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New generations of dihydropyridines for treatment of hypertension

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Abstract

Since the calcium channel blocker (CCB) has become one of the most prescribed agents for antihypertensive monotherapy in the world, this brief review will focus on the recent research and development of the dihydropyridine (DHP) CCB, addressing pharmacological mechanisms for the clinical efficacy of the third and fourth generations of the DHP CCBs, especially on their possible central mechanisms underlying lowering blood pressure.

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1 Background

Hypertension is a common medical and social problem leading to cardiovascular diseases worldwide.[1] Antihypertensive drugs are clinically applied to decrease the morbidity and mortality induced by hypertension itself and its complications. The 2014 hypertension guideline of the Eighth Joint National Committee (JNC8) for hypertension therapy in the United States has made several significant changes with respect to the clinical management of hypertension and the initiative medications, as compared with the previous guidelines.[1] In addition to the instructions that pharmacologic treatment should be initiated when blood pressure (BP) is 150/90 mmHg or higher in adults over 60 years, 140/90 mmHg in adults younger than 60 years, or 140/90 mmHg or higher (regardless of age) in patients with hypertension and diabetes, a thiazide-type diuretic,[1] calcium (Ca^{2+}) channel blocker (CCB), angiotensin-converting enzyme inhibitor (ACEI), or angiotensin receptor blocker (ARB) should be considered to start an initial antihypertensive medication in non-black population. In black population with or without diabetes, initial antihypertensive treatment should include a thiazide-type diuretic or CCB.[2] Thus, CCB has become one of the most important initial agents for antihypertensive monotherapy. Furthermore, since CCBs have been proved not to increase the risk of coronary events and stroke,[3,4] CCBs appear to be a favorable choice for monotherapy as well as for combination with other agent classes in the treatment of hypertension and may provide specific benefits beyond BP lowering.[5] Nowadays, dihydropyridine (DHP) CCBs are one group of most frequently prescribed antihypertensive medications in China and other Eastern Asian countries.[1,6,7]

2 Voltage-gated Ca^{2+} channel classification and its blockers

Voltage-gated Ca^{2+} channels in excitable cells such as cardiac myocytes, smooth muscle cells and neurons play important roles in excitement-contraction, excitement-transmission, and/or excitement-transcription couplings.[8] High voltage-gated Ca^{2+} channels are pharmacologically classified into at least five different subclasses (L-, N-, P-, Q-, and R-type), the characteristics of which are determined by the pore-forming α1 subunit.[9] The subunits α1S ($Ca_V1.1$), α1C ($Ca_V1.2$), α1D ($Ca_V1.3$), and α1F ($Ca_V1.4$) expressed in different tissues form the long-lasting (L) type Ca^{2+} channels. These α subunits bind DHP and non-DHPs including phenylalkylamines and benzothiazepines with high affinity. Other subunits such as α1B ($Ca_V2.2$), α1A ($Ca_V2.1$), and α1E ($Ca_V2.3$) form N-, P/Q-, and R-type Ca^{2+} channels, respectively, which show low affinities for these drugs.[8]

The first study of CCBs was reported by Fleckenstein, *et al*.[10] in 1969, who demonstrated that the inhibition of Ca^{2+} influx using verapamil into cells led to compromised cardiac

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function. In addition to non-DHP such as verapamil and diltiazem, Fleckenstein, *et al*.[11] found that the prototype of the DHP CCB nifedipine was a calcium antagonist, exclusively blocking L-type Ca^{2+} channels.[12] Since then, DHPs had been considered as selective blockers for L-type channels only. Recent studies, however, have demonstrated that several DHP compounds also block N-type Ca^{2+} channels. These novel findings indicate not only that DHPs are no longer considered as selective L-type blockers in heart and arterial vessels, but also that some DHPs may exert their clinical actions by blocking other subtypes of Ca^{2+} channels such as N-type Ca^{2+} channels in certain specific tissues or organs like the brain and peripheral nervous system.

Apart from DHP CCBs, two other major groups of structurally unrelated non-DHP CCBs, the phenylalkylamines (typified by verapamil) and the benzothiazepines (typified by diltiazem), are considered as the L-type Ca^{2+} channel blockers.[8] Although for many years all these three groups of CCBs form the mainstay of hypertension therapy, DHP CCBs have more potent vasodilators than the non-DHP CCBs, whereas the latter have more marked negative inotropic effects than DHP CCBs.

New Ca^{2+} channel antagonists have been recently developed, especially in the DHP compounds that have considerable higher vascular selectivity, slower onset and longer duration of hypotensive action than their prototype compound nifedipine. Those more lipophilic DHP CCBs may reach their receptor, the L-type Ca^{2+} channel, via a membrane pathway with a two-step process: first, these drugs bind and accumulate in the membrane lipid bilayer, and then diffuse within the membrane to its receptor site. As of now, there are four generations of DHPs clinically available. The first-generation DHPs nifedipine and nicardipine have proven efficacy against hypertension. However, because of their rapid onset and short half-life time, these drugs are more likely to be associated with adverse effects mainly baroreflex activation and their clinical use have been significantly limited.[12] Clinical side effects by nifedipine on sympathetic activity are related not only to baroreflex responses, but also to nifedipine-induced renin release and angiotensin II-induced sympathoexcitation. The second generation is characterized with slow release nifedipine and short-acting preparations like benidipine, which allows better control of the therapeutic effect and a reduced baroreflex activation. The third-generation DHPs, amlodipine and barnidipine,[6,13,14] exhibit more lipophilic with stable pharmacokinetics and long-term actions, are less cardio-selective and, consequently, well tolerated in patients with heart failure and beneficial for those with chronic kidney diseases.[13,15] The fourth-generation cilnidipine (L/N-type CCB) and lercanidipine are highly lipophilic DHPs and now available to provide a real degree of therapeutic comfort in terms of stable activity, reduced adverse effects and a broad therapeutic spectrum, especially in myocardial ischemia and potentially in congestive heart failure.[16]

3 Central mechanisms for DHP CCBs to lower BP

Clinical application of CCBs is dependent upon their following features, (1) anti-hypertensive and vasodilatory effects; (2) the duration of their effects; (3) their profiles of end organ-protective effects; and (4) the incidence of adverse effects. DHP CCBs are widely used clinically in the treatment of hypertension, angina pectoris, and cerebrovascular diseases. Introduced in the 1960s, DHP CCBs have undergone several changes to optimize their efficacy and safety. As a first generation of DHP CCB, however, quick vasodilation and negative inotropic effects induced by its prototype nifedipine via block of L-type Ca^{2+} channels in vascular smooth muscular and the heart are not only the major mechanisms for its clinical hypotensive effects but also the primary issues for its side effects by increased arterial baroreflex. The quick vasodilation induced by nifedipine, followed by a baroreceptor-mediated increase in sympathetic tone, however, results in indirect cardio-stimulation. In addition, arterial baroreflex mechanisms by means of sympathetic activation induced by nifedipine tend to limit its acute antihypertensive response.[17] Therefore, clinical use of the prototype of nifedipine is thwarted by its increased sympathetic actions caused by rapid onset of its vasodilating effect and the resulting baroreflex activation and tachycardia, which has been a long-term clinical issue that quick peripheral arterial dilation is a significant hurdle for use of the prototype of nifedipine, especially for those patients with coronary artery disease.

Though the controlled release formula of nifedipine was also developed, tachycardia, and efficacy to lower BP remains a significant challenge.[18,19] As such, slowly-acting DHP L-type Ca^{2+} channel blockers with long duration of hypotensive actions have been developed in 1990s, with an assumption that a slow onset but a long-lasting hypotensive effect of DHP CCBs may prevent patients from suffering side effects such as arteriodilation-mediated increases in baroreflex and sympathetic activity and tachycardia.[15] Amlodipine and barnidipine in the third generation of DHP L-type Ca^{2+} channel blockers are clinical examples for this scenario.[15,20,21]

Dihydropyridine CCB amlodipine is the most frequently prescribed and efficacious in the monotherapy for hyperten-

sion compared with other classes of CCBs.[6] Amlodipine has a unique pharmacokinetic profile,[15,22] that is, longer t1/2 (36 h) than second-generation agents and a slower induction of vasodilator action, greater vascular/cardiac effect ratios without significant fluid retention.[20] In addition, amlodipine can act centrally to decrease sympathetic outflow. Compared with nifedipine, amlodipine is more lipophilic and a long-term treatment with it was accompanied without changes or even modest decreases in plasma norepinephrine (NE).[15] However, studies in hypertensive patients show that while long-term treatment with the slow released formula of amlodipine lowered BP, it did not significant change NE and sympathetic nerve activity.[23]

Also, barnidipine is of interest as a relative new third generation DHP Ca^{2+} antagonist because it exerts a slow onset but strong and long-lasting vasodilatory effect without baroreflex activation in clinical practice.[13,15,24] While it has preferential effects on renal and mesenteric vasodilation,[25] barnidipine also significantly decreased both BP and plasma NE levels,[24] indicating that it lowered the BP by sympathoinhibition possibly via the central nervous system. Thus, it is evident that advantages of these two long-acting DHP CCBs are their pharmacokinetics with a slow onset of hypotensive actions without increase in sympathetic activities if low doses are applied for a long-term chronic treatment, due to their more lipophilic property allowing them easily to cross the blood-brain barrier (BBB). Furthermore, because of good tolerance, these medications could be taken for a long term.

DHP CCBs can lower blood pressure when administered directly into the brain.[26,27] However, even in the newly developed DHP compounds, significantly different actions on BP, reflex heart rate, end organs and renal sympathetic activities have been reported. Despite their central actions revealed above, pharmacological mechanisms of DHP CCBs on the subtypes of voltage-gated L-type Ca^{2+} channels, especially the central $Ca_V1.2$ and $Ca_V1.3$ subtype Ca^{2+} channels and neurotransmission, have yet to be determined.

4 Anti-hypertensive actions of dual L/N-type Ca^{2+} channel blockers

N-type Ca^{2+} channels are abundantly found in the nervous system particularly in peripheral and central sympathetic nerve endings and deeply involved in the fast release of norepinephrine and other neurotransmitters.[9,28] Inhibition of N-type Ca^{2+} channels in both peripheral and central nervous systems may provide a new strategy for the treatment of hypertension and other cardiovascular diseases. To ensure efficient coupling of Ca^{2+} influx to rapid vesicle release, N-type Ca^{2+} channels must be localized with the active zones of presynaptic nerve terminals.[8] Pharmacologically, N-type Ca^{2+} channels are typically inhibited by the selective peptidergic blocker ω-conotoxin GIVA.[9,28]

Recently, a highly lipophilic dihydropyridine compound cilnidipine, which belongs to the fourth-generation DPH CCB, is a dual L/N-type Ca^{2+} channel blocker used for hypertensive patients in Japan. This drug inhibits sympathetic N-type Ca^{2+} channels as well as the vascular L-type Ca^{2+} channels. It has been reported that cilnidipine reduces excessive excitation of the sympathetic nervous system and the release of norepinephrine from sympathetic nerve endings, and consequently suppresses reflective tachycardia and stress-induced BP elevation, which is more efficient than amlodipine.[29–31]

Cilnidipine also leads to less activation of the renin-angiotensin system (RAS) than amlodipine,[32] and thus, is expected to play a superior role in organ protection. While the L/N-type CCB cilnidipine suppresses renal injury in hypertensive patients, the L-type CCB amlodipine could not do so.[32] Furthermore, cilnidipine has excellent tolerance,[33] and therefore is able to resolve amlodipine-associated edema in hypertensive patients.[33] Cilnidipine is more beneficial than amlodipine in combined treatment for hypertensive patients with kidney disease and significant proteinuria who were also receiving treatment with a RAS inhibitor. The beneficial effect of cilnidipine might be attributable to the inhibition of renal sympathetic nerve activity, aldosterone secretion,[34] and reduction of glomerular hypertension (because of the vasodilation of efferent arterioles), which are consequences of the N-type calcium channel blockade. Because cilnidipine, which has marked lipophilicity, also showed significantly higher antioxidant activity in cultured human mesangial cells than did amlodipine and is associated with lower incidence of pedal edema,[35] it is therefore hypothesized that cilnidipine might exert its renoprotective effects by suppressing oxidative stress.

Since L-type Ca^{2+} channels are present primarily on afferent arterioles, the inhibition of these channels causes dilation of only afferent arterioles, resulting in an elevation of glomerular pressure. However, N-type Ca^{2+} channels, which are located in sympathetic nerve endings, control both afferent and efferent arterioles, thus resulting in well-balanced dilation of both arterioles.

5 DHP CCBs used for elderly patients with hypertension

DHP CCBs are also effective in the treatment of hypertension in the elderly.[19,36] As the aging population is rapidly

Table 1. Summary of the antihypertensive actions, pharmacokinetics and side effects of four generations of DHP CCBs.

DHP CCBs	First generation	Second generation	Third generation	Fourth generation
Antihypertensive actions:				
L-type Ca channel block	+++	+++	++	++
N-type Ca channel block	–	–	–	+
Onset	Rapid	Gradual	Slow	Slow
Strength	Strong	Strong	Strong	Strong
Duration	Short-acting	Moderate	Long-lasting	Long-lasting
Renoprotection	–	–	+	++
Vasodilation	+++	+++	+++	+++
Sympathoexcitation	+++	++	+	–
Blood norepinephrine	↑	↑	–	–
Tolerated	Badly	Badly	Well	Well
Pharmacokinetics				
Half-life, h	2	7	10–36	7.5–10
Lipophilic	+	+	++	+++
Side-effects				
Hypotension	+	+	+	+
Tachycardia	+++	++		–
Headache	+++	++	+	
Flushing	+++	++	+	+
Edema	++	++	+	–

CCB: calcium channel blocker; DHP: dihydropyridine. +: strong; ++: very strong; +++: strongest.

increasing, mainly due to medical advances and the control of chronic diseases, more and more attention has been paid to pharmacological management of hypertension in the geriatric patient using CCBs, since their long-term benefits as antihypertensive medications have been proved to prevent both cerebral and cardiac infarctions in the elderly. Based upon *in vitro* and *in vivo* experimental evidence, intracellular free Ca^{2+} accumulation plays a dominant role in age-related arterial stiffness, which are critically involved in hypertension and myocardia/brain ischemia, and cell death. CCBs have been shown to be effective in the treatment of hypertension in the elderly. CCBs block L-type Ca^{2+} channels, with the long-acting or latest generation dihydropyridines being the most effective of this group.

In geriatric hypertensive population, the most common drug classes prescribed were CCBs (37%) and ACE inhibitors (21%). The safety in the use of CCBs has been demonstrated for antihypertensive therapy in the elderly, with a level of effectiveness similar to other widely used drugs. Furthermore, favorable effects of CCBs on containing mental deterioration and neuroprotection in the elderly by DHP CCBs as a single class were also reported.[37]

In addition, the geriatric patients with chronic kidney disease are also at a high risk of hypertension and other cardiovascular diseases. New generation DHP CCB barnidipine is known to have both potent antihypertensive and renoprotective effects with anti-oxidative and neutral tolerability properties, especially when used in combination treatment with ACEIs or ARBs in geriatric hypertensive patients with chronic kidney diseases.

6 Perspectives and conclusion

Approaches for the pharmacological treatment of hypertension have varied widely over the modern medical history. Since 2014, the CCB, especially the DHP CCB, has been officially recommended by JNC 8 as an initial mono-therapy for the treatment of hypertension. The third and fourth generations of DHP CCBs have been proved to be effective and well tolerated antihypertensive agents without baroreflex activation in young and elderly hypertensive patients or in those patients with cardiovascular and/or renal complications. The concept with regard to the pharmacological mechanism underlying clinical efficacy by DHP CCBs has also been gradually updated by accumulating evidence from their traditional peripheral hypotensive actions to the involvement of the central pre-sympathetic neurons. Based upon the evidence in clinical trials on blood pressure, target organ protection, especially cardiovascular prevention in Eastern Asians who have relatively high salt intake, third and fourth generations of DHP CCBs could be recommended as preferred medications in the management of hypertension in this area.

References

1 Jarari N, Rao N, Peela JR, *et al*. A review on prescribing patterns of antihypertensive drugs. *Clin Hypertens* 2015; 22: 7.

2 Armstrong C, Joint National C. JNC8 guidelines for the management of hypertension in adults. *Am Fam Physician* 2014; 90: 503–504.

3 Mancia G, Fagard R, Narkiewicz K, *et al*. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2013; 34: 2159–2219.

4 Verdecchia P, Reboldi G, Angeli F, *et al*. Angiotensin-converting enzyme inhibitors and calcium channel blockers for coronary heart disease and stroke prevention. *Hypertension* 2005; 46: 386–392.

5 Kjeldsen SE, Aksnes TA, Ruilope LM. Clinical implications of the 2013 ESH/ESC hypertension guidelines: targets, choice of therapy, and blood pressure monitoring. *Drugs in R&D* 2014; 14: 31–43.

6 Wang JG, Kario K, Lau T, *et al*. Use of dihydropyridine calcium channel blockers in the management of hypertension in Eastern Asians: a scientific statement from the Asian Pacific Heart Association. *Hypertens Res* 2011; 34: 423–430.

7 Mori H, Ukai H, Yamamoto H, *et al*. Current status of antihypertensive prescription and associated blood pressure control in Japan. *Hypertens Res* 2006; 29: 143–151.

8 Hess EJ, Jen JC, Jinnah HA, Benarroch EE. Neuronal voltage-gated calcium channels: brief overview of their function and clinical implications in neurology. *Neurology* 2010; 75: 937; author reply 937–938.

9 Tsien RW, Ellinor PT, Horne WA. Molecular diversity of voltage-dependent Ca2+ channels. *Trends Pharmacol Sci* 1991; 12: 349–354.

10 Fleckenstein A, Tritthart H, Flackenstein B, *et al*. [A new group of competitive divalent Ca-antagonists (iproveratril, D 600, prenylamine) with potent inhibitory effects on electromechanical coupling in mammalian myocardium]. *Pflugers Arch* 1969; 307: R25. [Article in German].

11 Fleckenstein A, Tritthart H, Doring HJ, Byon KY. [BAY a 1040--a highly potent Ca^{++} antagonistic inhibitor of electro-mechanical coupling processes in mammalian myocardium]. *Arzneimittelforschung* 1972; 22: 22–33. [Article in German].

12 Coca A, Mazon P, Aranda P, *et al*. Role of dihydropyridinic calcium channel blockers in the management of hypertension. *Expert Rev Cardiovasc Ther* 2013; 11: 91–105.

13 Malhotra HS, Plosker GL. Barnidipine. *Drugs* 2001; 61: 989–996; discussion 97–98.

14 Liau CS. Barnidipine: a new calcium channel blocker for hypertension treatment. *Expert Rev Cardiovasc Ther* 2005; 3: 207–213.

15 van Zwieten PA. The pharmacological properties of lipophilic calcium antagonists. *Blood Press Suppl* 1998; 2: 5–9.

16 Chandra KS, Ramesh G. The fourth-generation Calcium channel blocker: cilnidipine. *Indian Heart J* 2013; 65: 691–695.

17 Kiowski W, Erne P, Bertel O, *et al*. Acute and chronic sympathetic reflex activation and antihypertensive response to nifedipine. *J Am Coll Cardiol* 1986; 7: 344–348.

18 Shimamoto K, Kimoto M, Matsuda Y, *et al*. Long-term safety and efficacy of high-dose controlled-release nifedipine (80 mg per day) in Japanese patients with essential hypertension. *Hypertens Res* 2015; 38: 695–700.

19 Ruzicka M, Coletta E, Floras J, Leenen FH. Effects of low-dose nifedipine GITS on sympathetic activity in young and older patients with hypertension. *J Hypertens* 2004; 22: 1039–1044.

20 Toal CB, Meredith PA, Elliott HL. Long-acting dihydropyridine calcium-channel blockers and sympathetic nervous system activity in hypertension: a literature review comparing amlodipine and nifedipine GITS. *Blood Press* 2012; 21 Suppl 1: S3–S10.

21 Rossetti G, Pizzocri S, Brasca F, *et al*. Antihypertensive effect of barnidipine 10 mg or amlodipine 5 to 10 mg once daily in treatment-naive patients with essential hypertension: A 24-week, randomized, open-label, pilot study. *Curr Ther Res Clin Exp* 2008; 69: 192–206.

22 Burges R, Moisey D. Unique pharmacologic properties of amlodipine. *Am J Cardiol* 1994; 73: 2A–9A.

23 Binggeli C, Corti R, Sudano I, *et al*. Effects of chronic calcium channel blockade on sympathetic nerve activity in hypertension. *Hypertension* 2002; 39: 892–896.

24 Argenziano L, Izzo R, Iovino G, *et al*. Distinct vasodilation, without reflex neurohormonal activation, induced by barnidipine in hypertensive patients. *Blood Press Suppl* 1998; 1: 9–14.

25 Janssen BJ, Kam KL, Smits JF. Preferential renal and mesenteric vasodilation induced by barnidipine and amlodipine in spontaneously hypertensive rats. *Naunyn Schmiedebergs Arch Pharmacol* 2001; 364: 414–421.

26 Huang BS, Leenen FH. Sympathoinhibitory effects of central nifedipine in spontaneously hypertensive rats on high versus regular sodium intake. *Hypertension* 1999; 33: 32–35.

27 Huang BS, Murzenok PP, Leenen FH. Sympathoinhibitory and depressor responses to long-term infusion of nifedipine in spontaneously hypertensive rats on high-salt diet. *J Cardiovasc Pharmacol* 2000; 36: 704–710.

28 Simms BA, Zamponi GW. Neuronal voltage-gated calcium channels: structure, function, and dysfunction. *Neuron* 2014; 82: 24–45.

29 Koike Y, Kawabe T, Nishihara K, *et al*. Cilnidipine but not amlodipine suppresses sympathetic activation elicited by isometric exercise in hypertensive patients. *Clin Exp Hypertens* 2015; 37: 531–535.

30 Takahara A. Cilnidipine: a new generation Ca channel blocker with inhibitory action on sympathetic neurotransmitter release. *Cardiovasc Ther* 2009; 27: 124–139.

31 Ogura C, Ono K, Miyamoto S, *et al*. L/T-type and L/N-type calcium-channel blockers attenuate cardiac sympathetic nerve activity in patients with hypertension. *Blood Press* 2012; 21: 367–371.
32 Konoshita T, Makino Y, Kimura T, *et al*. A new-generation N/L-type calcium channel blocker leads to less activation of the renin-angiotensin system compared with conventional L type calcium channel blocker. *J Hypertens* 2010; 28: 2156–2160.
33 Shetty R, Vivek G, Naha K, *et al*. Excellent tolerance to cilnidipine in hypertensives with amlodipine - induced edema. *N Am J Med Sci* 2013; 5: 47–50.
34 Tanaka M, Sekioka R, Nishimura T, *et al*. Effects of cilnidipine on sympathetic nerve activity and cardiorenal function in hypertensive patients with type 2 diabetes mellitus: association with BNP and aldosterone levels. *Diabetes Res Clin Pract* 2014; 106: 504–510.
35 Adake P, Somashekar HS, Mohammed Rafeeq PK, *et al*. Comparison of amlodipine with cilnidipine on antihypertensive efficacy and incidence of pedal edema in mild to moderate hypertensive individuals: A prospective study. *J Adv Pharm Technol Res* 2015; 6: 81–85.
36 Bucci C, Mamdani MM, Juurlink DN, Tu JV. Dihydropyridine calcium channel blockers and cardiovascular outcomes in elderly patients: a population-based study. *Can J Cardiol* 2008; 24: 629–632.
37 Chen GJ, Yang MS. The effects of calcium channel blockers in the prevention of stroke in adults with hypertension: a meta-analysis of data from 273,543 participants in 31 randomized controlled trials. *PloS one* 2013; 8: e57854.