

Monoamine oxidase inhibitors protect against coronary heart disease in rodent rat models: A pilot study

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Abstract: The present study designed to investigate the effect of monoamine oxidase inhibitor in the rat model of Coronary heart disease (cardiac hypertrophy). A total of 40 male adult Wistar rats having body weight 300-400 gram were equally distributed in two groups (Test group: Rats with Angiotensin II + monoamine oxidase inhibitor (Befloxatone); Reference group: Rats with cardiac hypertrophy induced by Angiotensin II). Rat model of cardiac hypertrophy were induced by Angiotensin II. Effect of Befloxatone on cardiac hypertrophy was evaluated by electrocardiography, hemodynamic and histological assessment. Vital signs such as pulse rate, and blood pressure were measured. Echocardiographic related variable including ejection fraction were also assessed in both the groups. Also, expression of monoamine oxidase was analyzed using by real-time-PCR and Western blot analysis. In results, we found following 1) monoamine oxidase inhibitor treatment prevents Angiotensin II induced increase in level of ANP and β -myosin, which are responsible for inducing cardiac hypertrophic responses; 2) monoamine oxidase inhibitor ameliorates Angiotensin II induced cell enlargement by reducing the surface area of cells; 3) monoamine oxidase inhibitor attenuates the hypertrophic response triggered by Angiotensin II; 4) monoamine oxidase inhibitor ameliorates increased heart rate and average arterial pressure induced by angiotensin II; 5) Overall finding suggested that monoamine oxidase inhibitor improves left ventricle hypertrophy and ejection fraction by inhibiting monoamine oxidase enzyme in heart. The finding of this study gives the new vision to cardiovascular researchers to develop anti- hypertrophy therapy based on monoamine oxidase inhibition.

Keywords: Hypertrophy, monoamine oxidase, angiotensin II, befloxatone, ejection fraction, rats.

INTRODUCTION

Monoamine oxidase (MAO) inhibitors are the first antidepressants available for clinical use. Early forms of have non-selective MAO inhibitors were disappeared due to 'cheese reaction' due to a build-up of dietary amines. Identification of two forms of MAO, known as MAOA and MAOB, led to the development of selective inhibitors that avoid this side effect. Further, establishing selective reversible MAO inhibitor offer better efficacy and safety profile in several neuro-degenerative diseases (Youdim, 2006).

Cardiac hypertrophy (CH) is a one of the leading causes of death worldwide, and associated with high healthcare cost, and one of the key reasons for cardiac mortality worldwide (Taegtmeyer, 1994; Stanley, 2002; Bing, 1954; Halestrap, 1998; Jennings, 1976; Lisa, 2007). World health organization data revealed that more than 30% of death (per year) occurred worldwide is mainly due to CH. It has been reported that monoamine oxidases (MAO) is present in various part of heart, and has been associated with oxidative stress which generate hydrogen peroxide, results in disruption of key biochemical parameters such as noradrenaline, adrenaline and dopamine (Bernardi,

2006; Weiss, 2003; Ide, 2001; Cucherat, 2007; Fox, 2008; Frey, 2004; Bianchi, 2005; Sturza, 2013). With the good understanding of monoamine oxidase involvement in CH, one can establish an accurate treatment for CH based on monoamine oxidase inhibition (Frey, 2004; Bianchi, 2005; Sturza, 2013; Pchejetski, 2007).

The monoamine oxidase enzyme in heart shows vital part in the ruling and preservation of several functions of cardiac system (Frey, 2004; Bianchi, 2005; Sturza, 2013). In heart, activation of monoamines enzyme has modulatory properties in heart which results in prolonged imbalance of neurotransmitter that led to hemodynamic trauma. This Increased heart rate results in decreased O₂ supply to myocardium, which is used as self-regulating markers of CV mortality among patients with CH. Besides, it has been revealed that oxidase stress induced by over activity of MAO that is facilitated mainly due to the stimulation of MAO enzyme (Fox, 2008; Frey, 2004; Bianchi, 2005; Sturza, 2013; Pchejetski, 2007).

It has been reported that the modulation of monoamine oxidase (MAO A and B enzyme) is related with dilation of arteries (Bianchi, 2005; Sturza, 2013; Pchejetski, 2007). Nevertheless, the association of MAO in CH remains mostly unknown. Hence, the present study designed to investigate the effect of monoamine oxidase

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inhibitor in the rat model of Coronary heart disease (cardiac hypertrophy). Rat model of cardiac hypertrophy were induced by Angiotensin II. Effect of befloxatone on cardiac hypertrophy was evaluated by ECG, hemodynamic assessment, and histological examination. The effect of befloxatone on expression of MAO enzymes was also analyzed by using real-time-PCR and western blot analysis.

MATERIALS AND METHODS

A total of 40 male adult Wistar rats having body weight 200–250 gram were equally distributed in two groups (Test group: Rats with Angiotensin II + MAO inhibitor [Befloxatone]; Reference group: Rats with cardiac hypertrophy induced by Angiotensin II). All rats were well-kept in quarantined cages at 30 °C with a 12-hour light–dark cycle, and rats were offered regular diet and liquid on SOS basis (as and when required) during the day. Before initiating any study related procedure, the study protocol was approved from ethics committee of The First Hospital of Jilin University vide reference no: AEC/FHJU-0134-18.

Rat model of cardiac hypertrophy were induced continuous infusion of Angiotensin II (0.6 mg per kg/day) for 2 weeks. Effect of MAO inhibitor (Befloxatone, 0.5 mg/kg per oral [p.o] for 14 days) on cardiac hypertrophy was evaluated by ECG, hemodynamic assessment, and histological examination. Also, effect of MAO inhibitor (Befloxatone, 0.5 mg/kg p.o) on expression of MAO enzymes was examined using by real-time-PCR and Western blot analysis. Total body weight including weight of heart was recorded. Also, vital signs such as pulse rate, blood pressure and ejection fraction were measured. Echocardiographic related variable were also assessed in both the groups.

Rats were sedated by pentobarbital 40mg/kg, i.p (a benzodiazepines class of drug). Subsequently, echocardiography was conducted using echocardiographic method. The diameter of LV and its wall width was also assessed. After administration of Angiotensin II and MAO inhibitor (Befloxatone) in respective group, rats of both the group were sedated by pentobarbital, and body temperature and ECG was recorded. Also, catheter with PV control was introduced in carotid blood vessel in order to measure blood pressure of arteries. Then, heart of each enrolled rats was dissected and then washed in chilled alkaline buffers. The ratio of body and heart weight was calculated; also weight of LV was also recorded. Sample was taken from ventricle, and then equally dispersed in 3 fragments. One fragment was stable with formaldehyde (4%), and was blemished with eosin/hematoxylin. Remaining two fragments was freeze in N₂O (liquid) and then kept at -20 °C for subsequent investigation. Western blot analysis was performed using protein sample, which

was removed from heart tissues/cells, and then fixed with gel electrophoresis and stimulated with cellulose sheath. Then, the cellulose sheath was dwindling at 4 °C during whole night along with antibodies with MAO. For real-time quantitative RT-PCR analysis, RNA was removed from heart tissues/cells using Trizol agents and cDNA sequencing was produced by reverse transcriptase equipment, using GAPDH as control, with selected primer series. Also, the effect of inhibition of MAO enzyme on inhibition of cardiac hypertrophic responses induced by Angiotensin II was tested using cell lines (H9c2 cells), measuring presence of ANP and β -myosin. Also, cell surface area after Angiotensin II was tested, and compared both the group.

As this study was intended as a pilot study, thus there is no formal sample size calculation was performed for this study. Therefore, we have planned to include at least 20 male rats in each group (total 40 rats in both groups). Echocardiographic assessment after administration of MAO inhibitor (Befloxatone) and Angiotensin II in rat model of CH was tested using unpaired t test. Comparison between test and reference for expression of MAO enzyme, hypertrophic response was analyzed using unpaired t test. Comparison between test and reference for expression of MAO enzyme, hypertrophic response was analyzed using unpaired t test. Effect of on mRNA levels of ANP and beta-myosin was compared between test and reference using Mann–Whitney test. P less than 0.005 indicates statsical significant different between test and reference. All the statistical analysis was performed using Statistica..

RESULTS

In this study, a total of 40 rats (test group: 20 rats; Reference group: 20 rats) were studied and, and data of all 40 rats were analyzed using appropriate statistical method. Echocardiographic assessment after administration of MAO inhibitor (Befloxatone) and Angiotensin II in rat model of CH showed that body weight, heart rate and average arterial pressure was significantly increased in rats treated with Angiotensin II when compared to rats those were treated with Angiotensin II + MAO inhibitor (Befloxatone) (table 1). This indicates that the MAO inhibitor (Befloxatone) ameliorates heart rate and average arterial pressure induced by Angiotensin II. Moreover, septum wideness of left ventricles during systole and diastole was considerably greater in rats that were treated with Angiotensin II when compared to rats treated with Angiotensin II + MAO inhibitor (Befloxatone). Moreover, posterior wall wideness of left ventricles during systole and diastole was significantly greater in rats that were treated with Angiotensin II as compared to rats that were treated with Angiotensin II + MAO inhibitor (Befloxatone). Also, LV diameter was meaningfully

Table 1: Echocardiographic assessment after administration of MAO inhibitor (Befloxatone) and Angiotensin II in rat model of CH

Variable	Angiotensin II + MAO inhibitor (Befloxatone) (Test) N=20 Mean (SD)	Angiotensin II (Reference) N=20 Mean (SD)
Septum wideness of ventricles during diastole, in mm	0.42 (0.11)*	1.21 (0.5)
Septum wideness of ventricles during systole, in mm	1.12 (0.5)*	2.89 (1.1)
Posterior wall wideness of ventricles during diastole, in mm	0.42 (0.13)*	1.7 (0.18)
Posterior wall wideness of ventricles during systole, in mm	1.32 (0.12)*	3.42 (0.63)
LV diameter, in Cm	0.78 (0.31)*	2.2 (0.17)
Ejection fraction (%)	0.58 (1.1)*	0.35 (1.5)
Body weight (gram)	300.18 (1.2)	389.12 (1.2)
Heart rate (beat per minute)	102 (2.9)*	179 (2.1)
Average arterial pressure (mmHg)	82.1 (1.3)*	192.3 (1.5)

* $P < 0.05$ using unpaired t test.

Test: Rats with Angiotensin II + MAO inhibitor (Befloxatone); Reference: Rats with cardiac hypertrophy induced by Angiotensin II.

larger in rats those were treated with Angiotensin II as compared to rats treated with Angiotensin II + MAO inhibitor (Befloxatone). Ejection fraction (%) was significantly lower in rats that were treated with Angiotensin II as compared to rats treated with Angiotensin II + MAO inhibitor (Befloxatone) (table 1). Overall, finding suggested that MAO inhibitor (Befloxatone) improves left ventricle hypertrophy and ejection fraction by inhibiting MAO enzyme.

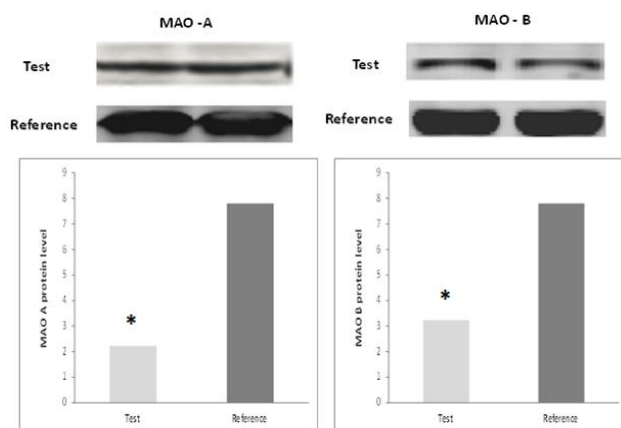


Fig. 1: Expression of MAO enzyme in rat model of hypertrophy after test and reference drug administration (N=20 in test group; N=20 in reference group). Test: Rats with Angiotensin II + MAO inhibitor (Befloxatone); Reference: Rats with cardiac hypertrophy induced by Angiotensin II). * $p < 0.005$ compared to reference, using unpaired t test. P value less than 0.05 indicates statistical significant difference.

Inactivation of MAO enzymes in ventricle after continuous infusion of Angiotensin II was also measured. After continuous infusion of Angiotensin II, the protein level of MAO-A & B enzymes in the hearts was meaningfully higher in rats treated with Angiotensin II compared to rats who received Angiotensin II + MAO

inhibitor (Befloxatone) after 2 weeks of treatment (fig. 1). Effect of overexpression of MAO enzymes on inhibition of cardiac hypertrophic responses induced by Angiotensin II was tested using cell line techniques. Also, surface area of cells after Angiotensin II was tested, the results showed that the surface area of cells was noticeably enlarged in rats treated with Angiotensin II as compared to the rats treated with MAO inhibitor (Befloxatone) plus Angiotensin II (fig. 2); this indicates that MAO inhibitor (Befloxatone) prevents Angiotensin II induced cell enlargement by reducing the surface area of cells. Also, we found that the levels of ANP and β -myosin were noticeably higher in rats treated with Angiotensin II as compared to the rats treated with MAO inhibitor (Befloxatone) plus Angiotensin II (fig. 3). This indicates that MAO inhibitor (Befloxatone) treatment prevents Angiotensin II induced increase in level of ANP and β -myosin, which are responsible for inducing cardiac hypertrophic responses.

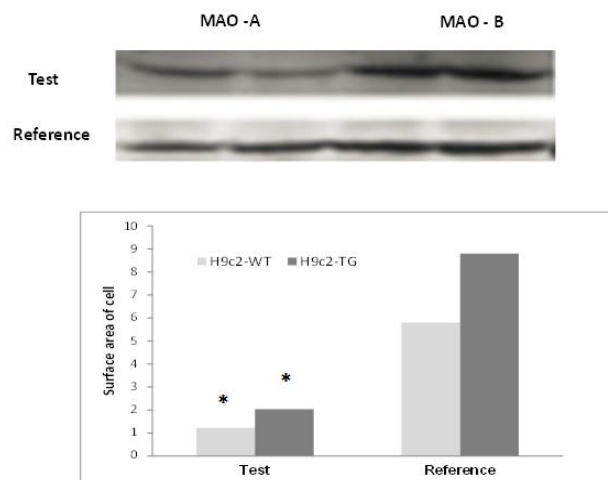


Fig. 2: Effect of MAO inhibitor (Befloxatone) on hypertrophic response induced by Angiotensin II (N=20 in test group; N=20 in reference group). Test: Rats with

Angiotensin II + MAO inhibitor (Befloxadone); Reference: Rats with cardiac hypertrophy induced by Angiotensin II. * $p < 0.005$ compared to reference, using unpaired t test. P value less than 0.05 indicates statistical significant difference.

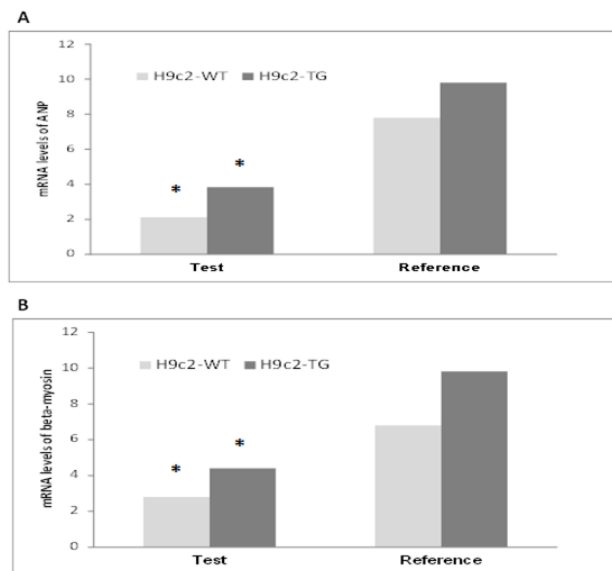


Fig. 3: Effect of MAO inhibitor (Befloxadone) on mRNA levels of ANP (A) and beta-myosin (B) in rat hypertrophic model induced by Ang II. (N=20 in test group; N=20 in reference group). Test: Rats with Angiotensin II + MAO inhibitor (Befloxadone); Reference: Rats with cardiac hypertrophy induced by Angiotensin II. * $p < 0.005$ compared to reference, using Mann-Whitney test. P value less than 0.05 indicates statistical significant difference.

Cardiac hypertrophic responses to Angiotensin II was significantly suppressed in test group (Angiotensin II + MAO inhibitor [Befloxadone]) compared reference group. Moreover, inhibition of MAO enzyme expression was greater in test group (Angiotensin II + MAO inhibitor [Befloxadone]) as compared to reference group (Angiotensin II). The results of this study showed that MAO inhibitor (Befloxadone) relieved the hypertrophic response triggered by Angiotensin II, by increased inhibiting overexpression of MAO enzyme at the time of hypertrophy (figs. 2 and 3).

DISCUSSION

Cardiac hypertrophy is a one of common type of CHD, responsible for cardiac mortality worldwide (Taegtmeier, 1994; Stanley, 2002; Bing, 1954; Halestrap, 1998; Jennings, 1976; Lisa, 2007; Bernardi, 2006). It has been reported that the MAO in heart shows vital part in the governing and protection of several functions of cardiac system (Frey, 2004; Bianchi, 2005; Sturza, 2013; Pchejetski, 2007; Gilsbach, 2010; Heineke, 2006;

Kasama, 2018). To establish an accurate treatment for CH, understanding of MAO involvement in CH is essential.

The results of this study showed that MAO inhibitor (Befloxadone) diminishes the hypertrophic response caused by Angiotensin II, by inhibiting MAO A enzyme. Overactivity of MAO enzyme (increased expression) after administration of Angiotensin II was noted, which results in hypertrophic responses induced by angiotensin II. MAO inhibitor (Befloxadone) treatment inhibits Angiotensin II induced increase in level of ANP and β -myosin, which are accountable for inducing CH responses. Moreover, MAO inhibitor (Befloxadone) ameliorates Angiotensin II induced cell enlargement by reducing the surface area of cells. These results offer new therapeutic target in the management of CH induced by Angiotensin II. Several lines of previous pre-clinical finding showed that the over activity of MAO enzymes in rat model of cardiovascular disorders (Fox, 2008; Frey, 2004; Bianchi, 2005; Sturza, 2013; Pchejetski, 2007). Similarly, the results of this study demonstrated that the protein level of MAO was upregulated during CH, after administration of Angiotensin II. Thus, the present study results indicate the convinced role of MAO in CH.

The most serious drug-food interaction of non-selective MAO inhibitors with is cheese reaction (Youdim, 2006), these results in stimulation of cardiovascular sympathetic nervous system activity that led to hypertensive crises due to increased levels of dietary amines (noradrenaline). Use of selective reversible MAO inhibitors prevents the occurrence of cheese reaction. Also, selective reversible MAO inhibitors are less likely to have drug-drug interaction compared to non-selective MAO inhibitors. Furthermore, selective reversible MAO inhibitor may offer better efficacy and safety profile in several diseases compared to non-selective MAO inhibitor. Based on this preliminary result, we encourage conducting a well-controlled clinical study to evaluate the efficacy and safety of selective reversible MAO-A inhibitor (Befloxadone) in patients with cardiac hypertrophy.

CONCLUSION

The results of present study showed that MAO inhibitor (Befloxadone) prevent progression of left ventricle hypertrophy and ejection fraction induced by Angiotensin II, by inhibiting MAO enzymes over activation in heart. The present study results suggested the need of targeting MAO enzymes in heart for developing effective treatment for cardiac hypertrophy. The finding of this study gives the new idea to cardiovascular researchers to develop anti- hypertrophy therapy based on MAO enzyme inhibition. The current study suggests conducting a well-designed clinical study to confirm the finding of this study in patients with cardiac hypertrophy.

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