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TRANSLATIONAL PERSPECTIVE

The CNP/NPR-B/cGMP Axis is a Therapeutic Target in Calcific Aortic Stenosis

David W.J. Armstrong, MD, PhD,[a] Deepak K. Gupta, MD, MSCI,[a] Brian R. Lindman, MD, MSCI,[a,b] W. David Merryman, PhD[c]

Calcific aortic stenosis (CAS) is a disease for which there is no effective medical therapy. The natriuretic peptide system (NPS) is well characterized in the context of heart failure, but it is increasingly clear that the NPS plays a role in the development of CAS. The overwhelming majority of clinical data regarding the NPS in CAS focuses on natriuretic peptides in risk stratification, but there are human and experimental data suggesting C-type natriuretic peptide (CNP) / natriuretic peptide receptor (NPR) B (also known as guanylate cyclase B or GC-B) signaling plays a direct role in the development of CAS (**Figure 1**). However, it remains to be shown that augmentation of the CNP pathway is a viable therapeutic strategy in CAS.

CNP REGULATES EXTRACELLULAR MATRIX AND FIBROSIS

CNP has been shown to be the most potent antifibrotic natriuretic peptide compared to atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). CNP also exerts direct effects on fibroblast, reducing fibroblast proliferation and collagen production in vitro, possibly via cyclic guanosine monophosphate (cGMP)-dependent and cGMP-independent mechanisms. The unique properties of CNP on regulating fibroblasts and extracellular matrix suggest that CNP may play a role in regulating matrix biology in aortic valve (AV) tissue.

DEFICIENCY OF CNP IN CAS

Initial reports identifying a possible causal role for the NPS in CAS come from basic studies in animal models. Using microarray analysis to examine gene expression in healthy porcine AVs, it was reported that *NPPC* (the gene encoding CNP) was more highly expressed on the disease-protected ventricular side of the valve (1). Immunostaining confirmed expression of CNP in freshly isolated valvular intestinal cells (VICs). In the fibrosa of sclerotic AVs there was also evidence of increased VIC myofibroblast differentiation (elevated smooth muscle alpha-actin immunostaining) associated with reduced or absent CNP (2). The first report of NPS expression in human AV tissue was from Finland in 2007 (3). In severe CAS there was significantly reduced expression of *NPPC*, *FURIN* (the gene encoding furin, which converts proCNP to biologically active CNP), *NPR1* (the gene encoding NPR-A, the receptor for ANP and BNP) and *NPR2* (the gene encoding NPR-B, the receptor for CNP), without changes in *NPPA* or *NPPB* (the genes encoding ANP and BNP, respectively). Expression levels of *CORIN* (the gene encoding corin,

From the [a]Division of Cardiovascular Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA; [b]Structural Heart and Valve Center, Vanderbilt University Medical Center, Nashville, Tennessee, USA; and the [c]Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee, USA.

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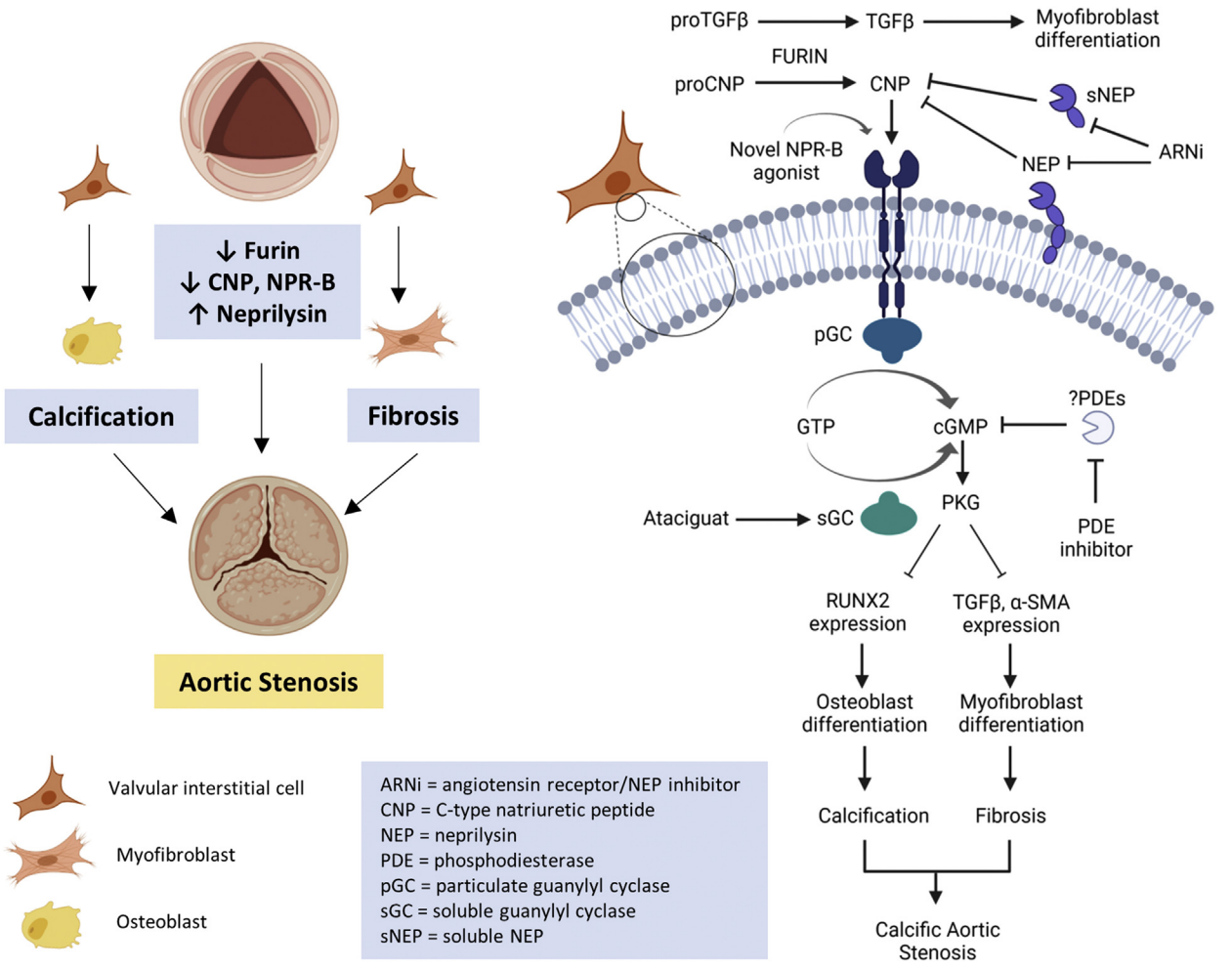
FIGURE 1 **The CNP/NPR-B/cGMP Axis in the Pathogenesis of Calcific Aortic Stenosis and Potential Therapeutic Targets**



The CNP/NPR-B/cGMP system is reduced in calcific aortic stenosis (CAS). Neprilysin (NEP), which degrades CNP, is increased in CAS. Collectively, these data suggest reduced CNP signaling may contribute to the development of CAS in humans. In mice, reduced CNP/NPR-B/cGMP signaling is associated with the development of CAS. Conversely, CNP administration prevents valvular interstitial cell transition to a myofibroblast/osteoblast phenotype *in vitro*. It is unknown if augmenting the CNP/NPR-B/cGMP pathway can attenuate the development of CAS *in vivo*. Potential strategies to augment CNP/NPR-B/cGMP include administering CNP analogs or novel NPR-B agonists, NEP inhibition, and phosphodiesterase inhibition. Although furin converts proCNP to biologically active CNP, it also converts proTGF-β to biologically active TGF-β, making furin a less attractive therapeutic strategy. NEP inhibitor or PDE inhibitor monotherapy may be ineffective in the presence of low CNP. Therefore, initial strategies should involve direct stimulation of NPR-B.

which converts proANP and proBNP to ANP and BNP, respectively) or *NPR3* (the gene encoding NPR-C) were also reduced (3). Immunohistochemistry in human AV with severe CAS showed that CNP was located in valvular endothelial cells and myofibroblasts, the latter being a component of VICs (3). Collectively, these data suggest that decreased CNP may contribute to or be a consequence of CAS, and that higher CNP may prevent CAS.

CNP DECREASES CHARACTERISTICS OF CAS IN VITRO

The first study reporting that augmentation of CNP prevents characteristics of CAS was in porcine VICs in vitro. Administration of CNP at supraphysiologic doses (0.1 to 100 nM) inhibited VIC calcification and myofibroblast and osteoblast differentiation and stiffening in vitro (2,4). CNP administration (0 to 100

nM) activated the natriuretic peptide receptor B (NPR-B)/cGMP/protein kinase G (PKG) pathway and inhibited transforming growth factor (TGF)-β-mediated proteoglycan synthesis in vitro (4). Use of specific NPR-B and NPR-C inhibitors showed that the antimyofibrogenic effect of CNP on porcine VICs was via the NPR-B/cGMP pathway rather than NPR-C.

DEFICIENCY IN CNP/NPR-B/cGMP/PKG SIGNALING IN MICE CONTRIBUTES TO THE PATHOGENESIS OF CAS

The most robust in vivo evidence confirming the role of the NPS in the pathogenesis of CAS has been reported in the *Npr2*$^{+/-}$ mouse model. There was a nearly 10% incidence of bicuspid aortic valve (all were right/noncoronary cusp fusion) in *Npr2*$^{+/-}$ mice compared to 0% incidence in wild-type mice.[4] Aged (8 to 10 months old) *Npr2*$^{+/-}$;*Ldlr*$^{-/-}$ mice exhibited increase AV leaflet calcification and fibrosis, which was accompanied by echocardiographic evidence of aortic stenosis all of which was worse in animals with bicuspid compared to tricuspid AVs (4). In AVs from *Npr2*$^{+/-}$;*Ldlr*$^{-/-}$ mice, there was evidence of decreased PKG activity, further suggesting the NPR-B/cGMP/PKG pathway is involved in the development of CAS (4). These data clearly show that impaired CNP/NPR-B/cGMP/PKG signaling contributes to the development of CAS in mice. However, the efficacy of augmenting the CNP/NPR-B/cGMP/PKG pathway to treat CAS has not been tested in vivo.

TRANSLATION OF CNP-BASED THERAPY FOR CAS

Options for augmenting the CNP/NPR-B/cGMP pathway include administration of exogenous CNP-based peptides, novel NPR-B agonists, neprilysin (NEP) inhibition, and phosphodiesterase (PDE) inhibition. Targeting FURIN (the convertase that activates CNP) is also a potential target, but FURIN is a ubiquitously expressed enzyme with multiples substrates, including the activation of pro-TGF-β to TGF-β.

Augmenting CNP/NPR-B/cGMP can be accomplished with CNP analogs or novel small molecules. C53 is a 53-amino acid intermediate of proCNP that has been shown to be resistant to NEP and exerts sustained antifibrotic actions in human cardiac and renal fibroblasts (5). However, the actions of C53 in VICs remains unknown. Other potential CNP-based therapies include Vosoritide (BioMarin Pharmaceuticals) and TransCon CNP. Vosoritide is a 39-amino acid analog of CNP that is approved for use in the European Union for children with achondroplasia. TransCon CNP (Ascendis Pharma) is a 38-amino acid CNP analog conjugated via a cleavable linker to a polyethylene glycol carrier molecule with substantially increased duration of action ($t_{1/2}$ = 90 hours) (6). TransCon CNP is currently under investigation for treatment of achondroplasia.

Sacubitril/valsartan is the only approved NEP inhibitor (combined with an angiotensin receptor blocker) currently indicated for patients with heart failure. Both *MME* (the gene encoding NEP) expression and NEP activity were increased in severe CAS in humans, and NEP was localized to valvular endothelial cells and myofibroblasts (7). Targeting NEP is a logical therapeutic objective given the fact that CNP is reduced in CAS, CNP is highly susceptible to degradation by NEP, and NEP is increased in CAS. Future studies are required to determine if NEP inhibition increases CNP in the circulation or AV tissue in animal models and humans with aortic stenosis. Retrospective analyses on the effect of sacubitril/valsartan on CAS may be possible from population studies. Time series analysis before and after the introduction of sacubitril/valsartan would be one method to determine if sacubitril/valsartan has an effect on incident CAS or the rates of intervention for CAS. Retrospective or prospective assessment the effect of sacubitril/valsartan on the progression of CAS in patients with mild to moderate aortic stenosis using serial echocardiographic data (transvalvular gradient, peak aortic velocity, valve area, or dimensionless orifice index) would be challenging given relatively slow progression of CAS and the potential confounding effect of reverse remodeling following initiation of sacubitril/valsartan (i.e., improved ejection fraction could increase peak aortic velocity for any given AV area). Patients with bicuspid valves represent the minority among patients with AS, but the more rapid progression of disease makes this an attractive population to test the hypothesis that sacubitril/valsartan can prevent or delay the progression of CAS.

Downstream inhibition of cGMP breakdown by PDE is a third approach. However, there are no reports describing the expression, activity, or basic biology of PDEs in AVs in humans or animal models. Despite the availability of numerous PDE inhibitors, addressing this considerable knowledge gap is obligatory before entertaining the use of PDEs in the treatment of CAS. Augmenting cGMP can also be accomplished through the parallel cGMP producing soluble guanylyl cyclase (sGC) pathway, which is beyond the scope of this perspective. However, the sGC/cGMP pathway is being tested in the trial Study Evaluating the Effects of Ataciguat (HMR1766) on Aortic Valve Calcification

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(CAVS), which randomized patients with mild to moderate CAS to the sGC activator Ataciguat versus placebo (NCT02481258). Preliminary data demonstrated significantly lower AV calcium by computed tomography in the Ataciguat arm. These data indicate that targeting cGMP signaling is a promising therapeutic target for CAS.

CONCLUSIONS

CNP/NPR-B/cGMP is decreased in humans and animal models of CAS. Data from in vitro studies have shown that CNP administration in VICs can prevent characteristics of CAS. However, future studies are required to determine if augmenting the CNP/NPR-B/cGMP pathway can prevent the development of CAS in vivo. Existing agents and innovate strategies targeting CNP/NPR-B/cGMP signaling provides the opportunity to develop medical therapy to prevent or attenuate the progression of CAS.

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ADDRESS FOR CORRESPONDENCE: Dr Merryman, Department of Biomedical Engineering, Vanderbilt University, Room 9445, MRB4 2213 Garland Avenue, Nashville, Tennessee 37232, USA. E-mail: david.merryman@vanderbilt.edu. Twitter: @merrymanlab.

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