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The Binding of Two Dihydropyridines to Isolated and Native Plasma Lipoproteins

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Abstract

The objectives of this study were to investigate the mechanisms involved in the interaction of two calcium antagonists, isradipine and amlodipine, with isolated lipoprotein fractions in-vitro and to determine the distribution of isradipine among individual plasma lipoproteins ex-vivo in healthy volunteers (n = 8) and in hypercholesterolaemic patients (n = 12).

The total binding affinity of isradipine to isolated low density lipoprotein (LDL) was markedly higher compared with amlodipine; total binding affinity (nK_a) of isradipine vs amlodipine was $1{\cdot}60 \pm 0{\cdot}08 \times 10^7$ vs $4{\cdot}14 \pm 0{\cdot}33 \times 10^6$ M^{-1}, respectively. Binding to high density lipoprotein (HDL) was also higher with isradipine, $nK_a = 1{\cdot}04 \pm 0{\cdot}04 \times 10^5$ M^{-1}, compared with that of amlodipine, $nK_a = 3{\cdot}82 \pm 0{\cdot}18 \times 10^4$ M^{-1}. The distribution study ex-vivo demonstrated the different relative affinity of isradipine for the plasma lipoproteins: HDL > LDL > very-low density lipoprotein (VLDL). Isradipine binding correlated linearly with the cholesterol levels in LDL and VLDL; however, binding to HDL did not correlate with the cholesterol level in this fraction. There was no significant competitive binding effect of cyclosporin A (1–5 $\mu g\,mL^{-1}$) on isradipine binding to individual lipoprotein fractions.

It is likely that, in addition to the structure of surface apoproteins, the factors determining the interaction of calcium antagonists with plasma lipoproteins also include the plasma level of each lipoprotein fraction as well as the lipophilicity of the drug.

The role of plasma lipoproteins as a transport system for lipophilic drugs is important for drug delivery to specific cells and tissues (via the lipoprotein receptor pathway) as well as for the degradation of drugs in plasma (Urien 1986; Prueksaritanont et al 1992). Moreover, a positive correlation between the total binding constant of the drug–low-density lipoprotein (LDL) complex and volume of distribution of various basic drugs has been observed (Glasson et al 1982; Albengres et al 1984; Urien et al 1984). The role of lipoproteins in the overall plasma binding of drugs varies widely, according to the ionization and structural profile of the drug: neutral compounds are more than 50% or totally bound to lipoproteins, whereas the degree of basic drug-binding ranges from 5 to 60%. Recently, it was reported that isradipine, in contrast to amlodipine, inhibits the platelet-activating effect of LDL and has a more pronounced platelet inhibitory effect in-vitro at higher LDL concentrations (Fetkovska 1992).

Recently it has been demonstrated that lipoproteins and lipids are important determinants of the plasma binding of various dihydropyridines (von Rosenkranz et al 1974; Urien et al 1985, 1987; Pinquier et al 1988). The aim of the present study was to investigate the association of isradipine with different plasma lipoproteins in healthy volunteers and in hypercholesterolaemic patients. In addition, the interaction of two calcium antagonists (isradipine and amlodipine) with isolated purified lipoprotein fractions was evaluated.

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Materials and Methods

Chemicals

[^{14}C]Isradipine (55 mCi $mmol^{-1}$) and unlabelled isradipine were provided by Sandoz Pharma Ltd (Basel, Switzerland). Amlodipine and cyclosporin A were supplied by Pfizer Central Research (Pfizer Ltd, Sandwich, Kent, UK) and Galena (Opava-Komarov, Czech Republic), respectively. Stock solutions of [^{14}C]isradipine were prepared in methanol (1 mg mL^{-1}). The radiochemical purity of [^{14}C]isradipine in stock solution was assessed by thin-layer chromatography with the following solvent systems: methanol/water/conc. ammonia (70:30:1) and *n*-hexane/diethylether/dioxane/water/conc. ammonia (40:40:20:0·5:0·5). The radiochemical evaluation of the chromatograms showed a purity > 97%. LDL (L-2139) and high density lipoprotein (HDL) (L-2014) were purchased from Sigma Chemical Corporation (St Louis, MO, USA). The concentrations were adjusted to 0·31 μM (1·09 $g\,L^{-1}$) in the case of LDL, and to 14·30 μM (3·33 $g\,L^{-1}$) in the case of HDL. All other chemicals used in this study were of analytical grade and purchased from the usual commercial sources.

Determination of drug-binding parameters in-vitro

Poor and variable results have been obtained using the conventional equilibrium dialysis technique. Thus, binding of the non-labelled isradipine and amlodipine to isolated LDL and HDL in-vitro was measured using a drug solution saturating a high-performance-size exclusion column according to the method of Hummel & Dreyer (1962) and described elsewhere (Sebille et al 1978). The high-performance liquid chromatographic apparatus consisted

of a high pressure pump (HPP 5001, Laboratorni pristroje, Prague, Czech Republic), a sample injector (Type 7125, Rheodyne, USA) equipped with a 50 μL sample loop, a compact glass cartridge column packed with LiChrosorb Diol (Merck, Darmstadt, Germany) and a variable wavelength UV/vis detector (SpectroMonitor 3200, LDC Analytical, USA) operating at 280 nm.

According to Scatchard (1949), several theoretical calculations were made to evaluate the amount of drug bound to the isolated LDL and HDL fraction. The analysis of the binding data revealed that both dihydropyridines interact with one class of binding sites with high capacity and low affinity, termed nonspecific binding by Taira & Terada (1985).

The following equation was used to describe the binding data:

$$B_i = (nK_a)_i \times F \quad (1)$$

where B_i and F are the bound ligand concentration to each lipoprotein of the ith class and the free ligand concentration, respectively, n is the number of binding sites per molecule of protein, and K_a is the corresponding association constant.

Experiments ex-vivo

Sera obtained from healthy volunteers (n = 8) or hypercholesterolaemic patients (n = 12; type IIa and IIb) were mixed with [^{14}C]isradipine. After 1 h incubation at room temperature (21°C), the lipoprotein fractions were isolated by sequential ultracentrifugation (50·3 Ti rotor L5-50 Beckman; 50°C; 36 000 rev min^{-1}) at increasing density (very low-density lipoprotein (VLDL): $d \ll 1{\cdot}066\,g\,mL^{-1}$; LDL: $d = 1{\cdot}006$–$1{\cdot}063\,g\,mL^{-1}$; HDL: $d = 1{\cdot}063$–$1{\cdot}210\,g\,mL^{-1}$); no precipitating agents were used. The concentration of cholesterol in serum lipoproteins, triacylglycerol levels and values of apoprotein (apo)-B and apo-A-I in sera were determined using commercial assay systems. [^{14}C]Isradipine in each lipoprotein fraction (VLDL, LDL, HDL and the lower protein-rich fraction) was re-extracted with toluene (Chellingsworth et al 1988) and evaporated to dryness under nitrogen. Its incorporation into individual lipoprotein fractions was measured using liquid scintillation spectrometry (Rack-Beta, LKB, Sweden; recovery 80–85%) and expressed as the percentage of the total amount of radioactivity obtained in serum.

Results and Discussion

Previous studies have shown that α_1-acid glycoprotein and lipoproteins are major determinants of the binding of dihydropyridine calcium antagonists in human plasma (Urien et al 1985, 1987; Pinquier et al 1988). It has been shown that nicardipine interacts preferentially with the lipid moiety of lipoproteins, and that triglycerides and phospholipids are the most important components determining its binding (Urien et al 1985). It should also be noted that lipoproteins can act as transport vehicles for the delivery of dihydropyridines, as lipoproteins bind to specific high-affinity receptors in different tissues (Havel & Hamilton 1988; Prueksaritanont et al 1992).

In the present study, the results obtained in-vitro with isolated lipoproteins were used to describe the binding mechanism of LDL and HDL over a range of therapeutic and supratherapeutic concentrations of isradipine and amlodipine (0·1–20 and 0·3–200 μM, respectively) (Kelly & O'Malley 1992; Meredith & Elliott 1992). The total binding affinity (nK_a)—the product of the number of binding sites multiplied by the association constant—of isradipine to isolated LDL was markedly higher than that of amlodipine being $1{\cdot}60 \pm 0{\cdot}08 \times 10^7$ and $4{\cdot}14 \pm 0{\cdot}33 \times 10^6\,M^{-1}$, respectively (Fig. 1a). The binding to HDL was also higher with isradipine ($nK_a = 1{\cdot}04 \pm 0{\cdot}04 \times 10^5\,M^{-1}$) compared with amlodipine ($nK_a = 3{\cdot}82 \pm 0{\cdot}18 \times 10^4\,M^{-1}$) (Fig. 1b). We were unable to confirm the findings reported by Pinquier et al (1988) on the saturable binding of isradipine to LDL ($n = 14{\cdot}9 \pm 1.5$; $K_a = 2{\cdot}32 \pm 0{\cdot}4 \times 10^5\,M^{-1}$).

The results from our ex-vivo experiments showed that the percentage of lipoprotein-bound isradipine in the healthy volunteers was approximately 10–12%. Further, the distribution study, including the samples of healthy volunteers and slightly hypercholesterolaemic patients, demonstrated the different relative affinities of isradipine for the individual plasma lipoproteins: HDL (7–13%) > LDL (2–4%) >

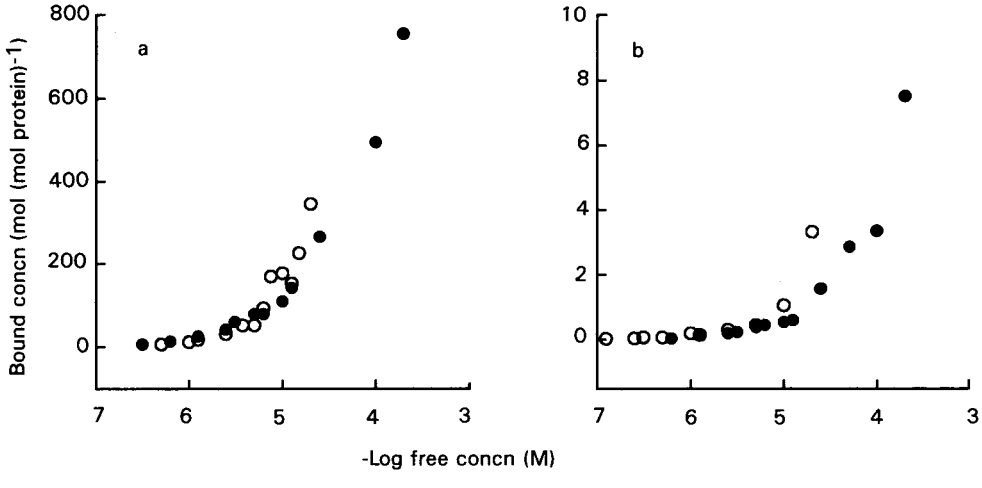

FIG. 1. Interaction of isradipine (○) and amlodipine (●) with isolated LDL (a) and HDL (b) fractions in-vitro as a direct plot of bound (B) vs free (F) drug concentration.

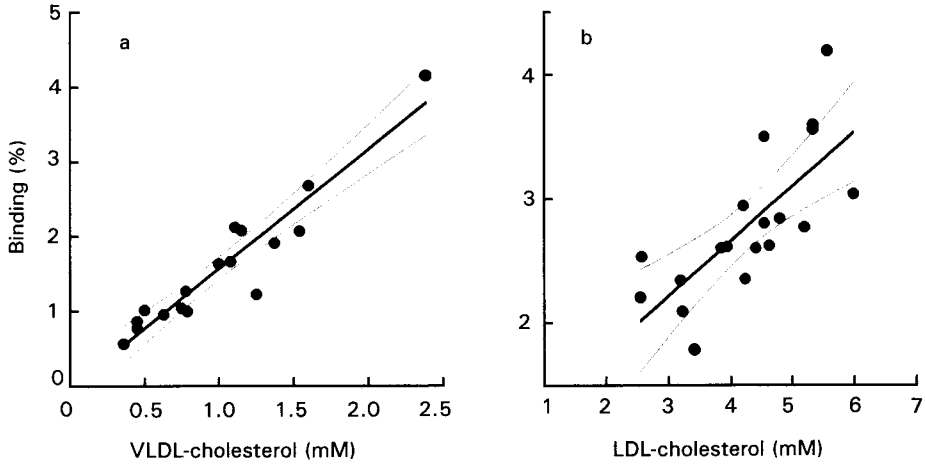

FIG. 2. Binding of [^{14}C]isradipine to VLDL (a) and LDL (b) fractions ex-vivo plotted against the cholesterol concentration of lipoproteins (percentage of total radioactivity recovered after ultracentrifugation). The values are the means of three separate experiments. Isradipine binding in all subjects correlated linearly with the cholesterol level in VLDL and LDL ($y = 0{\cdot}034 + 1{\cdot}599x$, $r = 0{\cdot}95$ and $y = 0{\cdot}855 + 0{\cdot}444x$, $r = 0{\cdot}74$, respectively; 95% confidence intervals).

VLDL (0·6–4%). Isradipine binding in all subjects correlated linearly with the cholesterol levels in VLDL and LDL ($y = 0{\cdot}034 + 1{\cdot}599x$, $r = 0{\cdot}95$ and $y = 0{\cdot}855 + 0{\cdot}444x$, $r = 0{\cdot}74$, respectively) (Fig. 2). However, isradipine binding to HDL did not correlate with the cholesterol concentration in this fraction ($y = 10{\cdot}831 - 1{\cdot}123x$, $r = 0{\cdot}22$) (Fig. 3).

Despite the fact that HDL and LDL are the strongest determinants of cyclosporin A plasma binding (Urien et al 1990; Hughes et al 1991; Prueksaritanont et al 1992) and cyclosporin A added in-vitro can qualitatively transfer from HDL to LDL (influencing thereby the access of other drugs to the relevant lipoprotein (Mraz et al 1986)), the addition of 1–5 $\mu g\,mL^{-1}$ cyclosporin A in our study did not influence the interaction of isradipine with human lipoproteins.

In human sera, the distribution of isradipine among the different lipoproteins did not correlate with the distribution of cholesterol or triglycerides among these fractions. Thus, in contrast to nicardipine (Urien et al 1985) or some lipophilic pollutants (Mohammed et al 1990), with isradipine, the cholesterol content of the individual lipoprotein fractions is only partly responsible for the overall distribution pattern of the agent in human serum. Although other particular components of individual lipoproteins may play an important role (mainly the type of surface apoprotein (Pinquier et al 1988)), further binding measurements of drugs with isolated apoproteins are needed to confirm this theory.

The factors of primary importance responsible for the interaction of dihydropyridines with plasma lipoproteins are related to the structure of the lipoprotein fraction (the surface apoproteins and the lipid composition of the lipid core) and the concentration of each lipoprotein fraction in plasma as well as the structure and lipophilicity of the drug. The different binding affinity of isradipine to the LDL fraction compared with that of amlodipine may be relevant to the specific cellular effects of these compounds (Fetkovska 1992). However, more experimental data are required to elucidate the role of some components of plasma lipoproteins in the tissue uptake and pharmacological effects of the dihydropyridine calcium antagonists.

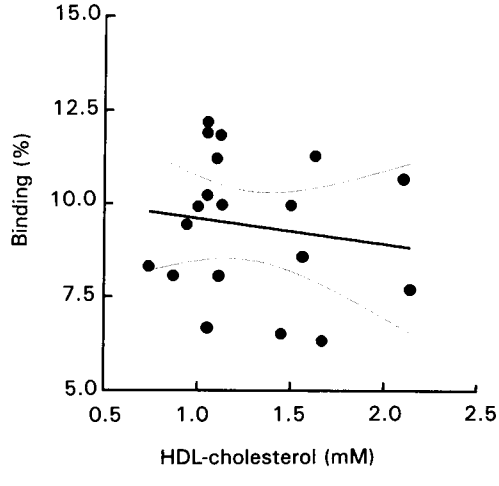

FIG. 3. Binding of [^{14}C]isradipine to HDL fraction ex-vivo ($y = 10{\cdot}831 - 1{\cdot}123x$, $r = 0{\cdot}22$; 95% confidence interval) plotted against the percentage of total radioactivity recovered after ultracentrifugation. The values are the means of three separate experiments.

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