Journal of Molecular and Cellular Cardiology 53 (2012) 134–144

Contents lists available at SciVerse ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Original article

Probenecid: Novel use as a non-injurious positive inotrope acting via cardiac TRPV2 stimulation

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ARTICLE INFO

Article history:
Received 15 March 2012
Received in revised form 28 March 2012
Accepted 16 April 2012
Available online 27 April 2012

Keywords:
TRPV2
Probenecid
Echocardiography
Inotropic agent
Calcium handling

ABSTRACT

Probenecid is a highly lipid soluble benzoic acid derivative originally used to increase serum antibiotic concentrations. It was later discovered to have uricosuric effects and was FDA approved for gout therapy. It has recently been found to be a potent agonist of transient receptor potential vanilloid 2 (TRPV2). We have shown that this receptor is in the cardiomyocyte and report a positive inotropic effect of the drug. Using echocardiography, Langendorff and isolated myocytes, we measured the change in contractility and, using TRPV2$^{-/-}$ mice, proved that the effect was mediated by TRPV2 channels in the cardiomyocytes. Analysis of the expression of Ca^{2+} handling and β-adrenergic signaling pathway proteins showed that the contractility was not increased through activation of the β-ADR. We propose that the response to probenecid is due to activation of TRPV2 channels secondary to SR release of Ca^{2+}.

1. Introduction

Probenecid is a highly lipid soluble benzoic acid derivative developed by Sharp and Dohme [1] to decrease the renal tubular excretion of penicillin [1–7] and since then has been used to increase the serum concentration of several antibiotics and antivirals [8]. It was also found to be a competitive inhibitor of active transport processes in the brain [9], liver [10] and eye [11] and was studied in these fields; however a clinical use was not established outside of its renal effects.

Similar to other drugs which were developed to increase serum levels of antibiotics, probenecid was initially administered via slow intravenous infusion, which caused local irritation. Subsequently, probenecid was found to be rapidly absorbed following oral administration with peak serum concentrations occurring in 1 to 5 h [3].

During the initial studies using probenecid (referred to as Benemid), it became clear that probenecid had a strong uricosuric effect; this was similar to, but greater than its predecessor carinamide, and it quickly became the standard treatment for gout. Roch-Ramel et al. discovered that probenecid decreased uric acid levels in the serum via inhibition of organic acid reabsorption in the renal proximal tube by acting as a competitive inhibitor of the organic anion transporter (OAT) and thus preventing OAT-mediated reuptake of uric acid from the urine to the serum [12]. Due to its capacity as an OAT inhibitor and its minimal adverse effect profile [5,6,13–15] it was studied for other indications including depression and glaucoma, though it was never approved for such uses. Therefore, its clinical use has declined significantly as other therapies for gout have shown better efficacy [16].

Research interest in probenecid has recently increased with the observation that it is a potent and selective agonist of transient receptor potential vanilloid 2 (TRPV2) channels [17]. The transient receptor potential (TRP) family of channels has been studied for many years in the nephrology and neurology literature. Several TRPs have also been shown to be important mediators of vascular tone (TRPC1, TRPC6 and TRPM4), cerebral blood flow (TRPM4), neointimal hyperplasia (TRPC1) and pulmonary hypertension (TRPC6) [18]. Until recently, only a few of the channels in this family have been found to have direct cardiac effects (e.g. TRPC3/6/7 in the development of cardiac hypertrophy in response to pressure overload [19]). With regards to the TRPV family, some members have been identified to carry a direct cardiac effect. The first study was reported by Iwata and colleagues who found that cardiac specific overexpression of TRPV2 (published as the Ca^{2+}-permeable growth factor-regulated channel) resulted in chamber dilation of all cavities of the murine heart [20]. This study, however, did not address whether endogenous TRPV2 plays any role in the heart. Subsequently, Huang et al. [21] discovered that

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doi:10.1016/j.yjmcc.2012.04.011

TRPV1$^{-/-}$ mice have an increased infarct size and a decreased survival rate after ligation of the left anterior descending artery in comparison to their WT littermates. Interestingly, several groups have found that TRPV1 activation with specific agonists results in protection against ischemia/reperfusion (I/R) injuries [22,23].

We performed a survey of murine and human myocardial tissue for expression of TRPV channels and established that TRPV2 was the highest of these expressed in whole heart samples and specifically in the left ventricle. This finding led us to study the cardiac effects of probenecid on the whole animal, isolated whole heart and the isolated ventricular myocyte with the hypothesis that it can modulate myocardial function.

2. Methods

2.1. Animals

All animal procedures were performed with the approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Cincinnati and in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH, revised 1996). All wild type (WT) mice (B6129SF2/JF2 and C57BL6J, Jackson laboratories) and TRPV2$^{-/-}$ mice (breeding pairs provided by Dr. M. Caterina, John's Hopkins, Baltimore, MD) were males at 12–16 weeks of age [24].

2.2. In vivo studies

2.2.1. Studies of contractility with intravenous administration of probenecid

2.2.1.1. Echocardiographic evaluation. In order to obtain a dose–response curve, male C57 WT (n = 39) mice 12–16 weeks of age were anesthetized with isoflurane while intravenous jugular access (IV) was obtained under a microscope as previously described [34]. Subsequently, an echocardiographic study with both M-mode and B-mode was obtained in parasternal long axis (PSLAX) as described below. Either saline or different doses of probenecid (increasing from 2 to 200 mg/kg) were injected (bolus IV) for the initial contractility studies in WT mice.

2.2.1.2. Invasive evaluation. Once a dose range was established, a separate group of WT mice was anesthetized with an intraperitoneal injection of ketamine (50 μg/g) and inactin (thiobutabarbital, 100 μg/g, Sigma, MA). A tracheotomy was performed (PE-90), and body temperature was monitored and maintained with a feedback-controlled heating table. The right femoral artery was cannulated with fluid-filled polyethylene tubing for measurement of blood pressure and connected to a low compliance pressure transducer (COBE Cardiovascular, Arvada, CO). The right femoral vein was cannulated for delivery of drugs. A high fidelity, 1.2-French SciSence pressure catheter (SciSence, London, ON, Canada) was inserted into the right carotid artery and advanced into the left ventricle to monitor cardiac performance. ECG leads were placed on the right and left arms, and left leg and connected to a BIOAmp (AD Instruments, Colorado Springs, CO). For carotid blood flow measurements, the left carotid artery was isolated and fitted with a 0.5-PSB perivascular flow probe connected to a TS420 flowmeter (Transonic Systems, Ithaca, NY). Experimental solutions of 100 μg/μl probenecid were delivered as a bolus via the femoral vein catheter at 30 and 100 mg/kg with 5 min interval between each dose. Hemodynamic variables were collected and analyzed using a MacLab 4/S system (AD Instruments, Colorado Springs, CO) and Chart software.

2.2.2. Contractility studies with WT, TRPV2$^{+/-}$ and TRPV2$^{-/-}$ mice

Based on the results of the above experiments, we determined that the dose of 100 mg/kg of probenecid gave a maximum contractility response. We injected the probenecid intraperitoneal (IP) to decrease the possible stress effects of surgery. WT, TRPV2$^{+/-}$, and TRPV2$^{-/-}$ mice were monitored by echo for 30 min after injection as described below.

2.2.3. Echocardiography

All echocardiographic studies were performed with a Vevo 2100 Ultrasound system (VisualSonics, Toronto, CA) with an MS400 probe (30 MHz centerline frequency) and were post-processed at a separate workstation with Vevostrain software (Vevo 2100, v1.1.1 B1455, VisualSonics, Toronto, Canada). Images were obtained from PSLAX and short axis (SAX) views at depths between 2 and 10 mm in both M-mode and B-mode. All studies on mice exposed to I/R injuries included M-mode, B-mode in PSLAX and strain imaging in SAX. While for the contractility studies, only M-mode measurements were obtained from the PSLAX. Strain imaging was performed from the B-mode images and regional radial strain and circumferential displacement were measured by regional wall and summed average from the SAX images. From the M-mode images, left ventricular cavity size and wall thickness were measured and the ejection fraction (EF) and fractional shortening (FS) calculations were obtained using the Vevo software.

The change in the EF, FS, as well as strain derived parameters was obtained by subtracting the baseline value from each individual subject against subsequent time-points. In addition, the average change from baseline for each time point between 5 and 30 min was determined for each mouse, with measurements being taken every 5 min. These averages were compared between the different groups.

2.2.4. In vivo electrophysiology

Electrocardiographic data was obtained during all echocardiographic studies. These studies were subsequently analyzed by an independent, blinded reader at all time points to evaluate for electrocardiographic changes and drug induced arrhythmias. The parameters PR interval, RR interval and QRS width were measured and reported as peak change while the presence of supraventricular or ventricular arrhythmias was measured as a total observed over all images obtained in 30 min.

2.3. Ex vivo (Langendorff) studies

Isolated heart experiments were performed as previously described [26,27] on WT mice. After the hearts achieved steady-state with pacing at 400 bpm, probenecid (10^{-6} M) was perfused using a syringe pump which was continued for up to 5 min. Measurements were taken every second. After the 5 min perfusion of probenecid, the hearts were removed from the cannula and flash frozen in N_2 for western blot analysis.

2.4. Molecular studies

2.4.1. Quantitative RT-PCR

Hearts (LV) obtained for RNA isolation and qRT-PCR from WT, TRPV2$^{+/-}$ and TRPV2$^{-/-}$ mice were flash frozen and stored at −80 °C. For assessment of TRPV2 transcript levels, total RNA was isolated (RNeasy kit; Qiagen, Valencia, CA) and cDNA synthesized (High Capacity RNA-to-cDNA kit; Applied Biosystems, Carlsbad, CA) per manufacturer's instructions, using the C-terminal located primers 5′-CTACTGCTCAACATGCTC-3′ (sense) and 5′-CTCATCAGGTATACCATCC-3′ (antisense) which generate a 198 base pair product. All samples were performed in triplicate with a minimum of 3 independent experimental replicates with expression differences calculated using the delta–delta Ct approximation method with 18S mRNA as a loading control [28]. Corrections for primer efficiency were made where appropriate using the Pfaffl method [29].