ORIGINAL ARTICLE

5-HT3 receptor antagonists protect against pressure overload-induced cardiac hypertrophy in murine

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Received 14 November 2011; revised 6 December 2011; accepted 13 December 2011

KEY WORDS
Cardiac hypertrophy; 5-HT3 receptor; 5-HT3 receptor antagonists

Abstract Activation of cardiac sympathetic afferent reflex results in the increase of sympathetic activity. Serotonin (5-HT) activates cardiac sympathetic afferent through stimulating 5-HT3 receptors, the aim of present study is to test whether 5-HT3 receptor antagonists protect against cardiac hypertrophy. Cardiac hypertrophy induced by TAC for 4 weeks in mice was significantly inhibited by administration of 5-HT3 receptor antagonists, ondansetron (2.5 mg/kg, ip.) or tropisetron (2.5 mg/kg, ip.). Histological analysis revealed that the increased cardiac fibrosis in hypertrophic heart was relieved by ondansetron or tropisetron treatment. Ondansetron or tropisetron reduced the elevated plasma level of noradrenalin in mice with cardiac hypertrophy. Ondansetron and tropisetron had no effect on cardiomyocte hypertrophy induced by phenylephrine treatment \textit{in vitro}. Finally, we took tropisetron as the representative drug and examined the effects of tropisetron on the desensitization of cardiac \(\beta\)-adrenergic receptor in rat treated with abdominal aortic banding (AB). Results showed that tropisetron restored the desensitization of cardiac \(\beta\)-adrenergic receptor in AB-treated rats. In conclusion, 5-HT3 receptor antagonists protected against cardiac hypertrophy and restored the desensitization of cardiac adrenergic responsiveness, the mechanism in which may be through reducing the sympathetic activity.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association. doi:10.1016/j.apsb.2011.12.010
1. Introduction

Cardiac hypertrophy involves a remodeling process of heart in response to diverse pathological stimuli. Although considered as a compensatory mechanism to maintain cardiac output under conditions of overload, cardiac hypertrophy eventually leads to heart failure and death\(^1\)\(^3\). Sympathetic activation is an important pathological factor in promoting heart hypertrophy to heart failure and the mechanism includes activation of renin-angiotensin system\(^4\)\(^5\), induction of cardiomyocyte apoptosis\(^6\)\(^7\), enhancement of cardiac oxidative stress\(^8\), etc. Therefore, antagonism of sympathetic nervous system is a valuable therapeutic method for cardiac hypertrophy and heart failure.

Serotonin (5-hydroxytryptamine, 5-HT), a neurohormone, is involved in a range of physiological or pathophysiological functions. 5-HT receptors include seven subtypes (5-HT\(_1\)–7). All other 5-HT receptors are G-protein coupled receptors, with the exception of the 5-HT\(_3\) receptor, which is a ligand-gated ion channel. 5-HT\(_3\) receptor plays an important role in activation of cardiac sympathetic afferent\(^9\)\(^10\). Cardiac sympathetic afferent reflex is a sympathoexcitatory reflex. The activation of this reflex results in an increase of sympathetic activity, which is responsible for the deteriorative progression of cardiac hypertrophy and heart failure\(^11\). Furthermore, activation of 5-HT\(_3\) receptors enhances noradrenaline release\(^12\)\(^13\). The above knowledge indicates that 5-HT\(_3\) receptor might be a target for inhibition of sympathetic activation, herein, it is anticipated that 5-HT\(_3\) receptor antagonists would protect against cardiac hypertrophy.

2. Materials and Methods

2.1. Animals

Kunming mice (22–26 g) and Wistar rats (220–250 g) were used. The animals were kept under standard animal room conditions (temperature 21 ± 1 °C; humidity 55–60%) with food and water continuously available for 1 week before the experiment. All the experimental procedures were approved by the Institutional Animal Care and Use Committee of Harbin Medical University, China.

2.2. Establishment of pressure-overload cardiac hypertrophy and drug treatment in mice

The pressure-overload cardiac hypertrophy model was established with mice. Detailed methods have been described in our previous study\(^14\). Three days after operation of transverse aortic constriction (TAC), the mice were randomly divided into four groups, sham group, TAC model group, TAC model treated with ondansetron (Ond) and tropisetron (Tro), named as TAC+Ond and TAC+Tro. Three days after operation, ondansetron (2.5 mg/kg) and tropisetron (2.5 mg/kg) were administered by intraperitoneal injection daily. After 4 weeks, surviving animals were sacrificed, the plasma was collected and the heart was quickly excised and weighed in cold buffer (4 °C). The left ventricle was fixed in paraformaldehyde for histological analysis or rapidly frozen in liquid nitrogen and stored at −80 °C for subsequent Western blot analysis.

2.3. Determination of plasma noradrenaline level

Plasma levels of noradrenaline were measured using noradrenaline research elisa Kit (BA 10-5200) (Labor Diagnostika Nord GmbH&Co KG, Nordhorn, Germany) according to the procedure specification.

2.4. Preparation of primary rat cardiomyocytes

Detailed methods are described in our previous study\(^14\). Briefly, cardiomyocyte cultures were prepared by dissociation of 1-day-old neonatal rat (Wistar) hearts and were differentially plated to remove fibroblasts. To induce the hypertrophic response, phenylephrine (PE) was added to cardiomyocyte cultures at 50 μM. The culture media containing PE was changed every 12 h for a period of 72 h. Ondansetron (50 μg/L) and tropisetron (20 μg/L) were present in the culture medium. The doses of ondansetron and tropisetron were referenced to the plasma concentrations in human\(^15\)\(^16\). Cardiomyocytes were prepared for immunocytochemistry. Monoclonal antibody against sarcomeric α-actinin (Sigma) was added at dilutions of 1:200. Nuclear staining was performed with 1.3 μM bisbenzimide (Sigma). The relative surface area of the cell was calculated from the number of pixels by using Image-Pro Plus Version (5.0.1).

2.5. Pressure analysis in anesthetized rats with abdominal aortic banding (AB)

The rats were subjected to abdominal aortic banding as described\(^17\). Rats were anesthetized with an intraperitoneal injection of 300 mg/kg of chloral hydrate. The suprarenal portion of the aorta was exposed and a blunted 22-gage needle placed adjacent to the aorta. A ligature (5-0 silk) was tied around both the aorta and the needle. The needle was then removed, leaving the internal diameter of the aorta approximately equal to that of the needle. Sham-operated animals had an untied ligature placed in the same location. One day after surgery, animals were randomized to three groups: sham, aortic banding (AB), AB+tropisetron (1.25 mg/kg daily). Forty days after treatment, rats were anesthetized and blood pressure was measured via a polyethylene catheter inserted left ventricle through the right carotid artery. The catheter was connected to a pressure transducer (Model YH-4; Institute of Space Medical-Engineering, China) connected to a multichannel acquisition and analysis system (Model BL-420E, Taimeng Technology Instrument, Chengdu, China). After 20 min stable recording, dobutamine (0.8 mg/kg) was injected (iv) to test the response of blood pressure to the stimulation of β-adrenergic receptor.

2.6. Histological analysis

The tissue samples were embedded in paraffin and subjected to standard hematoxylin and eosin (HE) staining or masson’s trichrome staining.

2.7. Data analysis

Data are presented as mean ± SEM. Significance was determined using one-way ANOVA in SigmaStat Analysis Software. \(P<0.05\) was considered significant.
3. Results

3.1. 5-HT₃ receptor antagonists protect against pressure overload-induced cardiac hypertrophy in mice

There was no difference of the body weight of animals among sham, TAC, TAC+Ond and TAC+Tro groups when the animals were sacrificed four weeks after surgery (Fig. 1A). TAC induced significant increases of the heart weight and left ventricle weight and these increases were significantly inhibited by the treatment of two 5-HT₃ receptor antagonists, ondansetron and tropisetron (Figs. 1B and C). Compared with the sham group, the heart weight and left ventricle weight of animals in TAC+Ond and TAC+Tro were still higher, although ondansetron and tropisetron showed inhibitory effects on cardiac hypertrophy (Figs. 1B and C). The results of heart weight index and left ventricle weight index coincided with the results of heart weight and left ventricle weight (Figs. 1D and E).

As shown in Fig. 2A, the representative masson’s trichrome staining showed that TAC induced marked cardiac fibrosis, which was significantly inhibited by the treatment of ondansetron and tropisetron, the summarized data was shown in Fig. 2B. Because the increase of cardiac ventricle pressure influences the reflux of pulmonary vein, therefore, we also observed the lung weight. TAC resulted in the increased lung weight and the lung weight index, which were inhibited by the treatment of ondansetron and tropisetron. The representative HE stainings of lung tissues and the analyzed data were shown in Fig. 3.

3.2. 5-HT₃ receptor antagonists have no effect on cardiomyocytes hypertrophy induced by phenylephrine treatment in vitro

In order to exclude the possibility that 5-HT₃ receptor antagonists inhibit cardiac hypertrophy through the direct

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Figure 1  5-HT₃ receptor antagonists, ondansetron and tropisetron, protect against pressure overload-induced cardiac hypertrophy in mice. (A) Comparison of body weight in each group. (B) Comparison of heart weight in each group. (C) Comparison of left ventricle weight in each group. (D) Comparison of heart weight/body weight (HW/BW) in each group. (E) Comparison of left ventricle weight/body weight (LVW/BW) in each group. TAC, transverse aortic constriction; BW, body weight; HW, heart weight; LVW, left ventricle weight; Ond, ondansetron; Tro, tropisetron. **P<0.01 vs sham; ###P<0.01 vs TAC; ¥¥P<0.01 vs sham.

Figure 2 (A) Representative fields of masson’s trichrome stained heart sections in sham, TAC, TAC+Ond, TAC+Tro animals. (B) The summarized data showed that TAC-induced cardiac interstitial fibrosis was inhibited by ondansetron and tropisetron treatment. **P<0.01 vs sham; ###P<0.01 vs TAC. TAC, transverse aortic constriction; Ond, ondansetron; Tro, tropisetron. n means the number of sections. The sections in each group were from 3 hearts. Fibrosis was colored blue.
action on cardiomyocytes, we examined the effect of 5-HT₃ receptor antagonists on cardiomyocytes hypertrophy induced by phenylephrine treatment in vitro. As shown in Fig. 4, ondansetron or tropisetron did not inhibit the increased cell area and elevated b-MHC mRNA level induced by phenylephrine treatment, suggesting that 5-HT₃ receptor antagonists have no direct inhibitory effects on cardiomyocyte hypertrophy. Previous study reported that tropisetron inhibited the transcriptional activity of NFAT18, the factor involved in phenylephrine induced cardiomyocyte hypertrophy. However, in their study, the concentration of tropisetron reached 50 μg/mL, which was much higher than the clinical concentration15,16.

3.3. 5-HT₃ receptor antagonists reduce the elevated plasma level of noradrenalin (NA) in mice with cardiac hypertrophy

Up-regulation of circulating 5-HT has been reported in patients with hypertensive heart disease19, heart failure20 and in rats with pressure-overload induced cardiac hypertrophy21. Moreover, it has been reported that increased in plasma 5-HT is responsible for valvular fibrosis, ventricular dysfunction22,23 and promotes cardiac hypertrophy by pressure overload24. Cardiac hypertrophy or congestive heart failure shows sympathetic activation and increased plasma noradrenaline25, which initiates cardiac hypertrophy and harmful consequences in the myocardium26,27. Based on the evidence that activation of 5-HT₃ receptors enhances noradrenaline release22,23.

Figure 3  (A) Representative fields of HE stained lung sections in sham, TAC, TAC+Ond, TAC+Tro animals. (B) The summarized data showed that TAC-induced lung weight increase was inhibited by ondansetron and tropisetron treatment. LW, lung weight; BW, body weight; **P<0.01 vs sham; ***P<0.01 vs TAC. TAC, transverse aortic constriction; Ond, ondansetron; Tro, tropisetron.

Figure 4  5-HT₃ receptor antagonists, ondansetron and tropisetron, have no effect on cultured cardiomyocytes hypertrophy induced by phenylephrine treatment in vitro. (A) The representative photographs of cardiomyocytes with immunohistochemical staining. Cardiomyocytes were identified (× 200) with α-actinin antibody (red signal) and nuclei were stained with bisbenzamide (blue). (B) Comparison of cell area in each group. (C) Comparison of β-MHC mRNA expression in each group. PE, phenylephrine; Ond, ondansetron; Tro, tropisetron. **P<0.01 vs control.

Figure 5  (A) 5-HT₃ receptor antagonists, ondansetron and tropisetron, reduce the elevated plasma level of NA in cardiac hypertrophy mice. *P<0.05 vs sham; ***P<0.01 vs TAC. (B) Ondansetron or tropisetron treatment does not change the plasma NA level in normal mice. TAC, transverse aortic constriction; Ond, ondansetron; Tro, tropisetron; NA, noradrenaline.
we hypothesize that blockade of 5-HT₃ receptors would decrease the elevated plasma NA. As shown in Fig. 5A, plasma NA level increased in mice with pressure-overload cardiac hypertrophy. Treatment with ondansetron or tropisetron reduced the increased plasma NA level. However, ondansetron or tropisetron treatment did not change the plasma NA level in normal mice (Fig. 5B).

3.4. Tropisetron restores the desensitization of cardiac β-adrenergic receptor in rat treated with abdominal aortic banding

The representative blood pressure recordings in sham, AB and AB rats treated with tropisetron were shown in Figs. 6A–C and the summarized data were shown in Figs. 6D and E. As expected, abdominal aortic banding was associated with a significant increase in systolic blood pressure and +dp/dt_max, indicating a hypercontractile state as previously described. Administration of tropisetron restored the changes of ±dp/dt but did not affect the increase of systolic blood pressure in AB-treated rats. It has been reported that desensitization and downregulation of β-adrenergic receptors occur before the development of overt cardiac dysfunction, we then examined whether tropisetron restored the contractile response to β-agonist stimulation in AB-treated rats. As shown in Fig. 6E, administration of tropisetron significantly attenuated the decreased contractile response to β-agonist dobutamine (0.8 mg/kg, iv) in AB-treated rats.

Figure 6 The representative blood pressure recordings in sham, AB and AB+TP rats were shown in (A–C) and the summarized data were shown in (D) and (E). AB, abdominal aortic banding; TP, tropisetron; P, pressure. *P<0.05, **P<0.01 vs sham; †P<0.05, ††P<0.01 vs AB.
Discussion

There is substantial evidence supporting that 5-HT induces cardiac hypertrophy22,24,30 and the major mechanism is through the activation of 5-HT3 receptor31,34. Blockade of 5-HT3 receptor inhibits cardiac hypertrophy33,35–37. Based on the role of 5-HT3 receptor in activation of cardiac sympathetic afferent9,10, we put forward the hypothesis that 5-HT3 receptor antagonists would protect against cardiac hypertrophy. Here, we found that 5-HT3 receptor antagonists protected against cardiac hypertrophy induced by pressure overload in mice, restored the desensitization of β-adrenergic receptors, and the mechanism might be through reducing the sympathetic activity.

We used two kinds of 5-HT3 receptor antagonists, ondansetron and tropisetron. Ondansetron and tropisetron showed different structure with similar parent nucleus. Both ondansetron and tropisetron inhibited pressure-overload induced cardiac hypertrophy, reduced the elevated plasma level of noradrenalin in cardiac hypertrophy mice, and had no direct inhibition on in vitro cardiomyocytes hypertrophy. Compared with sham group, the inhibitory effect of ondansetron and tropisetron on cardiac hypertrophy was not complete (Figs. 1B–E), suggesting that there were other factors in TAC-induced cardiac hypertrophy except the sympathetic activation.

In addition to protection against cardiac hypertrophy, the beneficial cardiovascular effects of 5-HT3 receptor antagonist have been reported previously. 5-HT3 receptor antagonist inhibits the 5-HT-induced cardiogenic hypertensive chemoreflex38, protects against cardiac arrhythmias without eliciting hemodynamic side effects39, and regulates the autonomic cardiac dysfunction in primary fibromyalgia syndrome patients40. The 5-HT3 receptors locate widely in the body, including the central nervous system, peripheral nervous system, and varieties of other cells. 5-HT3 receptors modulate the release of neurotransmitters and neuropeptides, like dopamine, acetylcholine, GABA, substance P10,41, therefore, it is no doubt that the effects of activation or inhibition of 5-HT3 receptors are complex. Although we demonstrated that 5-HT3 receptor antagonists reduced the elevated plasma level of noradrenalin in cardiac hypertrophy animals, we still could not elucidate the exact action site of 5-HT3 receptor antagonists, which was the limitation of the present study.

It has been reported that AB treatment in rats induced cardiac hypertrophy and desensitized cardiac adrenergic responsiveness42. In the present study, we found that AB treatment induced slight cardiac hypertrophy, but significantly reduced the cardiac adrenergic responsiveness, as shown in Fig. 6 that the response of cardiac function to β-adrenergic receptor agonist dobutamine was reduced in AB-treated rats. Tropisetron restores the desensitization of β-adrenergic receptor in AB-treated rats. The mechanism might be due to the reduction of elevated plasma level of noradrenalin in cardiac hypertrophy animals.

Heart failure, the result of cardiac hypertrophy, is a deadly condition. Sympathetic activation is an important pathological factor in promoting heart hypertrophy to heart failure. Therefore, antagonism of sympathetic nervous system is a valuable therapeutic method for cardiac hypertrophy and heart failure. β-Adrenergic receptor antagonists, for instance, carvedilol and nebivolol, are commonly used. In the present study, we found that 5-HT3 receptor antagonists, ondansetron and tropisetron, protected against pressure-overload cardiac hypertrophy. Both ondansetron and tropisetron reduced the elevated plasma noradrenalin level in hypertrophic mice, suggesting that the protective effects of 5-HT3 receptor antagonists on hearts were due to their inhibition of sympathetic activation. Unlike the direct adrenoceptor blockade, 5-HT3 receptor antagonists inhibit sympathetic nervous system through an indirect way. 5-HT3 receptor antagonists are clinically used in controlling the nausea and vomiting produced by cancer chemotherapy and show good tolerance43–45. We propose that they might be novel type of potential drugs for treatment of heart hypertrophy.

Acknowledgments

This work was supported by National Natural Science Foundation of China (30873064) and Foundation of Key Laboratory of Bio-pharmaceutical-engineering (Harbin Medical University), Ministry of Education (2010-07).

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