Inhibitory Effect of Antihypertensive Drugs on Calcineurin in Cardiomyocytes

Gus Q. Zhang¹, Zhiming Zhu² and Weiguo Zhang³

Calcineurin is a Ca²⁺–calmodulin-dependent serine/threonine phosphatase (also known as PP2B). Upon being activated, calcineurin dephosphorylates nuclear factor of activated T cells (also known as NFAT, a transcriptional factor). The dephosphorylated NFAT translocates into the nucleus and turns on interleukin-2 (IL-2) gene expression in T cells¹–³ (Figure 1). Calcineurin is abundant in lymphocytes and neurons, and it has also been found in cardiomyocytes.³–⁷

In recent years, a handful of research papers have presented experimental evidence that angiotensin-converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB), and calcium channel blocker (CCB, or calcium antagonist), which are all antihypertensive drugs, can inhibit calcineurin in myocardium.⁸–¹³ These investigators found that the antihypertensive agents prevented or attenuated cardiac hypertrophy, caused by various forms of hypertension in rodent models, such as surgical hypertension (abdominal aortic banding and 2-kidney 1-clip: 2K1C), salt-sensitive hypertension (Dahl rats), genetic hypertension (spontaneously hypertensive rats: SHR), and pulmonary hypertension (chronic hypoxia); while at the same time, cardiac calcineurin was concomitantly inhibited (see Table 1, which summarizes these studies). There is one exception though, where ACEI increased calcineurin protein expression in Dahl salt-sensitive rats in heart failure stage.¹⁴

Because myocardial calcineurin, when activated by mechanical and chemical stimulation, is believed to be one of several intracellular signal transduction pathways that cumulate in cardiac hypertrophy,¹⁵ some investigators attribute the antihypertrophic effect of these drugs to the calcineurin inhibitory effect seen in their experiments. Although these novel findings are indeed interesting, some more questions have arisen from these studies and need to be addressed.

CAN ACEI, ARB, AND CCB INHIBIT CALCINEURIN IN CARDIAC MUSCLE?

It is conceptually very difficult to understand and reconcile that both prohypertensive drugs, i.e., cyclosporine A (CsA) and tacrolimus (FK506), and antihypertensive drugs, i.e., ACEI, ARB, and CCB, can cause calcineurin inhibition. On the one hand, it has been repeatedly shown in numerous experimental and clinical investigations that conventional calcineurin inhibitors, CsA and FK506, are prohypertensive.¹⁶,¹⁷

In fact, a considerable number of patients who are on calcineurin inhibitors develop hypertension, therefore requiring antihypertensive drug therapy to control their high blood pressure.¹⁸ Two forms of hypertension by calcineurin inhibitors have been described. Acutely, the blood pressure–raising effect is largely mediated by a neural mechanism: calcineurin inhibition blocks the dephosphorylation of synapsin (a small vesicle associated phosphoprotein) in visceral afferent nerve endings from subdiaphragmatic area, stimulating these sensory nerves and causing reflex sympathetic activation and neurogenic (adrenergic) vasoconstriction.¹⁶,¹⁷ Chronically, the hypertension is multifactorial: in addition to calcineurin–synapsin–sympathetic pathway, renal (e.g., renal sodium retention) and vascular (e.g., increased expression of endothelin and endothelin receptors) mechanisms are all likely to play important roles.³,¹⁸ Since the clinical introduction of CsA in the early 1980s, calcineurin inhibitors have actually emerged as

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a new major cause of secondary hypertension in organ transplant recipients. On the other hand, these so-called newer calcineurin inhibitors (ACEI, ARB, and CCB) were originally designed, tested, and administered as antihypertensive drugs. Through two distinctive mechanisms, (i) interrupting the renin–angiotensin–aldosterone system by either inhibiting ACE activity or blocking angiotensin II type 1 (AT1) receptors and (ii) blocking calcium influx across the membrane of vascular small muscle cells, these classes of drugs cumulate in relaxation of vascular smooth muscle, therefore reducing peripheral arterial resistance and blood pressure. Thus, there exists an evident paradox: the immunosuppressive drugs (CsA and FK506) are prohypertension and anticalcineurin, whereas the antihypertensive drugs (ACEI, ARB, and CCB) are antihypertension and anticalcineurin (Figure 2).

Solving the puzzle may depend on discovering whether there is a cross-talk between the signaling pathway of antihypertensive drugs that mediates vascular smooth muscle relaxation and the signaling pathway of calcineurin–NFAT. It has been shown that heat shock proteins (HSPs) are components of the steroid receptor complex. After binding with aldosterone, HSPs are released into the cytosol, where they increase calcineurin activity in renal cortical collecting ducts and connecting tubules through a transcription-independent pathway in vitro. Both ACEI and ARB block the renin–angiotensin–aldosterone system, therefore reducing aldosterone formation, which could explain calcineurin inhibition. Mineralocorticoid receptors/HSPs are also expressed in the heart. It has been recently discovered that in cultured cardiomyocytes, aldosterone mediates a cascade of mitochondrial apoptotic responses, which are partially blocked by nifedipine and calcineurin inhibitors. In uninephrectomized rats treated with high salt diet and aldosterone, calcineurin mRNA expression was upregulated. However, it is unknown whether the nonepithelial effect of aldosterone stimulates cardiac calcineurin activity in clinically relevant models in vivo. It is possible that hypertrophic stimulus such as volume and pressure overload activates multiple, possibly redundant signal transduction pathways leading to ventricular hypertrophy.

There is a technical concern about calcineurin activity assay and its interpretation in these studies. As pointed out

Table 1 | Effect of ACEI, ARB, and CCB on cardiac hypertrophy and cardiac calcineurin expression

<table>
<thead>
<tr>
<th>Rat model</th>
<th>Drug</th>
<th>BP</th>
<th>Hypertrophy</th>
<th>Calcineurin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic banding8</td>
<td>Perindopril</td>
<td>NA</td>
<td>↓ (LV)</td>
<td>↓ (protein and activity)</td>
</tr>
<tr>
<td>Aortic banding8</td>
<td>Irbesartan</td>
<td>NA</td>
<td>↓ (LV)</td>
<td>↓ (protein and activity)</td>
</tr>
<tr>
<td>Hypoxia9</td>
<td>Amlodipine</td>
<td>NA</td>
<td>↓ (RV)</td>
<td>↓ (mRNA)</td>
</tr>
<tr>
<td>Dahl salt-sensitive10</td>
<td>Candesartan</td>
<td>←</td>
<td>↓ (LV)</td>
<td>↓ (activity and mRNA)</td>
</tr>
<tr>
<td>Renovascular hypertensive11</td>
<td>Perindopril</td>
<td>↓</td>
<td>↓ (LV)</td>
<td>↓ (protein and activity and mRNA)</td>
</tr>
<tr>
<td>Spontaneously hypertensive12</td>
<td>Nifedipine</td>
<td>↓</td>
<td>↓ (LV)</td>
<td>↓ (activity)</td>
</tr>
<tr>
<td>Uninephrectomized and aldosterone13</td>
<td>Losartan</td>
<td>↓</td>
<td>↓ (Heart)</td>
<td>↓ (protein and activity and mRNA)</td>
</tr>
<tr>
<td>Dahl salt-sensitive14</td>
<td>Temocapril</td>
<td>←</td>
<td>↓ (LV)</td>
<td>↑ (protein)</td>
</tr>
</tbody>
</table>

In Hayashida’s study, the rats were in heart failure stage. In Jiang’s study, the combination of ACEI and ARB resulted in additional reduction in LV hypertrophy, but no additional calcineurin inhibition.

←, no change; ↓, decreased; ↑, increased; LV, left ventricle; NA, not available from the paper presented; RV, right ventricle.
previously,\textsuperscript{3,4} calcineurin activity is often measured by a cell-lysed assay. In the presence of exogenous Ca\textsuperscript{2+} and calmodulin, it actually measures the maximal calcineurin activity regardless of whether the activity is increased, decreased, or unchanged \textit{in vivo}.\textsuperscript{4} In other words, the assay reflects the amount of calcineurin available for activation, but does not represent the amount of actually activated calcineurin. Without better methodology, the validity of any calcineurin data is undermined.\textsuperscript{3}

**IS THE ANTIHYPERTROPHIC EFFECT OF ACEI, ARB, AND CCB MEDIATED BY BLOOD PRESSURE REDUCTION OR CALCINEURIN INHIBITION?**

When validating these novel findings, it is important to determine whether the relationship between calcineurin inhibition and antihypertrophic effect of these antihypertensive drugs is causal or merely correlational. Although the causal relationship was proposed by some of these investigators, these studies were basically observational and lacked further exploration into the potential mechanism(s).

The development of ventricular hypertrophy is one of the consequences of persistent elevation of blood pressure,\textsuperscript{26} including high blood pressure caused by calcineurin inhibitors.\textsuperscript{27–37} Although it is known that blood pressure reduction can prevent, and to a certain degree, regress cardiac hypertrophy, it is still not completely clear whether ACEI and ARB provide any blood pressure–independent protection against the development of cardiac hypertrophy, particularly in clinical settings.\textsuperscript{38,39} If they do, would calcineurin inhibition constitute a potential target?

**CAN OTHER CLASSES OF ANTIHYPERTENSIVE DRUGS INHIBIT CALCINEURIN IN THE HEART?**

It is unknown whether the effect of calcineurin inhibition is limited to ACEI, ARB, and dihydropyridine CCB or can be extrapolated to all classes of antihypertensive drugs, including diuretics, central and peripheral sympathetic inhibitors, endothelin receptor antagonists, other direct vasodilators, and so forth. The modes of actions of antihypertensive drugs are quite diverse: some reduce systemic volume (preload), some reduce peripheral resistance (afterload), and some reduce cardiac contractility and output. A comparison among different classes of antihypertensive drugs can help in determining whether blood pressure reduction alone constitutes a hemodynamic trigger for suppressing cardiac calcineurin activity. As mentioned already, some previous investigations (particularly in animal experiments) have demonstrated that ARB and ACEI may have blood pressure–independent effects on cardiac hypertrophy.\textsuperscript{3,40} However, amlodipine, which was used in one of the studies and shown to inhibit cardiac calcineurin, is a selective L-type CCB. Unlike other dihydropyridine CCBS as well as phenylalkylamine and benzothiazepine, amlodipine does not have any pronounced antagonistic actions on calcium channels in cardiac muscle and its antihypertrophic effect should result primarily from direct peripheral arterial vasodilatation that leads to blood pressure reduction.\textsuperscript{41,42} This indicates that in the case of amlodipine, the antihypertrophic effect and any calcineurin inhibition effect (if it exists) may be due to blood pressure reduction alone.

Needless to say, blood pressure is not only controlled by these synthetic chemical drugs, but also affected by nonpharmacological interventions such as lifestyle changes.\textsuperscript{43,44} Would lifestyle modification (such as salt reduction, DASH diet, and body weight control) affect calcineurin as well?

**IS CALCINEURIN IN OTHER TISSUES AND ORGANS ALSO INHIBITED BY ANTIHYPERTENSIVE DRUGS?**

It is also unknown whether the inhibition of calcineurin by these antihypertensive drugs is specifically limited to cardiac muscle or is a generalized phenomenon that exists in other organs and systems. If it is in fact a generalized phenomenon, calcineurin in neural tissue and T cells could also be inhibited by antihypertensive drugs, and the expression of cytokines such as IL-2 from T cells might be reduced to some extent.\textsuperscript{2,45} The consequence is that patients on antihypertensive drugs might be subject to some potential risk of immunosuppression and immune deficiency, although available longer-term clinical trials with antihypertensive regimens have not yielded any evidence to support such possibility. It is not known whether such an effect is discernable, has been overlooked, or is simply nonexistent. On the other hand, because cyclosporine and FK506 cause hypertension, antihypertensive drugs have to be...
often administered to control clinical hypertension secondary to the use of calcineurin inhibitors in allograft recipients and patients with autoimmune disease. If these antihypertensive drugs do produce a generalized calcineurin inhibitory effect, then the dosage of calcineurin inhibitors could be reduced when antihypertensive drugs and calcineurin inhibitors are administered simultaneously to avoid excessive immunosuppression. Clinically, this would be beneficial as calcineurin inhibitors are relatively costly and have side effects, such as neural, renal, and cardiovascular toxicities, as well as increased infection and carcinogenesis effects that are generally not associated with any recommended antihypertensive drugs at the routine dose range. Once the relationship between antihypertensive drugs and general calcineurin inhibition is confirmed, it may have some impact on immunosuppressive therapy. In current clinical practice, antihypertensive drugs and calcineurin inhibitors are often coadministered.

Although antihypertensive therapy has conclusively reduced cardiovascular morbidity and mortality, a significant reduction of all-cause mortality has not been shown in all studies. The carcinogenic effect of some antihypertensive drugs (mainly diuretics) has been a source of concern for some time, although it is largely unproven and mechanistically unexplained. Because malignancy risk increases in subjects receiving immunosuppressive drugs CsA and FK506, if antihypertensive drugs modulate nerve activity as well; therefore, in vitro experiments such as cell culture or ex vivo experiments such as isolated working heart may help in elucidating whether the observed calcineurin inhibitory effect is in fact from cardiomyocytes or there is also a neuronal effect involved.

In summary, the new association between antihypertensive drugs and cardiac calcineurin appears perplexing at the moment. It will be scientifically interesting and clinically pertinent to have such claim empirically substantiated. In addition to some fundamental questions already asked, further experimental investigations may also ask, for instance, whether there is a dose–response relationship between ACEI, ARB, and CCB and calcineurin inhibition, whether there is an additive or synergistic effect on calcineurin inhibition from a combination of two or more classes of antihypertensive drugs, and whether the calcineurin inhibitory effect of these antihypertensive drugs is also seen in normotensive subjects who do not have cardiac hypertrophy. These questions, and the ones raised earlier in this review, must be addressed to understand fully the new mechanism (including pharmacokinetics and pharmacodynamics) of calcineurin inhibition by antihypertensive drugs, and to translate the basic research to clinical pharmacology and patient management.

Disclosure: W.Z. is now working for Nestle R&D Beijing, China/Nestle Research Center, Lausanne, Switzerland.
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