Br J clin Pharmac 1995; **39**: 536–538

Influence of metabolites on protein binding of verapamil enantiomers

JULIE A. JOHNSON & WENDELL S. AKERS
Department of Clinical Pharmacy, College of Pharmacy, University of Tennessee, Memphis, Memphis, Tennessee, USA

This study investigated the effect of verapamil metabolites on R-and S-verapamil protein binding in plasma samples collected from subjects prior to *rac*-verapamil dosing and following single dose and steady state *rac*-verapamil dosing. *In vitro* studies of the effects of norverapamil, D617 and D620 on R- and S-verapamil protein binding were also performed. Protein binding of R- and S-verapamil was unchanged following single and multiple doses of *rac*-verapamil as compared with protein binding in predose samples. *In vitro*, norverapamil had no effect on R- and S-verapamil protein binding up to 1000 ng ml^{-1}. Norverapamil 5000 ng ml^{-1} caused a 30% increase in free fraction of both R- and S-verapamil. D617 and D620 concentrations up to 5000 ng ml^{-1} had no effect on R- and S-verapamil protein binding. We conclude the metabolites of verapamil have no clinically significant effect on R- and S-verapamil protein binding.

Keywords verapamil enantiomers protein binding metabolites

Introduction

Verapamil is a slow calcium channel blocker used in the treatment of a variety of cardiovascular disorders. Currently available verapamil products are administered as a racemic mixture of its R- and S-enantiomers and it is well recognized that the enantiomers of verapamil differ in both their pharmacokinetic properties and pharmacological activity [1–3].

A recent study by Gross *et al.* [2] showed that R- and S-verapamil unbound fractions were two-fold higher in plasma samples collected from the subjects taking oral verapamil, than in the same subjects prior to dosing or following an intravenous dose. As a result of extensive first-pass metabolism, verapamil metabolite concentrations are higher following oral dosing than following intravenous dosing [1]. Thus, it is possible that metabolite accumulation during oral *rac*-verapamil therapy displaces verapamil enantiomers from their protein binding sites and leads to higher unbound fractions of R- and S-verapamil.

Other studies investigating the influence of metabolites on the protein binding of *rac*-verapamil have failed to show a consistent displacement interaction. Yong *et al.* [4] reported a 30% increase in the free fraction of *rac*-verapamil at an equal concentration of the major verapamil metabolite, norverapamil, *in vitro*. However, they failed to demonstrate verapamil displacement from plasma proteins in a patient being treated with chronic oral verapamil therapy. The addition of norverapamil at concentrations ranging from 200 ng ml^{-1} to 2000 ng ml^{-1} failed to displace *rac*-verapamil from plasma proteins [5].

The purpose of the current study was to investigate whether verapamil metabolites displace R- and/or S-verapamil from their protein binding sites.

Methods

Materials

Tritiated racemic verapamil was obtained from New England Nuclear (Lot #2994-04, Boston, MA). Norverapamil HCl was obtained from Research Biochemicals Incorporated (Natick, MA). Knoll Pharmaceuticals provided the metabolites D617 HCl and D620 HCl (Ludwigshafen, Germany). One millilitre plexiglass dialysis cells separated by Spectra/Por® 2 dialysis membranes (Spectrum,

Correspondence: Julie A. Johnson, Department of Clinical Pharmacy, University of Tennessee, Memphis, 26 South Dunlap Street, Suite 200, Memphis, Tennessee 38163, USA

Houston, TX) were used in equilibrium dialysis experiments. Fresh donor plasma, obtained from a healthy volunteer, was collected in glass blood collection tubes containing EDTA.

Characterization of the effect of oral verapamil dosing on protein binding

Plasma protein binding of R- and S-verapamil was determined in samples from eight healthy males who had participated in a bioavailability study. This study involved taking 240 mg of sustained release *rac*-verapamil (Isoptin®, Knoll Pharmaceuticals) once a day for 7 consecutive days. Protein binding was determined in plasma samples collected before the first dose and immediately prior to the second dose. Following the seventh dose, 12 samples were collected for a pharmacokinetic study. A 100 µl aliquot of plasma was taken from each of the 12 samples collected following the seventh dose and was pooled within subjects for determination of protein binding at steady state. Pooling of steady state samples within a subject was deemed appropriate for two reasons. First, protein binding was determined in each of the 12 samples from one subject and there was no change in R- or S-verapamil protein binding during the dosing interval. Secondly, we had shown no concentration-dependent binding in the concentration range of these samples (less than 230 ng ml^{-1} (0.5 µM) for each verapamil enantiomer). All samples analyzed were from the reference drug phase and were adjusted to pH 7.4 using phosphoric acid and/or sodium hydroxide.

In vitro *experiments*

In separate experiments, fresh plasma was collected from a donor. Plasma samples were adusted to pH 7.4, as described above, and spiked separately with the verapamil metabolites norverapamil, D617, and D620 and dialyzed against each tritiated verapamil enantiomer to examine their influence on protein binding of verapamil. Predialysis concentrations of metabolites were 0, 250, 500, 1000 and 5000 ng ml^{-1} (norverapamil: 0 to 10.5 µM; D617: 0 to 15.3 µM, D620: 0 to 16 µM). All three metabolites were also combined at various concentrations in fresh donor plasma and dialyzed against each tritiated verapamil enantiomer.

Tritiated R- and S-verapamil were separated from racemic [^{3}H]-verapamil by chiral h.p.l.c. with fluorescence detection, using a previously published assay [6]. Radiochemical purity exceeded 97%. Plasma concentrations of R- and S-verapamil and R- and S-norverapamil in all subject samples from the pharmacokinetic study were also determined using this assay.

A volume of 500 µl of plasma was dialyzed against 500 µl of Sorensen's phosphate buffer for 6 h at 37° C. Tritiated enantiomers were added to buffer, unlabelled metabolites were added to plasma. After equilibration, 300 µl aliquots of both the plasma and buffer solutions were pipetted separately into scintillation vials containing 5 ml of aqueous liquid scintillation cocktail (Bio-Safe II, Research Products International Corp., Mount Prospect, IL). Radioactivity was counted on a Beckman Model LS-6000 TA liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA).

Data analysis

The fraction unbound (*fu*) of each verapamil enantiomer was determined by: *fu* = B/P, where B and P refer to the disintegrations per minute contained in the buffer and protein solutions, respectively, at equilibrium. Sample volumes were measured before and after equilibrium dialysis and correction for volume shifts was made using previously published equations [7].

Unbound fractions of R- and S-verapamil in subject samples prior to verapamil administration, after a single dose, and at steady state were compared by repeated measures ANOVA. The effect of increasing concentrations of metabolites on verapamil enantiomer protein binding was also tested by repeated measures ANOVA. When a significant finding was noted, a *post-hoc t*-test was used to determine where the significant difference existed. Statistical significance was defined as $P < 0.05$. Statistical analyses were performed using Systat v5.2 (Systat, Inc., Evanston, IL).

Results

Protein binding of R- and S-verapamil in healthy volunteers prior to verapamil dosing was not different from that observed following a single oral *rac*-verapamil dose or during steady state *rac*-verapamil dosing (Table 1). The highest enantiomer and metabolite concentration attained in any subject was as follows: R-verapamil: 229 ng ml^{-1}, S-verapamil: 64 ng ml^{-1}, and R+S norverapamil: 212 ng ml^{-1}.

Results of *in vitro* metabolite displacement studies are shown in Table 2. R- and S-verapamil protein binding were not affected by norverapamil concentrations up to 1000 ng ml^{-1} (2.1 µM). However, norverapamil 5000 ng ml^{-1} (10.5 µM) caused approximately 30% increases in R- and S-verapamil unbound fractions. D617 or D620 concentrations up to 5000 ng ml^{-1} had no effect on the protein binding of R- or S-verapamil. Combinations of metabolites at various concentrations also had no affect on the free fraction of R- or S-verapamil (data not shown).

Table 1 Average free fractions (mean ± s.d.) of R- and S-verapamil in eight human subjects before, following a single dose, and after multiple doses of 240 mg sustained release racemic oral verapamil

Enantiomer	*Predose*	*Single dose*	*Steady state*
R-verapamil	0.051 ± 0.012	0.050 ± 0.008	0.051 ± 0.008
S-verapamil	0.097 ± 0.023	0.093 ± 0.017	0.094 ± 0.010

Table 2 Influence of norverapamil, D617, and D620 on the fraction unbound (mean ± s.d.) of S- and R-verapamil in fresh donor plasma

Metabolite concentrations (ng ml^{-1})	*Norverapamil*		*D617*		*D620*	
	S-V fu	*R-V fu*	*S-V fu*	*R-V fu*	*S-V fu*	*R-V fu*
0	0.125 ± 0.009	0.085 ± 0.005	0.127 ± 0.004	0.085 ± 0.002	0.119 ± 0.003	0.079 ± 0.004
250	0.109 ± 0.004	0.089 ± 0.006	0.130 ± 0.007	0.082 ± 0.012	0.125 ± 0.017	0.071 ± 0.009
500	0.129 ± 0.005	0.088 ± 0.005	0.128 ± 0.002	0.073 ± 0.007	0.112 ± 0.001	0.060 ± 0.001
1000	0.131 ± 0.009	0.084 ± 0.007	0.117 ± 0.005	0.074 ± 0.005	0.124 ± 0.008	0.084 ± 0.005
5000	0.162 ± 0.010*	0.111 ± 0.007*	0.128 ± 0.004	0.083 ± 0.004	0.129 ± 0.002	0.081 ± 0.001

Abbreviations: S-V, S-verapamil; R-V, R-verapamil; *fu*, fraction unbound.
*$P < 0.001$, compared with control (no metabolite).

Discussion

Verapamil enantiomer protein binding in samples from healthy volunteers prior to and following administration of a single dose and multiple doses of sustained release oral *rac*-verapamil was unchanged during *rac*-verapamil therapy in spite of the accumulation of metabolites in plasma. These data suggest there is no clinically significant protein binding displacement interaction between verapamil and its metabolites. *In vitro*, norverapamil concentrations up to 1000 ng ml^{-1} and D617 and D620 concentrations up to 5000 ng ml^{-1} caused no displacement of R- or S-verapamil from their protein binding sites. Only at norverapamil concentrations of 5000 ng ml^{-1} was a displacement interaction noted. The highest steady state norverapamil concentration observed in any subject was 212 ng ml^{-1}. Thus, standard dosing of verapamil would not be expected to produce high enough norverapamil concentrations to cause protein binding displacement. However, at the high doses of verapamil used as a chemosensitizer in cancer chemotherapy [8], or in verapamil overdose, norverapamil concentrations might accumulate to a level which would cause a displacement of verapamil from its protein binding sites.

Gross *et al.* [2] hypothesized that the two-fold increase in the free fraction of R- and S-verapamil following oral verapamil administration was due to metabolite accumulation. The results of the current study do not support either their findings or their hypothesis. Methodological differences seem an unlikely explanation for the discrepancy between the two studies because predose protein binding was essentially identical. In the current study, however, subjects received a commercially available 240 mg sustained release dose of *rac*-verapamil, while in the study by Gross *et al.* [2], subjects were given 80 mg d_2-(+)-verapamil and 80 mg unlabelled (−)-verapamil simultaneously. In their intravenous administration studies, labelled and unlabelled verapamil enantiomers were administered separately. Thus it is possible that a constituent in their oral dosage form displaced verapamil from protein binding sites. Alternatively, it is possible that concomitant administration of deuterium-labelled and unlabelled verapamil enantiomers affects verapamil protein binding. Since the current study utilized a commercially available product, it seems most likely that our results reflect the protein binding patterns in patients taking oral verapamil therapy.

References

1 Vogelgesang B, Echizen H, Schmidt E, Eichelbaum M. Stereoselective first-pass metabolism of highly cleared drugs: studies of the bioavailability of L- and D-verapamil examined using a stable isotope technique. *Br J clin Pharmac* 1984; **18**: 733–740.

2 Gross A, Heuer B, Eichelbaum M. Stereoselective protein binding of verapamil enantiomers. *Biochem Pharmac* 1988; **37**: 4623–4627.

3 Echizen H, Vogelgesang B, Eichelbaum M. Effects of D,L-verapamil on atrioventricular conduction in relation to its stereoselective first pass metabolism. *Clin Pharmac Ther* 1985; **38**: 71–76.

4 Yong C, Kunka R, Bates T. Factors affecting the plasma protein binding of verapamil and norverapamil in man. *Res Comm Chem Path Pharm* 1980; **3**: 329–339.

5 Keefe D, Yee Y, Kates R. Verapamil protein binding in patients and in normal subjects. *Clin Pharmac Ther* 1981; **29**: 21–26.

6 Hahn G, Spahn-Langguth H, Mohrke W, Mutschler E. Enantiospecific assay for verapamil and norverapamil in plasma (abstract). *Arch Pharm* 1991; **324**: 716.

7 Huang J. Errors in estimating the unbound fraction of drugs due to the volume shift in equilibrium dialysis. *J pharm Sci* 1983; **72**: 1368–1369.

8 Milroy R. A randomized clinical study of verapamil in addition to combination chemotherapy in small cell lung cancer. *Br J Cancer* 1993; **68**: 813–818.

(Received 5 September 1994, accepted 4 January 1995)