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PLASMA CONCENTRATIONS AND RELATIVE BIOAVAILABILITY OF NAFTIDROFURYL FROM DIFFERENT SALT FORMS

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ABSTRACT

The relative bioavailability of the vasodilator naftidrofuryl from formulations containing its oxalate or citrate salt has been estimated using a specific HPLC assay, and a less specific fluorimetric assay, to measure plasma drug concentrations. The conclusions of the study were the same irrespective of the assay employed. The relative rate, but not the extent, of bioavailability of naftidrofuryl from the citrate salt (peak 1096 ng ml^{-1} at 0·76 h) was marginally greater ($p = 0·003$) than that from the oxalate salt (peak 922 ng ml^{-1} at 0·94 h). The degree of intersubject variability was similar after administration of either salt form. The mean half-life of naftidrofuryl was 1·8 h and its mean residence time was 2·5 h.

KEY WORDS Naftidrofuryl Plasma concentrations Bioavailability

INTRODUCTION

Naftidrofuryl (nafronyl) is a vasodilator used in the treatment of cerebral and peripheral vascular disorders;[1–7] it is available for clinical use as a formulation of the oxalate salt (Praxilene®).

Although the drug has been in clinical use for several years, relatively little has been published concerning its pharmacokinetics in humans,[8,9] although reports of metabolism,[10–12] and more recently pharmacokinetic[13] studies in animals have appeared. This, together with the claim[14] that plasma naftidrofuryl concentrations and its relative extent of absorption in humans were at least two-fold greater when the drug was administered as the citrate salt than as the oxalate salt, led to the studies reported in this paper.

The claim[14] concerning the citrate salt was based on a fluorimetric assay for the measurement of the drug and consequently the results obtained using this assay, as well as a more specific high-performance liquid chromatography

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assay,[15] were compared. The published pharmacokinetic data on naftidrofuryl[8,9] were also based on the use of a non-specific fluorimetric assay for the measurement of the drug in plasma.

The pharmacokinetic study reported in this paper assessed the relative bioavailability of naftidrofuryl from formulations containing its oxalate or citrate salt. For drug administered on a continuous basis, the extent of absorption of a given dose is usually more critical than its rate of absorption, however, the rate of absorption may become critical if the body burden is excessive.

MATERIALS AND METHODS

Selection of subjects

Twelve healthy adult male subjects, age 20–25 years and body weights 63–76 kg, gave written consent to participate in this study after its nature and aim were explained. The subjects underwent medical examinations before and after the study, and on each occasion blood and urine samples were taken for extensive laboratory tests. The results of these examinations indicated that the subjects were in good health and that drug administration had no detected effect upon their health. The study was approved by the Hospital Ethics Committee. During the days of drug administration, the subjects remained ambulatory but under medical supervision; their activity was restricted and their diet was standardized.

Drug formulations

Naftidrofuryl was formulated as gelatin capsules containing 100 mg of the oxalate (Lot OF2784) or citrate (Lot OF2783) salts. Both formulations were provided by Lipha, Lyon, France.

Dosage schedule and samples

The subjects were fasted for 10 h before dosing and for 4 h afterwards. Administration was conducted as a complete two-way crossover in a repeated latin square design with 1 week between doses. On each day of administration, each subject received a single oral dose of three capsules of the appropriate formulation equivalent to 300 mg of each salt, together with 150 ml water. Blood samples (10 ml into heparin-fluoride tubes for fluorimetric assay and 10 ml into 'balanced oxalate' tubes (12 mg ammonium oxalate and 8 mg potassium oxalate per 10 ml blood) for high-performance liquid chromatographic assay, HPLC) were withdrawn before dosing and at several times during 12 h afterwards, centrifuged to remove cells which were discarded, and the plasma was stored at about −20° pending analysis.

Measurement of drug concentrations in plasma

Concentrations of naftidrofuryl in plasma were measured by both a specific HPLC procedure, described in detail elsewhere,[15] and a non-specific fluorimetric assay similar to that used by others[8,9,14] and outlined below.

Plasma (3 ml) from the heparinized tubes was mixed with ammonia solution (35 per cent w/v, 1 ml) and extracted with n-heptane (15 ml containing isoamyl alcohol, 3 per cent v/v) by rotary mixing for 30 min. The mixture was centrifuged and the organic phase (12 ml) re-extracted for 30 min with hydrochloric acid (4·5 ml, 0·1 M). After centrifugation, the emission of the acid extract was measured using a fluorescence spectrometer (Model LS-3, Perkin-Elmer, Beaconsfield, Bucks, England) at emission and excitation wavelengths of 335 nm and 286 nm, respectively.

In the HPLC assay, linear calibration lines were obtained over the concentration range 20–800 ng ml^{-1}. The coefficients of variation of the means of replicate analyses ($n = 5$) were ± 10 per cent at 20 ng ml^{-1} and ± 1 per cent at 300 ng ml^{-1}. The calibration data were similar regardless of which salt was taken as the source standard. The recovery of internal standard was 92 per cent ± 3 S.D., and the mean recovery of naftidrofuryl was 88 per cent ± 2 S.D. over the concentration range 100–500 ng ml^{-1}. No peaks were present at the positions of drug or internal standard in chromatograms of extracts of pre-dose control plasma, and for the purposes of this study, the limit of detection was arbitrarily set at 20 ng ml^{-1}, the lowest datum point on the calibration line. Metabolites of naftidrofuryl did not interfere with the parent drug or internal standard.[15]

In the fluorimetric assay, linear calibration lines were obtained over the range 20–1667 ng ml^{-1}. The coefficients of variation of the means of replicate analyses ($n = 5$) were ± 3 per cent at 333 ng ml^{-1} and ± 3 per cent at 1667 ng ml^{-1}. The mean recovery of drug was 82 per cent ± 2 S.D. over the concentration range 333–1667 ng ml^{-1}. Again, the calibration data were similar regardless of which salt was used. The limit of detection was considered to be 20 ng ml^{-1}, the lowest datum point on the calibration line, with a signal equivalent to approximately twice that produced from a control plasma sample.

Data processing

Areas (AUC) and first moments (AUMC[16]) under the plasma drug concentration–time curves were calculated by the log-linear trapezoidal rule[17] and extrapolated to infinite time. Terminal half-lives were calculated after least squares regression of $\log_e$ concentration–time data. Mean residence times were calculated as the ratio AUMC/AUC. Bioavailability parameters were logarithmically transformed to stabilize the variance and submitted to analyses of variance for crossover designs[18] with formulations (salts), 'method of analysis' and interactions of these effects, subjects, and order of dosing as factors in the analysis.

Table 1. Mean plasma concentrations of naftidrofuryl base in plasma after single oral doses of 300 mg of the oxalate and of the citrate salt. Results are expressed as ng ml^{-1} with coefficients of variation (per cent) in parentheses

Time (h)	HPLC assay		Fluorimetric assay	
	Oxalate salt	Citrate salt	Oxalate salt	Citrate salt
0·25	231(100)	206(105)	253(104)	233(109)
0·5	500(44)	521(47)	594(45)	669(56)
0·75	539(50)	594(32)	694(45)	769(32)
1	546(47)	553(54)	735 (45)	714(44)
1·5	476(57)	430(69)	664(50)	565(56)
2	409(90)	324(81)	567(80)	429(62)
3	230(84)	160(82)	323(77)	243(77)
4	133(76)	96(76)	202(85)	138(70)
5	79(68)	62(58)	108(85)	75(59)
6	49(57)	40(63)	60(83)	46(57)
8	ND(–)	ND(–)	ND(–)	ND(–)
10	ND(–)	ND(–)	ND(–)	ND(–)
12	ND(–)	ND(–)	ND(–)	ND(–)

ND = <20 ng ml^{-1}.

RESULTS AND DISCUSSION

Plasma drug concentrations

Concentrations of naftidrofuryl base were detectable during 6 h after dosing, when measured by either assay procedure. The peaks of mean drug concentrations in plasma measured by HPLC were 546 ng ml^{-1} and 594 ng ml^{-1} after administration of 300 mg of the oxalate and citrate salts respectively, occurring at 1 h and 0·75 h respectively (Table 1, Figure 1). Using fluorimetry, the peaks of mean 'drug' concentrations of 735 ng ml^{-1} and 769 ng ml^{-1} respectively, also occurred at these times after administration of the oxalate and citrate salts respectively. The coefficients of variation of mean drug concentrations were generally remarkably similar irrespective of the analytical technique used.

Bioavailability parameters

The means of the peak concentrations of naftidrofuryl base (adjusted to doses of 300 mg of the base) in plasma of individual subjects were 922 ng ml^{-1} ± 43 per cent CV and 1096 ng ml^{-1} ± 35 per cent CV after administration of the oxalate and citrate salts respectively (measured by HPLC), and the peak

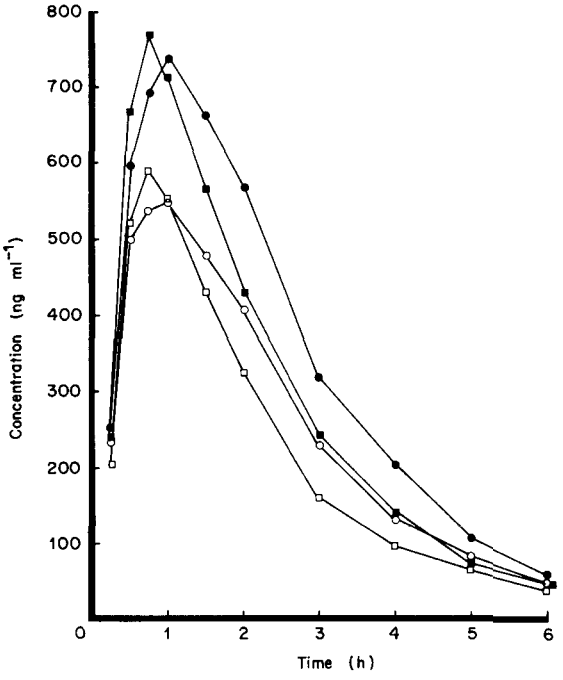

Figure 1. Mean concentrations of naftidrofuryl base in plasma measured by HPLC (open symbols) or fluorimetry (closed symbols) after single oral doses of 300 mg of the oxalate (○, ●) and citrate (□, ■) salts

levels occurred at mean times of 0·94 h ± 52 per cent CV and 0·76 h ± 53 per cent CV, respectively. Using fluorimetry, the mean peak 'drug' levels were higher, 1145 ng ml^{-1} ± 39 per cent CV and 1397 ng ml^{-1} ± 42 per cent CV respectively, but occurred at similar times, respectively (Table 2). The

Table 2. Mean bioavailability parameters of naftidrofuryl base after single oral doses of 300 mg of the oxalate and of the citrate salt, adjusted for doses of 300 mg base where appropriate. Coefficients of variation (per cent) in parentheses

	HPLC assay		Spectrophotofluorimetric assay	
Parameter	Oxalate salt	Citrate salt	Oxalate salt	Citrate salt
Peak level (ng ml^{-1})	922(43)	1096(35)	1145(39)	1397(42)
Time of peak (h)	0·94(52)	0·76(53)	0·98(47)	0·85(54)
AUC (ng h ml^{-1})	2022(49)	2144(50)	2680(50)	2765(41)
Half-life (h)	1·79(23)	1·88(25)	1·25(23)	1·45(26)
Mean residence time (h)	2·50(19)	2·39(19)	2·28(25)	2·22(19)

Table 3. Levels of significance of sources of variation in the analyses of bioavailability parameters adjusted, where appropriate, for doses of 300 mg naftidrofuryl base

Source of variation	Parameter				
	Area	Peak level	Peak time	Half-life	Mean residence time
Formulation	0·14	0·003	0·12	0·17	0·51
Method of analysis	<0·001	<0·001	0·25	<0·001	0·06
Interaction	1·00	0·94	0·91	0·42	0·78
Subject	<0·001	<0·001	<0·001	0·53	0·001
Order of dosing	0·44	0·16	0·69	0·50	0·26

formulation-related difference in peak plasma levels was statistically significantly different ($p = 0{\cdot}003$), but differences in times to peak were not ($p > 0{\cdot}05$), although the peak levels after administration of the citrate salt tended to occur earlier (Tables 1 and 2).

Formulation-related differences in the terminal half-life of naftidrofuryl (1·79 h ± 23 per cent CV and 1·88 h ± 25 per cent CV using HPLC, after administration of the oxalate and citrate salts respectively, and 1·25 h ± 23 per cent CV and 1·45 h ± 26 per cent CV, respectively, using fluorimetry) were not significant ($p > 0{\cdot}05$). However, 'method of analysis' effects were highly significant ($p < 0{\cdot}001$, Table 3). Since the fluorimetric assay included fluorescent metabolites of naftidrofuryl which were resolved from the parent drug by HPLC, it is probable that these metabolites may have been removed from plasma at rates greater than that of the unchanged drug, leading to the apparently shorter half-lives calculated from the fluorimetric data.

Mean residence times of naftidrofuryl after administration of each salt were not significantly different ($p > 0{\cdot}05$), and 'method of analysis' effects on this parameter were not significant.

The relative extent of bioavailability of naftidrofuryl from the salts was assessed by comparison of the areas under the plasma drug concentration–time curves (AUC). The relative bioavailability of drug from the citrate salt was approximately 106 per cent of that from the oxalate salt, calculated from log-transformed HPLC or fluorimetric data, and in this study this difference was not statistically significant ($p > 0{\cdot}05$), with 95 per cent confidence limits of 98 per cent to 116 per cent.

Highly statistically significant inter-subject differences were noted for all parameters except half-life. Comparisons of data obtained by HPLC and the non-specific fluorimetric method were statistically highly significantly different in the case of AUC, peak level, and half-life, and almost reached formal significance ($p = 0{\cdot}06$) in the case of mean residence times (Table 3).

The mean plasma half-life of naftidrofuryl measured in this study (1·8 h) was greater than that (41 min) observed by Lartigue-Mattei *et al.*,[9] who used a

less specific fluorimetric method, although not identical to that (1·2 h) reported by Brodie *et al.*[19] using HPLC and ultraviolet absorption detection in a limited study of two subjects. Furthermore, Lartigue-Mattei *et al.*[9] obtained a mean peak plasma drug concentration of 350 ng ml^{-1} at 66 min following a 50 mg oral dose of naftidrofuryl: this was disproportionately greater than 922 ng ml^{-1} at 56 min following a 300 mg oral dose. This difference in the relative magnitude of the peak plasma concentrations of naftidrofuryl can presumably be ascribed to differences in drug assay methodology, the more specific assay yielding the lower concentrations.

In conclusion, the results of this study refute the claim[14] that plasma drug concentrations obtained after administration of the citrate salt of naftidrofuryl to humans are at least two-fold greater than those obtained after administration of the clinically used oxalate salt. The rate, but not the extent, of absorption of naftidrofuryl from the citrate salt seems to be marginally greater than that from the oxalate salt, perhaps reflecting a difference in the physicochemical properties of the two salt forms. This would not be unusual — the chemical form of a drug is one factor that can affect its bioavailability to a greater or lesser extent. This study shows that no therapeutic benefit could be expected by substitution of the citrate salt of naftidrofuryl for the oxalate salt since the extent of bioavailability of naftidrofuryl from both salt forms is the same.

Interestingly, this study also shows that lack of specificity of a drug assay does not necessarily preclude its use in bioavailability studies, although clearly it is essential to employ a specific assay when determining pharmacokinetic parameters from which pharmacokinetic/pharmacodynamic correlations may be established, drug dosage regimens designed or drug concentrations predicted.

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