Sigma Chemical Co. (Poole, U.K.) except for 7-ethoxycoumarin which was synthesized as described previously [12]. Other chemicals used were of AR grade. Adult male Wistar rats (115–140 g) and adult male Mongolian gerbils (60–70 g) were obtained from the University of Nottingham Medical School Animal Unit. They had access to standard laboratory diet and tap water *ad lib*.

Liver microsomes were prepared by the calcium aggregation technique as outlined previously [13]. Separate microsomal fractions were obtained for each animal. They were stored at −196° until required. Protein content was measured by the method of Lowry *et al.* [14]. Cytochrome P450 [15] and cytochrome b_5 [16] contents, and NADPH-cytochrome *c* reductase activity [16], were determined by the methods quoted. COH [17], 7-ECOD [12], aniline 4-hydroxylase [16] and benzphetamine *N*-demethylase [16] activities were assayed by standard methods. In addition, the glutathione content of liver homogenates was measured [18].

Statistical analysis was performed by means of an unpaired Student's *t*-test.

* Abbreviations used: COH, coumarin 7-hydroxylase; 7-ECOD, 7-ethoxycoumarin *O*-deethylase; 7-HC, 7-hydroxycoumarin; GSH, glutathione; MMO, microsomal monooxygenase; P450, cytochrome P450.

Table 1. Comparison of measures of the hepatic microsomal monooxygenase system in the rat and gerbil

	Rat	Gerbil
Cytochrome P450 (nmol/mg protein)	0.70 ± 0.04	0.81 ± 0.03
Cytochrome b_5 (nmol/mg protein)	0.42 ± 0.04	0.60 ± 0.08
NADPH-cytochrome *c* reductase (nmol cytochrome *c* reduced/min/mg protein)	56.0 ± 3.2	49.9 ± 3.5
COH	0.0040 ± 0.0003	0.285 ± 0.030†
7-ECOD	0.87 ± 0.08	2.89 ± 0.33†
Aniline 4-hydroxylase	0.63 ± 0.04	1.16 ± 0.11*
Benzphetamine *N*-demethylase	7.05 ± 0.63	8.87 ± 0.79

MMO activities are expressed as nmoles of product formed/min/mg protein. Values are mean ± SE (6 rats; 6 gerbils).

Where indicated, values for the gerbil are significantly different from those of the rat at * $P < 0.01$, † $P < 0.001$.

Results and discussion

There were no statistically significant differences in cytochrome P450 and cytochrome b_5 contents, and NADPH-cytochrome *c* reductase activity, between rat and gerbil liver microsomes (Table 1). GSH contents (nmol GSH/g liver) of rat (3.67 ± 0.16) and gerbil (5.16 ± 0.35) liver homogenates were significantly different ($P < 0.01$). This could be due to differences in feeding habits, which may affect GSH synthesis.

COH, 7-ECOD and aniline 4-hydroxylase activities were significantly higher in gerbil liver microsomes (Table 1); benzphetamine *N*-demethylase activity was also higher in gerbil microsomes, but this was not statistically significant. The most marked difference was observed in COH activity, which was only just detectable in rat liver microsomes but was approximately 70-fold higher in gerbil liver microsomes. Preliminary results using HPLC to separate and quantify coumarin and its metabolites confirm this finding.

Statistically significant correlations were observed between the activities of COH and 7-ECOD ($r = 0.98$; $P < 0.001$), COH and aniline 4-hydroxylase ($r = 0.87$; $P < 0.025$), and 7-ECOD and aniline 4-hydroxylase ($r = 0.81$; $P < 0.05$) in gerbil liver microsomes. No such correlations were found for these monooxygenase activities in rat liver microsomes. Highly significant correlations between strains of mice in their abilities to metabolize 7-EC and coumarin have been reported [19], and it has previously been suggested that coumarin substrates are metabolized by the same P450 isozyme(s) [20]. A correlation between 7-ECOD and aniline 4-hydroxylase activities in human liver microsomes has also been reported [21]. The simplest explanation for this close correlation is that the COH, 7-ECOD and aniline 4-hydroxylase reactions are all carried out by the same P450 isozyme. It has recently been reported that, in the mouse, 7-EC is as good a substrate for $P450_{Coh}$ as coumarin, although it is also metabolized by other P450 isozymes [22].

Similarly, aniline is metabolized by $P450_{Coh}$, but only at a low rate. The close correlations observed between COH, 7-ECOD and aniline 4-hydroxylase activities in the gerbil suggest that these activities may also be catalysed by the same form of P450 in this species. In the standardized nomenclature of Nebert *et al.* [23] $P450_{Coh}$ is assigned to the P450IIB subfamily; more recently it has been reported to have a closer association with the P450IIA subfamily [24, 25].

Species differences in coumarin metabolism and toxicity have been well-documented [6], particularly with respect to 7-hydroxylation. In man coumarin is extensively metabolized to 7-HC, whereas in the rat, in which coumarin is hepatotoxic, 7-HC formation is negligible. On the basis of our experiments, and the findings of Ritschel and Hardt regarding coumarin pharmacokinetics [5], who report that the pharmacokinetic profile of coumarin, 7-HC and 7-HC glucuronide found in the blood of gerbils is similar to that observed in man, the gerbil would appear to be more appropriate than the rat as a model for man with respect to coumarin metabolism and toxicity [26]. COH activity (nmol 7-HC formed/min/mg protein) in gerbil liver microsomes (0.29 ± 0.03) agrees closely with that reported for human liver microsomes (0.33 ± 0.14) [27].

In summary, we have compared several hepatic microsomal monooxygenase activities in the Mongolian gerbil and in the rat. COH, 7-ECOD and aniline 4-hydroxylase activities were significantly higher in gerbil liver microsomes, although no significant differences were found in cytochrome P450 and cytochrome b_5 contents, nor in NADPH-cytochrome *c* reductase and benzphetamine *N*-demethylase activities. The most marked difference was in COH activity which was 70-fold higher in gerbil than in rat liver microsomes. Drug metabolism studies in the gerbil are of importance with regard to the many pharmacological investigations undertaken using this species as a model for the study of drugs in ischaemic stroke. The gerbil would also seem to be an appropriate species to use in further investigations of coumarin metabolism and toxicity due to its high COH activity; such studies are currently being undertaken.

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REFERENCES

1. Levine S and Sohn D, Cerebral ischemia in infant and adult gerbils. *Arch Pathol* **87**: 315–317, 1969.
2. Kahn K, The natural course of experimental cerebral infarction in the gerbil. *Neurology* **22**: 510–515, 1972.
3. Karpiak SE, Tagliavia A and Wakade CG, Animal models for the study of drugs in ischemic stroke. *Annu Rev Pharmacol Toxicol* **29**: 403–414, 1989.

4. Souhaili-El Amri H, Batt AM and Siest G, Comparison of cytochrome P450 content and activities in liver microsomes of seven animal species, including man. *Xenobiotica* **16**: 351–358, 1986.
5. Ritschel WA and Hardt TJ, Pharmacokinetics of coumarin, 7-hydroxycoumarin and 7-hydroxycoumarin glucuronide in the blood and brain of gerbils following intraperitoneal administration of coumarin. *Arzneimittel forschung* **33**(II): 1254–1258, 1983.
6. Cohen AJ, Critical review of the toxicology of coumarin with special reference to interspecies differences in metabolism and hepatotoxic response and their significance to man. *Food Cosmet Toxicol* **17**: 277–289, 1979.
7. Marshall ME, Mendelsohn L, Butler K, Cantrell J, Harvey J and Macdonald J, Treatment of non-small cell lung cancer with coumarin and cimetidine. *Cancer Treat Rep* **71**: 91–92, 1987.
8. Marshall ME, Mendelsohn L, Butler K, Riley LK, Cantrell J, Harvey J, Wiseman C, Taylor T and Macdonald J, Treatment of metastatic renal cell carcinoma with coumarin (1,2-benzopyrone) and cimetidine: a pilot study. *J Clin Oncol* **5**: 862–866, 1987.
9. Marshall ME, Butler K, Cantrell J, Wiseman C and Mendelsohn L, Treatment of advanced malignant melanoma with coumarin and cimetidine: a pilot study. *Cancer Chemother Pharmacol* **24**: 65–66, 1989.
10. Casley-Smith JR and Casley-Smith Judith R, *High Protein Oedemas and the Benzopyrones*. Lippincott, Sydney, 1986.
11. Jamal S, Casley-Smith JR and Casley-Smith Judith R, The effects of 5,6 benzo[*a*]pyrone (coumarin) and DEC on filaritic lymphoedema and elephantiasis in India. Preliminary results. *Ann Trop Med Parasitol* **83**: 287–290, 1989.
12. Paterson P, Fry JR and Horner SA, Influence of cytochrome P450 type on the pattern of conjugation of 7-hydroxycoumarin generated from 7-alkoxycoumarins. *Xenobiotica* **14**: 849–859, 1984.
13. Garle MJ and Fry JR, Detection of reactive metabolites *in vitro*. *Toxicology* **54**: 101–110, 1989.
14. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
15. Omura T and Sato R, The carbon monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
16. Gibson GG and Skett P, *Introduction to Drug Metabolism*. Chapman and Hall, London, 1986.
17. Jacobson M, Levin W, Poppers PJ, Wood AW and Conney AH, Comparison of the O-dealkylation of 7-ethoxycoumarin and the hydroxylation of benzo[*a*]-pyrene in human placenta. *Clin Pharmacol Ther* **16**: 701–710, 1974.
18. Sedlack J and Lindsay RH, Estimation of total, protein-bound and non-protein sulfhydryl groups in tissues with Ellman's reagent. *Anal Biochem* **25**: 192–198, 1968.
19. Lush I and Andrews KM, Genetic variation between mice in their metabolism of coumarin and its derivatives. *Genet Res* **31**: 177–186, 1978.
20. Rettie AE, Williams FM, Rawlins MD, Mayer RT and Burke MD, Major differences between lung, skin and liver in the microsomal metabolism of homologous series of resorufin and coumarin ethers. *Biochem Pharmacol* **35**: 3495–3500, 1986.
21. Beaune PH, Kremers PG, Kaminsky LS, De Graeve J, Albert A and Guengerich FP, Comparison of monooxygenase activities and cytochrome P450 isozyme concentrations in human liver microsomes. *Drug Metab Dispos* **14**: 437–442, 1986.
22. Kojo A, Honkakoski P, Jarvinen P, Pelkonen O and Lang M, Preferential inhibition of mouse hepatic coumarin 7-hydroxylase by inhibitors of steroid metabolizing monooxygenases. *Pharmacol Toxicol* **65**: 104–109, 1989.
23. Nebert DW, Nelson DR, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R and Waterman MR, The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA* **8**: 1–13, 1989.
24. Lang MA, Juvonen R, Jarvinen P, Honkakoski P and Raunio H, Mouse liver $P450_{Coh}$: genetic regulation of the pyrazole-inducible enzyme and comparison with other P450 isoenzymes. *Arch Biochem Biophys* **271**: 139–148, 1989.
25. Negishi M, Lindberg R, Burkhart B, Ichikawa T, Honkakoski P and Lang M, Mouse steroid 15α-hydroxylase gene family: identification of type II $P450_{15\alpha}$ as coumarin 7-hydroxylase. *Biochemistry* **28**: 4169–4172, 1989.
26. Lake BG, Gray TJB, Evans JG, Lewis DFV, Beamand JA and Hue KL, Studies on the mechanism of coumarin-induced toxicity in rat hepatocytes: comparison with dihydrocoumarin and other coumarin metabolites. *Toxicol Appl Pharmcol* **97**: 311–323, 1989.
27. Kratz F, Coumarin 7-hydroxylase activity in microsomes from needle biopsies of normal and diseased human liver. *Eur J Clin Pharmacol* **10**: 133–137, 1976.