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PHASE I METABOLISM OF IMIPRAMINE BY FOETAL LIVER OF MAN AND RHESUS MONKEY

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The metabolism of only a few drugs has been investigated as thoroughly as that of imipramine. The major metabolites in most species are desmethylimipramine (DMI) and 2-hydroxylated metabolites of imipramine and DMI, and their O-glucuronides (For a review see Bickel [1]). Tricyclic antidepressant drugs are used in pregnant women. Due to the high lipid solubility of these drugs it is probable that they reach the foetal compartment. Until now, the metabolism of only a limited number of drugs has been studied in human foetal liver microsomes. These include chlorpromazine, hexobarbital and meperidine (2-4), desmethylimipramine (5), ethylmorphine (6) and diazepam (7; 8). (For a review see Pelkonen [9]). The purpose of this study was to investigate the Phase I metabolism of imipramine in microsomes from foetal rhesus monkey liver and 10,000 xg supernatant from human foetal and adult liver.

Materials: Imipramine·HCl, DMI, didesmethylimipramine, imipramine-N-oxide, 2-hydroxy-imipramine (2-OH-IMP), 2-hydroxy-desmethylimipramine (2-OH-DMI) and iminodibenzyl were generously supplied by Dr. W. Theobald of Ciba-Geigy AG, Basel, Switzerland. ^{14}C-imipramine (specific activity 9.8 mCi/mmole) was purchased from Amersham Buchler. A human foetus (week 18 of gestation) was obtained by hysterotomy carried out for medical reasons. Human adult liver tissue was obtained from a 16 year old woman, who was killed in a road accident. Liver tissue was deep frozen and stored at -30° C. Two foetuses of rhesus monkeys (gestational age 95/165 days and 115/165 days respectively) were obtained from the Laboratorium für Pharmakologie and Toxikologie Prof. Leuschner, 2140 Hamburg. The rhesus foetuses were removed by caesarean section; the mother animals were healthy and not receiving any medications, except anaesthesia with hexobarbital (35 mg/kg i.v.). After surgery, the organs of the foetuses were kept in ice; microsomal fractions were prepared separately for each liver on the same day as follows: liver tissue was homogenized in Krebs-Ringer bicarbonate buffer, pH 7.4 (1+4; w/v). The homogenate was centrifuged at 14,000 g_{max} for 10 min; from the supernatant thus obtained, the microsomes were prepared by centrifugation at 105,000 g_{max} for 1 hr. The microsomal pellet was resuspended in the above mentioned

buffer and stored at -30° C. Cytochrome P 450 content was in the range of 0.057 nmoles per mg protein.

Human foetal liver tissue (1.3 g) was homogenized in Krebs-Ringer bicarbonate buffer, pH 7.4 (1+3; w/v) in a Potter-Elvehjem. The homogenate was centrifuged for 20 min at 10,000 xg. The supernatant was used immediately for incubations. Protein content was 25 mg per ml.

Human adult liver tissue (3.4 g) was first blended with an Ultra Turrax in 14 ml of Krebs-Ringer bicarbonate buffer, pH 7.4; and homogenized and centrifuged as described above. The supernatant protein content was 10 mg/ml.

Assays: final volume: 3.0 ml; imipramine; $NADP^+$ (2.4 µmoles), DL-isocitrate (24 µmoles), isocitrate dehydrogenase (EC 1.1.1.42) (0.2 U), Mg SO_4 (25 µmoles), microsomes (approx. 3.1 mg protein) or supernatant (1 ml) respectively, in Krebs-Ringer bicarbonate buffer pH 7.4. Imipramine concentrations: from 18 µmol/l to 480 µmol/l (rhesus monkey microsomes); 7.3 µmol/l; 14.6 µmol/l and 29.2 µmol/l (human adult liver); 8.5 µmol/l and 17 µmol/l (human foetal liver). The amount of radioactivity used per assay was usually 0.25 µCi. If not otherwise specified, the assays were performed for 20 min at 37° C in a Dubnoff metabolic shaker under air. The reaction was terminated by the addition of 0.5 ml of ammonia (25%). For blanks, indentical samples were incubated in parallel at 0° C. Imipramine and its metabolites were extracted twice with 2.5 ml of chloroform for 10 min. Pooled chloroform phases were evaporated to dryness under nitrogen at 40° C. The residue of each sample was dissolved in 0.3 ml of a chloroform/methanol mixture (2:1; v/v) and applied quantitatively to a thin layer plate (silica gel coated).

Thin layer chromatography and measurement of radioactivity: chromatograms were developed in the following solvent system: n-butanol/ethylmethylketone/ammonia (25%) (40 /50 /3; v/v). For scanning of radioactivity on TLC plates (quantitatively) a Berthold Thin-layer scanner II (Fa. Berthold, Wildbad, Germany) with integrator was used. Radioactivity of eluates was measured by liquid scintillation spectrometry (Packard model 2650).

Identification of imipramine metabolites: the bands were scraped off from thin layer plates and the compounds eluted twice with 3.0 ml of chloroform/methanol (2:1; v/v). After high speed centrifugation, the eluates were concentrated under nitrogen and aliquots were used for determination of radioactivity, rechromatography and derivatization. Desmethylimipramine and 2-hydroxy-desmethylimipramine were N-acetylated using a modification of a procedure described (10). To verify the identity of imipramine-N-oxide, it was reduced with titanous chloride to imipramine; 2-hydroxy-imipramine was rechromatographed in methanol/ammonia (25%) (50+50; v/v) using cellulose plates impregnated with peanutoil (10% in acetone). For details of identification see (11).

RESULTS

Foetal liver of rhesus monkey: microsomal fractions of both livers were made separately. Parallel experiments with identical assays and with five different

imipramine concentrations were performed. The following major metabolites were identified: imipramine-N-oxide>DMI>2-OH-IMP. The enzymatic reactions were linear with time for at least 30 minutes. N-Demethylation of imipramine exhibited saturation kinetics. The apparent Michaelis constant was in the range from 67 μM to 90 μM. These values correspond with those determined by Dvorchik et al. (12) for N-demethylation of meperidine and other drugs by microsomal preparations of foetal liver of the stump-tailed macaque (Macaca arctoides).

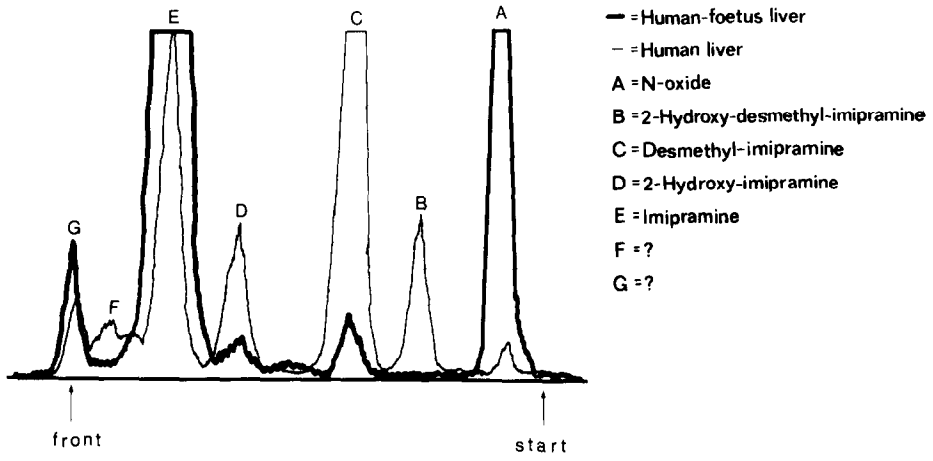

Fig. 1: Radiochromatograms of imipramine and metabolites from assays with supernatant from human foetal (imipramine conc. 17 μmol/l) and adult (imipramine conc. 14.6 μmol/l) liver

solid line: foetal liver; thin line: adult liver

Table 1: Metabolic pattern of imipramine; values presented as percentage of total radioactivity

	A IMP-N-oxide	B 2-OH-DMI	C DMI	D 2-OH-IMP	E Imipramine	F ?	G ?
rhesus monkey foetus liver	19		12.4	6.0	53.4		9.4
human foetus liver	28.5		2.9	2.9	61.1		4.7
human adult liver	1.4	9.6	47.5	9.6	22.9	4.6	4.3

imipramine concentrations: 18 μmol/l (rhesus monkey), 17 μmol/l (human foetus liver) and 14.6 μmol/l (human adult liver); incubation time 20 min.

Human foetal liver: at both imipramine concentrations a similar metabolic pattern was observed as with the microsomes from rhesus monkey foetus: imipramine-N-oxide was the main metabolite, followed by DMI and 2-OH-IMP (fig. 1 and table 1). An additional metabolite (G) can be seen in the radiochromatogram, but this metabolite was not identified.

Human adult liver: the metabolic pattern as well as the proportions were different from those observed with foetal liver (fig. 1 and table 1). The main metabolite was DMI, followed by 2-OH-IMP and 2-OH-DMI. The N-oxide formation is only of minor significance.

Conclusion: Human foetal liver possesses an active mixed function oxidase system as early as the eighth week of gestation (3). From our results and from the investigations of von Bahr et al. (5) it is evident that human foetal liver has a capacity for aromatic hydroxylation of imipramine and DMI. The existence of N-demethylating activity in foetal tissue has been established with various drugs (9) and has now been demonstrated with imipramine. The formation of imipramine-N-oxide was the predominant pathway with foetal liver from man as well as from rhesus monkey. This is in remarkable contrast to the findings with human adult liver, where N-demethylation is the major metabolic pathway. Smith and Caldwell (13) reviewed the metabolism of drugs in the rhesus monkey, rat, and other non-primates in relation to that of man. In the rhesus monkey, 72% of the compounds tested were found to show a good resemblance to their metabolism in man while only 19% showed resemblance in the rat. Certain metabolic reactions seem to be restricted in their occurrence to primates and appropriate animal models in foetal pharmacology should therefore be sought among primate species.

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