Antipyrine Metabolism in Female Lewis and Dark Agouti Strains of Rats, Which Are Extensive and Poor Metabolizers of Debrisoquine, Respectively

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Abstract—The metabolism of antipyrine (AP) was investigated in female rats of the Dark Agouti (DA) and Lewis (L) strains, which have been proposed as models for human poor and extensive metabolizers of debrisoquine (DB), respectively. Following i.p. injection of 50 mg/kg of AP, the rate of production of 4-hydroxyantipyrine in 24-hr urine was increased significantly in the L strain. The results suggested that different cytochrome P-450 isozymes were responsible for hydroxylation of AP and DB and showed interphenotype differences between the two strains.

The rate of oxidative metabolism of drugs is influenced by endogenous and exogenous factors including genetic and environmental ones (1, 2). From the preclinical point of view, it is very important to find a suitable animal model to screen drugs likely to be metabolized polymorphically by cytochrome P-450 in humans. It has been suggested that the Lewis (L) and Dark Agouti (DA) strains of rat provide models for human extensive (EM) and poor metabolizer (PM) phenotypes for debrisoquine (DB), respectively (3). Especially, female DA rats have a very low capacity to hydroxylate DB both in vivo and in vitro as compared to other strains of rats (3–5). Furthermore, the genetic mechanism of this low capacity has been described by Gonzalez et al. (6).

In the present study, we treated female DA and L rats with antipyrine (AP) as a model substrate for assessing hepatic drug-metabolizing capacity because it is metabolized by three distinctly different oxidative metabolic reactions which may involve more than one form of cytochrome P-450 isoenzyme. We have investigated further possible explanations for the differences between the DA and L strains of rat in the metabolism of AP. DB and 4-hydroxy DB (4-OHDB) were gifts from Dr. A.R. Boobis, Department of Clinical Pharmacology, Royal Postgraduate Medical School, London, UK. AP was purchased from Wako Pure Chemical Industries (Osaka, Japan). Three major metabolites of AP (norantipyrine: NORA, 4-hydroxyantipyrine: 4-OHA, 3-hydroxymethylantipyrine: 3-HMA) were obtained from Chiko Co. (Tokyo, Japan). Female DA (135–140 g) and L (140–150 g) rats, 7–8 weeks of age, were obtained from Clea (Tokyo, Japan). DB (10 mg/kg, p.o.) and AP (50 mg/kg, i.p.) were administered as a single dose. DA and L rats were fixed on a board (CFK Lab., Tokyo, Japan) only at the time (1, 2, 4, 8 and 12 hr) of blood sampling from the jugular vein after AP dosing. For collection of urine, AP- and DB-treated rats were individually placed in metabolic cages and allowed only water. Urine samples were collected for 24 hr. Serum and urinary concentrations of AP and its main metabolites and urinary concentration of DB and 4-OHDB were determined according to Teunissen et al. (7) and Harrison et al. (8), respectively. The half-life (t₁/₂) of AP was estimated by linear regression analysis. The apparent volume of distribution (Vd) was calculated from the ratio of dose to
serum drug concentration extrapolated to zero time. Total body clearance (CL) was calculated from: \( CL = \frac{0.693 \ Vd}{t_{1/2}} \). The rates of production of AP metabolites were expressed as CLm and calculated according to Danhof et al. (9). The results were statistically analyzed by Student's t-test.

The ratio of DB to its metabolite 4-OHDB in the urine was significantly (P<0.01) higher in 6 female DA rats (5.4±0.4, mean±S.D.) than in 6 female L rats (0.8±0.2). The two strains gave good recoveries of DB and 4-OHDB in the 24-hr urine samples: 72.0±9.2% (DA) and 64.6±4.8% (L), respectively. These results are similar to those reported by Al-Dabbagh et al. (3). As shown in Table 1, \( t_{1/2} \) and CL in the L strains were shortened and increased compared with the DA strain, respectively, whereas Vd was not changed.

The CLms of three metabolites of AP within 24 hr are shown in Fig. 1. There were no significant differences between the DA and L strains in the excretion of 3-HMA (3.63±0.28 ml/min/kg in DA and 3.30±0.30 ml/min/kg in L) and NORA (0.98±0.28 ml/min/kg in DA and 0.82±0.10 ml/min/kg in L). However, the CLm of 4-OHA (2.60±0.46 ml/min/kg in DA and 3.68±0.26 ml/min/kg in L, P<0.01) was significantly smaller in the DA rats than in the L rats. These results suggest that the cytochrome P-450 isozyme(s) responsible for the production of NORA (demethylation) or 3-HMA (hydroxymethylation) from AP is similar in DA and L rats. However, the large interstrain differences in 4-hydroxylation of AP and DB suggest that different cytochrome P-450 isozymes may be responsible for these reactions in DA and L rats. The present results are supported by in vitro experiments performed by Kahn et al. (5). They described that the DA rat differed from other strains in at least three different

### Table 1. Pharmacokinetic parameters of serum antipyrine in female Dark Agouti and Lewis rats after a single oral dose of antipyrine

<table>
<thead>
<tr>
<th>Strain</th>
<th>CL (ml/min/kg)</th>
<th>Vd (ml/kg)</th>
<th>( t_{1/2} ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Agouti</td>
<td>7.91±1.49</td>
<td>911±43</td>
<td>82.0±4.7</td>
</tr>
<tr>
<td>Lewis</td>
<td>10.34±1.17**</td>
<td>899±77</td>
<td>73.3±6.2*</td>
</tr>
</tbody>
</table>

Each value of the results is expressed as the mean±S.D. for 4 animals. *P<0.05, **P<0.01 vs. Dark Agouti.

isozymes of cytochrome P-450 that catalyze the 4-hydroxylation of DB.

Our results indicate that different cytochrome P-450 isozymes are responsible for 4-hydroxylation of AP and DB in female DA or L rats; however, further study will be required to see if this conclusion is supported by in vitro studies using liver microsomes.

### References


