is not required when designing the optimal dosage regimen with acebutolol.

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THE ELIMINATION OF PRACTOLOL BY TWO HUMAN SUBJECTS

Practolol was formerly in widespread use as a β -adrenoceptor blocking drug in the treatment of a number of cardiovascular disorders. However, an association between its use and the development of untoward effects resulted in its availability being restricted; at the present time it is available only as an injection form for hospital use in acute situations. Side-effects associated with the use of the drug include adverse changes of the skin, eyes, oral and nasal mucous membranes, ears and peritoneum (Rowland & Stevenson, 1972; Brown, Baddeley, Read, Davies & McGarry, 1974; Felix, Ive & Dahl, 1974; Wright, 1975; Thein & Asquith, 1977).

The mechanism whereby practolol produces these effects in some individuals remains obscure and research on the problem is hampered by the lack of a

suitable animal model. Various views have been put forward to explain drug toxicity including an immunological theory (Allison, Denman & Barnes, 1971) and the possible formation by some individuals of potentially toxic metabolites (see Jollow, Kocsis, Snyder & Vainio, 1977). The latter theory would require among other things the demonstration that people susceptible to practolol side-effects convert the drug to potentially toxic metabolites more extensively than do non-sensitive individuals. Previous studies, however, suggest that practolol is metabolically stable as oral and intravenous doses are cleared in the urine as the unchanged drug (Fitzgerald & Scales, 1968; Aellig, Prichard & Scales, 1970; Bodem & Chidsey, 1973; Kaye, Robinson & Turner, 1973).

We recently had an opportunity to investigate the

metabolism and elimination of orally administered [^{14}C]-practolol in two patients one of whom had developed dermatitis while on the drug. The extent of metabolism of the drug in the two subjects was determined by two techniques, namely, reverse isotope dilution and by quantitative radiochromatogram scanning. The results showed no detectable metabolism of the drug by either the apparently practolol sensitive or nonsensitive patient.

The first patient (R. E. male, aged 60 years) and suffering from mitral incompetence and angina pectoris had been treated with practolol (100 mg three times daily) for 4 years. The second patient (C.M. female, aged 53 years) suffering from cardiomyopathy had been treated with propranolol for 2 years and then changed to practolol in March 1973 in view of low blood pressure on propranolol. Nine months later the patient developed dermatitis of the hands and this was believed to be related to the use of practolol. The skin condition improved after the practolol had been replaced by propranolol. The latter was later stopped as the patient became hypotensive and she was put back on practolol 100 mg three times daily in February 1975 and had been on this drug for 16 months before the present study without recurrence of dermatitis.

Approval of the use of [^{14}C]-labelled practolol for the metabolic and elimination studies was obtained from the M.R.C. Radio-isotopes Advisory Panel and the Ethical Committee of the Hospital. The informed consent of the two patients was obtained. Each patient took [^{14}C]-ring-labelled practolol orally (100 mg; 14.6 μCi) dissolved in 0.1 M citric acid (50 ml) as part of their daily regimen of practolol. Blood samples (10 ml) were taken at 1, 2, 5, 8, 12 and 24 h after dosing by venous puncture. The blood was immediately transferred to tubes containing lithium-heparin coated granules and centrifuged to separate plasma from red blood cells. Urine and faeces were collected at intervals for up to 4 days after dosing. Radioactivity in urine and plasma samples was determined by liquid scintillation counting (Packard Model 3385) and quenching was corrected for by the channels ratio method. Faeces were bleached with alkaline hydrogen peroxide prior to counting as a suspension in thixotropic gel (Cab-O-Sil).

The nature of the urinary excretion products was determined by reverse isotope dilution and by chromatography as follows:

Reverse isotope dilution for practolol

Practolol (1g) was dissolved in urine (10 ml) made acidic with 2 M-HCl. The urine was then made strongly alkaline with 10 M-NaOH saturated with NaCl and extracted with chloroform (2 $\times$ 30 ml). The latter were combined, dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The residue of practolol was then recrystallized from

dioxan to constant specific radio-activity (m.p. 142°C). As a check on the procedure (to reduce the possibility of co-crystallization), the practolol that had been recrystallized to constant specific activity was then converted to its hydrochloride derivative and counted as such. For this purpose the practolol obtained above was dissolved in ethanol to which was added a slight excess of HCl in ethanol. The ethanol was evaporated and the residual hydrochloride recrystallized from aqueous acetone to constant specific activity (m.p. 145°C).

Chromatographic analysis

Urine samples were chromatographed on Whatman No. 1 paper and developed with either butan-1-ol saturated with 1.5 M-ammonia, ammonium carbonate buffer (Fewster & Hall, 1951) (Solvent A) or butan-2-ol: formic acid: water (100:12:10 by vol) (Solvent B). Some samples were further chromatographed on thin-layer silica gel pre-prepared plates (silica gel HF₂₅₄, 250 mm thick; E. Merck A. G. Darmstadt, Germany) using as solvent methanol: ammonium hydroxide (sp. gr. 0.88) (99: 1 v/v) (Solvent C). [^{14}C]-peaks were located by radiochromatogram scanning (Packard Radiochromatogram Scanner Model 3214). Desacetylpractolol and 3-hydroxypractolol could be detected by means of the colour reactions they afford when sprayed with diazotised 4-nitroaniline (Wickström & Salvesen, 1952). The chromatographic properties of practolol and related compounds are shown in Table 1.

The results showed that the two patients eliminated 79 and 80% of the dose respectively in the urine over a period of 3 days with a further 16% and 10% respectively in the faeces (Table 2). Most of the urinary elimination occurred within the first 24 h; both patients excreted 61% within this time. Radiochromatogram scans of paper and thin-layer chromatograms of the 0-24 h urine showed the presence of a single [^{14}C]-peak which corresponded to practolol. A similar picture was observed if the urine had been incubated at 37°C for 24 h with β -glucuronidase or arylsulphatase preparations suggesting that little if any conjugates of 3-hydroxypractolol were present. Reverse isotope dilution on serial urine samples showed that all the [^{14}C] could be accounted for as practolol, a finding which was further confirmed by quantitative radiochromatogram scanning (Table 3).

Plasma [^{14}C]-levels peaked at about 2 h after dosing; the plasma levels at this time were 660 and 510 ng/ml for subjects C.M. and M.E. respectively. These rapidly declined and at 10 h after dosing the plasma level of [^{14}C] was equivalent to less than 50 ng/ml. The approximate plasma half-life in the post-absorption phase was about 8 h for both subjects.

The findings are therefore in agreement with those

of other workers that practolol is rapidly excreted in the urine without apparently undergoing metabolic change (Fitzgerald & Scales, 1968; Bodem & Chidsey, 1973). There is, however, the possibility that part of the drug undergoes deacetylation followed by reacylation and this cannot be excluded on the basis of the present evidence available.

It is of particular interest that a patient who had previously shown a toxic response while on practolol did not differ significantly in her handling of the drug

compared to the other patient suggesting that the intact compound may be responsible for the untoward effects. It should be borne in mind however that when this patient was rechallenged with practolol she did not develop an adverse skin response. This might suggest that the original skin rash was not a manifestation of the typical oculocutaneous syndrome (Brown *et al.*, 1974; Felix *et al.*, 1974; Wright, 1975). It could also be speculated that practolol toxicity arises from the interaction of a number of factors

Table 1 Chromatographic properties of practolol and related compounds

Compound	R_f values (paper) in solvents		R_f value (thin layer)	Colour with diazotised 4-nitroaniline
	A	B	C	
Practolol	0.85	0.16	0.50	—
Desacetyl- practolol	0.70	0.25		reddish
3-hydroxypractolol	0.63	0.25	0.50	brownish

Table 2 Elimination of [^{14}C]-practolol in the urine and faeces of two patients

Day after dosing	Patient M E		Patient C M*	
	% dose urine	[^{14}C]-excreted in: faeces	% dose urine	[^{14}C]-excreted in: faeces
1	61	11	61	6
2	12	2.6	15	2.7
3	5.6	2.5	4	1.7
Total	78.6	16.1	80	10.4

Each patient received orally 100 mg (14.6 μCi) of [^{14}C]-practolol as part of their daily therapeutic regime.

* This patient had developed dermatitis while receiving practolol. On a subsequent occasion, when rechallenged with the drug, there was no repetition of the condition.

Table 3 Urinary excretion of [^{14}C]-practolol by two patients

Urine sample	% dose [^{14}C]-practolol excreted	
	Patient M E	Patient C M*
0–2 h	7.4 (6.6)	4.7 (4.9)
2–4 h	7.2 (8.7)	11.9 (12.4)
4–6 h	8.3 (7.1)	8.1 (8.5)
6–8 h	7.4 (7.1)	6.0 (6.3)
8–10 h	5.6 (6.1)	5.4 (5.6)
10–21 h	17 (17.7)	18 (18.7)
21–24 h	6.1 (6.7)	4.3 (4.3)
Total	59.0 (60.0)	58.4 (60.7)

The patients received orally 100 mg (14.6 μCi) of [^{14}C]-practolol as part of their daily therapeutic regime. The urine was collected at 2 h intervals and assayed for unchanged practolol both by reverse isotope dilution (RID) and by quantitative radiochromatogram scanning (RS). The values obtained by the latter techniques are shown in parentheses.

including the intact drug itself but that conditions during the second period of dosing were not such as to precipitate the reaction.

The present findings, however, do not exclude the possibility that toxic metabolite formation is responsible for the adverse effects of practolol, since these might be produced only in very small amounts, be highly reactive and covalently bound (Orton & Lowery, 1977) and not readily detectable by the methods used here.

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RADIOIMMUNOASSAY FOR FLUPHENAZINE IN HUMAN PLASMA

Fluphenazine (FPZ, Figure 1a) is a phenothiazine derivative. It is widely used in the maintenance treatment of schizophrenia as an intramuscular slow release preparation containing its decanoic acid ester (Figure 1b). Although treatment is generally effective (Ayd, 1975) there are individual differences in both clinical response and in side effects. Measurement of plasma fluphenazine levels would allow greater control of dosage and may improve therapeutic control. However, fluphenazine decanoate is given in low doses (typically 25 mg every 1–4 weeks) and plasma drug levels attained are below the range of current assay techniques (Larsen & Naestoft, 1973; Whelpton & Curry, 1976). The following account describes the development of a highly sensitive radioimmunoassay for fluphenazine in plasma which does not require

extraction and preliminary data from the analysis of clinical samples.

Antiserum was raised in an adult female sheep. The immunogen (Figure 1d) was prepared by covalent linkage of bovine serum albumin (BSA) to 7-carboxy flupenthixol (Figure 1c) employing the carbodiimide condensation reaction (Sheehan & Hlavka, 1956) followed by dialysis first against a citrate buffer (pH 3) and then saline (0.9%). The sheep was inoculated at 4-weekly intervals with 1 ml conjugate in saline (250 μ g flupenthixol) suspended in 4 ml of complete Freund's adjuvant, the dose being divided between four intramuscular sites. Venous blood samples taken on the same occasion were allowed to clot overnight at 4°C and serum, separated by centrifugation, was stored at –20°C.