Serum binding of nifedipine and verapamil in patients with ischaemic heart disease on monotherapy

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Serum free fractions of nifedipine, verapamil and some of their metabolites were measured in patients with ischaemic heart disease receiving single oral dose and chronic monotherapy and were compared with those obtained *in vitro*. The percentages of unbound nifedipine and verapamil *in vitro* (concentration range 50–200 and 150–400 ng ml⁻¹, respectively) were 2.51 and 7.23%, respectively, and did not differ from those found on monotherapy with these drugs (2.05 and 8.08%, respectively), and after single dosing. It is suggested that, during treatment with nifedipine or verapamil, their serum metabolites do not affect binding of the parent drugs to serum proteins.

Introduction

It is well known that only drug that is unbound to plasma proteins may reach the sites of pharmacological action (Meijer & Van der Sluijs, 1987). Serum binding is usually assessed *in vitro* by the addition of the drug under study to plasma or serum taken from patients or volunteers without any medication. The unbound fraction and the physico-chemical constants of drug-protein interaction may be estimated in this way. However, the results obtained may not reflect the clinical situation, since the blood of subjects given drugs may contain metabolites which could alter the binding of the parent drug. Because of this, more reliable binding data may be obtained using the serum of patients under drug treatment. Comparing such *ex vivo* data with those found *in vitro* may detect the influence of metabolites (known and unidentified) and other possible factors which are absent under *in vitro* conditions. There are data on the plasma binding of nifedipine (Von Rosenkrantz *et al.*, 1974; Kleinbloesem *et al.*, 1985; Otto & Lesko, 1986) and verapamil (Schomerus *et al.*, 1976; Yong *et al.*, 1980; Keefe *et al.*, 1981; McGowan *et al.*, 1983) *in vitro* and

after intravenous administration (McGowan *et al.*, 1983; Kleinbloesem *et al.*, 1985). The concentration of metabolites is low after intravenous dosing (Waller *et al.*, 1984) and might not influence the binding of unchanged nifedipine. It has been found that verapamil binding *in vitro* is independent of concentration up to concentrations of more than 1500 ng ml⁻¹ (Keefe *et al.*, 1981) and that of nifedipine up to concentrations of 1200 ng ml⁻¹ (Otto & Lesko, 1986).

In this study we compared the binding of nifedipine and verapamil in patients' serum before and during a period of 3–6 months of monotherapy. The binding of some metabolites of nifedipine and verapamil which are present in serum at high concentrations was also assessed.

Methods

Male patients (mean 53 years old; range 42–59 years) with ischaemic heart disease and frequent attacks of angina pectoris took part in the study. The history and appropriate clinical tests showed

no signs of liver or kidney diseases. Nifedipine pure substance and tablets (Corinfar) were obtained from Germed (Dresden, GDR), verapamil pure substance was supplied by LEK (Ljubljana, Yugoslavia), and verapamil tablets (Finoptin) were obtained from Orion Pharmaceutica, (Helsinki, Finland).

The drug-free patients' sera were spiked with known amounts of verapamil as 20–50 μl of an aqueous solution. Nifedipine was added to empty tubes in 20–50 μl of methanol. The serum was added after evaporating the solvent in a gentle stream of nitrogen. Serum samples were maintained at 37° C for 1 h before dialysis. Binding of verapamil and its *N*-demethylated metabolite, norverapamil, and of nifedipine and its primary metabolite B-4759 (the pyridine analogue of nifedipine) were measured by equilibrium dialysis against an isotonic phosphate buffer (KH_2PO_4 – 0.024 M; Na_2HPO_4 – 0.107 M; pH 7.4) at 37° C. The dialysis system EMD-101 using membranes EMD-103 and polyacrylate dialysis units with 1 ml cells (Hoeffer, San Francisco, CA, USA) was used for nifedipine and the same membranes and self-made PTFE units with 0.7 ml cells were used for verapamil, as it had been found that polyacrylate units adsorb verapamil strongly. The duration of dialysis was 6–7 h. The extent of binding of the drugs and their metabolites was estimated as a mean of two–three replicate measures.

Serum concentrations of nifedipine and its metabolite B-4759 before and after dialysis and in dialysates were measured by gas chromatography with electron-capture detection (Rumiantsev *et al.*, 1986b). Serum concentrations of verapamil and its three *N*-dealkylated metabolites (norverapamil, D-617 and D-620) before dialysis were measured by cation-exchange high-performance liquid chromatography with fluorescence detection (Piotrovskii *et al.*, 1983). After dialysis only unchanged verapamil and norverapamil could be measured in serum and dialysate since the samples contained unknown substances which interfered with analysis of D-617 and D-620. The percentage unbound of drugs and metabolites in serum was calculated as follows:

$$\% \text{ unbound} = \frac{C_u}{C} \times 100$$

where: *C* is the equilibrium drug concentration in serum and *C_u* is the equilibrium drug concentration in dialysate.

The following binding experiments were performed:

1. Verapamil (150–400 ng ml^{–1}) was added to

sera from eight patients who had been without medication for at least 2 weeks.

2. Verapamil and norverapamil binding was studied in sera from two patients taking a single 120 mg dose of verapamil.

3. Verapamil and norverapamil binding was studied in sera from 24 patients treated with verapamil (80–120 mg 3–4 times daily) for 3–7 months.

4. Nifedipine and B-4759 (50–200 ng ml^{–1}) were added separately to sera from 14 patients without medication for 2 weeks or more.

5. Nifedipine binding was measured in sera from 35 patients who had received the drug as a single dose of 20 or 30 mg.

6. The binding of nifedipine (in 44 patients) and B-4759 (in 28 patients) were measured in sera from patients on monotherapy with nifedipine (20–40 mg 3–4 times a day) for 3–6 months.

Blood samples were collected in siliconized tubes 2–3 h after the administration of the drugs. Serum was separated by centrifugation 40–45 min thereafter and stored at –20° C until assay. Prior to the dialysis the pH of serum samples was adjusted to 7.4 by bubbling with carbon dioxide.

Results

The values for the percentage unbound for nifedipine and verapamil showed extremely wide inter-individual variations. Figure 1 shows as an example the frequency histogram for the per cent unbound of nifedipine in serum of patients on monotherapy. The distribution is clearly asymmetric, and similar patterns were found in all of the studies. Because of this a geometric mean was used to characterize the average values. The nonparametric Wilcoxon-Mann-Whitney test (Zar, 1984) was used to assess the significance of the differences.

Figure 1 Frequency histogram of the percentage of nifedipine unbound in serum from patients on nifedipine monotherapy.

After single dose administration the serum concentrations of nifedipine ranged from 29 to 73 ng ml⁻¹, and the concentrations of the metabolite (B-4759) were from 26 to 67 ng ml⁻¹; during nifedipine monotherapy the concentrations were 36–122 and 27–98 ng ml⁻¹, respectively.

Before dialysis verapamil and norverapamil serum concentrations in patients on verapamil regular treatment were in the range of 97–595 and 150–768 ng ml⁻¹, respectively.

The percentage unbound values of nifedipine, B-4759, verapamil, and norverapamil measured under various conditions are shown in Table 1. There were no significant differences between the extent of binding of nifedipine and verapamil measured after a single intake and after monotherapy, or between those values and the values measured *in vitro*.

In all cases the per cent of the nifedipine metabolite B-4759 unbound was higher than that of the unchanged drug ($P < 0.001$) and consistent in the three studies. In contrast, the free fraction of norverapamil on verapamil monotherapy was lower than that of the unchanged drug ($P < 0.01$).

Discussion

After oral administration, the metabolites of nifedipine and verapamil were present in patient's serum at concentrations comparable with or exceeding those of the unchanged drugs (Raemisch & Sommer, 1983; Waller *et al.*, 1984; Barbieri *et al.*, 1985; Piotrovskii *et al.*, 1986). It might be anticipated that the degree of serum protein binding of nifedipine and verapamil in patients receiving these drugs differ from that

measured *in vitro* due to the displacement of the parent drugs by their metabolites. However, we found no difference in the binding of the parent drugs measured after single dosing, during chronic treatment, and *in vitro* (Table 1).

The extent of nifedipine binding measured in our study (Table 1) was within the limits reported previously in plasma *in vitro*: 0.8–4% unbound (Von Rosenkrantz *et al.*, 1974; Otto & Lesko, 1986; Kleinbloesem *et al.*, 1985).

It was shown that the oxidized metabolite of nifedipine (B-4759) was less extensively bound to serum proteins than the unchanged drug. This may explain why the metabolite does not displace nifedipine from its binding sites.

The percentage of verapamil unbound (Table 1) was also within the limits reported previously from *in vitro* studies: 8–12% (Schomerus *et al.*, 1976; Yong *et al.*, 1980; Keefe *et al.*, 1981; McGowan *et al.*, 1983; Eichelbaum, *et al.*, 1984). Yong *et al.* (1980) found that at a concentration of 50 ng ml⁻¹ norverapamil increased verapamil free fraction from 0.10 to 0.13. However, this effect was not confirmed by others (Keefe *et al.*, 1981), who showed that norverapamil did not affect verapamil binding until its concentration was greater than 1,557 ng ml⁻¹. Our results demonstrate that serum verapamil binding in patients after single and chronic administration does not differ from that *in vitro*, thereby supporting the *in vitro* findings of Keefe *et al.* (1981). Thus, despite the higher binding of norverapamil compared to verapamil, it does not displace the parent drug in the concentration range associated with clinical doses. Moreover, our data indicate that none of the metabolites of verapamil in serum influence the binding of the unchanged drug.

There is evidence that chronic verapamil administration is associated with a decrease in its

Table 1 Percentage unbound values of nifedipine, verapamil and certain of their metabolites in the serum of patients with ischaemic heart disease.

		No drug (in vitro)	Treatment	
			Single dose	Multiple dose
Nifedipine	G	2.51 (1.60)	1.95 (1.34)	2.05 (1.43)
	M	2.20 (1.20–6.49)	1.80 (1.30–4.20)	1.90 (1.20–4.90)
B-4759	G	8.55 (1.06)	–	9.33 (1.20)
	M	8.60 (5.90–14.5)	–	9.80 (4.00–16.1)
Verapamil	G	7.23 (1.35)	10.2*;8.2*	8.08 (1.59)
	M	7.65 (4.00–11.2)	–	9.44 (3.50–16.4)
Norverapamil	G	–	4.7*;2.3*	3.34 (1.48)
	M	–	–	3.28 (3.09–11.2)

G – Geometric mean (standard error of geometric mean); M – Median (range);

* – Individual data

oral clearance (Kates *et al.*, 1981; Freedman *et al.*, 1981; Shand *et al.*, 1981; Wagner *et al.*, 1982; Schwartz *et al.*, 1982, 1985; Rumiantsev *et al.*, 1986a). It is known that the clearance after oral administration of drugs such as verapamil with high hepatic extraction ratio (Woodcock *et al.*, 1981) may be approximated as the product of intrinsic clearance and free fraction in blood (Wilkinson & Shand, 1975). Our work shows that verapamil free fraction is not changed on long-term administration as compared to that determined before the start of treatment and

after single dosing. In view of this, the decrease in the oral clearance of verapamil on chronic administration seems to be due to impairment of the activity of the enzymes responsible for drug metabolism. This is consistent with data showing inhibition of nonspecific microsomal mixed function oxidases as a result of chronic verapamil administration (Rumiantsev *et al.*, 1986; Bach *et al.*, 1986; Bauer *et al.*, 1986).

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