Nov 1, 2023

Dr. Julie Stacey
Editor-in-Chief *eBioMedicine*

Dear Dr. Stacey:

Attached please find our manuscript entitled “The SK4 channel allosteric blocker, BA6b9, reduces atrial fibrillation substrate in rats with reduced ejection fraction after myocardial infarction”, which we would like to submit for publication in *eBioMedicine*.

In this study, we present solid proof-of-concept evidence that long-term inhibition of calcium-activated SK4 K+ channels using a novel allosteric blocker of these channels (BA6b9) represents a promising new therapeutic strategy that targets the atrial overexpression of these channels in the setting of heart failure (HF) after myocardial infarction (MI). Moreover, this treatment dramatically inhibits the deleterious atrial remodeling and increased atrial fibrillation (AF) susceptibility that develops in this setting over time.

Atrial fibrillation (AF) and heart failure (HF) are the two most prevalent cardiovascular diseases of this century. AF is a progressive, age-related disease affecting millions of patients worldwide and is associated with severe complications such as thromboembolic events, impaired cognitive function, and increased mortality. HF occurs as frequently as AF and the co-existence of these two diseases is very common. Failure rates of AF therapy in HF patients are high, in large part because HF induces the structural remodeling of the atrial myocardium, which in turn impairs the electrical activation of the atria, predisposing HF patients to AF.

We previously identified a new druggable target in the heart in the form of the calcium-activated SK4 K+ channels, whose existence was previously overlooked in cardiac tissues ([1](#_ENREF_1" \o "Weisbrod, 2013 #18)). More recently, we demonstrated that the SK4 channel protein is widely expressed in the atria of rat and human hearts, with somewhat lower expression levels in the ventricles. In addition, we designed a novel allosteric blocker, BA6b9, intended to target the calmodulin-PIP2 binding domain, a previously untargeted region of SK4 channels, thereby leading to the potent and specific inhibition of these SK4 K+ channels ([2](#_ENREF_2" \o "Burg, 2022 #15)). SK4 K+ channels are also expressed in fibroblasts and macrophages, which, like atrial cardiomyocytes, possess the inflammatory signaling machinery that is activated during atrial remodeling and AF progression. We thus presumed that SK4 K+ channels are pro-arrhythmic and pro-inflammatory such that blocking them may prevent atrial remodeling and AF progression, especially in the post-MI setting.

In the present study, we examined the ability of BA6b9 to alter AF susceptibility and atrial remodeling in a HF rat model following MI. We found that the atrial levels of SK4 K+ channels were indeed markedly upregulated after MI, in conjunction with increased NLRP3 inflammasome expression, atrial connexin Cx43 lateralization, and collagen deposition. Treatment with BA6b9 attenuated all of these detrimental changes, and markedly inhibited susceptibility to AF, indicating that the BA6b9-mediated blockade of SK4 K+ channels not only favors rhythm control but can also inhibit structural remodeling, which is an activity that is highly desirable, particularly in the setting of HF. We believe that our findings should be of strong interest to many basic scientists and clinicians.

This manuscript is original, no part of it has been published previously, nor is any part of it under consideration for publication elsewhere. We have no conflict of interest to disclose. All authors have approved the written content and figures in the submitted manuscript.

Thank you for your consideration of this manuscript.

Sincerely,

Yoram Etzion

Bernard Attali

**References**

1. Weisbrod D, Peretz A, Ziskind A, Menaker N, Oz S, Barad L, et al. SK4 Ca2+ activated K+ channel is a critical player in cardiac pacemaker derived from human embryonic stem cells. Proc Natl Acad Sci U S A. 2013;110(18):E1685-94.

2. Burg S, Shapiro S, Peretz A, Haimov E, Redko B, Yeheskel A, et al. Allosteric inhibitors targeting the calmodulin-PIP2 interface of SK4 K(+) channels for atrial fibrillation treatment. Proc Natl Acad Sci U S A. 2022;119(34):e2202926119.