Effects of ACE inhibitor on diabetic cardiovascular complications: cardiac and vascular hypertrophy.

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Pongseepera Suwangool*  Suthiluk Patumraj*


Using streptozotocin-induced diabetic rats (STZ-rats), the effects of angiotensin-converting enzyme inhibitor (ACEI) on cardiac and vascular hypertrophy were studied. Light micrographs of heart specimens of controls (CON), STZ-rats, and STZ-rats treated with ACEI (STZ-C) were obtained at 8, 12, and 16 weeks after the streptozotocin injections. Thicknesses of the right ventricular wall (RV), left ventricular wall (LV), and interventricular septum wall (IVS) were measured randomly using a micrometer in a light microscope with a 4 x -objective. In addition, the thickness of the intramural coronary arterial wall of each heart was also assessed. The results indicate that at 8–16 weeks the LVs of STZ-rat hearts were significantly thicker than those of CON (p<0.05). The thickening of the intramural coronary arterial wall was observed to be significant at 12 weeks. Interestingly, ACEI was able to attenuate these cardiovascular abnormalities. It is concluded that the results of pathophysiological studies indicate that ACEI might be of benefit in the prevention of diabetic cardiovascular complications.

Key words: Diabetes, Cardiac and vascular hypertrophy, ACEI.

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ผลของตัวบันทึกแสงจีโอเทคโนโลยีขั้นตอนแนวเศรษฐกิจเข้มข้นของทั่วไปและหลอดเลือดในแนวทาง: การขยายขนาดของทั่วไปและหลอดเลือด.

การศึกษาครั้งนี้เป็นการศึกษาผลของตัวบันทึกแสงจีโอเทคโนโลยีขั้นตอนแนวเศรษฐกิจ (ACEI) ต่อการขยายขนาดของทั่วไปและหลอดเลือด โดยใช้หนูที่ถูกทำให้เป็นเบาหวานด้วย streptozotocin ภาพจาก light microscope ของขั้นตอนทั่วไปของหลอดเลือด (CON) หนูเบาหวาน (STZ) และหนูเบาหวานที่ได้รับ ACEI (STZ–C) จะนำมาศึกษาที่ช่วงเวลา 8,12 และ 16 สัปดาห์หลังการฉีด streptozotocin ผลการศึกษาพบว่าขนาดของทั่วไปอย่างล่งช่วยว่า (RV) และช่วย (LV) และของหนึ่งครั้งที่ (IVS) รวมทั้งขนาดของหลอดเลือด intramural coronary arteries ซึ่งทำให้การวัดด้วยไมโครเมนูที่ต่อทับ light microscope และใช้ 4 x objective นั้นมั่นคงกว่า LV ของกลุ่ม STZ มีขนาดมากกว่ากลุ่ม CON อย่างมีนัยสำคัญทางสถิติ (p<0.05) ที่ช่วงเวลา 8-16 สัปดาห์ ส่วนความยาวของหลอดเลือด intramural coronary arteries ของ STZ นั้นมีขนาด

มากกว่า CON อย่างมีนัยสำคัญทางสถิติ (p<0.05) ที่ช่วงเวลา 12-16 สัปดาห์ เป็นนั่นสูงกว่า ACEIสามารถป้องกันการขยายขนาดของทั่วไปและหลอดเลือดได้จากการศึกษาครั้งนี้อาจสรุปว่า ACEI น่าจะเป็นประโยชน์กับการป้องกันการเกิดภาวะแทรกซ้อนของทั่วไปและหลอดเลือดในแนวทางได้
Cardiomyopathy as well as other cardiovascular diseases, such as hypertension, atherosclerosis, and arteriosclerosis, are major causes of diabetic deaths.\(^{(1-2)}\) However, the pathogenesis of diabetic cardiomyopathy has not yet been confirmed. Some investigators believe that diabetic cardiomyopathy might occur as a secondary effect of intramural coronary artery disease.\(^{(2)}\)

It appears that the risk of atherosclerosis and myocardial dysfunction in diabetes mellitus involves multi-factors, such as hyperglycemia, hypertension, dyslipidemia, abnormal platelet function and endothelial functional changes as well as abnormality of the renin–angiotensin system. In particular, an increase of serum angiotensin-converting enzyme (ACE) activity in diabetes has recently been reported by many investigators.\(^{(3-4)}\) Interestingly, the role of Ang II is not only that of a potent vasoconstrictor but also as a growth-promoting factor of both vascular smooth muscle cells\(^{(5)}\) and myocardial cells.\(^{(6)}\) At present, the newly documented vascular effect of ACE inhibitors has been studied extensively, especially in the vascular injury model.\(^{(6-12)}\) By contrast, there are no reports in the literature which address the effects of ACE inhibitor on coronary vascular changes in diabetes. Therefore, the objective of this investigation is to study the effects of ACE inhibitor on intramural coronary vascular walls and on myocardial hypertrophy in a diabetic animal model.

**Materials and Methods**

Twenty-seven male Wistar-Furth rats weighing 100-150 g (4-5 weeks old) were used in this investigation. Diabetes was induced by the i.p. injection of STZ (65 mg/kg bw). Controls rats (CON) (n=9) were sham injected with normal saline. The duration of diabetic state was defined as 8, 12, and 16 weeks after the STZ injections. Blood glucose was determined by hemoglocostrip and Glucometer (Reflolux S). The criterion used to establish the diabetic state was a blood glucose concentration of 400 mg/dl. Nine of eighteen STZ-rats (STZ-C, n=9) received oral daily feeding with cilazapril (10 mg/kg bw) starting one day after the STZ injection until the day of the experiment. After mean arterial pressure (MAP) was determined from the cannulated common carotid arteries, using a Nikhon Model TP-300T pressure transducer, the hearts were removed and weighed. The hearts were then soaked with a 10% formalin solution and cut into six pieces. The first piece, i.e. a piece taken from the top of the heart was 2 mm thick, the same as the sixth piece which was from the apex. The other four pieces were cut equally in to pieces about 3-4 mm thick. In this study, the second piece, which was the largest one, was used for further pathological examinations. Some of heart specimens were obtained by Eosin Hematotoxylin fixation for the measurement of sizes of the left ventricular wall (LV), right ventricular wall (RV), and interventricular septal wall (IVS). With different methods of elastic fixation, some heart specimens were used for the determination of intramural coronary vascular wall thickness. For taking measurements of either the ventricular or the vascular wall thickness, the micrometer of a light microscope with a 4x-objective was used.

**Results**

The injection of STZ (65 mg/kg bw) into the 100-150 g rats resulted in polyuria, polydipsia, polyphagia, and stable hyperglycemia within 24-48 hours. From the pathological examination, wall
thicknesses of LV, RV and IVS were estimated for each group at each time point (8, 12 and 16 wks) as shown in Table 1. Such results indicated that STZ-rat hearts were hypertrophic. The pathological studies showed that the left ventricle became hypertrophic from the eighth week following STZ injection, and there was no significant change in the size of IVS at all three monitored time points. Interestingly, the STZ-C hearts were significantly smaller in size compared with those of STZ-rats at all three time points. Light photomicrographs of 16-week STZ-rat hearts compared with the age-matched controls and STZ-C hearts are shown in Fig. 1A, Fig. 1B and Fig. 1C, respectively.

Figure 1. Cross sections of hearts of CON (A), STZ-rat (B), and STZ-C (C) at 16 weeks of experiment. RV = right ventricular wall, LV = left ventricular wall, and IVS = interventricular septum. H&E, x4.

The thickening of the vascular wall of the intramural coronary arteries of STZ-rats was clearly observed in all three hearts at 12 weeks after STZ injection (Table 2). However, this thickening of the coronary arterial wall was not observed in the three hearts of the 12 week STZ-C rats. At 16 weeks after STZ injection, these morphological changes of the coronary arterial wall became more remarkable in the STZ-rats as compared with the CON. In contrast, the 16-week STZ-C coronary arterial walls were not as thick as those of the STZ-rats, as shown in Fig. 2A, Fig. 2B, and Fig. 2C. And this difference was consistent for all three hearts obtained in this study.
Table 1. Sizes (um) of left (LV) and right (RV) ventricular wall and interventricular septum wall (IVS) of controls (CON), streptozotocin–induced diabetic rats (STZ-rats), and STZ-rats treated with ACEI (STZ-C) at 8, 12, and 16 weeks of experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Thickness (um)</th>
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<tbody>
<tr>
<td></td>
<td>LV</td>
</tr>
<tr>
<td><strong>8-WK</strong></td>
<td></td>
</tr>
<tr>
<td>CON (n=3)</td>
<td>2153.33 ± 94.52</td>
</tr>
<tr>
<td>STZ-rats (n=3)</td>
<td>2406.66 ± 48.04*</td>
</tr>
<tr>
<td>STZ-C (n=3)</td>
<td>2071.66 ±120.86**</td>
</tr>
<tr>
<td><strong>12-WK</strong></td>
<td></td>
</tr>
<tr>
<td>CON (n=3)</td>
<td>2278.33 ± 38.19</td>
</tr>
<tr>
<td>STZ-rats (n=3)</td>
<td>2513.33 ± 128.67*</td>
</tr>
<tr>
<td>STZ-Z (n=3)</td>
<td>2236.66 ± 177.78**</td>
</tr>
<tr>
<td><strong>16-WK</strong></td>
<td></td>
</tr>
<tr>
<td>CON (n=3)</td>
<td>2376.66 ± 80.20</td>
</tr>
<tr>
<td>STZ-rats (n=3)</td>
<td>2613.33 ± 63.70*</td>
</tr>
<tr>
<td>STZ-C (n=3)</td>
<td>2220.00 ± 171.09**</td>
</tr>
</tbody>
</table>

*Statistical difference compared to controls (p<0.05)

**Statistical difference compared to STZ-rats (p<0.05)

ns = non significantly different than controls or STZ-rats (p<0.05).

Table 2. Thickness of intramural coronary arterial wall (um) of controls (CON), streptozotocin–induced diabetic rats (STZ-rats), and STZ-rats treated with ACEI (STZ-C) at 8, 12, and 16 weeks of experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Thickness (um)</th>
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<tbody>
<tr>
<td></td>
<td>8-wk</td>
</tr>
<tr>
<td>CON (n=3)</td>
<td>23.96 ± 3.44</td>
</tr>
<tr>
<td>STZ-rats (n=3)</td>
<td>31.00 ± 4.38</td>
</tr>
<tr>
<td>STZ-C (n=3)</td>
<td>23.13 ± 2.25</td>
</tr>
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</table>

* Statistical difference compared to CON (p<0.05).

** Statistical difference compared to STZ-rats (p<0.05).
Discussion

Hearts from diabetic rats induced by STZ demonstrated depressed cardiovascular functions, as reported in the previous study. Concomitantly, the alterations of cardiovascular morphology were also observed during these cardiovascular func-
tional changes (8–16 weeks). At eight weeks after STZ injection, the decrease in cardiac contractility was observed together with an increase in BP and hypertrophy of LV. However, thickening of the intramural coronary arterial wall was later observed at 12 weeks after the STZ injection. Therefore, the abnormalities of cardiac function and hypertrophy observed in STZ-rats were unlikely the result of coronary vascular changes. In 1981, Factor and his co-workers\textsuperscript{14} showed that diabetic myocardium had demonstrated deterioration of the ultrastructure as the duration of the diabetic state reached its eighth week. In their study, myocytolysis in hypertensive-diabetic rats was observed.

Hypertension is well-known as a possible cause of vascular and cardiac hypertrophy. Valentovic et al. (1987)\textsuperscript{6} indicated that the presence of diabetes could accelerate damage to the myocardium caused by hypertension. In our study, we observed both events, i.e. hypertension, and vascular and cardiac hypertrophy, in our STZ-rats. Moreover, our results show that ACE-inhibitor could attenuate these diabetic cardiovascular complications, since all observed STZ-C rats had no hypertrophy of their vascular and left ventricle walls as the duration of the diabetic state progressed to 16 weeks.

The mechanism of this effect of ACE inhibitor might be simply that ACE inhibitor could prevent further cardiovascular complications in the STZ-rats, since ACE inhibitor directly decreased the activity of ACE that was often higher than normal.\textsuperscript{4}

In diabetes, the exaggerated pressor response to Ang II and other vasoconstrictors was reported by Weidmann et al. 1985\textsuperscript{15}. It is believed that ACE inhibitors could effectively lower blood pressure in cases of hypertensive diabetes, because
these agents not only lower circulating and tissue levels of Ang II, but they also raise local levels of vasodiating agents such as kinins and prostaglandins.\(^{(16)}\)

Recently, many investigations have shown that Ang II was a potent direct mediator of protein synthesis in chick-heart cell culture.\(^{(17)}\) This action of Ang II as a growth-promoting agent was also suggested as being a mediator of hypertrophy in vascular smooth muscle cells.\(^{(17)}\) Thus, it is reasonable to speculate that besides the hypotensive effect, the abilities of ACE inhibitor in preventing diabetic cardiovascular complications shown in our study might be also linked to the inhibition growth-promoting effect of Ang II.

In summary, our study has shown that ACE inhibitor could prevent cardiovascular complications in STZ-induced diabetic rats during the progression of the diabetic state reached 8-16 weeks. However, further studies are needed for explaining the mechanisms of this agent. A also more clinical studies are required in order to evaluate the therapeutic effect of this agent which may provide further benefit to diabetic patients.

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References


9. Powell JS, Clozel JP, Muller RKM, Kuhn H, Hefti F, Hosang M, Baumgartner HR. Inhibitors of angiotensin-converting en-


13. Udayachalerm W, Jariyapongsakul A, Suwangool P, Patumraj S. Effects of ACE inhibitor on diabetic cardiovascular com-

plications: cardiac and vascular hypertro-


