Effect of muscarinic receptors agonist in the rat model of coronary heart disease: A potential therapeutic target in cardiovascular diseases

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Abstract: Cardiac hypertrophy is one of a common type of CHD, responsible for cardiac mortality worldwide. The present study designed to investigate the effect of muscarinic receptors agonist in the rat model of cardiac hypertrophy. A total of 30 male adult Wistar rats having body weight 300-400 gram were equally distributed in two groups (Test group: Rats with Angiotensin II + M3 receptor agonist [acetylcholine]; Reference group: Rats with cardiac hypertrophy induced by Angiotensin II). Rat model of cardiac hypertrophy were induced by Angiotensin II. Effect of M3 receptor agonist on cardiac hypertrophy was evaluated by electrocardiography, hemodynamic and histological assessment. Also, expression of M3 receptor was analyzed using by real-time-PCR and Western blot analysis. Also, vital signs such as pulse rate, and blood pressure were measured. Echocardiographic related variable including ejection fraction were also assessed in both the groups. The results of this study showed acetylcholine attenuates the hypertrophic response triggered by Angiotensin II, by upregulation of M3 receptor. Upregulation of M3 receptor after administration of acetylcholine ameliorates hypertrophic responses induced by angiotensin II. Also acetylcholine treatment prevents Angiotensin II induced increase in level of ANP and β-myosin, which are responsible for inducing cardiac hypertrophic responses. Moreover, acetylcholine ameliorates Angiotensin II induced cell enlargement by reducing the surface area of cells. Overall finding suggested that acetylcholine improves left ventricle hypertrophy and ejection fraction by activating M3 receptor in heart. The finding of this study gives the new vision to cardiovascular researchers to develop anti- hypertrophy therapy based on M3 receptor.

Keywords: Hypertrophy, muscarinic receptor, Angiotensin II, acetylcholine, ejection fraction.

INTRODUCTION

Coronary heart disease (CHD) is one of the foremost causes of death worldwide, and associated with high healthcare cost (Frey, 2004; Heineke, 2006; Gilsbach, 2010). WHO data revealed that more than 30% of death (per year) occurred worldwide is mainly due to CHD. Cardiac hypertrophy (CH) is a one of common type of CHD, and key reason for cardiac mortality worldwide (Heineke, 2006; Gilsbach, 2010; Kasama, 2007; Ohshima, 2005; Shi, 1999). It has been reported that muscarinic receptor type 3 (M3) is present in various part of heart. With the good understanding of cholinergic involvement in CHD, one can establish an accurate treatment for CH (Kasama, 2007; Ohshima, 2005; Shi, 1999; Wang, 1999; Wang, 2004).

The muscarinic receptor type 3 in heart shows vital part in the ruling and preservation of several functions of cardiac system (Liu, 2008; Wang, 2004). In heart, activation of M3 receptor showed positive inotropic effect and negative chronotropic effects. Increased heart rate results in decreased O2 supply to myocardium, which is used as self-regulating markers of CV mortality among patients with CHD. Besides, it has been revealed that vasodilatation induced by acetylcholine in coronary arties was facilitated mainly due to the stimulation of M3 receptor (Wang, 2012; Liu, 2009; Liu, 2001).

It has been reported that the modulation of muscarinic receptor (M2 and M3 receptor) is related with dilation of arteries (Liu, 2009; Liu, 2001; Fox, 2008; Cucherat, 2007; Lamping, 2004). Nevertheless, the association of M3 receptor of muscarinic receptor in CH remains mostly unknown. Hence, the present study designed to investigate the effect of muscarinic receptors agonist in the rat model of Coronary heart disease/ Coronary hypertrophy. Rat model of cardiac hypertrophy were induced by Angiotensin II. Effect of M3 receptor agonist (acetylcholine) on cardiac hypertrophy was evaluated by ECG, hemodynamic assessment, and histological examination. Also, effect of M3 receptor agonist (acetylcholine) on expression of M3 receptor was analyzed using by real-time-PCR and western blot analysis.

MATERIALS AND METHODS

A total of 30 male adult Wistar rats having body weight 300-400 gram were equally distributed in two groups (Test group: Rats with Angiotensin II + M3 receptor agonist [acetylcholine]; Reference group: Rats with

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cardiac hypertrophy induced by Angiotensin II). All rats were preserved in quarantined cages with 12-day and night cycle, and rats were offered regular diet and liquid as and when required throughout the day. Ethics committee approval was obtained from Xi’an No.3 Hospital before conducting study.

Rat model of cardiac hypertrophy were induced continuous infusion of Angiotensin II (0.6 mg per kg/day) for 2 weeks. Effect of M3 receptor agonist (acetylcholine) on cardiac hypertrophy was evaluated by ECG, hemodynamic assessment and histological examination. Also, effect of M3 receptor agonist (acetylcholine) on expression of M3 receptor was analyzed 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 benzodiazepines class of drug (pentobarbital). Thereafter, echocardiography was conducted using echocardiographic method. The diameter of LV and its wall width was also assessed. After administration of angiotensin II and acetylcholine in rat model of cardiac hypertrophy was induced by ECG, hemodynamic assessment and histological examination. Also, effect of M3 receptor agonist (acetylcholine) on expression of M3 receptor was analyzed using 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.

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 15 male rats in each group (total 30 rats in both groups). Appropriate statistical test was applied to compare both the groups for selected variables. All the statistical analysis was performed using Statistica (a type of statistical analysis software).

RESULTS

In this study, a total of 30 rats (test group: 15 rats; Reference: Rats with cardiac hypertrophy induced by Angiotensin II).

Table 1: Vital and echocardiographic assessment after administration of acetylcholine and Angiotensin II in rat model of hypertrophy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Angiotensin II (Reference)</th>
<th>Angiotensin II + Ach (Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (gram)</td>
<td>23.12 (1.2)</td>
<td>21.18 (1.8)</td>
</tr>
<tr>
<td>Heart rate (beat per minute)</td>
<td>333 (2.1)</td>
<td>231 (1.9)*</td>
</tr>
<tr>
<td>Average arterial pressure (mmHg)</td>
<td>178.8 (1.3)</td>
<td>93.2 (2.8)*</td>
</tr>
<tr>
<td>Septum wideness of ventricles during diastole (IVSd), in mm</td>
<td>1.69 (0.98)</td>
<td>0.62 (0.12)*</td>
</tr>
<tr>
<td>Septum wideness of ventricles during systole (IVSs), in mm</td>
<td>2.29 (1.18)</td>
<td>1.12 (0.8)*</td>
</tr>
<tr>
<td>Posterior wall wideness of ventricles during diastole (LVPWd), in mm</td>
<td>1.9 (0.38)</td>
<td>0.42 (0.13)*</td>
</tr>
<tr>
<td>Posterior wall wideness of ventricles during systole (LVPWs), in mm</td>
<td>3.29 (0.78)</td>
<td>1.02 (0.72)*</td>
</tr>
<tr>
<td>LV diameter, in Cm</td>
<td>2.19 (0.37)</td>
<td>0.98 (0.21)*</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>0.54 (2.3)</td>
<td>0.79 (1.3)*</td>
</tr>
</tbody>
</table>

Abbreviations: IVSd: diastolic intraventricular septum thickness, IVSs: systolic intraventricular septum thickness, LVPWd: diastolic left ventricular posterior wall thickness, LVPWs: systolic left ventricular posterior wall thickness; LV: left ventricular. Values are expressed as Mean (SD). *p <0.005 compared to Angiotensin II group.

Test: Rats with Angiotensin II + M3 receptor agonist [acetylcholine]; Reference: Rats with cardiac hypertrophy induced by Angiotensin II.)
Reference group: 15 rats) were studied and, and data of all 30 rats were analyzed using appropriate statistical method. Upregulation of M3 receptor in ventricle after continuous infusion of Angiotensin II was measured. After continuous infusion of Angiotensin II, the protein level of M3 receptor in the hearts was approx. 2 fold higher in rats treated with Angiotensin II compared to rats who received vehicle (0.6% CMC) after 2 weeks of treatment (fig. 1). Nevertheless, the protein level of M2 receptor in the hearts was similar in rats treated with Angiotensin II and vehicle (0.6% CMC), respectively, after 2 weeks of treatment (fig. 1).

Effect of overexpression of M3 receptor 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 considerably enlarged in rats treated with Angiotensin II as compared to the rats treated with acetylcholine plus Angiotensin II (fig. 2); this indicates that acetylcholine prevents Angiotensin II induced cell enlargement by reducing the surface area of cells. Also, we found that the levels of ANP and βeta-mysin were considerably higher in rats treated with Angiotensin II as compared the rats treated with acetylcholine plus Angiotensin II (fig. 3). This indicates that acetylcholine treatment prevents Angiotensin II induced increase in level of ANP and βeta-mysin, which are responsible for inducing cardiac hypertrophic responses.

**Fig. 1: Upregulation of muscarinic receptor (M2 and M3 receptor) in rat model of hypertrophy after test and reference drug administration.** Fig. 1A, 2B: Western blot analysis of muscarinic receptor (M2 and M3 receptor) protein levels in tissues from rat with chronic angiotensin II (Ang II) (0.6 mg/kg per day) infusion for 2 weeks. Muscarinic receptor (M2 and M3 receptor) protein level was incubated with isoproterenol (10 μM) for 48 hours. GAPDH aided as loading control. Values were expressed as mean ± SEM and normalized to the Reference group. **P < 0.05 vs. Reference group; #P > 0.05 vs. Reference group**

**Fig. 2: Effect of M3 receptor agonist on hypertrophic response of Angiotensin II in H9c2 cells of rat model.** Values were expressed as means ± SEM (n = 15 in each group). P<0.05 for all comparisons for H9c2 – WT and H9c2 – TG cells. M3-mAChR over-expression inhibited the increase of cell surface area induced by angiotensin II (Ang II).
Cardiac hypertrophic responses to Angiotensin II was significantly suppressed in test group (Angiotensin II + Acetylcholine) compared reference group. Moreover, expression of M3 receptor was greater in test group (Angiotensin II + Acetylcholine) as compared to reference group (Angiotensin II). Increased M3 receptor considerably diminished the increased mRNA levels of ANP and βeta-myosin induced by Angiotensin II. The results of this study showed acetylcholine relieved the hypertrophic response triggered by Angiotensin II, by increased expression of M3 receptor at the time of hypertrophy (figs. 2 and 3).

Also, body weight, heart rate and average arterial pressure was significantly increased in rats that were treated with Angiotensin II as compared to rats that were treated with Angiotensin II + acetylcholine (table 1). This indicates that the acetylcholine ameliorates increased heart rate and average arterial pressure induced by angiotensin II. Moreover, septum 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 + acetylcholine. In addition, 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 + acetylcholine. Also, LV diameter was significantly larger in rats that were treated with Angiotensin II as compared to rats that were treated with Angiotensin II + acetylcholine. Ejection fraction (%) was significantly lower in rats that were treated with Angiotensin II as compared to rats that were treated with Angiotensin II + acetylcholine (table 1). Overall, finding suggested that acetylcholine improves left ventricle hypertrophy and ejection fraction by activating M3 receptor.

**DISCUSSION**

Cardiac hypertrophy is a one of common type of CHD, responsible for cardiac mortality worldwide (Frey, 2004; Heineke, 2006; Gilsbach, 2010). It has been reported that the muscarinic receptor type 3 in heart shows vital part in the ruling and preservation of several functions of cardiac system (Liu, 2008; Wang, 2004). To establish an accurate treatment for cardiac hypertrophy, understanding of cholinergic involvement in cardiac hypertrophy is important.

The results of this study showed acetylcholine attenuates the hypertrophic response triggered by Angiotensin II, by up-regulation of M3 receptor. Up-regulation of M3
receptor after administration of Acetylcholine ameliorates hypertrophic responses induced by angiotensin II. Also acetylcholine treatment prevents Angiotensin II induced increase in level of ANP and β-myosin, which are responsible for inducing cardiac hypertrophic responses. Moreover, acetylcholine ameliorates Angiotensin II induced cell enlargement by reducing the surface area of cells. These results provide novel insight into the mechanisms underlying the cardioprotective effects of M3-mAChR in cardiac hypertrophy and indicate that M3-mAChR is a potential therapeutic target. Several line of previous pre-clinical finding showed that the expression of M3 receptor was up-regulated in rat model of atrial fibrillation and ischemia (Wang, 2012; Liu, 2009; Liu, 2001). Similarly, the results of this study showed that the protein level of M3-mAChR was up-regulated during cardiac hypertrophy. Thus, the present study results indicate the specific role of M3-mAChR in cardiac hypertrophy.

In summary, the current study provides new insight into the role of M3-receptor up-regulation in the development of cardiac hypertrophy induced by Ang II. Our findings suggest that M3-mAChR functions as an endogenous negative regulator of hypertrophic response, thus representing a novel therapeutic target for cardiac hypertrophy. Further studies to reveal the molecular mechanisms of the anti-hypertrophic properties of cardiac M3-mAChR will promote the application of M3-mAChR agonist in clinical use. The finding of this study provides a new vision to cardiovascular researchers to develop anti-hypertrophy therapy based on M3 receptor activation. We encourage conducting well designed clinical study to confirm the finding of this study among patients with cardiac hypertrophy.

CONCLUSION

Overall this study results suggested that acetylcholine improves left ventricle hypertrophy and ejection fraction by activating M3 receptor in heart. Our study results suggest, there is need to target M3 receptor in heart for developing effective treatment for cardiac hypertrophy. The finding of this study gives the new vision to cardiovascular researchers to develop anti-hypertrophy therapy based on M3 receptor activation. We encourage conducting well designed clinical study to confirm the verdict of this study in patients with cardiac hypertrophy.

REFERENCES


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