Amidopyrine disposition in rat

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Amidopyrine is extensively used as a model substrate for in vitro and in vivo investigations into the various factors influencing drug metabolism. Although most of these studies are carried out in animal species, the published information on amidopyrine (AP) disposition relates mainly to man. Brodie & Axelrod (1950) have demonstrated that AP is metabolized via two sequential demethylations to give first, monomethylaminoantipyrine (MAP) and then, aminoantipyrine (AA). The primary amine is acetylated to form acetylaminoantipyrine (AAA). The importance of this metabolic route in man has been confirmed by Goromani et al (1976) using gas chromatography-mass spectrometry. The above three metabolites account for up to 50% of an administered dose in man; no corresponding percentage has been reported for rat.

The methods used for assessing AP *N*-demethylation are based upon metabolite production. For in vitro microsomal studies, the rate of formaldehyde production is measured (Mazel 1972). For in vivo studies [N-$^{14}CH_3$]-AP is used and exhalation of $^{14}CO_2$ is monitored to assess the rate of *N*-demethylation (Lauterburg & Bircher 1976). Since MAP and AP are both substrates for the demethylase enzyme, the results obtained from formaldehyde and $^{14}CO_2$ production must be interpreted with care. Gram et al (1968) have investigated this problem in vitro. However, there has been no attempt to assess the consequences of this situation in vivo. Characterization of the pharmacokinetics of AP and MAP (possibly also AA and AAA) would be essential for such studies.

We report a high performance liquid chromatographic (h.p.l.c.) method of estimating simultaneously AP, MAP, AA and AAA in plasma and urine together with preliminary data on the disposition of AP and metabolites in rat.

The h.p.l.c. system used was:—Pye Unicam LC3 pump and u.v. detector (set at 260 nm) attached to a J.J. CR100 recorder. A 200 × 5 mm (i.d.) stainless steel column packed with octadecyl silane-bonded silica (Partisil 10, Whatman) with a septum injector. A mobile phase of 10% acetonitrile (Rathburn Chemicals, h.p.l.c. grade), 4% glacial acetic acid in distilled water at a flow rate of 2·5 ml min^{-1}.

The sample preparation involved basifying a plasma or diluted urine sample, adding internal standard (antipyrine) and extracting into chloroform (phase volume ratio, 1:5). The organic layer was transferred to a clean test tube, evaporated to dryness under nitrogen and reconstituted in methanol. Ten μl samples of methanol solution were injected on to the column.

Symmetrical peaks were obtained for all compounds (see Fig. 1) and calibration graphs are linear over the following ranges: 0·1–10 (AP), 0·1–4·0 (MAP), 0·1–3·0 (AA) and 0·1–6·0 (AAA) μg when an initial volume of 200 μl of biological sample is carried through the assay. Replicate assays (n = 6) showed a coefficent of variation of 4 (AP), 3 (MAP), 4 (AA) and 7% (AAA) for a final injected mass of 0·3 μg.

The application of the h.p.l.c. assay to analysis of urinary excretion products of AP is illustrated in Table 1. Eight rats (Sprague-Dawley, male, 200–250 g) were individually housed in glass metabolism cages and allowed free access to food and water. Following AP administration (30 mg kg^{-1} i.p.), urine was collected in ice-cooled flasks over 24 h.

Renal excretion of AP amounted to a minor route of elimination. Demethylation of AP accounted for, on average, 81% of the administered dose. Thus, in contrast to man, the demethylation-acetylation pathway is essentially the only metabolic route in rat. Considerable variation was observed between animals with respect to the individual percentages of AP, MAP, AA and AAA in the urine (see Table 1). However, replicate studies showed small intra-animal variation.

The predominance of the demethylation pathway was confirmed in studies where rats were administered [*N*-dimethyl-^{14}C]AP (30 mg kg^{-1}, 3 μCi kg^{-1} i.p.) and exhaled $^{14}CO_2$ trapped in ethanolamine–methanol (1:4). From the urinary excretion products it is possible to

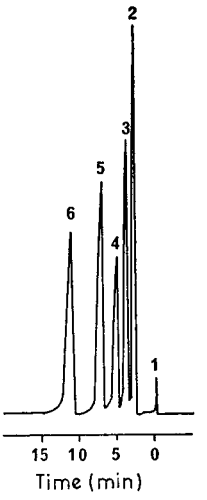

FIG. 1. A typical high performance liquid chromatogram. 1, Injection; 2, aminoantipyrine; 3, monomethylaminoantipyrine; 4, amidopyrine; 5, acetylaminoantipyrine; 6, antipyrine (internal standard).

* Correspondence.

estimate the percentage dose of ^{14}C converted to $^{14}CO_2$, using the equation

$$\% ^{14}CO_2 = 100 \left[\frac{fm^{AP}}{2} + \frac{(fm^{AP} fm^{MAP})}{2} \right] fc$$

fm^{AP} is the fraction of AP dose metabolized by demethylation (calculated by the sum of the MAP, AA and AAA fractions in urine) and $fm^{AP}/2$ is the fraction of radioactive dose losing one methyl-^{14}C group. fm^{MAP} is the fraction of MAP demethylated (calculated by the sum of the AA and AAA fractions in urine) and $(fm^{AP} fm^{MAP})/2$ is the fraction of radioactive dose losing a second methyl-^{14}C group. fc is the fraction of formaldehyde-^{14}C ultimately exhaled as $^{14}CO_2$ and equals 0·82 (Neely 1964).

The equation may underestimate the % $^{14}CO_2$ since it does not allow for alternative metabolic routes for MAP, AA and AAA. However, the data presented in Table 1 would suggest the contribution of these routes would be minimal. For the animals studied the equation predicts 59% (s.d. 13) of AP-^{14}C would be exhaled as $^{14}CO_2$. Observed values are in good agreement with this prediction 63% (s.d. 5) was exhaled as $^{14}CO_2$.

Fig. 2 shows a set of typical plasma concentration-time curves for AP, MAP, AA and AAA obtained from a rat dosed with AP (30 mg kg^{-1} intravenously via a cannula in the jugular vein). The rat was anaesthetized with urethane and the plasma samples (200 μl at each time point) were obtained from arterial blood collected via a carotid cannula.

The elimination of AP from the plasma is biphasic with a terminal half-life of 49 min (s.d. 17, n = 6). The volume of distribution is 1300 ml kg^{-1} (s.d. 350, n = 6) calculated by the area under the curve method. The plasma clearance is 21 ml min^{-1} kg^{-1} (s.d. 10, n = 6) which will be approximately equal to the blood clearance since AP has a blood concentration: plasma concentration ratio of close to unity (Brodie & Axelrod 1950). Therefore AP would be classed as a lowly cleared drug (Wilkinson & Shand 1975) since its blood clearance is only 25% of hepatic blood flow. AP clearance in the rat would be a sensitive measure of hepatocellular activity being insensitive to hepatic blood flow changes and not appreciably bound to plasma proteins (Brodie & Axelrod 1950).

Plasma concentrations of the primary metabolite MAP peak within 40 min of AP administration and thereafter are maintained at a higher concentration than AP. The terminal half-life for MAP is 154 min (s.d. 48, n = 6).

Table 1. Urinary excretion products of amidopyrine in rats.

Urinary excretion product	% Dose
Amidopyrine (AP)	4·0 (3·1)
Monomethylaminoantipyrine (MAP)	3·2 (3·2)
Aminoantipyrine (AA)	19·4 (9·5)
Acetylaminoantipyrine (AAA)	58·0 (14·0)

Mean (s.d., n = 8).

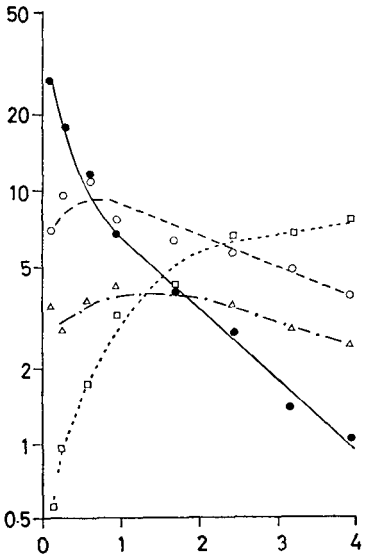

FIG. 2. A typical plasma concentration-time profile for amidopyrine (●), monomethylaminoantipyrine (○), aminoantipyrine (△) and acetylaminoantipyrine (□) after intravenous administration of aminopyrine (30 mg kg^{-1}) to a rat. Ordinate: plasma concentration (μg ml^{-1}). Abscissa: time (h).

Tsuzuki et al (1974) have reported AP half-lives of greater than 120 min in rat. However, their assay would not distinguish between AP and MAP and therefore the half-lives quoted (Tsuzuki et al 1974) represent composite values of AP and MAP. Similarly, earlier estimates of the volume of distribution of AP are erroneously low.

On the basis of the above preliminary data, AP would appear to be a particularly good model substrate for studying the factors influencing drug disposition in vivo. Using a newly developed h.p.l.c. assay, the composition of AP urinary metabolites can be established and the plasma concentration-time profiles for AP and its three metabolites can be followed.

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