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Protein binding of nifedipine

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The protein binding of nifedipine in concentrations up to 1200 ng ml^{-1} has been measured in serum, pure human albumin solution and pure human α_1-acid glycoprotein (AAG) solutions by ultrafiltration. The drug was extensively bound in serum from four healthy volunteers with a mean (±s.d.) fraction bound of 0·992 ± 0·008. In albumin solution (40 g $litre^{-1}$) the mean (±s.d.) fraction bound 0·970 ± 0·012, was not significantly different ($P > 0·05$) from that in serum, suggesting that albumin is the major, but not necessarily the only, binding protein for nifedipine in serum. The binding of nifedipine in solutions of AAG was proportional to the AAG concentration and ranged from 0·514 ± 0·059 to 0·755 ± 0·035 in solutions containing 50 and 150 mg % AAG, respectively. Binding of nifedipine in all protein solutions was linear.

Nifedipine is a calcium channel blocker and a potent vasodilator which is widely used in the treatment of ischaemic heart disease and hypertension. We were aware of only one study on the extent of binding of nifedipine in serum and to selected serum protein fractions (Rosenkranz et al 1974). The binding in pooled serum was concentration-dependent decreasing from 98% at 0·2 μg ml^{-1} to 92% at 20 μg ml^{-1}. Nifedipine was highly bound to albumin (88%) and to α_1-lipoprotein (86%) but only moderately bound to β-lipoprotein (46%), β-globulin (42%) and to γ-globulin (10%). No information was available on the binding to the α_1-globulin electrophoretic fraction, which includes α_1-acid glycoprotein (AAG).

We have therefore evaluated the intersubject variability of nifedipine binding in serum, to confirm albumin as the major binding protein in serum, and to assess the extent of nifedipine binding to AAG.

Materials and methods

Blood was collected by direct venepuncture from four healthy, male volunteers, aged 26 to 49 years (mean: 36 years). After transfer to a glass culture tube, the blood was allowed to clot at room temperature and serum was harvested after centrifugation. The total protein and albumin concentrations in each serum were determined on a Dupont ACA discrete clinical analyser. AAG concentrations were not measured in these serum specimens but were assumed to be normal. Protein solutions were prepared to contain 40 g $litre^{-1}$ ($5·8 \times 10^{-4}$ M) human albumin (fraction V, 98% pure, #12666, Calbiochem-Behring, San Diego, CA), 50 mg/100 ml ($1·1 \times 10^{-5}$ M) human AAG (fraction VI, 99% pure, #G-9885, Sigma Chemical Co., St. Louis, Mo.), and 150 mg/100 ml ($3·4 \times 10^{-5}$ M) human AAG, respectively, in 50 mM phosphate buffer (pH 7·4) whose ionic strength (μ) was adjusted to 0·16 with sodium chloride.

Protein binding studies of nifedipine were conducted at 22 ± 1 °C by ultrafiltration. All procedures were performed under gold fluorescent lights (General Electric #F20T12–60) to prevent photodecomposition of nifedipine. Immediately before ultrafiltration the pH of each specimen was measured and adjusted to 7·4 if necessary. A 1 ml aliquot of each sample was placed in the reservoir of a disposable ultrafiltration unit (Centrifree Micropartition System, Amicon Corporation, Lexington, MA) equipped with a YMT membrane capable of retaining 99·9% of protein with a molecular weight cut-off of 10000 daltons. The reservoir was capped to prevent sample evaporation and pH changes during ultrafiltration. Ultrafiltrate was collected in a removable cup attached to the base of the unit by centrifuging the device for 10 min at 2000*g*. Each filtrate was tested for protein leakage by treating an aliquot with an equal volume of acetonitrile. If the filtrate became turbid or if a precipitate formed, indicating protein leakage, the binding determination was repeated. We had determined that acetonitrile could be used to detect leakage of less than 0·05% of serum protein concentrations.

In preliminary studies to determine the recovery of nifedipine and the extent of non-specific binding to the ultrafiltration unit, phosphate-buffered (50 mM, pH 7.4, $\mu = 0·16$), protein-free solutions containing 100–1200 ng ml^{-1} of nifedipine were ultrafiltered. 1200 ng ml^{-1} is several times higher than the serum nifedipine concentrations anticipated in antianginal or antihypertensive therapy. For serum protein binding measurements, individual serum samples containing 400, 800 or 1200 ng ml^{-1} of nifedipine were ultrafiltered. The binding to albumin and AAG was determined by ultrafiltering the respective protein solutions to which 100 to 1200 ng ml^{-1} of nifedipine had been added. The determinations were in duplicate and the extent of protein binding was expressed as a mean ± s.d. over the concentration range studied. The concentration of nifedipine in all solutions and ultrafiltrates was determined by GLC (Lesko et al 1983); the sensitivity of the method was 3 ng ml^{-1} in ultrafiltrate and the interday precision was <±11% at this concentration.

The fraction bound, β, was calculated as $[(C_T - C_{UF})/C_T]$ where C_T was the total (bound + unbound) concentration (ng ml^{-1}) of nifedipine in each sample before ultrafiltration and C_{UF} was the ultrafiltrate nifedipine concentration (ng ml^{-1}). An estimate of the average equilibrium (association) constant, K_a (litre

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mol^{-1}), for albumin, AAG and serum was obtained by solving the rearranged and modified Sandberg/Rosenthal equation (eqn 1) algebraically for

$$\frac{(C_T - C_{UF})}{C_{UF}} = nK_aP_T - \frac{K_a(C_T - C_{UF})}{1000 \times 346} \quad (1)$$

where n (assumed to be 1·0) was the number of binding sites per protein molecule, P_T was the total molar concentration of protein and 346 was the molecular weight of nifedipine. For calculating K_a in isolated protein solutions the molecular weights of albumin and AAG were taken as 69 000 and 44 100, respectively, and for serum, albumin was assumed to be the major binding protein. Estimates of K_a were insensitive to changes in C_T over the concentration range of 100–1200 ng ml^{-1} so all calculations of K_a were made using 600 ml^{-1} for C_T and a corresponding concentration value for C_{UF} based on the value of β in the respective protein solutions.

Statistical analysis was conducted using a paired *t*-test.

Results and discussion

The recovery of nifedipine after ultrafiltration of phosphate buffered solutions in the absence of protein was constant over the range 100–1200 ng ml^{-1}. Loss of nifedipine apparently due to non-specific adsorption to the YMT membrane or walls of the filtration unit averaged less than 8% and loss from protein-free solutions containing 100 ng ml^{-1} of nifedipine would account for less than a 0·1% error in the observed value of β.

The interindividual variability in the binding of nifedipine was studied using serum from four healthy volunteers. The total protein and albumin concentrations in each serum were within the normal ranges of 6·0–8·4 g dl^{-1} and 3·5–5·0 g dl^{-1}, respectively. The values of β in all sera were independent of the nifedipine concentration up to 1200 ng ml^{-1}. Rosenkranz et al (1974) showed that serum protein binding of nifedipine was saturable but only when the concentration was increased to 20 mg ml^{-1}. The steady state serum concentrations observed after therapeutic doses are less than 400 ng ml^{-1} so saturable protein binding should not be a clinically relevant issue. The mean ± s.d. value of β in the present study was 0·992 ± 0·008 (n = 12) which is similar to the value of 0·98 reported by Rosenkranz et al (1974). The small coefficient of variation (about 1%) in serum protein binding is evidence of negligible intersubject variability.

The extent of nifedipine binding to albumin was 0·970 ± 0·012 (n = 15), not significantly different from that in serum ($P > 0·05$). A plot of log bound concentration as a function of log free concentration was linear (r^2 = 0·999) with a slope of 1·0 and a zero intercept indicating that binding was independent of nifedipine concentration up to 1200 ng ml^{-1}. The similarities of the values in serum and buffered albumin solutions confirm that albumin is a major binding protein for nifedipine in serum.

The extent of binding to AAG was independent of nifedipine concentration and averaged 0·514 ± 0·059 or 0·755 ± 0·035 in solutions of 50 or 150 mg % of AAG, respectively. The binding ratio (bound/free concentrations) of nifedipine was directly proportional to the AAG concentration. Since the molar concentration of AAG and the extent of nifedipine binding is markedly lower than with albumin, circulating AAG levels probably contribute little to the total binding of nifedipine in serum.

Because binding of nifedipine was constant over the concentration range of 100–1200 ng ml^{-1} in all protein solutions, it was not possible to estimate graphically the apparent binding parameters n and K_a. However, by assuming n = 1, it was possible to obtain algebraically average estimates of K_a. The apparent affinity of nifedipine for protein binding sites was greatest in serum ($K_a = 2·12 \times 10^5$ litre mol^{-1}) and lowest in albumin solutions ($K_a = 0·55 \times 10^5$ litre mol^{-1}). The difference in K_a values between serum and albumin suggest either the presence of more than one class of binding sites per molecule of albumin in pure solution, or that nifedipine is more preferentially bound in serum to a macromolecule with a lower molecular weight and/or lower molar concentration than albumin (e.g. lipoproteins). The value of K_a for nifedipine in AAG solution was $1·00 \times 10^5$ litre mol^{-1} indicative of avid binding of nifedipine. This value was in agreement with the value of K_a obtained from the slope of the line (assuming n = 1) formed by plotting the binding concentration ratio (bound/free) of nifedipine versus the molar AAG concentration. This indicates that the assumption of one equivalent binding site (n = 1) per AAG molecule was correct.

We have confirmed that nifedipine is bound to a large extent in serum, mainly to albumin, and that β is constant over a wide range of nifedipine concentrations (100–1200 ng) and although the drug is moderately bound to AAG with higher affinity than albumin, changes in circulating AAG levels are unlikely to influence the absolute or relative extent of protein binding in patient sera.

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