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THE APPLICATION OF HEPATOCYTE CULTURE TO THE IDENTIFICATION OF PATHWAYS OF DRUG METABOLISM: STUDIES WITH PINDOLOL AND FLUPERLAPINE.

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INTRODUCTION In the study of drug metabolism a multiplicity of systems, e.g. plasma samples, urine and bile collection, tissue homogenates or subcellular fractions fortified with cofactors, are often needed to identify all of the metabolites produced from a compound. Liver cell culture could represent a good model system for studying pathways of drug metabolism since the liver contains high activities of those enzymes that metabolise drugs. Furthermore cell culture is a closed system and metabolites normally excreted from the whole animal in the bile or urine should accumulate in the culture medium. Accordingly we have determined whether hepatocyte culture can reduce the multiplicity of systems required to identify pathways of drug metabolism by studying the metabolism of Pindolol, a β-blocker in current use, and Fluperlapine, a new neuroleptic agent, since both compounds are extensively metabolised *in vivo*.

MATERIALS ^{14}C-Pindolol, 4-(2-hydroxy-3-[^{14}C]isopropylamino-propoxy) indole and ^{14}C-Fluperlapine, 3-Fluor-6-[^{14}C]-(4 methyl-piperazinyl)- 11H dibenz-[b,e]azepine were synthesised in the Labelling Laboratory of Sandoz Ltd.

METHODS Hepatocytes were isolated from Wistar rats by the method of Seglen(1).

Studies in hepatocyte suspensions: 10^7 hepatocytes were suspended in 5 ml of Seglen's incubation buffer (1) containing 28 μM Pindolol and incubated at 37°C in a shaking incubator in 50 ml conical flasks under air. The reaction was terminated by addition of 20 ml of methanol.

Studies in hepatocyte monolayers: Hepatocytes were cultured as previously described (2). 4 and 48 hours after commencing the cultures the culture medium was replaced with medium containing 10 μM ^{14}C-Pindolol or 100 μM ^{14}C-Fluperlapine and incubated at 37°C for the next 20-24 hours when the medium was removed. The hepatocyte monolayer was washed with 3 ml methanol for 5 minutes to extract the metabolites associated with the cells.

Administration of ^{14}C-Pindolol and ^{14}C-Fluperlapine to rats: 300 gm male Wistar rats received an oral dose, by gastric intubation, of 30 mg ^{14}C-Pindolol/kg and 6 mg or 12 mg ^{14}C-Fluperlapine/kg. The rats were housed in metabolism cages that allowed the separate collection of urine, faeces and bile via a bile fistula. The rats had free access to water and rat chow (NAFAG No.850/194) during the experiment.

Determination of metabolite patterns: The separation of metabolites was done by HPLC and the methods will be published elsewhere (Maurer & Von Wartburg, in preparation).

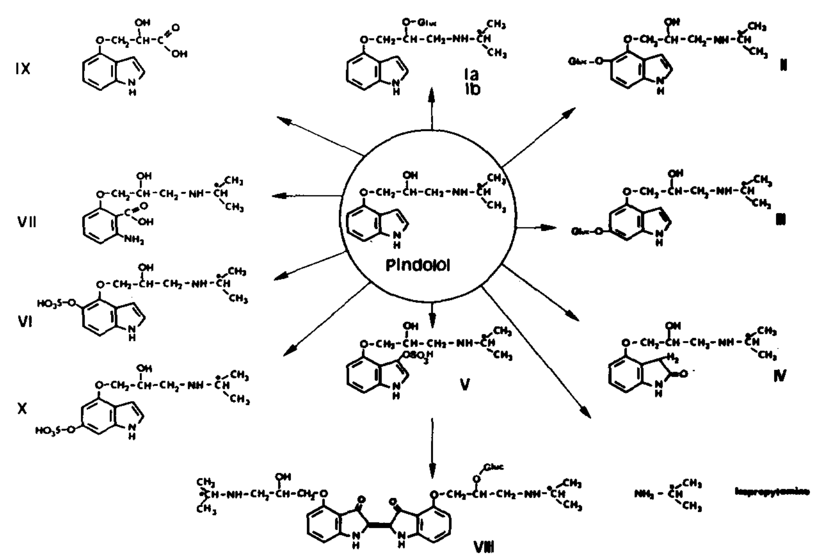
RESULTS AND DISCUSSION

a) Studies with Pindolol The oral administration of ^{14}C-Pindolol to rats results, 2 hours later, in the production of 9 metabolites detected in the plasma (Table 1) which are mainly excreted in the urine (70.5% of the dose). Fig.1 demonstrates the proposed metabolic pathway of pindolol (3). In rats the major urinary metabolites arise by hydroxylation or oxidation and conjugation of the indole ring (metabolites II, III, IV, V, VI and X), oxidative ring scission (metabolite VII) and side-chain deamination and oxidation (isopropylamine and metabolite IX). Metabolite IX will not be detected in this study since it does not contain the radioactively labelled carbon atom.

Table 1 shows that pindolol is extensively metabolised in suspensions of isolated rat liver cells and all the metabolites detected in urine are found in a 2 hour incubation of isolated liver cells. Similarly Phase I and Phase II metabolism of pindolol was found in cultures of rat liver cells (Table 1). The culture conditions employed, eg "P-450 medium" and Williams Medium E + 0.5 mM metyrapone, are known to prevent the loss of cytochrome P-450 that occurs in hepatocyte culture (2) and indeed metabolism of pindolol was observed for up to 3 days. Although metyrapone is a known inhibitor of cytochrome P-450 mediated drug metabolism (4) the metabolite patterns obtained with cultured hepatocytes compare qualitatively well with those found in plasma, urine and isolated liver cells. The apparent lack of inhibitory effects of metyrapone on pindolol metabolism may be due to the metabolism of metyrapone (5) to products which are either non-inhibitory or are excreted from hepatocytes into the culture medium. Higher concentrations of Pindolol (50 and 100 µM) in the culture medium resulted in a similar metabolite profile (data not shown) and metabolites I and VII became detectable (Table 1). Although the age of the culture, in P-450 medium, did not affect the total amount of Pindolol metabolised it would appear that glucuronidation may decrease with age and this may coincide with an increase in sulphation as demonstrated by the decrease in metabolites II and III and an increase in the proportions of metabolites V, VI and X (Table 1). The work of Paterson et al. (6) suggests

FIG.1

Proposed pathways for the biotransformation of Pindolol in rats.
*Indicates position of ^{14}C label.



that such changes in glucuronidation and sulphation could result from changes in the rate of phase I metabolism. However, the maximum 9 metabolites of pindolol anticipated can be detected in hepatocyte culture (Table 1). These metabolites account for 95.8% of the radioactivity of the sample. Metabolites were not detected when ^{14}C-Pindolol was incubated in the absence of hepatocytes. 96 ± 3% of the metabolites were found in the culture medium and 4 ± 1% were associated with the cells (extracted with methanol). Whether the amount associated with the cells is due to the incubation medium adhering to the cells was not determined.

Table 1. Percentages of ^{14}C-Pindolol and its metabolites in biological samples of rat.

Sample and Sampling time	Pindolol	I	II	III	IV	V	VI	X	VII	Isopropyl-amine
Plasma (2 h)	1.8	0.5	8.1	10.0	0.2	4.5	1.0	3.0	36.5	16.0
Urine (0-24 h)	17.7	*	8.0		10.2	17.6	7.2	1.3	14.6	7.3
Isolated liver cells (2 h)	41.2	*	4.6	2.3	12.1	14.0	3.0	1.8	2.5	5.3
Cultured hepatocytes										
4-24 h a)	2.4	c)	7.8	22.0	*	17.2	17.3		c)	19.3
48-72 h a)	2.9	*	5.7	14.6	2.0	32.2	23.6		*	10.6
4-24 h b)	6.8	*	6.4	5.5	*	27.8	19.9		*	11.8
48-72 h b)	10.3	*	4.9	5.4	*	34.7	26.9		*	2.9

a) = P-450 medium, b) = Williams E + 0.5 mM metyrapone, c) = metabolite detectable in incubations with 100 µM Pindolol. *indicates not detected.

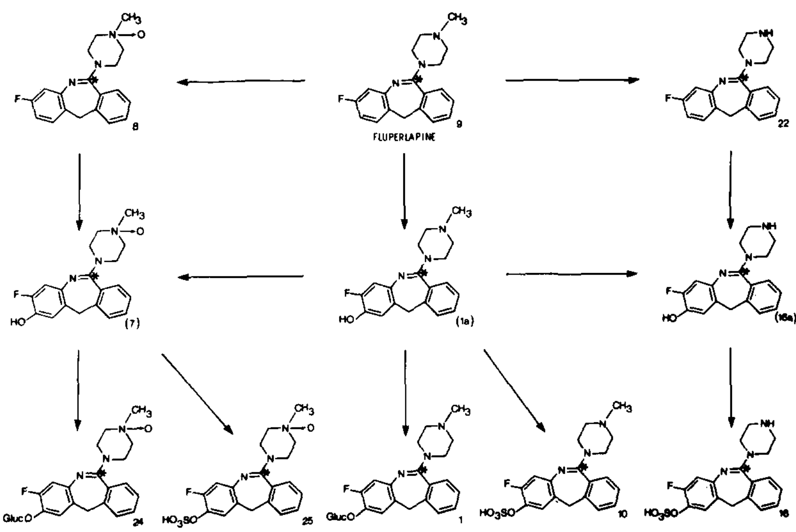
b) Studies with Fluperlapine The oral administration of ^{14}C-Fluperlapine to rats results in the formation of 7 different metabolites detectable in the plasma, urine and bile (Table 2). Fig.2 demonstrates the proposed metabolic pathway of Fluperlapine. Based on the identified metabolites the following main routes appear to be operative in the biotransformation of Fluperlapine in rats: Formation of N-oxides (metabolite 8), oxidative N-demethylation, (metabolite 22) aromatic oxidation to yield phenols (1a, 7, 16a) and transformation of the phenols to yield conjugates such as sulphates (10, 16, 25) and glucuronides (1, 24).

Table 2 shows that Fluperlapine is extensively metabolised in rat liver cell culture. As with the incubations with Pindolol the metabolite patterns of Fluperlapine are similar when cells are incubated in P-450 medium or Williams medium E + 0.5 mM metyrapone. Whilst the age of the culture did not significantly alter the amount of Fluperlapine metabolised, it did, especially in "P-450 medium", markedly change the proportion of polar metabolites (1, 10, 16, 24) to non polar metabolites (8, 22) formed. Thus the polar metabolites accounted for 65% of the total at 24 hours and 29% at 72 hours. Nevertheless 6 out of the 7 known metabolites can be identified in hepatocyte culture.

In conclusion the present work shows that 9 out of the 11 known metabolites of Pindolol and 6 out of the 7 known metabolites of Fluperlapine can be identified in hepatocyte culture, a single system, whereas a number of systems were previously needed to detect all of the metabolites. Accordingly hepatocyte culture could be a useful model system in the identification of pathways of drug metabolism, especially when the pharmacological action of the drug (eg Lysergide) does not permit high doses to be administered to experimental animals.

FIG.2

Proposed pathways for the biotransformation of Fluperlapine in rats



*Indicates position of ^{14}C label. Parenthesis indicate metabolite not detected.

Table 2. Percentage of ^{14}C-Fluperlapine and its metabolites in biological samples of rat.

Sample and sampling time	Fluper-lapine	Metabolites 1	8	10	16	22	24	25
Plasma (0.5h)	3.5	*	53.7	5.7	14.4	1.1	14.9	*
Urine (0-24h)	1.1	3.1	4.5	< 1	33.4	*	13.9	14.2
Bile (0-24h)	*	8.8	*	22.6	11.4	*	6.1	6.9
Cultured hepatocytes								
4-24h[a)]	1.4	4.5	9.9	4.8	13.1	16.3	42.6	*
48-72h[a)]	5.7	4.6	42.6	4.4	0.9	17.9	19.3	*
4-24h[b)]	-	4.8	18.7	4.0	5.4	26.2	39.1	*
48-72h[b)]	-	9.1	51.9	10.2	0.5	5.9	19.5	*

a) = P-450 medium, b) = Williams E + 0.5 mM metyrapone. *indicates not detected Plasma samples from rats dosed with 12 mg/kg, urine & bile from rats dosed with 6 mg/kg.

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