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PLASMA BINDING OF DISOPYRAMIDE AND MONO-*N*-DEALKYLDISOPYRAMIDE

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1 Measuring total plasma levels of disopyramide (DP) and the main metabolite mono-*N*-dealkyldisopyramide (MND) in patients on maintenance therapy with DP has shown concentrations of MND comparable with those of DP, with wide intersubject variations.

2 A method which permits simultaneous measurement of unbound fraction of DP and MND has been developed.

3 In healthy subjects the unbound fraction of both DP and MND was concentration dependent, i.e. increased with higher concentrations of DP or MND.

4 The plasma protein binding of DP is altered by varying concentrations of MND. Clinically relevant concentrations of MND may increase the unbound fraction of DP approximately twofold.

5 The plasma protein binding of MND is also altered by varying concentrations of DP. Variation in the concentration of DP from the lower to the upper part of the therapeutic range may cause a 1.5-fold increase in the unbound fraction of MND.

6 In the assumed therapeutic range of 6–15 μmol DP/L, the interpatient variance of unbound DP concentration might be ten-fold or even higher. The present findings indicate the need for monitoring unbound drug concentrations in any attempt to establish plasma concentration/effect relationship.

Introduction

Disopyramide (DP) is an antiarrhythmic drug with an assumed therapeutic range of 6–15 μmol/l (for review, see Koch-Weser, 1979; Brown & Shand, 1982). Thus monitoring total plasma concentration of disopyramide is tentatively used by some clinicians to adjust the dosage in problem patients. DP is almost completely absorbed with peak plasma levels occurring about 2 h after an oral dose. On average 55% of the administered dose of DP is eliminated unchanged, mainly in the urine. Dealkylation to mono-*N*-dealkyldisopyramide (MND) is the most important pathway and about 25% of DP appears in the urine as MND. The major metabolite possesses some electrophysiological activity, but the serum concentration was until recently thought to be only about 10% of that of the parent drug. However, recent studies show that the serum concentrations of MND in patients on maintenance treatment with DP, may become high enough to be taken into account (Bredesen, 1980; Aitio, 1981).

The value of measuring total plasma concentrations of DP can be further questioned by the fact that its plasma protein binding shows a wide intersubject and concentration dependent variability already within the assumed therapeutic range (Meffin *et al.*, 1979; David *et al.*, 1980; Lima *et al.*, 1981).

Accordingly, the purpose of the present study was to investigate the variation of the DP/MND concentration ratio in patients on maintenance treatment with DP. In order to elucidate whether DP and MND compete for similar sites in their binding to plasma proteins, *in vitro* studies were also performed on plasma from non-treated healthy subjects.

Methods

The total plasma concentration of disopyramide and mono-*N*-dealkyldisopyramide was measured in single routine samples from 42 male, aged 21–78 (62 ± 15) years, and 28 female patients, aged 27–74 (56 ± 13) years, all on maintenance therapy. All patients had used DP for more than 2 weeks, and the samples were drawn just before the dose of the drug. None of the patients had severe renal dysfunction or were taking drugs known to possess liver microsomal enzyme inducing capability.

Plasma from five healthy subjects, three male aged 37–47 years, and two female aged 29 and 31 years, was analysed over a wide range of concentrations to determine whether the plasma binding of DP or MND was concentration dependent. Duplicate

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plasma samples containing 1, 5, 10, 15, 20, 50 and 100 μmol/l of either DP or MND, were examined, using equilibrium dialysis (Pike & Skuterud, 1982) and gas chromatographic determination (Bredesen, 1980). Equilibrium dialysis took place for 4 h at 37° C using 0.7 ml plasma against an equal volume of Krebs Ringer bicarbonate buffer at pH 7.4. Preliminary studies showed that equilibrium was obtained after 3 h and remained fairly constant until at least 6 h. The equilibrium concentration of both DP and MND were measured simultaneously in 0.5 ml aliquots from each cell. The unbound fractions were calculated as the concentration quotient of DP and MND between buffer and plasma.

Results

The plasma concentration of DP and MND in the 70 patients showed wide variation (Figure 1), 19 of which having a MND/DP ratio ≧ 1 (range 0.06–4.0, mean 0.62 ± 0.51). The correlation between DP and MND was 0.41.

The reproducibility of the method for measuring the protein binding of DP and MND was tested by analysing 20 parallel samples of a plasma standard containing 10 μmol/l of both. The coefficient of variation was 4.2 and 4.8% respectively for DP and MND. The analytical sensitivity in the procedure described, using 0.5 ml volumes for extraction, as calculated from a signal corresponding to twice the noise level at attenuation $16 \cdot 10^2$ was 0.05 μmol/l for DP and 0.1 μmol/l for MND (for further details, see Bredesen, 1980). Table 1 shows the unbound fraction of DP and MND in the plasma from five healthy subjects at the various initial concentrations used, as well as the subsequent equilibrium concentrations.

As seen there was a 1.4 to 2.0-fold range in the unbound fraction of DP and a 1.2 to 1.7-fold range in the unbound fraction of MND. The effect of adding the major metabolite MND, in clinically relevant concentrations, to plasma containing DP, and *vice versa*, is shown in Table 2. The reason for the varying equilibrium concentration of DP seen when the MND concentration ranges from 0 to 14.4 μmol/l is that the initial concentrations of DP were equal in all the experiments. When the unbound fraction increases the equilibrium plasma concentration will decrease. However, we have not been able to measure any difference in the protein binding of DP in concentrations between 4.0 and 3.8 or of MND between 3.2 and 2.8 μmol/l.

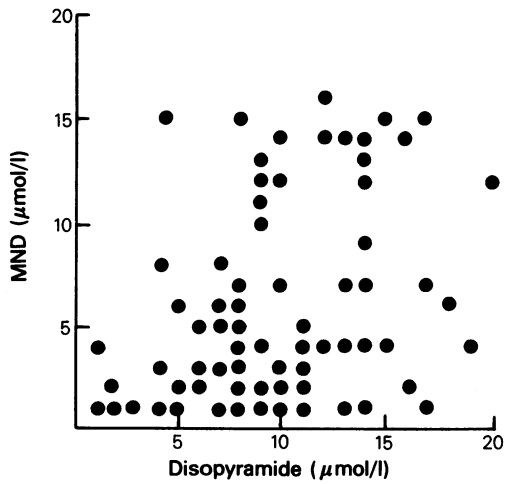

Figure 1 Plasma levels of disopyramide (DP) and mono-*N*-dealkyldisopyramide (MND) in 70 patients on maintenance therapy with DP ($r = 0.41$).

The addition to normal plasma of various drugs (chlorprothixene, carbamazepine, amitriptyline, diphenylhydantoin, digitoxin, quinidine, procainamide, diazepam, propranolol and salicylic acid) in concentrations known to occur during treatment, did not significantly affect the plasma protein binding of DP and MND.

Discussion

Relatively high concentrations of MND as compared to DP are observed in patients receiving enzyme in-

Table 1 Unbound fraction, mean and range of disopyramide (DP) and mono-*N*-dealkyldisopyramide (MND) in plasma from five healthy subjects at different initial and equilibrium concentrations.

Initial concentration (μmol/l)	*Mean equilibrium concentration (μmol/l)*		*Unbound fraction (%)*			
			DP		*MND*	
	DP	*MND*	*Mean ± s.d.*	*Range*	*Mean ± s.d.*	*Range*
1	0.8	0.7	12 ± 2	(10–14)	31 ± 4	(25–35)
5	4.0	3.2	19 ± 5	(14–28)	42 ± 9	(29–52)
10	7.0	7.1	28 ± 4	(22–31)	46 ± 10	(37–62)
15	10.6	10.4	34 ± 5	(29–41)	50 ± 11	(41–69)
20	12.8	12.9	43 ± 8	(34–50)	53 ± 10	(47–71)
50	29.9	33.2	59 ± 9	(48–70)	64 ± 11	(52–79)
100	58.4	57.0	68 ± 12	(59–88)	73 ± 8	(68–83)

Table 2 Effect of mono-*N*-dealkyldisopyramide (MND) on disopyramide (DP) plasma protein binding (a) and *vice versa* (b) at different equilibrium concentrations in five healthy subjects.

(a) *Mean equilibrium concentrations (μmol/l)*		*Unbound fraction (% DP)*		(b) *Mean equilibrium concentrations (μmol/l)*		*Unbound fraction (% MND)*	
DP	*MND*	*Mean ± s.d.*	*Range*	*MND*	*DP*	*Mean ± s.d.*	*Range*
4.0	0	19 ± 5	(14–28)	3.2	0	42 ± 9	(29–52)
4.0	3.0	22 ± 7	(17–34)	3.0	4.0	51 ± 11	(38–64)
3.8	14.4	35 ± 10	(25–46)	2.8	12.8	65 ± 13	(44–80)
12.8	0	42 ± 8	(34–50)	14.9	0	59 ± 10	(42–69)
12.8	2.8	45 ± 10	(35–58)	14.4	3.8	65 ± 10	(52–75)
12.4	14.3	54 ± 10	(40–64)	14.3	12.4	76 ± 12	(65–88)
12.1	20.8	59 ± 11	(48–74)	20.8	12.1	82 ± 10	(68–95)

ducing drugs and in patients with renal insufficiency (Aitio, 1980), whereas the plasma concentration of MND in patients without renal insufficiency was thought to be some 10% of that of the parent drug (Koch-Weser, 1979). Our present (Figure 1) and previous studies (Bredesen, 1980), based upon samples obtained under maintenance treatment, clearly demonstrate that much higher, and a wider range of MND plasma concentrations can be expected, as also recently reported by others (Aitio, 1981). The discrepancy between our finding that the mean MND/DP plasma concentration ratio is twice as high as that reported by Aitio (1981), might be due to differences in the patient populations. However, both studies show that the concentration of DP cannot be used to predict that of MND in plasma, and the need for concomitant determination of both should be thoroughly considered.

Concentration dependent plasma protein binding and interindividual binding variations for DP are reported by a number of authors (Chien *et al.*, 1974, Hinderling *et al.*, 1974; Cunningham *et al.*, 1977; Meffin *et al.*, 1979; David *et al.*, 1980; Aitio, 1981; Johnston & Hamer, 1982). Very little is known about the main metabolite MND which also possesses some antiarrhythmic and electrophysiological activity (for review, see Koch-Weser, 1979; Brown & Shand, 1982). Our study confirms the concentration dependent plasma protein binding of DP. Previous studies show wide variation in DP binding, the reason for which might be related to the different techniques used in the various studies (David *et al.*, 1980). In some studies it is not clear whether the protein binding of DP refers to initial or equilibrium concentrations of the drug. However, our results are in fair agreement with those of Meffin *et al.* (1979) and David *et al.* (1980), using a similar technique. DP shows the greatest variation in the protein binding within 'the therapeutic range'. The plasma binding of MND is also clearly concentration dependent (Table 1).

When emphasizing the high concentrations of MND demonstrated in patients (Figure 1) the potential clinical significance of our finding that MND and DP are competing for similar binding sites at concentrations regularly obtained during DP therapy (Table 2), seems obvious. A previous report showed that at high concentrations of MND (508 μmol/l) and low molar ratios of the parent drug to metabolite the binding capacity of the plasma proteins for DP was significantly reduced (Hinderling *et al.*, 1974). No significant interaction between parent drug and metabolite at clinically relevant concentrations could be demonstrated, however, Aitio (1981) found in patients a somewhat reduced binding of MND at higher total drug concentrations. As evident from our results (Figure 1) an equilibrium concentration of about 4.0 μmol DP/l could reflect a concentration of MND in patients ranging from nearly zero to about 15 μmol/l. Accordingly (Table 2) the mean unbound fraction of DP may vary from 19 to 35%, depending of the concomitant concentration of MND. In addition the intersubject variability in DP binding must be taken into account (Table 1). Thus, at a total concentration of about 4.0 μmol/l, the unbound fraction of DP may range from 14 to 46%. When considering the assumed therapeutic range of 6–15 μmol DP/l, the inter-patient variance of unbound DP concentration might be ten-fold or even higher. In addition DP also changes the protein binding of MND. For instance at a MND concentration of about 3.0 μmol/l unbound fraction may range from 20 to 80% at DP concentrations of 0 and 12.8 μmol/l respectively (Table 2b).

Hardly any other drug is hitherto known to be subject to such a pronounced interindividual variability in steady state concentration and plasma binding as DP and MND. Evidently all brave efforts to establish plasma concentration/effect relationship for DP will fail if they are not seen in the full context of the binding variations now demonstrated, and also taking the potential cardiovascular effect of MND into account.

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