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SHORT COMMUNICATIONS

Assessment of the mouse as an experimental model for studying polymorphic oxidation of the sparteine/debrisoquine type

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The oxidative metabolism of about 25 drugs exhibits genetic polymorphism of the debrisoquine-type in humans [1]. The female Dark Agouti (DA) rat was the first animal model to be proposed for the human poor metabolizer phenotype [2]. However, *in vitro* kinetic analyses [3] and studies at the protein and gene level [4] have subsequently indicated that this rat model does not completely reflect the human polymorphism. Furthermore, observations that the metabolism of drugs such as antipyrine [5] and benzphetamine [6], which are not affected by the debrisoquine polymorphism, is decreased in the DA rat suggest that this strain may have a more generally lowered capacity to metabolize drugs. An animal model has also been sought among non-human primates and there is evidence that debrisoquine oxidation is polymorphic in crab-eating macaque (*Macaca fascicularis*) [7]. Furthermore, *in vitro* data have shown that the form of cytochrome P450 responsible for this oxidation in African Green monkeys (*Cercopithecus aethiops*) is indistinguishable both catalytically and immunologically from the human form of the enzyme (P450IID6) [8]. The mouse is a more easily obtainable species than the monkey and also has advantages over the monkey and the rat for biochemical genetic studies. In view of the successful development of mouse models for studying human polymorphic *N*-acetyltransferase [9] and thiopurine methyltransferase [10], we have investigated the α-hydroxylation and O-demethylation of metoprolol in hepatic microsomes from 12 strains of mice, chosen to reflect a wide range of genetic pedigrees. Debrisoquine oxidation phenotype is the major determinant of the metabolism of metoprolol in humans [11].

Materials and Methods

Studies were performed using the following inbred strains of 8-week-old male mice (25–30 g): A2G/Ola, BALB/c/Ola, BXSB/Mp/Ola, CBA/Ca/Ola, C3H/He/Ola, C57BL/6J/Ola, C57BL/10/ScSn/Ola, DBA/1/Ola,DBA/2/Ola, NIH/Ola, NZB/Ola and 129/Ola [12] obtained from Olac Harlan Ltd (Bicester, U.K.). All strains were inbred for at least 20 consecutive generations of brother–sister matings. Mice were housed 10 to a cage on dust-free sawdust (grade 10) (Datesand, Cheshire, U.K.). Animals were maintained on a 12 hr light–dark cycle and fed on a commercially available small mammal diet (Clark Rat and Mouse Diet, Labsure Foods). They were allowed access to food and water *ad lib*. All mice were kept for 1 week prior to the experiments in identical cage conditions at the University of Sheffield Animal House to allow recovery from transit and acclimatization to the new environment.

Five or six animals from each strain were used in these experiments, which were performed over 6 days. One mouse from every strain was killed by cervical dislocation at the same time on each day and the microsomes from individual livers were prepared by a standard procedure [13]. The microsomal pellets were resuspended in 6 mL of a solution of glycerol (30% v/v) in phosphate buffer (0.25 M, pH 7.25). This suspension (0.2 mL) was incubated with racemic metoprolol dissolved in 1.15% (w/v) KCl (0.2 mL), $MgCl_2$ (100 mM, 0.2 mL) solution and an NADPH generating system comprising of 4 μmol glucose-6-phosphate, 0.4 μmol NADP, 0.4 units glucose-6-phosphate dehydrogenase dissolved in potassium phosphate buffer (0.2 M, pH 7.4, 0.4 mL). The incubations were carried out for 5 min at 37°. The rates of formation of both metabolites were linear over this time period and with respect to protein concentration. The reaction was terminated by addition of sodium hydroxide solution (1.2 M, 0.2 mL). Sample preparation and analysis of α-hydroxymetoprolol and *O*-demethylmetoprolol were carried out as described previously using high performance liquid chromatography with fluorescence detection [10]. Microsomal protein concentration was measured as described previously [13]. Values for the apparent K_m and V_{max} were calculated from graphical extrapolation of the Hanes plot [14]. Lines of best fit were obtained by the method of least squares analysis.

Results and Discussion

Hepatic microsomes from all of the strains of mice catalysed the formation of both metoprolol metabolites. The average ratio of *O*-demethylmetoprolol to α-hydroxymetoprolol appearance was 5:1 (substrate concentration 30 μM). This value was similar to that found for human liver (4:1) [15] but contrasts with the pattern of metabolism in rat liver microsomes in which α-hydroxylation predominates (0.4:1). Mouse liver microsomes metabolized metoprolol between five and nine times more rapidly (per mg protein) than do human liver microsomes. Over the concentration range studied (10–160 μM) the kinetics of both reactions were monophasic in all strains, suggesting the presence of a single class of enzyme sites. Considerable variation in K_m and V_{max} values was observed within each strain (Fig. 1). With respect to α-hydroxylation there were no significant differences between any of the strains for either parameter (analysis of variance test for a one factor, independent group design). Similarly, no significant differences were observed in V_{max} for O-demethylation. Inter-strain differences in K_m values for this pathway reached marginal statistical significance (F ratio = 2.15 with a critical F value of 1.92). Thus, none of the 12 strains studied showed a decreased ability to metabolize metoprolol. This contrasts markedly with the clear bimodal distribution of hepatic thiopurine methyl transferase activity observed by Otterness *et al.* [10] in 10 strains of mouse, in which three of the strains had 25–32% of the activity of the other seven with no overlap. In the present work strains genetically similar to those found to be deficient in thiopurine methyltransferase activity were used. These did not differ from the other nine strains with respect to metoprolol metabolism. When the data from all of the strains were pooled a significant correlation was observed between the V_{max} values for O-demethylation and α-hydroxylation (Spearman rank correlation coefficient r_s = 0.62, $P < 0.001$), but not between corresponding K_m values (r_s = 0.12, not significant) (N = 65).

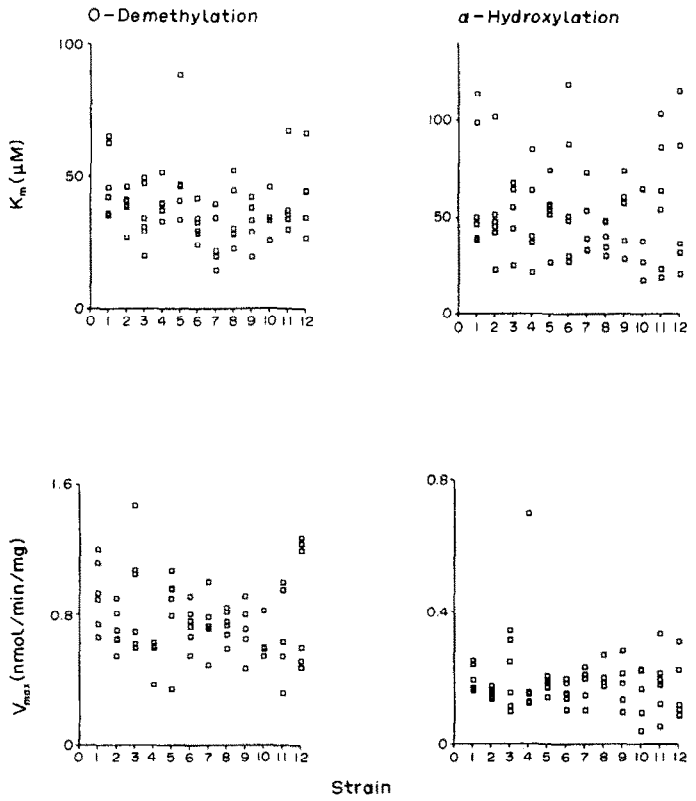

Fig. 1. Individual values of K_m and V_{max} for the α-hydroxylation and O-demethylation of metoprolol by hepatic microsomes from 12 inbred strains of mice. Key to strains: 1 = A2G/Ola, 2 = BALB/c/Ola, 3 = BXSB/Mp/Ola, 4 = CBA/Ca/Ola, 5 = C3H/He/Ola, 6 = C57BL/6J/Ola, 7 = C57BL/10/ScSn/Ola, 8 = DBA/1/Ola = DBA/2/Ola, 10 = NIH/Ola, 11 = NZV/Ola and 12 = 129/Ola. Mean ± SD values of K_m (μM) for α-hydroxylation and O-demethylation, respectively, were 64 ± 32 and 48 ± 13 (strain 1), 52 ± 26 and 39 ± 6 (strain 2), 54 ± 17 and 35 ± 11 (strain 3), 49 ± 25 and 40 ± 6 (strain 4), 53 ± 15 and 50 ± 19 (strain 5), 60 ± 36 and 32 ± 6 (strain 6), 46 ± 17 and 26 ± 11 (strain 7), 40 ± 8 and 34 ± 11 (strain 8), 49 ± 17 and 32 ± 9 (strain 9), 42 ± 22 and 33 ± 8 (strain 10), 58 ± 34 and 41 ± 15 (strain 11), 59 ± 37 and 43 ± 13 (strain 12). Mean ± SD V_{max} values (nmol/min/mg protein) for α-hydroxylation and O-demethylation, respectively, were 0.20 ± 0.04 and 0.92 ± 0.21 (strain 1), 0.15 ± 0.02 and 0.71 ± 0.13 (strain 2), 0.21 ± 0.11 and 0.92 ± 0.35 (strain 3), 0.25 ± 0.25 and 0.57 ± 0.10 (strain 4), 0.18 ± 0.03 and 0.83 ± 0.26 (strain 5), 0.15 ± 0.03 and 0.73 ± 0.13 (strain 6), 0.18 ± 0.05 and 0.74 ± 0.19 (strain 7), 0.20 ± 0.04 and 0.73 ± 0.09 (strain 8), 0.17 ± 0.07 and 0.71 ± 0.17 (strain 9), 0.15 ± 0.08 and 0.63 ± 0.11 (strain 10), 0.18 ± 0.09 and 0.69 ± 0.29 (strain 11), 0.17 ± 0.10 and 0.88 ± 0.39 (strain 12).

In conclusion, we found no evidence of polymorphic drug oxidation in the 12 mouse strains examined using metoprolol as the substrate. Thus, none of the strains appear suitable for use as a model of the human debrisoquine poor metabolizer phenotype. The potency and selectivity of quinidine as an inhibitor of human debrisoquine 4-hydroxylase is absent in the mouse and rat [15, 16] but is retained in the monkey [8]. Thus, in spite of its disadvantages the monkey may be superior to the rat and mouse as an animal model for the polymorphic oxidation of debrisoquine.

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Metabolism of T-2 toxin by rat brain homogenate

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T-2 toxin is one of the most poisonous trichothecenes, produced by *Fusarium* species [1]. Its metabolism has been studied in the liver, in various internal organs [2, 3] and in whole blood and its components [4]. Ohta *et al.* [5] included rat brain in their experiments. Microsomal preparations of this organ converted T-2 toxin into HT-2 toxin in 30 min, but further metabolic changes were not studied, in spite of the extensive metabolic reactions found with liver. Furthermore, it was not stated whether the brain was used with its physiological blood content.

Recently we reported that direct administration of T-2 toxin to the rat brain causes death with doses that are only a low percentage of those required for systemic applications [6]. These observations led to the question of whether cerebral T-2 toxin metabolism differs from its metabolism following peripheral administration. This problem was approached by treatment of the toxin with rat brain homogenate. Since whole blood degrades T-2 toxin effectively [4], we have prepared this homogenate by two procedures: either by direct decapitation, extraction of the brain and its homogenization; or first exhaustive saline perfusion to remove all the blood from the brain before decapitation and homogenization.

Materials and Methods

Chemicals (see Fig. 1). The toxin and neosolaniol were isolated from *Fusarium sporotrichioides* [7]. HT-2 toxin, T-2 triol, T-2 tetraol, 4-deacetylneosolaniol (DANS) and 15-monoacetylscirpenol (MAS) were prepared according to the literature [8]. Identification was performed by NMR and mass spectroscopy, purity was checked by GLC. For analysis by GLC with a ^{63}Ni electron capture detector, all trichothecenes were converted into their heptafluorobutyric esters [9]. Heptafluorobutyric (HFB) anhydride was purchased from Aldrich (Milwaukee, WI, U.S.A.) and trimethylamine (TMA) from BDH (Poole, U.K.).

Brain homogenates. Male albino rats of the Hebrew University Sabra strain, of 250 ± 50 g body weight, were used. The animals were anesthetized with pentobarbitone-sodium (40–50 mg/kg i.p. or s.c.) and were perfused via the heart with 37° warm saline, until the perfusate was practically colorless. The skull was rapidly opened, the brain was removed and homogenized with 10 mL of 0.1 M phosphate buffer pH 7.4, per gram of tissue, using an Ultra Turrax homogenizer.

Homogenates were also prepared from whole rat brain without previous perfusion. Such preparations contain about 40 μL of blood per gram of brain [10].

Analysis. The apparatus used for gas chromatography and the conditions of the analytical procedure, including the method of derivatization with HFB-anhydride, have been described previously [9].

Incubation. In a 100 mL Erlenmeyer flask, 20 mL of brain homogenate were thoroughly mixed with 5–10 μg/mL of substrate in dimethyl sulfoxide solution.

Penicillin and streptomycin were added to suppress the growth of airborne microorganisms. The vessels were shaken at 37° for 90 min, and samples of 3 mL were