Japanese Herbal Medicine Toki-shakuyaku-san (TJ-23) Enhances Cardiac Contractile Function in Isolated Ventricular Cardiomyocytes

Nicholas S. Aberle II¹, Midori Hiramatsu² and Jun Ren¹,*

¹Department of Pharmacology, Physiology, and Therapeutics, University of North Dakota School of Medicine and Health Sciences, Grand Forks, ND 58203, USA
²Division of Medical Science, Institute for Life Support Technology, Yamagata Technopolis Foundation, 2-2-1 Matsuei, Yamagata 990-2473, Japan

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Abstract. Toki-shakuyaku-san (TJ-23), a Japanese traditional herbal medicine, has a long history in Asia for the treatment of neurodegenerative, immune, and airway diseases. However, the effect of TJ-23 on heart function has not been elucidated. This study was designed to examine the effect of TJ-23 on ventricular contractile function at the single cardiomyocyte level. Ventricular cardiomyocytes from adult rat hearts were stimulated to contract at 0.5 Hz, and mechanical properties were evaluated using an IonOptix Myocam system. Contractile properties analyzed included peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR90), and maximal velocity of shortening/relengthening (±dL/dt). TJ-23 (10⁻⁸ – 10⁻⁵ mg/ml) exhibited significant augmentation in PS, with a maximal response of 27.2%. TJ-23 at 10⁻⁷ – 10⁻⁵ mg/ml also increased ±dL/dt, shortened TR90, while had no effect on TPS. Pretreatment with the Na⁺/K⁺-ATPase inhibitor ouabain (1 μM), removal of extracellular sodium from contractile buffer (which inhibits Na⁺/Ca²⁺ exchanger), or both concurrently abolished the positive effect of TJ-23 in cell shortening without inhibiting the baseline cell shortening. This study demonstrated a direct cardiac stimulatory action of TJ-23 at the cardiomyocyte level, which may be related to, at least in part, a Na⁺/K⁺-ATPase and/or Na⁺/Ca²⁺ exchanger-dependent mechanism.

Keywords: cardiomyocyte, cell shortening, Na⁺/K⁺-ATPase, Na⁺/Ca²⁺ exchanger

Introduction

Toki-shakuyaku-san (TJ-23) is a Japanese traditional herbal medicine with a long history in the treatment of neurodegenerative disease (1, 2), immune problems (3), and asthma (4, 5). It is a mixture of six galenicals including Angelicae radix, Hoelen, Cnidii rhizoma, Alismatis rhizoma, Paeoniae radix, and Atractylodis lanceae rhizoma (6). Investigations have demonstrated that TJ-23 may help to prevent the development of neurological disorders, particularly Alzheimer’s disease. This is in part through antioxidant mechanisms such as free radical scavenging and an increased production of superoxide dismutase in neurons (2). Evidence has also demonstrated that TJ-23 attenuates choline acetyltransferase activity and acetylcholine binding to nicotinic receptor (7), and enhancement of cytokine release, such as interleukin-1β and tumor necrosis factor-α, as well as platelet-activating factor from neutrophils (3). TJ-23 has also shown an efficacy in the alleviation of symptoms of asthma through its action on bronchial smooth muscle cells (4, 8). However, the effect of TJ-23 on heart function has not been elucidated. With increasing use of TJ-23, information regarding the profile of TJ-23 on cardiac contractile function is especially essential in patients with a preexisting heart condition. The aim of the present study was to elucidate the effect of TJ-23 on cardiac contractile function at the cellular level by evaluating cardiomyocyte shortening in isolated ventricular cardiomyocytes. The sarcolemmal sodium-potassium adenosine triphosphatase (Na⁺-K⁺ ATPase) and sarcolemmal sodium-calcium (Na⁺/Ca²⁺) exchanger have
been implicated in TJ-23-induced relaxant responses of airway and vascular smooth muscle (8), and both have been shown to affect cardiac contractility through Ca\(^{2+}\) transport as well as action potential generation and propagation (9, 10). Therefore, the role of sarcolemmal Na\(^+/K^+\) ATPase and Na\(^+/Ca^{2+}\) exchanger in TJ-23-induced cardiac response was also examined using the selective Na\(^+/K^+\) ATPase inhibitor ouabain or removal of extracellular Na\(^+\) ion.

Materials and Methods

Isolation of ventricular cardiomyocytes

The experimental procedures described in this study were approved by the animal investigation committee of the University of North Dakota School of Medicine and Health Sciences (Grand Forks, ND, USA). Single ventricular cardiomyocytes were isolated from adult male Sprague-Dawley rats (200 – 225 g) as described previously (11). Briefly, hearts were rapidly removed and perfused (at 37°C) with oxygenated (5% CO\(_2\)-95% O\(_2\)) Krebs-Henseleit bicarbonate (KHB) buffer (118 mM NaCl, 4.7 mM KCl, 1.25 mM CaCl\(_2\), 1.2 mM MgSO\(_4\), 1.2 mM KH\(_2\)PO\(_4\), 25 mM NaHCO\(_3\), 10 mM \(\text{N}^{-}[2\text{-hydroxyethyl}]-\text{piperazine\,-}\,[2\text{-ethanesulfonic acid}]\) (HEPES), 11.1 mM glucose, pH 7.4). Hearts were subsequently perfused with a nominally Ca\(^{2+}\)-free KHB buffer for 2 – 3 min followed by a 20-min perfusion with Ca\(^{2+}\)-free KHB containing 223 U/ml collagenase (Type II; Worthington Biochemical Corporation, Freehold, NJ, USA) and 0.1 mg/ml hyaluronidase (Sigma Chemical, St. Louis, MO, USA). After perfusion, the left ventricle was removed, minced, and further digested with trypsin (Type IX, Sigma) at 37°C for 5 min before being filtered through a nylon mesh (300 \(\mu\)m) and collected by centrifugation. Cells were initially washed with Ca\(^{2+}\)-free KHB buffer to remove remnant enzyme and extracellular Ca\(^{2+}\) was added incrementally back to 1.25 mM.

Cardiomyocyte shortening and relengthening

Mechanical properties of ventricular cardiomyocytes were assessed by an IonOptix Myocam® system (IonOptix, Inc., Milton, MA, USA). Cells were placed in a chamber mounted on the stage of an inverted microscope (model IX-70; Olympus, Tokyo) and superfused (at 25°C) with a buffer containing 131 mM NaCl, 4 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), 10 mM glucose, 10 mM HEPES, at pH 7.4. In some experiments, equal molar choline chloride salt was used to replace NaCl to remove the extracellular Na\(^+\) ion in order to inhibit the forward mode of the Na\(^+/Ca^{2+}\) exchanger. The cells were field stimulated with a suprathreshold (50%) voltage (approx. 40 – 80 volts) and at a frequency of 0.5 Hz, 3-ms duration, using a pair of platinum wires placed on opposite sides of the chamber connected to a FHC stimulator (Brunswick, NE, USA). The polarity of the stimulatory electrodes was reversed frequently to avoid possible build up of electrolyte by-products. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time-to-90% PS (TPS), time-to-90% relengthening (TR\(_{90}\)), maximal velocities of shortening (+dL/dt), and relengthening (−dL/dt) (11). To test the effect of TJ-23 on cardiac contraction, cell shortening was recorded before drug administration and after each concentration was administered at 3-min intervals. TJ-23 was obtained from Tsumura & Co. (Tokyo) and dissolved in H\(_2\)O.

Data analyses

Eight rats were used in this study and the sample collected was distributed among all the animals. Data were presented as the mean ± S.E.M. Statistical significance (\(P<0.05\)) for each variable was estimated by analysis of variance (ANOVA).

Results

Effect of TJ-23 on cardiomyocytes shortening (PS)

The average cell length used in this study was 106.6 ± 2.6 \(\mu\)m (from 101 cardiomyocytes). Acute exposure of TJ-23 did not affect resting cardiomyocyte cell length over the range of concentrations tested. A representative trace depicting the effect of TJ-23 (10\(^{-5}\) mg/ml) on cardiomyocyte shortening (PS) is shown in Fig. 1A. TJ-23 (10\(^{-8}\) – 10\(^{-5}\) mg/ml) elicited a significant increase of PS, with a maximal augmentation of 27.2% (Fig. 1B). Also, TJ-23 between 10\(^{-7}\) and 10\(^{-5}\) mg/ml enhanced TR\(_{90}\) and ±dL/dt. TPS was unaffected throughout the concentration ranges tested (Table 1). The threshold of TJ-23-induced mechanical response was between 10\(^{-7}\) and 10\(^{-5}\) mg/ml (Fig. 1B).

Effect of TJ-23 on cardiomyocyte shortening in the presence of the Na\(^+/K^+\) -ATPase inhibitor ouabain, in the absence of extracellular Na\(^+\) ion or both

To examine the potential mechanism of action for TJ-23, the effect of TJ-23 on cardiomyocyte shortening was re-examined in the presence of the Na\(^+/K^+\) -ATPase inhibitor, ouabain (1 \(\mu\)M) (12) or in the absence of extracellular Na\(^+\) ion to inhibit Na\(^+/Ca^{2+}\) exchanger, or both concurrently. Ouabain alone significantly enhanced cell shortening (PS) by 65.9 ± 18.2% (\(n = 11\), \(P<0.05\) vs baseline), consistent with the notion that Na\(^+/K^+\) -ATPase is crucial in the maintenance of the resting membrane potential. Interestingly, administration of TJ-23 at concentrations of 10\(^{-7}\) and 10\(^{-6}\) mg/ml, which
significantly enhanced PS, failed to elicit any further augmentation of PS in the presence of ouabain. In a different set of experiments, removal of the extracellular $\text{Na}^+$ ion (replaced with choline to maintain the osmolality) also abolished TJ-23-induced augmentation of PS without significantly affecting the baseline PS (data not shown). Simultaneous addition of ouabain and removal of extracellular $\text{Na}^+$ ion from the contractile buffer abolished the TJ-23-induced positive response on PS (Fig. 2). Removal of extracellular $\text{Na}^+$ ion did not affect the positive effect on PS elicited by ouabain itself (data not shown).

**Discussion**

Our study has provided evidence, for the first time, that the Japanese herbal medicine, TJ-23 directly enhances ventricular contraction in isolated cardiomyocytes. TJ-23 also increased $\frac{\text{d}L}{\text{d}t}$, shortened $\text{TR}_{90}$, but exhibited no effect on TPS. The $\text{Na}^+$/K$^+$-ATPase inhibitor ouabain and inhibition of $\text{Na}^+$/Ca$^{2+}$ exchanger with removal of extracellular $\text{Na}^+$ ion, either alone or combined, abolished the TJ-23-induced cardiac contractile response, suggesting involvement of these ion transport machineries in TJ-23-induced cardiac function.

In our current study, TJ-23 significantly enhanced PS,
revealing its force enhancing property in the heart. The pro-contraction effect of TJ-23 may work synergistically with its antioxidant property to provide beneficial effects for the heart. Our results indicated that the Na'/K'-ATPase inhibitor ouabain abolished the TJ-23-induced cardiac response, suggesting an involvement of the Na pump in the TJ-23-induced cardiac effect. The distribution of Na⁺ and Ca²⁺ ions across the sarcolemmal membrane is tightly regulated by the concerted action of ion channels, pumps, and exchangers. The Na'/K'-ATPase generates the electrochemical concentration gradient for Na⁺. This concentration gradient of Na⁺ ion then becomes the driving force for Ca²⁺ removal from the cytosolic space via the Na⁺/Ca²⁺ exchanger. Inhibition of the membrane-bound Na'/K'-ATPase such as inbition by cardiac glycosides and ouabain leads to an elevation of the intracellular Na⁺ concentration. Elevated intracellular Na⁺ may produce a positive cardiac inotropic effect by either shifting the reversal potential of the Na⁺/Ca²⁺ exchanger to more negative potentials, therefore limiting the outward transport of Ca²⁺ at resting membrane potentials or by increasing intracellular Ca²⁺ load during depolarization when the Na⁺/Ca²⁺ exchanger is operating in its reversal mode (i.e., Na⁺-efflux/Ca²⁺ influx). The increased Ca²⁺ load in the sarcoplasmic reticulum may cause augmented intracellular Ca²⁺ release (13, 14). This is supported by our observation that ouabain enhanced myocyte shortening. It is possible that TJ-23 promotes cardiac contraction by an antagonistic action against Na⁺/K'-ATPase, similar to that of ouabain. Our further experimental finding that removal of extracellular Na⁺ ion itself could abolish the TJ-23-induced cardiac contractile response seems to suggest that Na⁺/Ca²⁺ exchanger is also involved in the TJ-23-induced contractile response and may be in fact, downstream of the Na⁺/K'-ATPase. This is consistent with the aforementioned notion that Na⁺/K'-ATPase is required to generate the electrochemical concentration gradient for Na⁺, which is the driving force for Na⁺/Ca²⁺ exchanger (13, 14). It is worthy mentioning that neither ouabain nor removal of extracellular Na⁺ directly inhibited the baseline myocyte shortening (PS) amplitude, validating the involvement of Na⁺/K'-ATPase and/or Na⁺/Ca²⁺ exchanger in TJ-23-induced positive cardiac response. TJ-23 (10⁻⁷ – 10⁻³ mg/ml) significantly increased ±dL/dt and shortened TR₉₀ without any effect on TPS. This observation indicates that TJ-23 may disparate facilitate the intracellular Ca²⁺ release (systole phase) and clearing (diastole phase) by the intracellular Ca²⁺ pool-sarcoplasmic reticulum (SR), with the effect on intracellular Ca²⁺ clearing (or SR Ca²⁺ re-uptake) being predominant. Further study is warranted to investigate the effect of TJ-23 on intracellular Ca²⁺ regulatory machineries.

In conclusion, our study demonstrates a direct cardiac enhancement of TJ-23 at the ventricular cardiomyocytes level, possibly through a Na⁺/K' ATPase and/or a Na⁺/Ca²⁺ exchanger-associated mechanism. The precise nature of cardiac contractile effects of TJ-23 is still far from clear. Future studies should focus on identifying the effective component(s) among the six galenicals in TJ-23 and characterizing their action on components of cardiac excitation-contraction coupling (such as Na⁺/K'-ATPase and Na⁺/Ca²⁺ exchanger) and membrane ion channels. These approaches will be essential to understand the cellular effects and pharmacological profiles of this herbal compound.

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