Journal of the American College of Cardiology
© 2013 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 62, No. 4, 2013
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2013.04.041

PRE-CLINICAL RESEARCH

Provocation of an Autoimmune Response to Cardiac Voltage-Gated Sodium Channel $Na_V1.5$ Induces Cardiac Conduction Defects in Rats

Sevil Korkmaz, PhD,*† Edgar Zitron, MD,†‡ Anna Bangert,†‡ Claudia Seyler, PhD,†‡ Shiliang Li, MD,*† Peter Hegedüs, MD,*† Daniel Scherer, MD,†‡ Jin Li, MD,†‡ Thomas Fink,†‡ Patrick A. Schweizer, MD,†‡ Evangelos Giannitsis, MD,†‡ Matthias Karck, MD,*† Gábor Szabó, MD, PhD,*† Hugo A. Katus, MD,†‡ Ziya Kaya, MD†‡

Heidelberg, Germany

Objectives This study sought to test the hypothesis that inducing an autoimmune response against the cardiac sodium channel ($Na_V1.5$) induces arrhythmias.

Background Sporadic evidence supports the concept that autoantibodies may cause cardiac arrhythmias but substantial experimental investigations using in vivo models have been lacking to date. The $Na_V1.5$ is essential for cardiac impulse propagation and its dysfunction has been linked to conduction disease.

Methods Rats were immunized with a peptide sequence derived from the third extracellular loop of the first domain of $Na_V1.5$. After 28 days, we evaluated in vivo both the electrical and mechanical parameters of cardiac function. Histopathology, myocardial gene and protein expression were assessed. Whole-cell patch-clamp was used to measure sodium current (I_{Na}) density in isolated cardiomyocytes.

Results $Na_V1.5$-immunized rats had high titers of autoantibodies against $Na_V1.5$. On ECG recording, $Na_V1.5$-immunized animals showed significantly prolonged PR-intervals. During Holter ECG-monitoring we observed repeated prolonged episodes of third-degree atrioventricular and sinoatrial block in every $Na_V1.5$-immunized animal, but not in controls. Immunization had no effect on cardiac function. In comparison to controls, myocardial $Na_V1.5$ mRNA and protein levels were decreased in immunized rats. I_{Na} density was reduced in cardiomyocytes incubated with sera from $Na_V1.5$-immunized rats and from patients with idiopathic atrioventricular block (AVB) in comparison to sera from respective controls. In patients with idiopathic AVB, we observed autoantibodies against $Na_V1.5$ that were absent in sera from healthy controls.

Conclusions Provocation of an autoimmune response against $Na_V1.5$ induces conductance defects probably caused by a reduced expression level and an inhibition of $Na_V1.5$ by autoantibodies, resulting in decreased I_{Na}. (J Am Coll Cardiol 2013;62:340–9) © 2013 by the American College of Cardiology Foundation

Cardiac arrhythmias contribute substantially to morbidity and mortality and have consequences that range from asymptomatic to life-threatening disorders. Among different pathophysiological mechanisms involved in arrhythmogenesis, a heterogeneous group of sporadic experimental and clinical studies have supported the hypothesis that autoantibodies may play a pathogenetic role in arrhythmogenesis (1). Various antiheart antibodies, including those targeting $beta_1$-adrenergic receptors, muscarinic M2-acetylcholine receptors, Na^+/K^+-ATPase, cardiac troponin I, and cardiac myosin heavy chain, can be detected in the serum of patients with dilated cardiomyopathy and have been suggested to play a pivotal part

From the *Department of Cardiac Surgery, University of Heidelberg, Heidelberg, Germany; †DZHK (German Centre for Cardiovascular Research), partner site Heidelberg/Mannheim, University of Heidelberg, Heidelberg, Germany; and the ‡Department of Internal Medicine III, Cardiology, University of Heidelberg, Heidelberg, Germany. This work was supported in part by grants from Else Kröner-Fresenius-Stiftung (to Z.K.), DZHK (German Centre for Cardiovascular Research), BMBF (German Ministry of Education and Research), and the Medical Faculty of the University of Heidelberg, Germany (postdoctoral fellowships to S.K. and D.S.). The authors have reported that they have no other relationships relevant to the contents of this paper to disclose.

Manuscript received January 25, 2013; revised manuscript received April 27, 2013, accepted April 30, 2013.

in the pathomechanism of dilated cardiomyopathy (2). Among these, antibodies against beta$_1$-adrenergic receptors and muscarinic M2-acetylcholine receptors have been associated with cardiac arrhythmias (3). Whereas the inflammatory process may itself be arrhythmogenic, changes in the electrophysiological properties of the myocardium also play a crucial role. In line with this notion, autoantibodies targeting the cardiac $KCNH_2$ ion channel (human *ether-a-go-go*–related gene) have recently been associated with an exclusively electrophysiological phenotype with QT prolongation in a case report (4).

Most of the currently available data rely on clinical associations between the occurrence of distinct autoantibodies and arrhythmias (5,6). Unlike for cardiomyopathy phenotypes, a causative experimental investigation in an animal model has not been performed to date that evaluates whether autoimmunity against a specific cardiac target may suffice to monocausally induce an electrophysiological phenotype. However, such evidence may be highly relevant to motivate and guide future clinical investigations.

In cardiomyocytes, voltage-gated sodium channels are crucial for the initiation and conduction of action potentials. The alpha subunit of the cardiac sodium channel $Na_V1.5$ is encoded by the *SCN5A* gene and is the predominant isoform in the heart. Heterozygous mutations in *SCN5A* leading to cardiac sodium channelopathies have been associated with a range of cardiac phenotypes including progressive cardiac conduction defect, sick sinus syndrome, long QT syndrome type 3, Brugada syndrome, atrial fibrillation, and even dilated cardiomyopathy (7–9). $Na_V1.5$ is located in the sarcolemma of cardiomyocytes and consists of four homologous domains (DI-DIV), with each domain composed of six transmembrane segments (S1–S6) connected to each other by alternating extracellular and cytoplasmic loops.

Hence, we studied the functional effects of antibodies induced against the third extracellular loop of the first domain of the $Na_V1.5$.

Methods

See the Online Appendix for further details.

Animals. Male Lewis rats (8 weeks old; Charles River, Sulzfeld, Germany) were housed at 22 ± 2°C under 12-h light/dark cycles and were fed a standard laboratory rat diet and water ad libitum. The rats were acclimatized for at least 1 week before experiments. All animals received humane care in compliance with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research; the Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (publication no. 86-23, revised 1996); and the German animal protection code. Approval was also granted by the local ethics review board (G-135/10).

$Na_V1.5$-peptide. The $Na_V1.5$ peptide (GNLRHKCVRN FTELNGTNGSVEADGLVW) corresponding to the third extracellular loop of the $Na_V1.5$ (residues 275–302), between the fifth and sixth transmembrane segment of the first domain was synthesized and purified through high-performance liquid chromatography with a purity of >90% (Peptide Specialty Laboratories, Heidelberg, Germany).

Experimental groups and immunization of animals. Rats were randomly assigned to one of two groups: the $Na_V1.5$-peptide immunized group (n = 10) or the control group (n = 11). Immunization (on days 0, 7, and 14) was performed through a subcutaneous injection of 0.1 mL of emulsion containing 500 μg of $Na_V1.5$ peptide dissolved in complete Freund's adjuvant supplemented with 5 mg/mL of *Mycobacterium tuberculosis* H37Ra (Sigma, St. Louis, Missouri). Control rats received buffer with supplemented complete Freund's adjuvant. The experiment was terminated on day 28.

Abbreviations and Acronyms

AVB = atrioventricular block

dP/dt_{max} = maximal slope of the systolic pressure increment

dP/dt_{min} = maximal slope of the diastolic pressure decrement

ECG = electrocardiography

H&E = hematoxylin and eosin

I_{Na} = cardiac voltage-gated sodium-channel current

I_{to} = cardiac transient outward potassium current

LV = left ventricular

MMP = matrix metalloproteinase

$Na_V1.5$ = cardiac voltage-gated sodium channel α-subunit

SCN5A = cardiac voltage-gated sodium channel α-subunit gene

TIMP = tissue inhibitor of metalloproteinase

Enzyme-linked immunosorbent assay for autoantibodies detection. Serum samples were obtained after centrifugation of blood collected from rats on days 0, 7, 14, 21, and 28. Antibody levels were determined using the enzyme-linked immunosorbent assay technique, as described elsewhere (10).

Electrocardiography. Standard 12-lead electrocardiography (ECG) was recorded for 1 h on days 21 and 28.

Telemetry ECG. On day 25, rats were implanted with transmitter devices (ECG Device, Data Sciences International, St. Paul, Minnesota) for ECG recordings for a period of 24 h.

In vivo hemodynamic measurements. On day 28, left ventricular (LV) pressure–volume analysis of cardiac function was performed using a 2-Fr microtip pressure–volume catheter (SPR-838, Millar Instruments, Houston, Texas), as described elsewhere (11).

Histopathology. The heart was removed, and the right atrium and atrioventricular septum were cut away from the ventricles. The right atrium was opened by a longitudinal incision through the tricuspid valve and into the superior vena cava (n = 4–6). Additionally, 5 or 6 hearts were dissected longitudinally into 2 symmetric sections. Formalin-fixed, paraffin-embedded tissues were stained with hematoxylin and eosin (H&E) and Masson's trichrome to determine the extent of inflammation and fibrosis, respectively, as described elsewhere (12).

Quantitative polymerase chain reaction. Myocardial gene expression of $Na_V1.5$, a tissue inhibitor of metalloproteinase (TIMP)-1 and matrix metalloproteinases (MMP)-2, -9, and -14