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SINGLE-DOSE PHARMACOKINETICS OF PERHEXILINE ADMINISTERED ORALLY TO HUMANS

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SUMMARY

A high-performance liquid chromatographic method for the simultaneous determination of perhexiline and its major metabolites, the *cis*- and *trans*-monohydroxyperhexilines M_1 and M_3, respectively, in human plasma or urine has been developed. Perhexiline and its metabolites are extracted from plasma or urine and derivatized with 1-fluoro-2,4-dinitrobenzene. The extracted dinitrophenyl derivatives of drug and metabolites are separated on a Spherisorb S5 ODS column by gradient elution. The limits of detection for perhexiline and its monohydroxy metabolites were 15 and 3 ng/ml, respectively. The inter-assay coefficients of variation for 100 ng/ml perhexiline, 100 ng/ml M_1 and 400 ng/ml M_3 were 10.5, 7.6 and 5.6%, respectively (n = 9).

The method has been employed in a limited kinetic study with five healthy adult male volunteers who received 150-mg and 300-mg Pexid tablets at an interval of one week. In four subjects perhexiline exhibited marked first pass effects, with plasma M_1 levels higher than unchanged perhexiline; in the urine M_1 was the predominant metabolite except in one subject who had higher M_3 than M_1 in the 300-mg Pexid study. The fifth subject exhibited a defective capacity to hydroxylate perhexiline; M_1 and M_3 were not detectable in plasma, and the urinary excretion of the monohydroxyperhexilines was relatively less, with M_3 present in higher amounts than M_1

INTRODUCTION

Perhexiline maleate (Pexid) is an effective prophylactic agent in angina pectoris [1]. Analytical methods presently available for measuring perhexiline are either insufficiently sensitive for single-dose kinetic studies, such as the gas—liquid chromatographic methods of Singlas et al. [2] and Cooper and Turnell [3], or do not permit the determination of the major metabolites of perhexiline as in the high-performance liquid chromatographic (HPLC) method of Horowitz et al. [4] which uses fluorescence detection. We now report a sensitive UV spectrophotometric HPLC method for the simultaneous deter-

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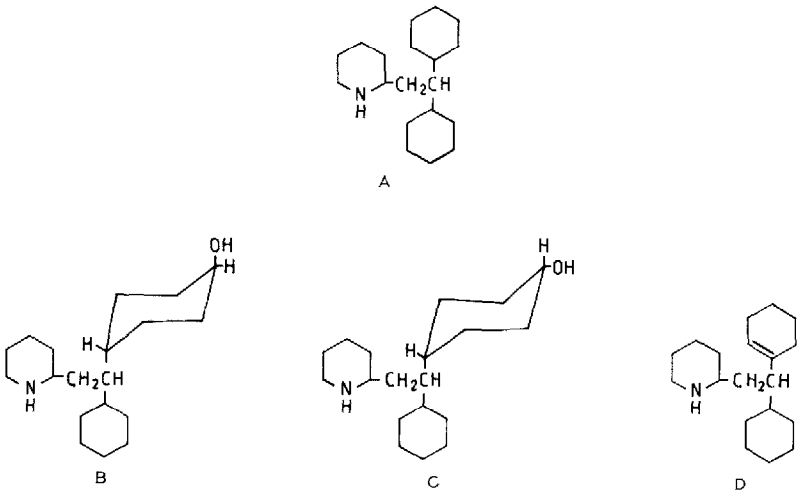

Fig. 1. Chemical structures of perhexiline (A), its monohydroxylated metabolites M_1 (B) and M_3 (C), and the HPLC internal standard (D).

mination of perhexiline and its major metabolites, the *cis*- and *trans*-monohydroxyperhexilines M_1 and M_3, respectively (Fig. 1). This method is suitable for single-dose kinetic studies with human plasma or urine.

MATERIALS AND METHODS

Reagents

All aqueous solutions were prepared with deionized water that had been passed through a 0.45-μm MF Millipore filter (Millipore U.K., London, U.K.). Reagent-grade 1-fluoro-2,4-dinitrobenzene, disodium tetraborate and sodium hydroxide (B.D.H. Chemicals, Poole, U.K.), diethyl ether (analytical grade, May and Baker, Dagenham, U.K.) and cyclohexane (analytical grade, B.D.H. Chemicals) were purchased. Methanol (analytical grade, James Burrough, London, U.K.) was passed through a 0.5-μm FH Millipore filter before use. Perhexiline, monohydroxyperhexilines M_1 and M_3 (1:4 mixture) and internal standard [2-(2′-cyclohexenyl-2-cyclohexylethyl)piperidine hydrochloride] (see Fig. 1) were gifts from Merrell National Labs. (Cincinnati, OH, U.S.A.).

Chromatographic system

A chromatographic system incorporating a Gradient Master and two Constametric I pumps, both from Laboratory Data Control (Riviera Beach, FL, U.S.A.), a Rheodyne syringe loading sample injector (Model 7120, Rheodyne, Berkeley, CA, U.S.A.) with a 100-μl sample loop, and a Spectro Monitor III (Laboratory Data Control) set at 360 nm was used in the analysis. Chromatographic separation of drug and metabolites was achieved with a 250 × 4.6 mm steel column packed with Spherisorb ODS, particle size 5 μm (Phase Separations, Queensferry, U.K.).

Chromatographic conditions

The mobile phase was contained in two reservoirs, A (methanol—water,

85:15) and B (methanol). Elution was performed at 20°C with a flow-rate of 1.0 ml/min. The gradient time and gradient exponent were 20 min and 3, respectively (Service Manual Gradient Monitor Model 1601, Laboratory Data Control). The initial mobile phase composition was 95% A and 5% B with a final composition of 40% A and 60% B. The final mobile-phase composition was maintained for a further 5 min when the run was terminated. The mobile phase was restored to its initial composition and allowed to equilibrate with the column for 10 min before another sample was injected into the chromatograph.

Sample preparation

Plasma or urine samples (1.0 ml) containing 250 ng of internal standard, 0.1 ml of 4 *M* sodium hydroxide and 20 ml of diethyl ether were placed into 25-ml screw-capped tubes (Sovirel, Paris, France). Extraction was carried out on a rotary mixer for 30 min and the organic phase removed; the extraction was repeated and the combined organic extracts evaporated to dryness at 40°C. N-Dinitrophenyl derivatives of perhexiline, its metabolites and the internal standard were prepared by a modification of the method of Cox [5] for secondary amines. The dried residue of the ether extract was heated at 80°C for 15 min with 1.5 ml of 26.2 m*M* disodium tetraborate and 0.5 ml of 0.16 *M* 1-fluoro-2,4-dinitrobenzene, and heating continued for a further 1 min after addition of 0.2 ml of 4 *M* sodium hydroxide. The reaction mixture was then cooled and the N-dinitrophenyl derivatives extracted with 20 ml of cyclohexane on a rotary mixer for 30 min. The cyclohexane layer was removed, evaporated to dryness under vacuum at 20°C and the residue redissolved in 100 μl of methanol—water (85:15) for injection into the chromatograph.

A calibration curve was prepared from blank plasma or urine to which known amounts of perhexiline, and metabolites M_1 and M_3 had been added. The peak height ratio of drug or metabolite relative to the internal standard was then plotted as a function of drug and metabolite concentration. The drug and metabolite concentrations of samples were determined from the calibration curve.

For recovery studies, known amounts of drug and metabolites were added to normal human blood plasma of which 1.0 ml was extracted with two 20-ml portions of diethyl ether, as previously described.

For precision studies 100 ng/ml perhexiline, 100 ng/ml M_1 and 400 ng/ml M_3 were added to normal human plasma, which was stored at −20°C until required for analysis.

Clinical studies

Following the approval of the ethical committees of the University of Surrey and St. Luke's Hospital, Guildford, five consenting adult male volunteers aged 23—46 years (Table I) were accepted for the clinical study on the basis of a normal medical examination and routine investigations and screening for liver disease. After an overnight fast, each volunteer received 150 mg Pexid orally; fasting was continued for a further 3 h before the subjects were allowed a light lunch. Blood samples were withdrawn over an 8-h period from an intravenous catheter inserted into a forearm vein. A two-hourly urine

TABLE I

HUMAN VOLUNTEERS PARTICIPATING IN THE SINGLE-DOSE PERHEXILINE STUDIES

None of the volunteers was taking other drugs during the study. All volunteers were males.

Volunteer	Age (years)	Weight (kg)	Low-dose study (mg perhexiline base per kg body weight)	High-dose study (mg perhexiline base per kg body weight)
J.D.	32	70	2.1	4.2
J.B.	33	83	1.8	3.6
A.D.	31	55	2.7	5.4
S.G.	23	68	2.2	4.4
E.E	46	83	1 8	3.6
Mean	33	71.8	2.1	4.2

collection was made for the first 8 h after drug ingestion, with a final pooled collection from 8—24 h.

The study was repeated a week later (when perhexiline and metabolites were undetectable in plasma and urine) with 300 mg of Pexid. An additional blood sample was taken at 24 h after drug administration. Plasma and urine samples were analysed by HPLC.

RESULTS

The recoveries from normal blood plasma were for perhexiline (0.1—10 μg/ml) 95—100%; M_1 (0.1—8.0 μg/ml) 90—110%; and M_3 (0.4—10 μg/ml) 94—110%. For mixtures of all three (0.5—10 μg/ml of each) the recoveries were 87—118% with a coefficient of variation of 10.5% (n = 9). Chromatograms of blank blood plasma and plasma containing perhexiline, monohydroxyperhexilines M_1 and M_3, and internal standard are shown in Fig. 2. Plasma samples from patients receiving perhexiline were similar to the spiked plasma (Fig. 2B) and no other peaks were present. The retention times of metabolite M_3, M_1, the internal standard and perhexiline were 10.75, 12.25, 23.5 and 24.5 min, respectively. The limit of detection for plasma perhexiline was 15 ng/ml and that for monohydroxyperhexilines (M_1 and M_3) was 3 ng/ml. The calibration curve was linear over the concentration range 15 ng/ml to 10 μg/ml for perhexiline, and over the range 5 ng/ml to 10 μg/ml for the monohydroxyperhexilines. The coefficients of variation of replicate assays performed over a 28-day period for 100 ng/ml perhexiline, 100 ng/ml M_1 and 400 ng/ml M_3 were 10.5, 7.6 and 5.6%, respectively (n = 9).

Clinical studies

Following the administration of 150 mg of Pexid, perhexiline was undetectable in the plasma of subject J.D. over the 8-h sampling period; this subject had the highest plasma M_1 levels (Fig. 3A). Three subjects (A.D., J.B. and S.G.) had significant plasma perhexiline levels with relatively lower M_1

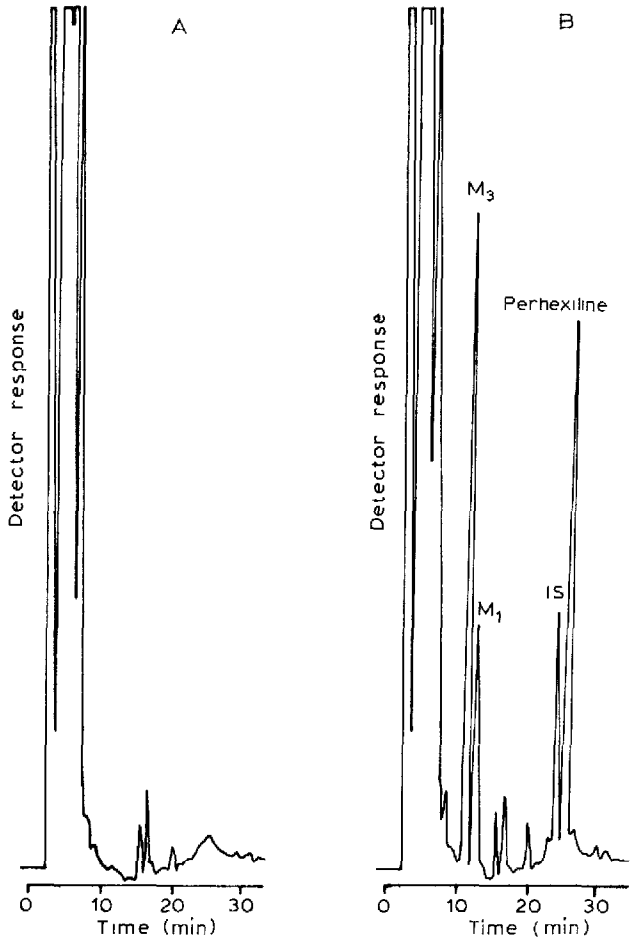

Fig. 2. High-performance liquid chromatograms of ether extracts of (A) human blood plasma and (B) human blood plasma with added perhexiline, monohydroxyperhexilines and internal standard. Perhexiline (500 ng/ml), metabolite M_1 (100 ng/ml), metabolite M_3 (400 ng/ml) and internal standard (IS) (250 ng/ml) were added to the blood plasma (1.0 ml) before extraction. HPLC conditions were: column, 250 × 4.6 mm Spherisorb ODS; mobile phase, A (methanol—water, 85:15), B (methanol), flow-rate, 1 ml/min; gradient time/exponent, 20 min/3; initial mobile phase, 95% A and 5% B; final mobile phase, 40% A and 60% B.

levels following the 150-mg dose of Pexid. M_3 was either undetectable or very low in the plasma of the above four subjects (Fig. 3). The highest plasma perhexiline levels were observed in the fifth volunteer (E.E.); at 8 h the plasma concentration of perhexiline was still rising (Fig. 3E); metabolites M_1 and M_3 were undetectable in the plasma. At 2 h following drug administration metabolites M_1 and M_3 were present in the urines of four of the subjects (J.D., J.B., A.D., S.G.) albeit in small amounts (Fig. 4); at 24 h M_1 was present in higher amounts than M_3. No unchanged perhexiline was detected in any of the urines of the four subjects. In the fifth subject (E.E.) metabolite M_1 was not detectable in the urine, M_3 was present in relatively small amounts, and significant amounts of unchanged perhexiline were also present (Fig. 4E).

At the higher dose study of Pexid, low concentrations of perhexiline were

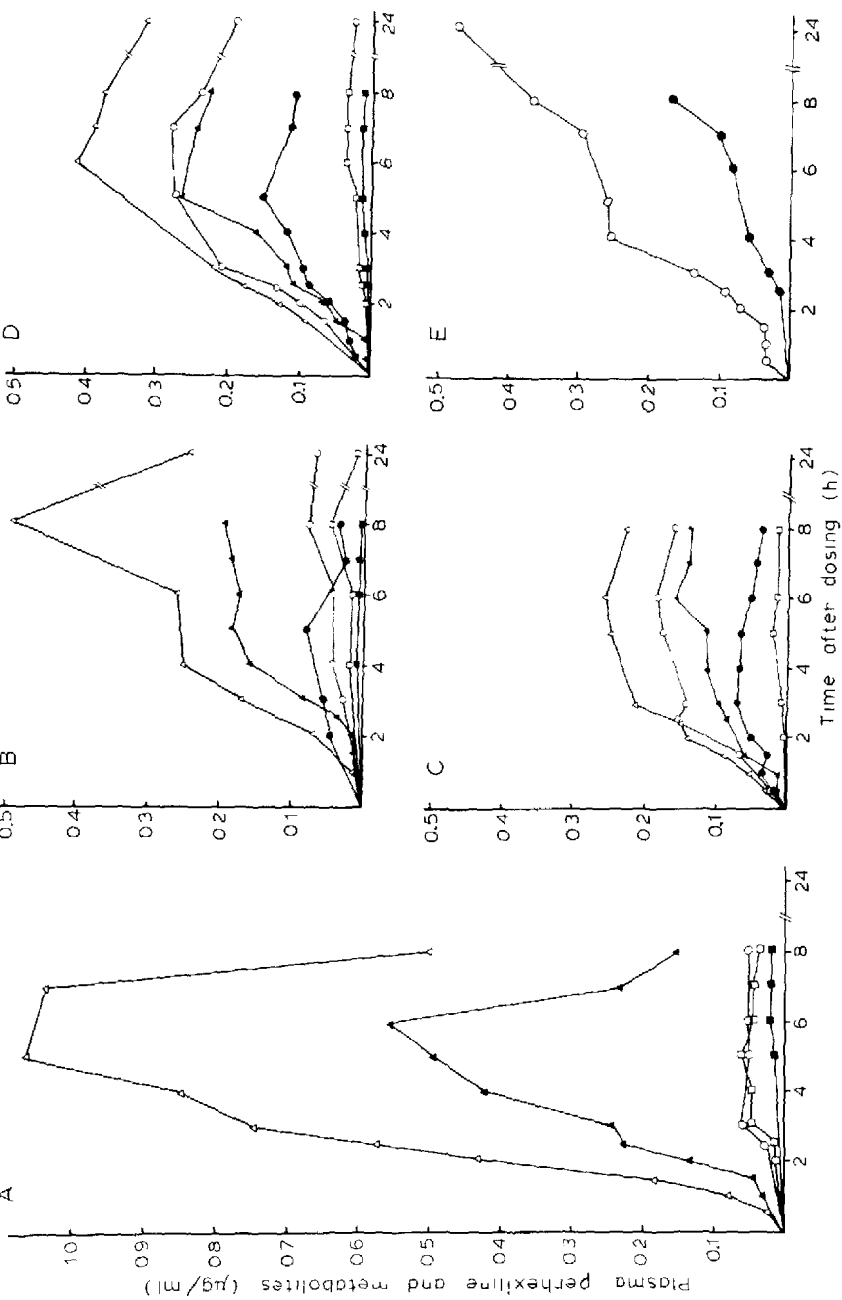

Fig. 3. Plasma concentration–time curve for perhexiline and its monohydroxylated metabolites after oral administration of perhexiline to human volunteers. (A) subject J.D.; (B) subject J.B.; (C) subject A.D.; (D) subject S.G.; and (E) subject E.E. Unchanged perhexiline (•), metabolite M_1 (▲), and metabolite M_2 (■), are shown after oral dosage with 150 mg of perhexiline; and unchanged perhexiline (○), M_1 (△), and M_2 (□), after 300 mg of perhexiline.

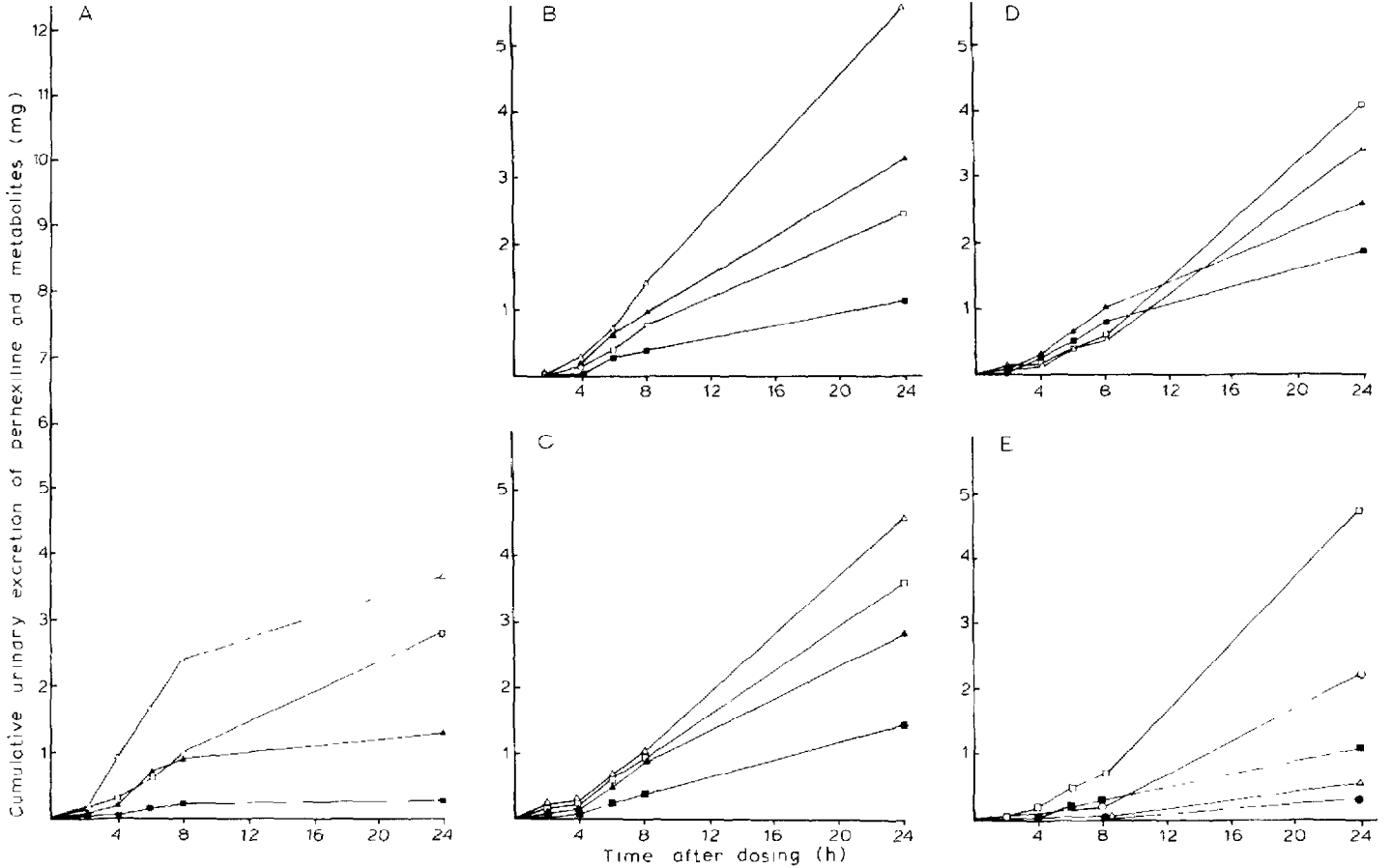

Fig. 4. Cumulative urinary excretion of perhexiline and its monohydroxylated metabolites after oral administration of perhexiline to human volunteers (A) subject J.D.; (B) subject J.B.; (C) subject A.D.; (D) subject S.G. and (E) subject E.E. Unchanged perhexiline (●), metabolite M_1 (▲), and metabolite M_3 (■), are shown after oral dosage with 150 mg of perhexiline; and unchanged perhexiline (○), M_1 (△), and M_3 (□), after 300 mg of perhexiline

observed in the plasma of subject J.D., with very high levels of metabolite M_1 (Fig. 3A). Plasma concentrations of perhexiline and metabolites M_1 and M_3 were higher at the higher dose of Pexid in subjects J.B., A.D. and S.G. (Fig. 3B–D); at 8 h after dosing, the plasma perhexiline M_1 and M_3 concentrations were still rising in J.B. (Fig. 3B). Monohydroxyperhexiline metabolites M_1 and M_3 were again undetectable in the plasma of the fifth subject E.E.; this subject had the highest plasma perhexiline concentration. At 24 h after dosing, metabolite M_1 was present in greater amounts than M_3 in the urines of three volunteers (Fig. 4). One subject (S.G.) had approximately the same amounts of metabolites M_3 and M_1 in the urine (Fig. 4D). In the fifth subject (E.E.), M_3 was the predominant hydroxylated species present in the urine; metabolite M_1 was excreted in the urine, in small amounts, but only at the higher dose; unchanged perhexiline was also present in substantial amounts in the urine of this subject (Fig. 4E).

DISCUSSION

Despite the proven efficacy of perhexiline maleate in angina pectoris and its reported superiority over some β-adrenergic blocking drugs in the treatment of angina pectoris [6–8] the prescription of perhexiline remains limited because of the frequency and severity of some adverse reactions to the drug.

The mechanism of perhexiline-induced toxicity is still not clear, although a disorder in perhexiline metabolism has been suggested as a possible factor [9]. Singlas et al. [2] have provided evidence in support of this view in that thirteen neuropathic patients showed slower hepatic metabolism of perhexiline, with longer plasma perhexiline half-life and higher mean plasma perhexiline concentration (3.8 μg/ml), than did fourteen patients with no neuropathy (mean plasma perhexiline concentration, 1.0 μg/ml).

The HPLC method now described, with its added advantage of the simultaneous determination of the unchanged drug and two monohydroxy metabolites, has permitted us to carry out a preliminary examination of the single-dose kinetics of perhexiline in human volunteers. In this limited study we observed considerable individuality with respect to the metabolism and clearance of perhexiline which supports the earlier studies using [^{14}C]perhexiline [10].

In four subjects (J.D., J.B., A.D., S.G.) perhexiline exhibited marked first-pass effects with plasma metabolite concentrations higher than unchanged perhexiline (Fig. 3). This might explain the observed initial delay between perhexiline administration and the onset of drug effect [11]. The major metabolite in both plasma and urine was M_1 in three volunteers. In the fourth subject (S.G.) M_1 was also the major metabolite in the plasma, and in the urine at the lower-dose study but at the high-dose study M_3 became the major metabolite in the urine at 24 h (Figs. 3 and 4). Perhexiline metabolism could thus be subject to saturable kinetics with respect to metabolite M_1. The concentration of free M_3 in the urines of all five subjects is greater than might have been expected from the blood concentrations of this metabolite (Figs. 3 and 4).

The fifth subject (E.E.) appeared to have a defective capacity to hydroxylate perhexiline. Metabolites M_1 and M_3 were not detectable in the blood plasma and

the concentration of unchanged perhexiline in the plasma was much higher than in the four other subjects. Smaller amounts of metabolites were excreted in the urine and there was significant excretion of unchanged perhexiline. The major metabolite in this subject was M_3. It is interesting to note that the neuropathic patients of Singlas et al. [2] also formed greater amounts of metabolite M_3 than the non-neuropathic patients.

It thus appears that single-dose kinetics of perhexiline may be of value in identifying those subjects at risk of toxic accumulation of perhexiline on conventional dosage regimes. However, further studies are needed to elucidate the complexities of perhexiline metabolism and kinetics, and to fully evaluate the use of single-dose kinetics in predicting potential adverse reactions to perhexiline.

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