นิพนธ์ต้นฉบับ

Effects of nicardipine and nicardipine combined with cilazapril on cardiovascular complications of diabetic rats

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Objective : *To study the effects of nicardipine and nicardipine combined with cilazapril on cardiovascular complications in diabetic rats.*

Setting : *Department of Physiology, Faculty of Medicine, Chulalongkorn University*

Research design : *Experimental design*

Materials : *Fourty eight male Wistar-Furth rats were used and separated into four groups :*

Control group (NSS) *The animals received an intravenous injection of normal saline solution (NSS) instead of STZ (n=12).*

Diabetic group (STZ) *The animals received a single intravenous injection of streptozotocin (STZ) (55 mg/kg BW). This group was treated daily with an NSS oral feeding starting 1 day after the STZ injection until the day of performing the isolated heart experiment (n=12).*

Diabetic with nicardipine treatment group (STZ-N) *These animals received the STZ injection as same as the diabetic group and were treated with nicardipine (10 mg/kg BW/day) via oral feeding starting 1 day after the STZ injection until the day of performing the isolated heart experiment (n=12).*

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Diabetic with nicardipine combined with cilazapril treatment group (STZ-NC) These animals received the STZ injection as same as the diabetic group and were treated with nicardipine and cilazapril (10 mg/kg BW/day) via oral feeding starting 1 day after the STZ injection until the day of performing the isolated heart experiment (n=12).

Methods : *By using a modified Langendroff's method, the cardiovascular parameters were monitored for all four groups for experimental periods of 8 and 16 weeks.*

Results : *The cardiovascular parameters, including aortic and coronary flow rates, and left ventricular isotonic contractions of the STZ-rats were significantly decreased as compared to the controls at 16 weeks ($p < 0.05$). Both treatments of nicardipine and nicardipine combined with cilazapril significantly attenuated these abnormalities. However, there was no synergistic effects between these two medicinal treatments. It is concluded that there was a beneficial effect of combined nicardipine and cilazapril for prevention of diabetic cardiovascular complications. Nevertheless, calcium ions should be the important central factor for the effects demonstrated by these two medicines.*

Key words : *Diabetic cardiovascular complications, Nicardipine, Cilazapril, STZ-rat.*

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วัตถุประสงค์ : *เพื่อศึกษาผลของ nicardipine และ nicardipine ร่วมกับ cilazapril ต่อภาวะแทรกซ้อนของหัวใจและหลอดเลือดในหนูแร็ทที่เป็นเบาหวาน*

สถานที่ที่ทำการศึกษา : *ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย*

รูปแบบการวิจัย : *การวิจัยเชิงทดลอง*

วัสดุที่ใช้ : *หนูตัวผู้พันธุ์ Wistar-Furth จำนวน 48 ตัว แบ่งออกเป็น 4 กลุ่ม คือ: หนูควบคุม (NSS) คือหนูที่ได้รับการฉีดน้ำเกลือปกติแทนการฉีด STZ (n=12 ตัว) หนูเบาหวาน (STZ) คือหนูที่ได้รับการฉีด streptozotocin จำนวน 55 มิลลิกรัม/กิโลกรัมน้ำหนักตัวทางหลอดเลือดดำ 1 ครั้ง จนกระทั่งถึงวันที่นำมาศึกษา (n = 12 ตัว) หนูเบาหวานที่ได้รับ nicardipine (STZ-N) คือหนูที่ได้รับ STZ และได้รับการป้อน nicardipine ในปริมาณ 10 มิลลิกรัมต่อน้ำหนักตัวหนึ่งกิโลกรัมทุกวัน วันละ 1 ครั้ง จนกระทั่งถึงวันที่นำมาศึกษา (n = 12 ตัว) หนูเบาหวานที่ได้รับ nicardipine ร่วมกับ cilazapril (STZ-NC) คือหนูเบาหวานที่ได้รับ nicardipine และ cilazapril ในปริมาณอย่างละ 10 มิลลิกรัมต่อน้ำหนักตัวหนึ่งกิโลกรัมทุกวัน วันละ 1 ครั้ง (n = 12 ตัว)*

วิธีการทดลอง : *โดยใช้วิธี modified Langendroff เพื่อศึกษาประเมินค่า parameters ต่าง ๆ ของหัวใจและหลอดเลือดของทุกกลุ่มที่ช่วงเวลา 8 และ 16 สัปดาห์*

ผลการทดลอง : *ค่าอัตราการไหลของเลือดใน aortic และ coronary และค่าแรงหดตัวของหัวใจห้องล่างข้างซ้ายของหนูเบาหวานมีค่าลดลงอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับหนูปกติที่ช่วงเวลา 16 สัปดาห์ ($p < 0.05$) การให้ nicardipine ร่วมกับ cilazapril พบว่าสามารถช่วยป้องกันการเกิดความผิดปกตินี้ได้ แต่อย่างไรก็ตามไม่พบความสัมพันธ์ที่เป็นลักษณะช่วยเสริมกันของยาทั้งสองตัวนี้*

จึงสรุปได้ว่า ผลของ nicardipine และ cilazapril คือช่วยป้องกันการเกิดภาวะแทรกซ้อนของหัวใจและหลอดเลือดในหนูเบาหวานได้ อย่างไรก็ดี calcium ions อาจเป็นปัจจัยกลางที่สำคัญของการออกฤทธิ์ของยาทั้งสองตัวนี้

คำสำคัญ : *ภาวะแทรกซ้อนของหัวใจและหลอดเลือดในภาวะเบาหวาน, Nicardipine, Cilazapril, หนู streptozotocin*

Cardiovascular complications are the most important cause of morbidity and mortality in diabetes mellitus. The pathophysiology of vascular disease may involve the enhancement of vasoconstriction, increase of platelet vessel wall interaction, adherence of monocytes, migration and production. [1] Several studies have demonstrated that vascular functions, including vasomotion, proliferation of vascular smooth muscle cells and oxidizing LDL (low density lipoprotein) on endothelial cells mediated by increasing intracellular calcium, are response to local changes such as cells and their environments. [2-4] In 1985 Anthony and co-workers[5] studied effects of a calcium antagonist (nicardipine) which suppresses the formation of atherosclerotic lesions and accumulation of cholesterol in rabbits. The rabbits were fed with a 2% cholesterol diet for 8 weeks. Calcium antagonist effects antiatherosclerotic mechanisms through antihypertensive activity or suppression of lipid uptake by scavenger cells. The possibility exists that this calcium antagonist promotes cholesterol ester catabolism and cholesterol clearance by reducing monocyted adhesions to activated endothelium. [6]

However, some other studies indicated that nicardipine in clinical trials has no effect on advanced coronary atherosclerosis but may retard the progression of minimal lesions.[7] Later, in 1989, Naftilan[8] demonstrated an effect of angiotensin II which led to a rapid rise in the steady-state mRNA levels of the proto-oncogenes c-fos, c-myc and c-jun. Also, angiotensin II induce platelet derived growth factor that increase smooth muscle cell protein synthesis. [9]

Several observations have highlighted the coincidence of the diabetic cardiomyopathys and atherosclerosis. However, calcium channel blockers have been only tested for their effects on human atherosclerosis. Nifedepine and nicardipine prevented the progression of small atherosclerotic lessions, but large plaques were unchanged. [10, 11] Unfortunately, the effect of angiotensin converting enzyme inhibitors (ACEI) such as captopril that retard the progression of atherosclerosis had been demonstrated only in hyperlipidemia rabbits. [12]

In the previous study conducted in our laboratory,[13] the prevention of diabetic cardiovascular complications by using daily oral feedings of cilazapril (10 g/kg/BW) was demonstrated in 16-week STZ-rats. It was hypothesized for our study that cilazapril could retard these diabetic cardiovascular complications according to their actions as an inhibitor of vascular growth promoting factor and also on its effect on bradykinin synthesis.

Interestingly, angiotensin converting enzyme inhibitors and calcium channel blockers have a complementary profile, both in their hemodynamic and local vascular actions. Hence, combination therapy with these two classes of drugs appears particulary useful in hypertensive patients for reducing blood pressure.[14] However this combined therapeutic effect on diabetic cardiovascular complications has not yet been demonstrated. Moreover, the recent studies have showed that angiotensin II might play a key role among hyperlipidemia, endothelial dysfunction, atherosclerosis, and also myocyte apoptosis .[15-20]

Therefore, the objective of this investigation were to determine the effects of nicardipine with and without cilazapril on cardiovascular complications in diabetic rats.

Materials and Methods

Male Wistar Furth rats, weighing about 100-150 g (aged 4-5 weeks) were used in this study (n=48). All animals were fasted overnight before the diabetic induction using streptozotocin (STZ) (55 mg/kg BW, iv.) The animals were separated into four groups:

Control group (NSS) The animals received an intravenous injection of normal saline solution (NSS) instead of STZ (n=12).

Diabetic group (STZ) The animals received a single intravenous injection of STZ (55 mg/kg BW). This group was treated daily with an NSS oral feeding starting 1 day after the STZ injection until the day of performing the isolated heart experiment (n=12).

Diabetic with nicardipine treatment group (STZ-N) These animals received the STZ injection as same as the diabetic group did. The animal were treated with nicardipine (10 mg/kg BW/day) via oral feeding starting 1 day after the STZ injection until the day of performing the isolated heart experiment (n=12).

Diabetic with nicardipine combined with cilazapril treatment group (STZ-NC) These animals received the STZ injection as same as the diabetic group did. They were treated with nicardipine and cilazapril (each of 10 mg/kg BW/day) via oral feeding starting 1 day after the STZ injection until the day of performing the isolated heart experiment (n=12).

On the day of the isolated heart experiment, the animal was weighed and then anesthetized by intraperitoneal injection of 45 mg/kg body weight of sodium pentobarbital. After tracheostomy, the animal was ventilated with a small animal respirator (Harvard Rodent model 683). Blood pressure was measured by catheter (PE 180) inserted into a common carotid artery and connected to the pressure transducer (Nikhon model TP-300T), then shown on the polygraph (Nikhon RM 6000). The chest was opened in order to remove the pericardial sac and the heart. Two vessels, the right subclavian artery and the ascending aorta, were then loosely ligated.

Before the isolation of the heart, the flow probe (Nikhon model FE-020T) was placed around the ascending aorta and measured the values of aortic flow rate. After these measurements, the right subclavian artery was done and 150 units of heparin were injected into the right atrium. The common carotid artery catheter was then connected to the perfusate system and the right atrium was then quickly cut open. The ligature on the ascending aorta was then tightened, directing the perfusate flow retrograde to the coronary circulation. The heart was then carefully removed from the animal. After the heart was left equilibrating for 15 minutes, the coronary flow rate was measured as the volume of fluid that flowed out from the cut right atrium per unit time. The left ventricular isotonic contraction was recorded through the wire hooked at the apex of the left ventricle and connected to the isotonic transducer. The patterns of contraction were recorded on the polygraph (Nikhon RM 6000).

Statistics

Data are reported as mean $\pm$ SD. Statistical analysis was performed on the raw data by using analysis of variance (ANOVA) followed by Unpaired T-tests between groups.

Results

The streptozotocin treated rat was used as an experimental model of diabetes mellitus in this

study. The results shown in Table 1 indicated that all three groups (8, 16 weeks) of six STZ-rats, six STZ-N rats and six STZ-NC rats had severe hyperglycemia. There was no significant difference of plasma glucose among the three groups. The body weights of the STZ-rats, STZ-N rats and STZ-NC rats were significantly decreased as compared to the controls. However, the ratio of heart weight per 100 grams body weight in 16 week STZ-rats, STZ-N rats, and STZ-NC rats were significantly higher than those of the control group.

Cardiovascular functions including heart rate (HR), mean arterial pressure (MAP), aortic flow rate (AFR), coronary flow rate (CFR), and left ventricular isotonic contractions (LVIC) were determined for the three groups (8,16 weeks) of six controls, six STZ-N rats and six STZ-NC rats, as demonstrated in Fig. 1-4.

At eight weeks after treatment, the heart rate of the treated rats were slower than those of the control rats. However, the STZ-N rats had heart rates much lower than the others. In the same manner, the sixteen week treatment rats showed that all STZ-treatment rats had heart rates lower than those of the controls.

At the eight week experimental point the mean arterial pressure of the STZ-rats, STZ-N rats

Table 1. Plasma glucose, body weight, ratio of heart weight per100grams body weight, and heart rate of controls (NSS), streptozotocin-treated rats (STZ), nicardipine treated STZ-rats (STZ-N), and cilazapril combined with nicardipine-treated STZ-rats (STZ-NC) at 8 and 16 weeks of experimental periods. a Significant difference as compared to controls ($p<0.05$). b Significant difference as compared to STZ-rats. ($p<0.05$).

Parameters	NSS (n = 6)	STZ (n = 6)	STZ-N (n = 6)	STZ-NC (n = 6)
Plasma glucose (mg/dl)				
8 week	104.67 ± 49.46	499.33 ± 23.52	514.00 ± 10.26	526.33 ± 97.17
16 week	99.00 ± 15.11	490.33 ± 47.54	477.00 ± 35.65	487.00 ± 63.69
Body weight (g)				
8 week	347.00 ± 40.02	229.83 ± 30.20[a]	250.00 ± 28.06[a]	270.00 ± 35.38[a]
16 week	456.00 ± 39.81	266.45 ± 33.91[a]	255.76 ± 21.75[a]	235.50 ± 23.15a
Ratio of heart wt. per 100g BW				
8 week	0.32 ± 0.03	0.40 ± 0.12[a]	0.36 ± 0.02[a]	0.38 ± 0.03[a]
16 week	0.26 ± 0.01	0.45 ± 0.08[a]	0.36 ± 0.03[a,b]	0.38 ± 0.05[a,b]
Heart rate (beat per min)				
8 week	362.50 ± 36.02	320.00 ± 39.87[a]	270.66 ± 17.22[a]	325.00 ± 39.87[a]
16 week	375.00 ± 26.83	295.00 ± 48.06[a]	295.00 ± 18.16[a]	262.50 ± 32.51[a]

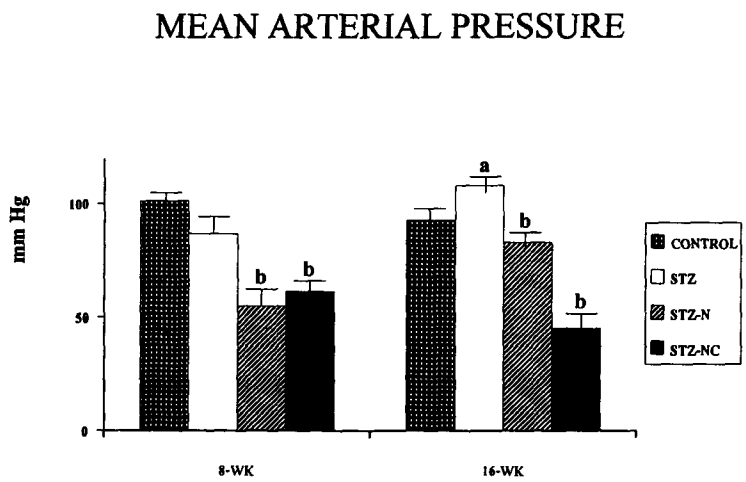

Figure 1. Mean arterial pressure (mm Hg) of controls , streotozotocin-treated (STZ), nicardipine treated STZ-rats, (STZ-N) , and cilazapril combined with nicardipine treated STZ-rats (STZ-NC) were monitored at 8 and 16 weeks of experimental periods.

a Significant difference as compared to controls ($p < 0.05$).

b Significant difference as compared to STZ-rats ($p < 0.05$).

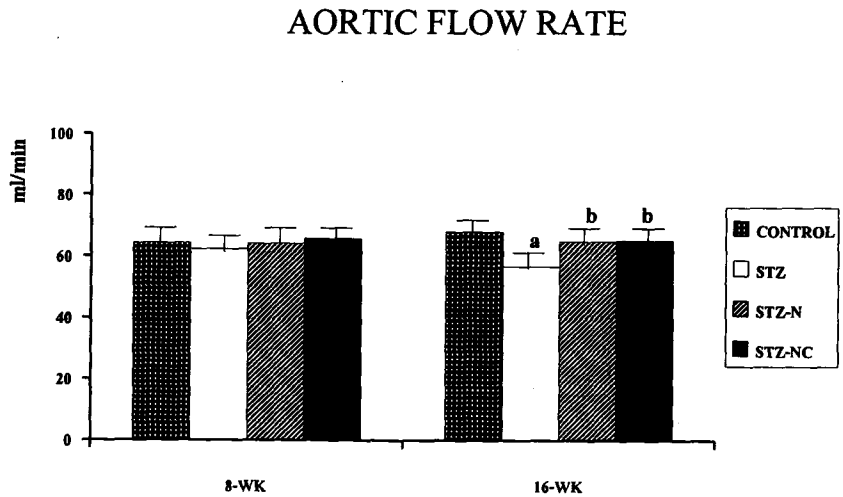

Figure 2. Aortic flow rates (ml/min) of controls, streotozotocin-treated rats (STZ), nicardipine treated STZ-rats, (STZ-N), and cilazapr il combined with nicardipine treated STZ-rats (STZ-NC) were monitored at 8 and 16 weeks experimental periods.

a Significant difference as compared to controls ($p < 0.05$).

b Significant difference as compared to STZ-rats ($P < 0.05$)

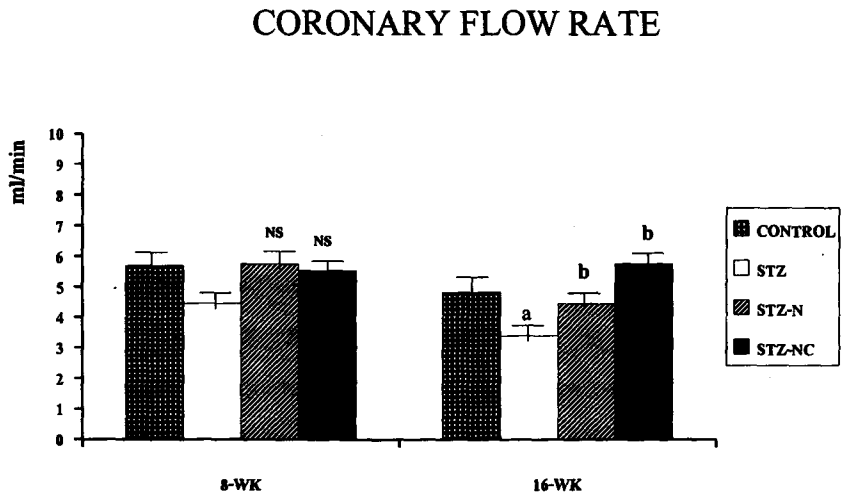

Figure 3. Coronary flow rates (ml.min) of controls, streotozotocin-treated rats (STZ), nicardipine treated STZ- rats, (ST Z- N), and cilazapril combined with nicardipine treated STZ- rats (STZ- NC) were monitored at 8 and 16 weeks of experimental periods.

a Significant difference as compared to controls ($p < 0.05$).

b Significant difference as compared to STZ - rats ($p < 0.05$).

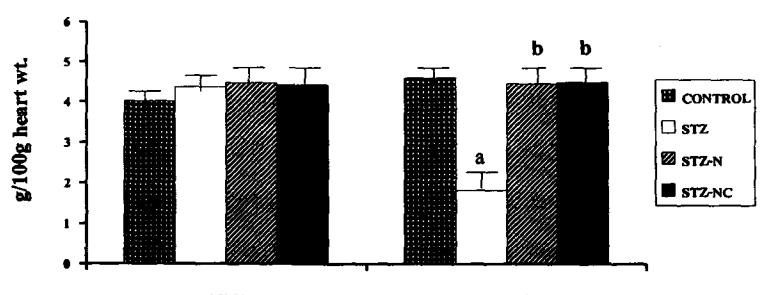

Figure 4. Left ventricular isotonic contraction (g/100g heart wt). of controls, streotozotocin - treated rats (STZ), nicardipine treated STZ - rats, (STZ - N), and cilazapril combined with nicardipine treated STZ - rat (STZ - NC) were monitored at 8 and 16 weeks of experimental periods.

a Significant difference as compared to controls ($p < 0.05$).

b Significant difference as compared to STZ - rats ($p < 0.05$).

and STZ-NC rats were significantly less than those of the controls. Moreover, the mean arterial pressure of the nicardipine treated rats was lower than those of the STZ- rats at the same experimental periods. After sixteen weeks of the experiment the mean arterial pressure of the STZ- N and STZ-NC rats were significantly lower than those of the control rats and the STZ-rats. In addition, the mean arterial pressure of the STZ-NC rat group was the lowest.

The aortic flow rate of all six control rats, STZ-rats, STZ-N rats and STZ-NC rats were not different at 8 weeks. But at 16 weeks the aortic flow rate of the STZ-N rats and STZ-NC rats were significantly increased as compared to the STZ-rats.

At eight weeks, the coronary flow rates of the STZ-rats, STZ-N rats and STZ-NC rats were not different as compared to the control rats. At 16 weeks into the experiment, the coronary flow rates of the STZ-N and STZ-NC rats were not different compared to the control group. However, the coronary flow rates of the STZ-rats seemed to be less than those of the other groups. In other words the coronary flow rates of the STZ-N rats and STZ-NC rats were significantly higher than in the STZ-rats.

At eight weeks, the results of left ventricular isotonic contractions (LVIC, gm/100 gm of heart weight) showed that there was no significant difference among the three groups of STZ-rats, STZ-N rats and STZ-NC rats, and also with that of the controls. However, the results after sixteen weeks of the experiment showed a decrease of LVIC in STZ-rat groups as compared to those of the controls. Interestingly, at 16 weeks, there were no significant difference of LVIC between the STZ-Ns rat and STZ-NC rats as compared to the control group.

Discussion

The results of this study demonstrated that abnormalities of cardiovascular functions such as mean arterial pressure, aortic flow rate, coronary flow rate and left ventricular isotonic contractions had been observed in streptozotocin treated rats. Similarly, decreases of cardiovascular functions such as cardiac output and coronary blood flow in STZ-rats were reported together with the observation of ventricular wall thickening by Penpargkul et al. (1980).[21]

Recently, many studies have also shown the high incidence of abnormal renin-angiotensin systems in association with diabetes. Liberman, et al (1980) [22] reported that an increasing of serum angiotensin-converting-enzyme (ACE) was associated with diabetes mellitus. In their study, the mean value of ACE activity in the diabetic group was significantly higher than those of the healthy and the hypertensive groups. Since ACE is an enzyme that converts angiotensin I to angiotensin II, the increasing of serum ACE will cause the increase of serum angiotensin II synthesis. Angiotensin II is a potent vasoconstrictor and is likely to promote hypertension in diabetic patients. [23]

After 24 hours of diabetic induction by using STZ, rats were treated with cilazapril 10 mg/kg BW for 16 weeks. Hypertension, myocardial hypertrophy and coronary arterial wall thickening, which are usually observed in STZ-treated rats, could be protected by the oral treatment of cilazapril.[13] They suggest that the possible role of angiotensin II is likely to be a growth factor/modulator which relates to the development of cardiovascular hypertrophy. The previous study also showed that angiotensin II acts through AT1 (angiotensin II subtype I) receptors

to increase c-fos expression and phosphoinositide turnover in vascular smooth muscle cells.[24] Furthermore, angiotensin-II enhances the activation of the voltage-gate channel of calcium ion; that makes it easier for the channel to open in response to a wave of depolarization. This effect of angiotensin II is probably mediated by a GTP-binding protein.[25] In addition, calcium ions may be involved in the regulation of muscle growth. This reasonable speculation could be factors which temporarily act as vasoconstrictors in a short term by increasing the level of cytosolic calcium during a long and continued stimulation of vascular smooth muscle which leads to myointimal proliferation.

Thus, a calcium channel blocker and a calcium channel blocker combined with angiotensin converting enzyme inhibitor (ACEI) were used in this study to prevent cardiovascular complications in diabetic rats. In our study, the result supported an observation that chronic diabetic hearts could cause the reduction of both aortic and coronary flow rates, including the diminishing of ventricular contractility. In contrast, our results also showed that a calcium channel blocker and a calcium channel blocker combined with angiotensin converting enzyme inhibitor can improve this defect.

Calcium movement from extracellular to intracellular regions can be regulated by a numbers of mechanisms; for example, through plasma membranes via voltage or receptor operated channels. Previous studies demonstrated that the mechanism of dihydropyridine calcium channel blocker (nicardipine) helps to reduce the accumulation of arterial cholesterol in dietary-induced atherosclerosis in an animal model. They also found that a calcium channel blocker enhanced lysosomal and cytoplasmic cholesterol ester hydrolytic activity. Moreover, the releasing of prostaglandin I_2(PGI_2) was increased in response to nicardipine treatment. In addition, There is the implication of increased PGI_2 by nicardipine could block platelet deposition and activation, which causes early pro-atherosclerotic events in the genesis of vascular lessions.

Feron and co-worker (1996)[26] demonstrated that a dihydropyridine calcium antagonist exerted inhibition of ventricular hypertrophy and reduced cardiac pre-proendothelin-1 mRNA expression in hypertensive rats. The effects of endothelin was involved in vascular hypertrophy.[27] Other studies showed that the heart weight/body weight ratios (represent to cardiomegaly) of diabetic rats were significantly larger than for normal rats but were reduced in calcium channel blocker treated rats.

In our study, we found that the increase of heart weight/body weight ratio, together with the decrease of the left ventricular isotonic contractions and coronary flow rate, could be improved by using either nicardipine or nicardipine combined with cilazapril.

In other words, from this study we propose that cardiovascular complications in diabetic rats could be partly initiated by the increase of angiotensin II. Especially, angiotensin II could not only act as a growth promoting factor but also as an enhancer for calcium ions released from sarcoplasmic reticulum. These cytosolic calcium ions act as a mediator, and could cause further physiological processes including vasoconstriction, vascular and ventricular hypertrophy through the mechanisms previously mentioned. Moreover, the effects of calcium channel blockers have

recently been demonstrated to reduce the mortality in diabetic patients with systolic hypertension.[28] In conclusion, it is believed that calcium channel blockers might be a beneficial therapeutic agent for use as a preventor for diabetic cardiovascular complications.

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