Lercanidipine: Short Plasma Half-life, Long Duration of Action and High Cholesterol tolerance

Updated Molecular Model to Rationalize its Pharmacokinetic Properties

LEO G. HERBETTE,[1,2] MICHELLE VECCHIARELLI,[2] ABRAHAM SARTANI[3] AND AMEDEO LEONARDI[3]

From the Departments of [1]Radiology and Medicine, and the [2]Biomolecular Structure Analysis Center, University of Connecticut Health Center, Farmington, CT, USA; [3]Pharmaceutical R & D Division, Recordati, S.p.A., Milan, Italy

Herbette LG, Vecchiarelli M, Sartani A, Leonardi A. *Lercanidipine: Short plasma half-life, long duration of action and high cholesterol tolerance. Updated molecular model to rationalize its pharmacokinetic properties.* Blood Pressure 1998; 7 (Suppl 2): 10–17.

Calcium-channel antagonist drugs of the 1,4-dihydropyridine type have been shown to bind to the L-type calcium channel. These drugs are not only amphiphilic, but new molecular designs have become increasingly lipophilic and can readily transport across cell membranes, accessing both hydrophilic and hydrophobic environments, despite becoming more soluble in the membrane bilayer. This biophysical understanding appears not only to define the molecular pathways for drug binding to the calcium-channel receptor, but also to explain differences in the overall clinical pharmacokinetics observed for different drugs in this class. The pharmacokinetic profile of calcium antagonists, although influenced to some degree by interactions with their target calcium-channel receptor, appears to be largely dictated by their interactions with cell membranes at the molecular level. There appears to be a correlation between the duration of action of such membrane-active drugs and the membrane partition coefficient in conjunction with the washout rate. This class of drugs has evolved from a drug such as amlodipine, with a long duration of action related to prolonged plasma half-life, to lercanidipine, which has the shortest plasma half-life relative to its intrinsically long duration of action. Recently, it was discovered that membrane cholesterol reduces the amount of calcium-channel antagonist that can partition into the membrane. Atherosclerotic disease results in increased levels of membrane cholesterol in smooth muscle cells. Latest generation calcium antagonists, which have a long duration of action, can better overcome this negative effect. Lercanidipine has now been shown to have one of the highest measured tolerances to cholesterol, which may indicate its ability to treat a broad range of hypertensive patients with varying degrees of progressive atherosclerotic disease. On what criteria should the effectiveness of calcium antagonists be evaluated? A good calcium antagonist needs to exhibit a placebo-like side-effect profile, thus ensuring good patient compliance. However, an intrinsically long-lasting, once-a-day dose is also pharmacokinetically desirable. To be a truly optimal calcium antagonist, it should function and be efficacious over a broad range of hypertensive patients. It should be able to control blood pressure in light of other complications such as progressive atherosclerotic disease. Recent studies indicate that during the progression of atherosclerosis, cholesterol levels within cell membranes of the arterial wall increase, a process that can reduce the effective concentration of calcium antagonists in these membranes. What is needed is a calcium antagonist that is slow acting to reduce vasodilatory induced side-effects and intrinsically long lasting to ensure once-a-day dosage, and that possesses a high cholesterol tolerance factor to overcome the molecular and compositional changes taking place in the arterial wall, so that it can treat effectively a broad range of hypertensive patients. *Key words: cholesterol tolerance, lercanidipine, lipid bilayer, lipophilicity, residence time.*

INTRODUCTION

The clinical efficacy of 1,4-dihydropyridine (DHP) calcium-channel antagonists (blockers) results from their physiological effects on vasodilating the arterial network. This depends on their being highly soluble in vascular smooth muscle membranes, which is dictated by molecular interactions of these drugs at the membrane level

[1]. These drugs bind to calcium-channel receptors, block calcium ion movement into the cell and result in relaxation of smooth muscle cells and concomitant dilation of blood vessels, thus lowering peripheral resistance and blood pressure. The equilibrium binding of these drugs to membranes is a critical parameter to determine and can be quantitated by determination of the membrane partition coefficient ($Kp_{[mem]}$). In addition to this equi-
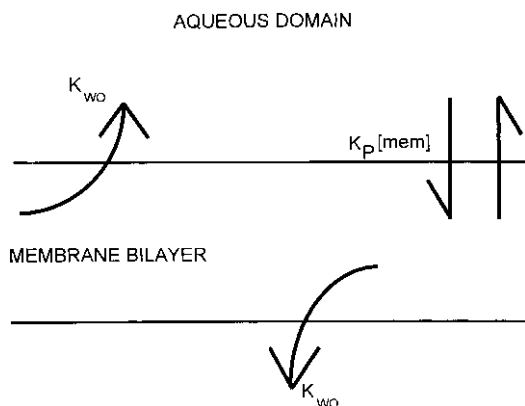
Fig. 1. Membrane partition coefficient $Kp_{[mem]}$ is an equilibrium value expressing the relative amount, by mass, of a drug in the membrane versus the aqueous domain. k_{wo} = time-dependent measurement of this drug's release or washout from the membrane into the aqueous domain.

Fig. 2. Structure of lercanidipine with its bulky bis-phenyl side-chain (boxed). Lercanidipine's analogue Rec 2520 has this side-chain changed for a hydrogen atom (structure without the boxed side-group). This hydrophobic and bulky bis-phenyl side-chain acts like a 'membrane anchor', making lercanidipine much more lipophilic than its analogue.

librium binding, the duration that a drug resides in a membrane, characterized by its washout rate (k_{WO}), may also contribute to its biological half-life (Fig. 1). Thus, a calcium antagonist has a plasma half-life and duration of action which can be understood in terms of molecular events at the level of the cell membrane [2, 3].

The rate at which a calcium antagonist is able to enter the membrane (k_{WI}) and be available for binding to its target site of action will dictate, to some degree, the time course for dilation of blood vessels and lowering of the blood pressure. Alternatively, the on-rate of binding may be controlled at the calcium channel site of action, which may be dependent on the state of the calcium channel, as dictated by the membrane potential. Nevertheless, the ideal calcium antagonist would provide a gradual lowering of blood pressure and reduce the incidence of associated side-effects such as ankle oedema, headaches and flushing, so long as it possesses a slow rate of onset.

Studies have shown that most calcium antagonists of the dihydropyridine type interact with calcium channels from a location near to the surface of the cell membrane [1]. Amlodipine, nimodipine and nitrendipine, for example, are primarily located at the hydrocarbon core/water interface of the membrane bilayer. In certain membranes containing a low amount of cholesterol, lacidipine is located much deeper in the membrane [2], owing to its hydrophobic side-chain on the phenyl moiety. Lercanidipine, also designed with a unique membrane anchor side-chain (Fig. 2), penetrates into the hydrophobic core of the lipid bilayer structure of membranes, but also has a hydrophilic moiety, a charged amine which, together with its membrane-soluble anchor, may provide for a unique combination of membrane and pharmacokinetic properties. For this class of drugs, interaction with the hydrocarbon core of the lipid bilayer appears to dominate.
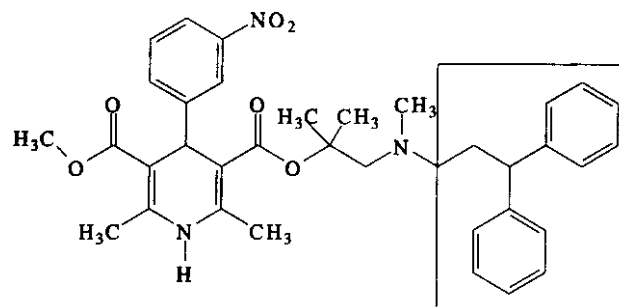
The amount of cholesterol in cell membranes where the calcium channels are located varies for different membranes found in vascular and cardiac tissue and possibly with the progression of atherosclerosis [4]. Membrane cholesterol can affect the binding of calcium antagonists to membranes [5]. For all calcium antagonists, the presence of increasing amounts of cholesterol in membranes decreases the concentration of these drugs within the membrane bilayer compartment. Some calcium antagonists, such as nimodipine, are greatly affected by membrane cholesterol [6]. Amlodipine's partition coefficient demonstrates a dependency on the presence of cholesterol in membranes and a plasma half-life that may be exorbitantly long in elderly patients with renal impairment. This dependency of the membrane interaction on the cholesterol content of the membrane has also been observed for lacidipine. Thus, the cholesterol content in a target membrane can be one determinant of a drug's biological and clinical half-life and may be important in light of the finding that hypertension is often associated with atherosclerosis and elevated cholesterol levels in membranes [7].

What is needed is a calcium antagonist that remains in the therapeutic window of concentration regardless of the cholesterol content of the target membrane. Such is the case for the long-lasting calcium-channel antagonist lercanidipine, which is characterized by one of the highest known membrane partition coefficients for a drug of this class, coupled with the slowest washout from biological membranes. These properties at the membrane molecular level may correlate with its overall long-lasting biological and clinical half-life. The $Kp_{[mem]}$ for lercanidipine is dependent on the cholesterol content of the membrane, like other drugs in its class. $Kp_{[mem]}$ and k_{wo} may work together in maintaining lercanidipine in the therapeutic window for activity over a much longer period, while reducing its sensitivity to membrane

cholesterol changes, in contrast to other calcium-channel antagonists (see Table V). This potentially provides for unique and favourable pharmacokinetics for lercanidipine in the treatment of hypertension in patients with a moderate to severe degree of progressive atherosclerotic disease.

POTENTIAL MOLECULAR PATHWAYS FOR CALCIUM-CHANNEL DRUG BINDING TO MEMBRANE-BOUND RECEPTORS

In general, it has been previously thought that the mechanism for drug binding to a plasma membrane receptor could be considered analogous to that of endogenous ligands such as hormones, growth factors and neurotransmitters, i.e. through an aqueous pathway outside the membrane. Generally, such ligands are water soluble and are thought to bind to an extracellular portion of the receptor. A classical example of this is the binding of the charged acetylcholine neurotransmitter and its competitive antagonist to an extracellular portion of the alpha-subunit near the opening of the ion channel [8]. Even this classical case has come under scrutiny and anaesthetics binding to this membrane-bound receptor could take multiple pathways, including one using the membrane lipid bilayer [9].

In contrast to ligand binding directly from the aqueous, extracellular environment, there is experimental evidence that highly lipophilic drugs bind via the membrane bilayer. Hille [10] suggested that various local anaesthetics utilize a membrane bilayer pathway in their interaction with target sodium channels (see also Ref. [9]). Although the experiments examined drug binding to neural membrane bilayers, it was hypothesized that "a modulated receptor with alternative hydrophobic and hydrophilic pathways (for drug binding) is probably applicable to other cases," [10].

An intrabilayer receptor site has also been implicated for the β-adrenergic receptor, which contains seven hydrophobic domains that have been modelled as seven transmembrane-spanning segments [11]. The seventh membrane-spanning domain appears to be necessary for ligand binding to an intrabilayer receptor site based on deletion mutation studies [11]. Small-angle neutron diffraction experiments have determined the drug's time-averaged location to be in the hydrocarbon core of biological membranes, near the glycerol backbone [12]. Propranolol's location at this depth in the membrane bilayer is consistent with the proposed site suggested by the deletion mutation approach and may define a region for lateral diffusion to an intrabilayer receptor site.

In the case of the binding of DHP calcium-channel antagonists to the L-type calcium channel, support for a receptor site buried in the membrane bilayer is based on the DHP receptor subunit being heavily labelled by a hydrophobic photoaffinity probe, indicating multiple transmembrane helices [13]. The primary structure of the DHP receptor subunit from rabbit skeletal muscle has been deduced from its DNA sequences. The polypeptide is structurally similar to the voltage-dependent sodium channel, with four regions of homology that putatively comprise six transmembrane alpha-helices and may serve as the channel for calcium [14]. In light of the high homology of the hydrophobic domains of calcium channels with sodium channels, it is interesting that DHPs have been shown to bind with high affinity and stereoselectivity to the cardiac sarcolemmal sodium channel [15]. These data suggest that the specific receptor site for the DHPs common to both the calcium and sodium channel is a hydrophobic, transmembrane domain. Using a similar strategy, deletion mutation studies of the receptor support a putative location of the DHP binding site at the level of the calcium channel, which is consistent with the time-averaged location of these drugs using X-ray and neutron diffraction [16].

These static structural and molecular biology studies can only infer the possibility of a membrane pathway for drug binding. Consistent with these findings are patch-clamp studies by Kokubin and Reuter [17] with neonatal rat ventricular cells, which showed that DHP derivatives added to the medium outside the patch were still capable of binding and blocking single calcium channels within the patch. These authors suggest that the drug must have access to the calcium channel through the lipid phase of the membrane, since no drug was present in the pipette and the hydrophobic nature of the drug makes it unlikely that it enters the channel from the cytoplasm. Drug diffusion within the membrane was also demonstrated with reconstituted calcium channels in lipid planar bilayers [18]. This study showed that when the DHP Bay K 8644 was added to one side of the bilayer, it was still able to bind specific sites in asymmetrically arranged calcium receptors. These data highlight the fact that DHPs may access their receptor sites via a membrane bilayer pathway.

This biophysical model of DHP binding makes sense in light of the physical properties of these drugs [1, 3]. This membrane pathway model of calcium-channel drug binding may help to rationalize the clinical pharmacokinetic properties of different drugs in this class.

CLASSIFICATION OF CALCIUM ANTAGONISTS BASED ON PHARMACOKINETIC PARAMETERS

It now appears that calcium antagonists can be classified according to their pharmacokinetic parameters, as described in Table I.

Table I. *Classification of calcium antagonists*

Clinical half-life	Plasma half-life	Compartment controlled
Short	Short	Plasma
	Long	Plasma
Long	Intermediate	Plasma/tissue
	Short	Tissue

Short clinical half-life

First-generation calcium antagonists were characterized by having a short duration of action, in the order of a few hours, which was related to an equally short plasma half-life [19, 20]. In other words, the activity of a calcium antagonist with these properties was strongly coupled to how long it remained in the plasma compartment. These first-generation calcium antagonists were also characterized by a fast onset of action. That is, they rapidly reached a peak plasma concentration in less than 2 h and a peak activity with respect to smooth muscle cell relaxation and concomitant vasodilation with a similar time course. In retrospect, work in our laboratory has shown that these calcium antagonists, typified by nimodipine, nitrendipine, nifedipine, etc., also had very rapid equilibration times with tissue cellular membranes and rapid washout from membranes, and were easily transported across membranes. In other words, these drugs were somewhat lipophilic, as measured by their membrane partition coefficients, with log $Kp_{[mem]}$ being in the order of approximately 3. These drugs were also shown to be highly amphiphilic in that they could rapidly transport into, out of and across cellular membranes with ease. In other words, these drugs could move effectively from a water compartment to a membrane compartment with little apparent kinetic control. Hence, it is expected that drug activity would be largely controlled by the plasma compartment, whereby drug in the plasma compartment is in equilibrium with drug in the arterial wall compartment. The problems with drugs in this category, with both a short plasma half-life and a fast onset of action, include an elevated degree of side-effects, including headaches, nausea, ankle oedema and flushing.

Long clinical half-life

New, intrinsically long-acting calcium antagonists, namely amlodipine, lacidipine and lercanidipine, are now available. Their duration of action is apparently derived from distinct molecular mechanisms. Amlodipine has been shown to have a long plasma half-life which is equal to its duration of action and, as such, appears to be controlled in the plasma compartment [21]. Amlodipine appears to have a rate-limiting transport across membranes, which is much slower than any of the first-generation calcium antagonists [22]. It exhibits a slow or gradual onset of action, which may be the result of more than one mechanism. At the same time, it equilibrates to relatively high levels in membranes, as measured by the membrane partition coefficient (log $Kp_{[mem]} = 4.3$). The drawback of a long duration of action being controlled primarily in the plasma compartment is that in elderly patients with renal impairment the half-life can increase to 50–60 h, resulting in apparent accumulation of the calcium antagonist over time as has been shown for amlodipine.

Lacidipine is clearly different from amlodipine in that its pharmacokinetics is controlled primarily by the tissue cell membrane compartment via a membrane kinetic control mechanism. In addition, lacidipine's plasma half-life is intermediate, with a terminal plasma half-life in the order of 14 h (Boehringer Ingelheim, personal communication). Its duration of action classifies it as a once-a-day calcium-channel antagonist with a slow washout from membranes, as does that of amlodipine. Its partitioning in cellular membranes is high, comparable to that observed for lercanidipine.

Lercanidipine, also classified as a once-a-day calcium antagonist, is the first calcium antagonist to demonstrate a short plasma half-life, in the order of 3–5 h, with a long duration of action. It has one of the highest measured membrane partition coefficients to date (Tables II and III). Thus, lercanidipine combines a relatively short plasma half-life, a gradual onset of action and an intrinsically long duration of action. Apparently, its duration of action is controlled not by the plasma compartment, but by the arterial tissue wall compartment, where lercanidipine may be stored over a long period and from which it can gradually interact with calcium channels in arterial smooth muscle cells. This combination of a significantly reduced plasma half-life, yet a long duration of action, coupled with a gradual onset of action, may provide an explanation for lercanidipine's good safety profile.

Table II. *Membrane partition coefficients in cardiac model membranes at varying cholesterol to phospholipid (C:L) mole ratios*

Drug	0:1 C:L	0.3:1 C:L
Lercanidipine	$315\,600 \pm 7700$	$230\,900 \pm 8400$
Amlodipine	$21\,300 \pm 1200$	$19\,300 \pm 1300$

Values are $\pm$ SEM; pH 7.0.
Data taken from assorted studies performed by R. P. Mason and L. G. Herbette.

Table III. *LogKp$_{[mem]}$ and logd at pH 7.4 for lercanidipine versus its analogue Rec 2520**

Drug	LogKp$_{[mem]}$ neutral	logD pH 7.4†
Lercanidipine	6.140 ± 0.084	5.85
Rec 2520	2.428 ± 0.044	0‡

* Methyl *N*,1,1-trimethyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate.
† Predicted value using the experimentally derived log$Kp_{[mem]}$ by titration using the PCA101 pK_a LogP Analyser (Sirius Analytical, East Sussex, Forest Row, UK).
‡ Analysis indicates a lack of partitioning for the charged species.

Table IV. *DHP* calcium channel drug partition coefficients in biological membranes*

Drug	Biological membranes† (sarcoplasmic reticulum)
Bay P 8857	125 000
Iodipine	26 000
Amlodipine	19 000
Nisoldipine	13 000
Bay K 8644	11 000
Nimodipine	6300
Nifedipine	3000

* 1,4-dihydropyridine.
† Similar values were obtained with lipid extracts, indicating a primary interaction of the drug with the membrane bilayer component of these biological membranes.

INTERACTIONS OF CALCIUM ANTAGONISTS WITH MEMBRANES

Membrane partition coefficient

The binding of calcium antagonists to membranes can be measured directly as the equilibrium membrane partition coefficient ($Kp_{[mem]}$). Based on these and other studies, it is reasonable to generalize that calcium antagonists are membrane-soluble drugs. The data in Tables III and IV clearly demonstrate a range of membrane solubilities, with lercanidipine having one of the highest membrane interactions compared with other drugs in its class. These membrane solubilities seem to correlate directly with the duration of action.

Washout kinetics from membranes

Figure 3 shows the washout kinetics of the intrinsically long-lasting calcium antagonist lercanidipine. The washout rate was characterized by a half-life of 84 min. Comparison with other calcium antagonists showed that lercanidipine's washout is the slowest measured to date. Washout of lacidipine under the same conditions shows a monotonic exponential release characterized by a half-life of 45 min but which could be as high as 61 min. Thus,
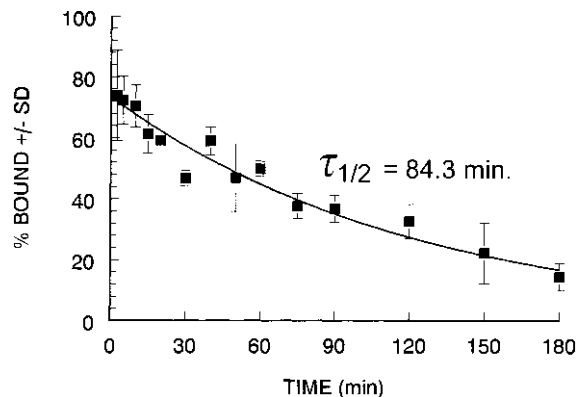

Fig. 3. Washout of lercanidipine from eggPC LUV (Large Unilamellar Vesicles) (0.3:1, cholesterol:eggPC) over 180 min at pH 7.0. An exponential fit determined a half-life ($\tau_{1/2}$) = 84 min ($r^2 = 0.95$) with $N \geq 3$ at each time point.

lercanidipine washout is 40% slower, but may be as much as 80% slower than lacidipine. This would mean that incorporation of lercanidipine into the membrane compartment results in its retention there for a long period, which may be reflected in a longer duration of action. This experiment was designed to measure the ability of the drug to cross several membranes in succession and, hence, give some idea of the transport kinetic capability of each drug across biological membranes. Thus, lercanidipine's slow transport and eventual washout from membranes would be indicative of a model in which the pharmacokinetics of its intrinsic duration of action is controlled by its interaction with the arterial wall compartment and not the plasma compartment.

FACTORS DETERMINING THE ENHANCED MEMBRANE INTERACTION FOR LERCANIDIPINE

All calcium antagonists have a similar backbone chemical structure with a phenyl ring covalently linked to a dihydropyridine ring, each ring being decorated with different chemical moieties. The primary reason for the enhanced membrane properties of lercanidipine is the unique bis-phenyl moiety structure built into a well-designed side-chain. The hydrophobic and bulky bis-phenyl side-chain makes lercanidipine highly lipophilic and sterically bulky (Fig. 2). The membrane partition coefficient for lercanidipine is three orders of magnitude higher than that of the corresponding analogue (Rec 2520), which has this side-chain removed (Table III). Thus, lercanidipine partitions into the hydrocarbon core of the membrane, where it can be stored over a period of time and act on calcium channels. Since it appears that this side-chain may have little to do with calcium-channel receptor-binding requirements, it is tempting to refer to this unique chemical moiety of lercanidipine as the

membrane-modulating or membrane-control moiety. By contrast, most calcium antagonists have a more compact and less lipophilic structure. Although lercanidipine and lacidipine both have lipophilic moieties, lercanidipine also contains a protonated amine moiety which must further combine to give lercanidipine different plasma compartment properties from lacidipine.

PLASMA VERSUS ARTERIOLE WALL (MEMBRANE) COMPARTMENT

Within the calcium-antagonist class of drugs, there are clear differences in the kinetic and equilibrium interactions with membranes. In addition, these drugs bind differently to particles in the plasma. The way in which a drug interacts with these plasma particles in the plasma compartment and with membranes of the arteriole wall compartment can define critical clinical properties of these drugs.

It has been shown clinically that calcium antagonists bind to plasma particles, predominantly to lipoproteins and less so to red blood cells [23]. For example, preliminary studies in our laboratory have shown that calcium antagonists will bind in large amounts to a low-density lipoprotein (LDL)-enriched plasma fraction *in vitro*. The protein, free cholesterol and phospholipid composition (weight ratio) of these particles was measured as 2.7:0.44:1 (free cholesterol/phospholipid mole ratio of 0.85:1). Different calcium antagonists have different washout kinetics for this LDL-enriched plasma fraction.

Clinically, nimodipine has a terminal plasma half-life of 3–4 h, while lacidipine's terminal plasma half-life is approximately 14 h, consistent with their different kinetics for binding to the LDL-enriched fractions. Moreover, the clinical duration of action of these two drugs may depend differently on a combination of their plasma behaviour, non-specific membrane affinity and association/dissociation kinetics with both plasma particles and membranes. This hypothesis is supported by observations that nimodipine rapidly partitions into membranes and has a relatively low $Kp_{[mem]}$ and a relatively fast washout. It thus appears that the distinct pharmacological profiles of these two calcium antagonists may be distinguished by significant differences in their non-specific plasma particle and membrane interactions.

The measured terminal half-life of lacidipine of 14 h is, however, shorter than that of amlodipine, which can exceed 30 h [24]. Both lacidipine and amlodipine are considered to be intrinsically long lasting [24–26]. By contrast, lercanidipine has a plasma half-life in the order of 3–5 h, similar to that of the short-acting calcium antagonists, yet it maintains a long duration of action. This may be a distinct advantage for lercanidipine to clear the plasma compartment quickly and bind to the tissue membrane compartment. This unique property of lercanidipine may be related to it having both a lipophilic anchor group, so that it binds efficiently to the tissue wall compartment (long duration of action), and a protonated amine group that allows for easier exchange between particles in the plasma and tissue wall compartment (short plasma half-life). Hence, the unique pharmacokinetics for this calcium antagonist may be due, in part, to its interactions with plasma particles, although the intrinsic long duration of action for lercanidipine, as an example, may be more related to cell membrane interactions. Thus, lercanidipine quickly clears the plasma compartment and accumulates in smooth muscle cell membranes where the calcium channels are located. Once in this membrane compartment, lercanidipine can act on calcium channels over a relatively long period (high membrane tissue binding and slow washout) according to the membrane bilayer hypothesis for drug binding to membrane-bound receptors [27]. This provides an explanation at the molecular level for the short plasma half-life yet long duration of action of lercanidipine.

LERCANIDIPINE IN THE THERAPEUTIC WINDOW

Lercanidipine is unique amongst the calcium antagonists in that it exhibits both a high $Kp_{[mem]}$ and the slowest k_{wo}. These two parameters have the combined effect of keeping lercanidipine within the therapeutic window for activity for a long period. The high $Kp_{[mem]}$ establishes a high concentration of lercanidipine within the membrane relative to other calcium antagonists. Presumably, according to the membrane bilayer hypothesis [1], this lercanidipine in the membrane bilayer is in equilibrium with that which binds to the calcium-channel receptor, and so an excess of lercanidipine is available to its target site for binding. The slow k_{wo} ensures that the membrane-partitioned lercanidipine resides in the membrane bilayer for a longer period then other calcium antagonists. Thus, this model at the membrane molecular level may, in part, explain the long duration of action of lercanidipine by a unique mechanism involving the environment of the target membrane-bound protein receptor. This molecular model may also explain, in part, the high tolerance to membrane cholesterol, observed for lercanidipine.

CHOLESTEROL TOLERANCE OF LERCANIDIPINE

Cholesterol has been shown to elevate in smooth muscle cells, possibly as one of the initiating events in the eventual formation of atherosclerotic plaques in the arterial wall [2, 4, 7]. There appears to be a correlation between circulating plasma levels of cholesterol and the amount of cholesterol found in cell membranes of the arterial wall (T. Tulenko, Medical College of Pennsylva-
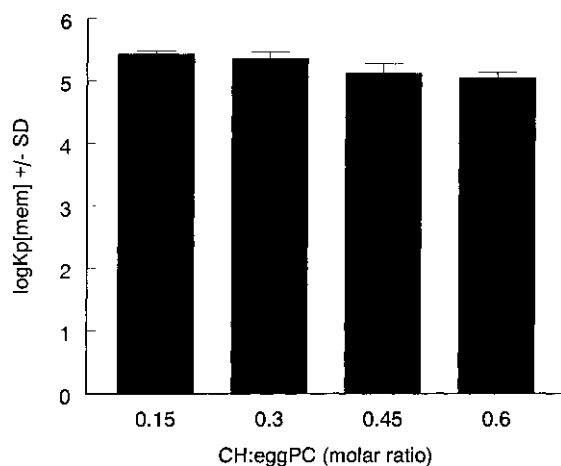
Fig. 4. Log$Kp_{[mem]}$ for lercanidipine with varying molar ratios of cholesterol (CH):eggPC LUV (Large Unilamellar Vesicles) at pH 7.0 at a 1 h time point with $N \geq 4$ for each cholesterol ratio, showing lercanidipine's high tolerance for cholesterol.

Table V. *Cholesterol tolerance for calcium-channel antagonists*

Drug	Cholesterol tolerance factor*
Lercanidipine	63,218
Lacidipine	47,775
Amlodipine	5544
Isradipine	62
Nimodipine	62

* The cholesterol tolerance factor is calculated as the inverse of the slope ($\times 10^{-3}$) of the partition coefficient's dependence on the cholesterol content of the cell membrane. y-intercept of this curve, x-washout rate.

nia, personal communication). The present authors' work has shown that as the cholesterol level in cell membranes increases, less calcium antagonist is able to enter and reside in these cell membranes, effectively reducing the concentration of calcium antagonist available to interact with the calcium channel receptor. This may be especially detrimental to first-generation, less lipophilic calcium antagonists which, at higher cholesterol levels, are effectively excluded from membranes, thus lowering their concentration below threshold levels, becoming ineffective in blocking calcium channels and hence less efficacious in controlling blood pressure.

Lercanidipine, which has a high membrane partition coefficient, shows a dependence on the cholesterol levels in membranes, as shown in Fig. 4. Even at the highest cholesterol levels established, indicative of the most progressive state of atherosclerotic disease, lercanidipine is still able to enter and reside in the cell membrane to act on calcium channels, effectively overcoming this negative cholesterol effect. Thus, at the highest level of cholesterol, there is more than sufficient lercanidipine to inhibit calcium channels. Lercanidipine has the highest cholesterol tolerance factor of any calcium antagonist measured to date (Table V). This may be a distinct advantage for this calcium-channel antagonist in treating hypertension in a broad range of patients with varying degrees of progressive atherosclerosis.

CONCLUSIONS

Initial efforts in the design of calcium antagonists focused on increasing potency, which ultimately was related to chemically designing a stronger binding affinity to the calcium channel. Once sufficient potency was achieved,

the next phase of refinement in the design of calcium antagonists was to introduce intrinsic pharmacokinetic properties that are deemed favourable, such as gradual onset and long duration of action. These properties can be dependent, to some degree, on the receptor-binding mechanism, but their control can be shifted to interactions with the immediate environment of the calcium channel, namely the cell membrane bilayer. These chemical design features can be built into the base structure of the DHP/ phenyl backbone and provide intrinsic control over the rate of entry into, accumulation in, transport across and exit from the cell-membrane structure. These intrinsic equilibrium and kinetic membrane-dependent properties distinguish some of the second-generation calcium antagonists from the first generation.

One clear example of this 'membrane dependency' is with the calcium antagonist lercanidipine. The long duration of action of lercanidipine is apparently not due to its interactions within the plasma compartment of the small arterioles, since lercanidipine has the shortest plasma half-life of all the known intrinsically long-lasting calcium antagonists. The unique clinical pharmacokinetics of lercanidipine are thus mainly due to its interactions with membranes of the arteriole wall, where it is stored over a long period. Lercanidipine's pharmacokinetic membrane dependency may have several associated benefits, especially in managing side-effects.

Lercanidipine's unique chemical structure, which provides its unique membrane properties, may help to rationalize its ability to overcome the negative effects of membrane cholesterol. This high tolerance to elevated levels of membrane cholesterol may allow lercanidipine to be an effective antihypertensive agent over a broad range of patients exhibiting a mild to severe degree of progressive atherosclerotic disease. Thus lercanidipine could prove to be an effective agent for the treatment of hypertension in patients with progressive atherosclerotic disease who do not respond well to other calcium antagonists.

REFERENCES

1. Herbette LG, Rhodes DG, Mason RP. New approaches to drug design and delivery based on drug–membrane interactions. Drug Design Delivery 1991; 7: 75–118.
2. Herbette LG, Gaviraghi G, Tulenko T, Mason RP. Molecular interaction between Lacidipine and biological membranes. J Hypertens 1993; 11 (Suppl 1): S13–9.
3. Herbette LG. Calcium antagonists: membrane binding, duration of action, and anti-atherosclerotic potential effect. JAMA 1994; SEA (Suppl.) 10: 2–8.
4. Tulenko TN. Atherogenic activity of excess membrane cholesterol in arterial smooth muscle and endothelial cells. J Am Coll Cardiol 1991; 17: 24A.
5. Mason RP, Moisey DM, Shajenko L. Cholesterol alters the binding of Ca^{2+} channel blockers to the membrane lipid bilayer. Mol Pharmacol 1992; 41: 315–21.
6. Herbette LG, Mason PE, Sweeney KR, Trumbore MW, Mason RP. Favorable amphiphilicity of nimodipine facilitates its interactions with brain membranes. Neuropharmacology 1994; 33: 241–9.
7. Herbette LG, Mason RP, Tulenko T. Calcium antagonists and membrane binding—the unique properties of lacidipine. In: Van Zwieten PA, editor Research and clinical forums, vol. 16. Kent, England. Wells Medical, 1994: 17–28.
8. Changeux J, Devillers-Thiery A, Chemouilli P. Acetylcholine receptor, an allosteric protein. Science 1984; 225: 1335–45.
9. Blanton M, McCardy E, Gallaher R, Wang HH. Noncompetitive inhibitors reach their binding site in the acetylcholine receptor by two different paths. Mol Pharmacol 1988; 33: 634–42.
10. Hille KB. Local anesthetics, hydrophilic and hydrophobic pathways for the drug receptor reaction. J Gen Physiol 1977; 69: 497–515.
11. Kobilk BK, Kobilka TS, Daniel K, Regan JW, Caron MG, Lefkowitz RJ. Chimeric alpha$_2$-, beta$_2$-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity. Science 1988; 240: 1310–6.
12. Herbette LG, Katz AM, Sturtevant JM. Comparisons of the interactions of proparanol and timolol with model and biological membrane systems. Mol Pharm 1983; 24: 259–69.
13. Takahashi M, Seagar MJ, Jones JF, Reber BFX, Catterall WA. Subunit structure of dihydropyridine-sensitive Ca^{+2} channels from skeletal muscle. Proc Natl Acad Sci USA 1987; 84: 5478–82.
14. Tanabe TH, Takeshima A, Mikami V, et al. Primary structure of the receptor for Ca^{+2} channel blockers from skeletal muscle. Nature 1987; 328: 313–8.
15. Yatani AD, Kuntze DL, Brown AM. Effects of dihydropyridine Ca^{+2} channel modulators on cardiac sodium channels. Am J Physiol 1988; 254: H140–7.
16. Tanabe T. Voltage-dependent calcium channel–from structure to function. Tanpakushitsu Kakusan Koso 1991; 36: 861–8.
17. Kokubun S, Reuter H. Dihydropyridine derivatives prolong the open state of Ca^{++} channel in cultured cardiac cells. Proc Natl Acad Sci USA 1984; 81: 4824–7.
18. Affolter H, Coronada R. The sidedness of reconstituted calcium channels from muscle transverse tubules. Biophys J 1986; 48: 341–7.
19. Janis RA, Silver PJ, Triggle DJ. Drug action and calcium regulation. Adv Drug Res 1987; 16: 309–591.
20. Janis RA, Triggle DJ. Pharmacology of calcium channels. In: Hurwitz L, Partridge LD, Leach JK, editors. Calcium channels — their properties, functions, regulation and clinical relevance. Boca Raton, FL: CRC Press, 1991: 195–249.
21. Abernethy DR, Gutkowaka J, Winterbottom LM. Effects of amlodipine, a long acting dihydropyridine calcium antagonist in aging hypertension: pharmacodynamics in relation to disposition. Clin Pharmacol Ther 1990; 48: 76–86.
22. Mason RP, Campbell S, Wang S, Herbette LG. A comparison of bilayer location and binding for the charged 1,4-dihydropyridine Ca^{+2} channel antagonist amlodipine with uncharged drugs of this class in cardiac and model membranes. Mol Pharmacol 1989; 36: 634–40.
23. Herbette LG. Membrane pathways for drug/ion channel interactions: molecular basis for pharmacokinetic properties. Drug Dev Res 1994; 33: 214–24.
24. Abernethy DR. The pharmacokinetic profile of amlodipine. Am Heart J 1989; 118: 1100–3.
25. Micheli D, Collodel A, Semeraro C, Gaviraghi G, Carpi C. Lacidipine: a calcium antagonist with potent and long-lasting antihypertensive effects in animal studies. J Cardiovasc Pharmacol 1990; 15: 666–75.
26. Lombardo D, Raimondi F. Efficacy and safety: evaluation of lacidipine compared to amlodipine in mild to moderate hypertensive patients. J Cardiovasc Pharmacol 1994; 23 (Suppl 5): S98–100.
27. Rhodes DG, Sarmiento JG, Herbette LG. Kinetics of binding of membrane-active drugs to receptor sites. Diffusion limited rates for a membrane bilayer approach of 1,4-dihydropyridine Ca^{+2} channel antagonists to their active site. Mol Pharmacol 1985; 27: 612–23.

Address for correspondence:

Leo Herbette
Biomolecular Structure Analysis Center
University of Connecticuit Health Center
Farmington
CT 06030
USA
Tel: +1 860 679 2951
Fax: +1 860 679 1989