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EDITORIAL COMMENT

Ancient Remedy for a Modern Disease

Will Celastrol Become a Treatment for Aortic Valve Stenosis?*

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Calcific aortic valve disease (CAVD) is characterized histologically by progressive aortic valve fibrocalcific changes that lead to stenosis of the valve and obstruction to blood flow. It is becoming more apparent that progression of aortic stenosis (AS) is not simply a disease of aging, but rather that its pathophysiology involves several active pathways, including lipid disorders such as accumulation of lipoprotein(a) and low-density lipoprotein particles, pro-oxidant processes such as accumulation of proinflammatory oxidized phospholipids that may lead to fibrosis, and procalcifying genes and proteins that lead to mineralization of the valve leaflets (1). Progression of AS generally occurs predictably, but there is wide clinical variability, in that there are both slow and fast progressors. Contemporary management of AS is limited to monitoring by echocardiography until stenosis becomes severe or symptomatic, at which time invasive valve replacement or implantation is performed. Approximately 12% of the population over 75 years of age has AS, and no effective medical therapies are currently available to prevent disease progression.

In this issue of *JACC: Basic to Translational Science*, Liu et al. (2) evaluated the role for celastrol, a naturally occurring pentacyclic triterpenoid compound that is derived from the roots of *Tripterygium wilfordii*, also known as the thunder god vine. Celastrol is a small molecule that has promiscuous pharmacologic activities (3) that include antioxidant properties via inhibition of NADPH oxidase-2 (Nox2), which is a major source of reactive oxygen species (ROS) and anti-inflammatory, anticancer, and antiobesity properties.

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In a series of experiments in human valve leaflets, cell culture studies in porcine valvular interstitial cells (VICs) and a vitamin D2/hypercholesterolemic (25000 IU/day/0.5% cholesterol-enriched chow) rabbit CAVD model treated for only 18 weeks, Liu et al. (2) convincingly demonstrated that celastrol has effects on aortic valve pathology that are worth pursuing in further studies for potential clinical translation. First, they showed that Nox2 proteins were significantly upregulated in human aortic valves with CAVD. Then, they demonstrated upregulation of Nox2, ROS production, and calcium nodule formation in VICs with osteogenic medium containing ascorbic acid, β-glycerophosphate sodium, and dexamethasone. In parallel, knockdown of endogenous Nox2 or celastrol treatment significantly inhibited GSK3β/β-catenin signaling, leading to attenuation of fibrogenic and osteogenic responses of VIC. Last, in the rabbit model, celastrol significantly reduced aortic valve ROS production, fibrosis, and calcification and improved aortic valve hemodynamics. It also led to less left ventricular dilatation and better preserved

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contractile function, although this was difficult to predict from the modest effects on the valve hemodynamics. These in vivo changes were quite modest, with attenuation of baseline peak jet velocities, which averaged ~1.3 m/s, by 22%. Overall, the authors demonstrated that ROS are a significant contributor to fibrocalcific changes in valve leaflets, and within the limitations of the current CAVD rabbit model of very early disease, that celastrol attenuated development of CAVD. However, Liu et al. (2) did not perform studies to assess whether celastrol affects preexisting CAVD, which would have been more clinically relevant.

How might celastrol affect CAVD? One plausible explanation is by directly antagonizing Nox2 mediated signaling pathways and ROS generation in the aortic valve, although the specific mechanisms were not directly assessed in this study. Consistent with this hypothesis, pharmacologic and genetic inhibition of Nox2 in VIC with celastrol and specific short hairpin RNA, respectively, attenuated calcification and expression of procalcific and pro-osteogenic Runx2 in vitro. Expectedly, adenovirus-mediated Nox2 overexpression in VICs resulted in increased calcification in vitro. Because treatment with the antioxidant SOD was insufficient to phenocopy the effect of Nox2 inhibition on VICs, celastrol likely mediates its effects via additional pathways, such as the GSK3B/B-catenin pathway proposed in this study.

These observations not only provide insights into the potential use of celastrol in AS, but also suggest viable targets with other compounds to inhibit Nox2 and its downstream pro-oxidant metabolites. For example, several studies have shown plasma levels of oxidized phospholipids on apolipoprotein B-100, which can be generated by ROS such as superoxide generated by Nox2, are associated with higher rate of progression of preexisting aortic stenosis (1). In addition, an antibody targeting oxidized phospholipids reduced echocardiographically determined aortic valve gradients in an *Ldlr*$^{-/-}$ mouse model (4).

Although celastrol is convincingly anticalcific in vitro, evaluation of potential therapies for CAVD is best suited for in vivo studies, and the authors should be commended for extending their study to a rabbit model of CAVD. However, preclinical CAVD models have only had modest success in reflecting human disease (5). CAVD risk is multifactorial disease, including advanced age, hypertension, hypercholesterolemia, elevated lipoprotein(a), metabolic syndrome and diabetes, and chronic kidney disease, which require modeling in vivo. In the present study, one limitation of this model is a concomitant reduction in total cholesterol and low-density lipoprotein cholesterol by ~50%. Multiple hypercholesterolemic animal models as well as patients with familial hypercholesterolemia develop CAVD, suggesting a causal role of hypercholesterolemia in the development of this disease. Thus, it cannot be excluded that this effect led to some of the salutatory effects of celastrol on valve pathology independent of or in addition to inhibition of Nox2. Whether celastrol will also offset other clinically relevant risk factors not present in this rabbit model remain to be determined. For example, lipoprotein(a) is a common and likely causal risk factor for CAVD in humans that is not endogenously expressed in rabbits (nor in other nonprimate models) (1). Although Liu et al. (2) demonstrated that there was no liver or renal toxicity with short-term celastrol administration, a full toxicologic assessment will also be important before translation to human trials.

As the population ages, developing medical therapies to prevent the development of CAVD is will be critically important, and cost-effective therapies will need to be directed toward preventing progression of disease. The early stage of CAVD, aortic sclerosis, has no hemodynamic consequences, but has a prevalence of 25% in individuals >65 years of age. The risk of progression from aortic sclerosis to AS is only approximately 2% per year. However, once AS is established, the vast majority of patients experience hemodynamic progression and are at risk of developing severe AS. Therefore, the true test of a clinically useful therapy would be one that effectively prevents progression of established AS and not simply development of CAVD. Statins, although effective at low-density lipoprotein cholesterol lowering, failed to show efficacy in preventing progression of mild-to-moderate AS in randomized clinical trials (6). Whether celastrol will live up to the promise of being the first effective medical therapy for AS depends on whether it can: 1) prevent progression of established AS; 2) be effective for patients who have risk factors other than hypercholesterolemia; and 3) demonstrate safety. Because of the lack of currently available medical therapies for AS and the significant unmet clinical need that will become even more urgent as our population ages, this study provides an impetus to further evaluate celastrol preclinically to assess its safety and efficacy in the hope that it can eventually enter clinical trials.

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