นิพนธ์ต้นฉบับ

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

Wasan Udayachalerm* Amporn Jariyapongsakul*
Pongsepeera Suwangool* Suthiluk Patumraj*

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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 intramular 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.*

Reprint request: Udayachalerm W, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

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*Department of Physiology, Faculty of Medicine, Chulalongkorn University.

วสันต์ อุทัยเฉลิม, อัมพร จาริยะพงศ์สกุล, พงษ์พีระ สุวรรณกูล, สุทธิลักษณ์ ปทุมราช. ผลของตัวยับยั้งแองจิโอเทนซินคอนเวอร์ติ้งเอนไซม์ต่อภาวะแทรกซ้อนของหัวใจและหลอดเลือดในเบาหวาน : การขยายขนาดของหัวใจและผนังหลอดเลือด. จุฬาลงกรณ์เวชสาร 2538 มกราคม; 39(1): 29-36

การศึกษาครั้งนี้เป็นการศึกษาผลของตัวยับยั้งแองจิโอเทนซินคอนเวอร์ติ้งเอนไซม์ (ACEI) ต่อการขยายขนาดของหัวใจและผนังหลอดเลือด โดยใช้หนูที่ถูกทำให้เป็นเบาหวานด้วย streptozotocin ภาพถ่ายจาก light microscope ของชิ้นเนื้อหัวใจของหนูควบคุม (CON) หนูเบาหวาน (STZ) และหนูเบาหวานที่ได้รับ ACEI (STZ-C) จะนำมาศึกษาที่ช่วงเวลา 8,12 และ 16 สัปดาห์หลังการฉีด streptozotocin ผลการศึกษาพบว่าขนาดของหัวใจห้องล่างข้างขวา (RV) และซ้าย (LV) และของผนังตรงกลาง (IVS) รวมทั้งขนาดของผนังหลอดเลือด intramural coronary arteries ซึ่งทำการวัดด้วยไมโครมิเตอร์ที่ต่อกับ light microscope และใช้ 4 x objective นั้น แสดงว่า LV ของกลุ่ม STZ มีขนาดหนากว่ากลุ่ม CON อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ที่ช่วงเวลา 8-16 สัปดาห์ ส่วนความหนาของผนังหลอดเลือด intramural coronary arteries ของ STZ นั้นมีขนาดหนากว่า CON อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ที่ช่วงเวลา 12-16 สัปดาห์ เป็นที่น่าสังเกตว่า ACEIสามารถป้องกันการขยายขนาดของหัวใจและหนังหลอดเลือดนี้ได้จากการศึกษาครั้งนี้อาจสรุปว่า ACEI น่าจะเป็นประโยชน์กับการป้องกันการเกิดภาวะแทรกซ้อนของหัวใจและหลอดเลือดในเบาหวานได้

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 beleive that diabetic cardiomyopathy might occur as a secondary effect of intramural coronary artery disease.[3]

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.[4-6] 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[7] and myocardial cells.[8] At present, the newly documented vascular effect of ACE inhibitors has been studied extensively, especially in the vascular injury model.[9-12] 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.

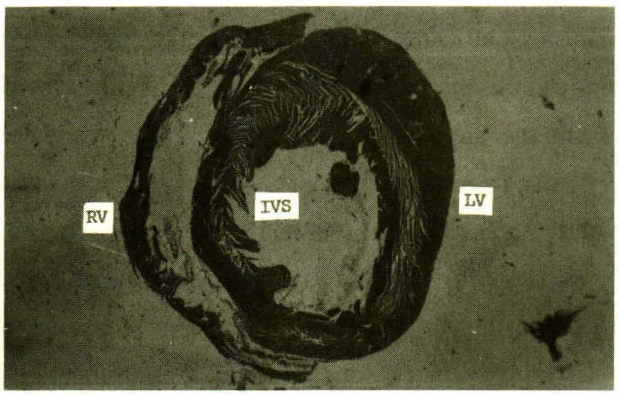
Materials and Methods

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 hemoglucostrip 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 can-nulated 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.



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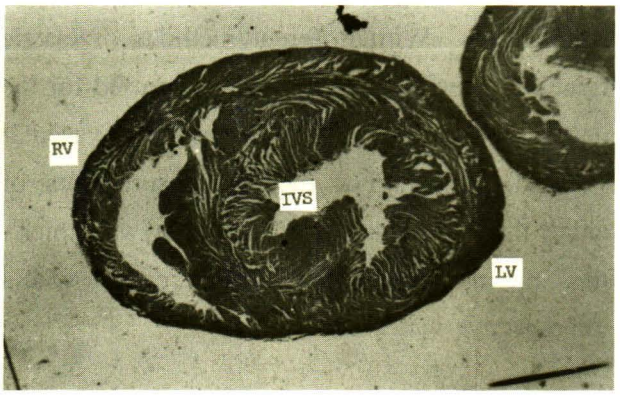

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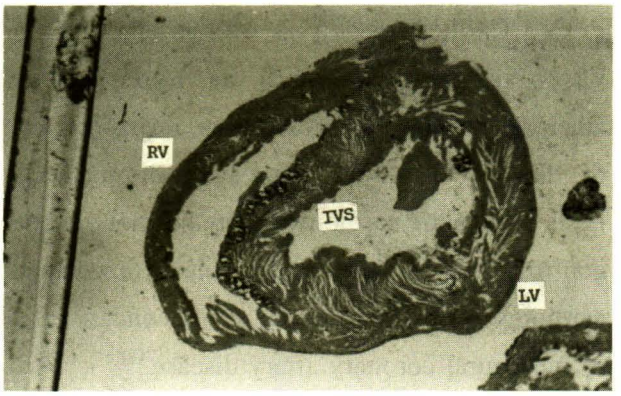

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Figure 1. Cross sections of hearts of CON (A), STZ-rat(B), and STZ-C (C) at 16 weeks of experiment. RV = right ventricular wall, LV = left ventricular wall, and IVS = interventricular septum. H&E, x4.

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 reptum 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.

Group	Thickness (um)		
	LV	RV	IVS
8-WK			
CON (n=3)	2153.33 ± 94.52	723.16 ± 28.15	1771.66 ± 38.83
STZ-rats (n=3)	2406.66 ± 48.04*	766.66 ± 65.25^{ns}	2000.00 ± 117.57*
STZ-C (n=3)	2071.66 ±120.86**	756.66 ± 59.23^{ns}	1730.00 ± 257.34^{ns}
12-WK			
CON (n=3)	2278.33 ± 38.19	645.00 ± 55.67	1800.00 ± 157.87
STZ-rats (n=3)	2513.33 ± 128.67*	881.66 ± 115.79*	2113.33 ± 363.05^{ns}
STC-Z (n=3)	2236.66 ± 177.78**	673.35 ± 49.32**	1613.33 ± 125.53^{ns}
16-WK			
CON (n=3)	2376.66 ± 80.20	790.00 ± 75.47	1710.00 ± 72.62
STZ-rats (n=3)	2613.33 ± 63.70*	1078.33 ± 205.93*	1838.33 ± 607.99^{ns}
STZ-C (n=3)	2220.00 ± 171.09**	713.33 ± 72.85**	1430.00 ± 242.53^{ns}

*Statistical difference compared to controls ($p<0.05$)

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

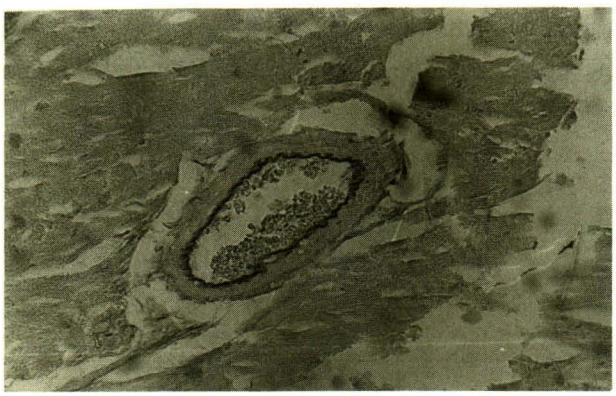
ns = non significantly different than controls or STZ-rats ($p<0.05$).

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.

Group	Thickness (um)		
	8-wk	12-wk	16-wk
CON (n=3)	23.96 ± 3.44	30.63 ± 3.31	27.50 ± 7.21
STZ-rats (n=3)	31.00 ± 4.38	41.67 ± 5.81*	52.71 ± 6.88*
STZ-C (n=3)	23.13 ± 2.25	26.77 ± 5.95**	33.33 ± 5.67**

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

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



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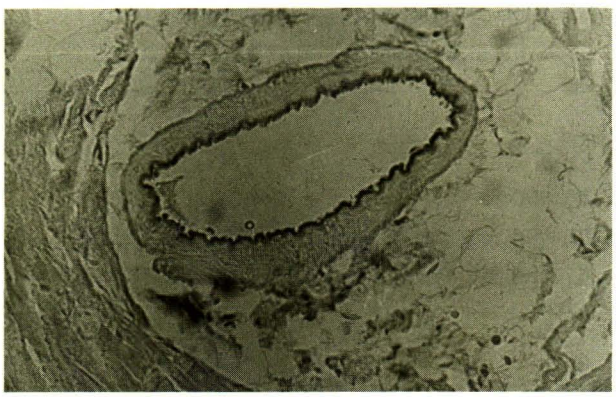

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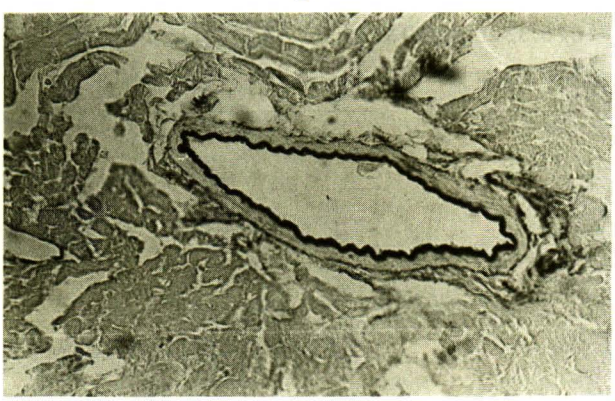

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Figure 2. Light photomicrographs of intramural coronary arteries of CON (A), STZ-rat (B), and STZ-C (C) at 16 weeks of experiment. Elastic, x40.

Discussion

Hearts from diabetic rats induced by STZ demonstrated depressed cardiovascular functions, as reported in the previous study.[13] Concomitantly, the alterations of cardiovascular morphology were also observed during these cardiovascular functional 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[14] showed that diabetic myocardium had demonstrated diterioration 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)[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.[4]

In diabetes, the exaggerated pressor response to Ang II and other vasoconstrictors was reported by Weidmann et al. 1985[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 medicator 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.[7] Thus, it is resonable to speculate that besides the hypotensive effect, the abilities of ACE inhibitor in preventing diabetic cadiovascular 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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