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BASIC PHARMACOLOGY

Taurine May Prevent Diabetic Rats from Developing Cardiomyopathy also by Downregulating Angiotensin II Type2 Receptor Expression

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Summary. Objective: **In diabetes, intracellular accumulation of sorbitol resulting from the high extracellular levels of glucose leads to depletion of intracellular compounds including taurine. This is associated with the development of late diabetic complications such as cardiomyopathy. The development of myocyte hypertrophy has been largely attributed to angiotensin II, whose growth properties are antagonized by taurine. However, the interaction between taurine, angiotensin II type2 receptor (AT2) and cardiomyopathy related to angiotensin II is still unknown. This study investigates the roles of taurine and AT2 in rats with streptozotocin (STZ)-induced diabetic cardiomyopathy.**

Methods: **Of 60 female 4-week-old Wistar rats, 8 were treated with common diet and the other 52 with high sugar/fat diet (during the whole experiment) to induce insulin resistance. At the 4th week, of the 52 rats, 7 treated with sodium citrate buffer (pH = 4.5) were grouped into control group1 (con1) and the other 45 were treated by intraperitoneal injection (I.P) with STZ to develop type 2 diabetes. At the 28th week, the maximal velocity decrease of pressure per second in left ventricle within the period of isovolumic relaxation ($-dp/dt_{max}$) was detected by a cannula through right carotid artery. After the cannula operation, of the 45 rats, all the living 24 with $-dp/dt_{max} \leq 5250$ mmHg/s, who had developed diabetic cardiomyopathy, were grouped as follows: 7 treated with double distilled H2O (I.P) were grouped into control group2 (con2). 8 treated with AT2 agonist (CGP42112A) (I.P) were grouped into experimental group1 (exp1). Another 9 treated with taurine (I.P) were grouped into experimental group2 (exp2). All injections lasted 4 weeks (Q.D) and the heart weight (HW) was recorded. To examine cardiomyocyte apoptosis index (CAI), mRNA and protein of AT2 and Bcl-2 in cardiomyocytes, methods of terminal-deoxynucltidyl transferase mediated nick end labeling (TUNEL), reversal transcription polymerase chain reaction (RT-PCR) and immunoblot (Western Blot) were used, respectively.**

Results: **Values of $-dp/dt_{max}$ in exp1, exp2 or con2 were much less than those in con1, respectively ($p < 0.01$). CAI (= stained cell number/total cell number $\times$ 100%) and AT2 values both in mRNA and protein levels in con1 were less than those in the other three groups, respectively ($p < 0.01$). The three parameters above were more in exp1 but less in exp2 than those in con2, respectively ($p < 0.01$).The three parameters and HW in exp1 were much higher than those in exp2, respectively ($p < 0.01$). Changes of Bcl-2 were opposed to those of AT2.**

Conclusions: **A high expression of AT2 may accelerate the apoptosis of cardiomyocytes in diabetic rats and play a role in precipitating diabetic cardiomyopathy; taurine may protect diabetic rats from developing cardiomyopathy also by downregulating AT2 receptors.**

Key Words. **taurine, diabetic cardiomyopathy, AT2 agonist, apoptosis, AT2 receptors, angiotensin II**

1. Introduction

Diabetic cardiomyopathy has been catching more and more people's eyes since the concept was put forward

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in 1972 by Hansen et al. [1]. However, it is extremely difficult to treat diabetic cardiomyopathy, although carvedilol is being used with increasing success. One of the reasons may be that our knowledge of the mechanisms underlying type2 diabetic cardiomyopathy is lacking. Several possible mechanisms of diabetic cardiomyopathy have been put forward by now. Streptozotocin (STZ)-induced diabetes may selectively reduce cardiac Na^+, K^+-ATPase concentration by 25%, which reduces the capacity of the heart to maintain K^+-Ca^{2+}-homeostasis [2]. This may be associated with heart failure in diabetic cardiomyopathy. Advanced glycation end products on sarco (endo) plasmic reticulum Ca^{2+}-ATPase (SERCA2a) may slow cardiac relaxation in diabetic rats [3]. Also, decreased Hsp60 expression and subsequent decline of insulin-like growth factor-1 receptor (IGF-1R) signaling could be a fundamental mechanism underlying the development of diabetic cardiomyopathy [4]. It has been suggested that the energy metabolism disorder might play an important role in the pathogenesis of diabetic cardiomyopathy [5]. The increase in phospholamban, decrease in Na^+/Ca^{2+} exchanger, together with unchanged L-type Ca^{2+}channel activity are features of rats with diabetic cardiomyopathy [6]. In diabetes, intracellular accumulation of sorbitol resulting from the high extracellular levels of glucose leads to depletion of taurine, which is associated with the development of diabetic late complications such as cardiomyopathy [7]. As is known to all, that endocrinal maladjustment of heart (usually excessively) is the common rationale of all kinds of heart failure and diabetic cardiomyopathy is of no exception. In fact, the renin-angiotensin-aldosteron system (RAS) has been activated before damage was done to diabetic rats' hearts, which is reason or result of cardiomyopathy and has been certified by clinic facts and our experiment. Angiotensin II is the key step of RAS, whose roles are carried out through angiotensin II type1 receptor (AT1) or/and angiotensin II type2 receptor (AT2). On the one hand, functions of AT1 are understood well: promote the proliferation of vascular muscle and endothelial cells, hypertrophy and fibrosis of cardiomyocytes etc; on the other hand, specificity and function of AT2 has not been understood well. To date, we know that AT2 distributes in embryonic tissue in a great amount and decreases largely in adult animals. It is interesting that heart failure, myocardial infarction, trauma healing and vascular intima injuring make AT2 reactivate and express highly. The development of myocyte hypertrophy has been largely attributed to angiotensin II, whose growth properties are antagonized by taurine [8]. The mechanisms of taurine antagonizing the myocyte hypertrophy due to angiotensin II and the interaction between AT2 and angiotensin II related to Cardiomyopathy, however, are still unknown. This study was therefore to investigate the roles of taurine and AT2 in rats with STZ-induced diabetic cardiomyopathy.

2. Methods

2.1. Experimental animal preparation

60 4-week-old female Wistar rats with with body weights ranging from 120 g to 150 g were included and 7 or 8 rats were kept in one cage with free access to drinking water and with a 12 hour illumination period per day. This study conforms to *the Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication No. Revised1996).

2.2. Model establishing and grouping of rats

Of 60 rats, 8 were treated with common diet (common group) and the 52 were treated with uncommon diet (i.e., uncommon group1, mixed with 10% lard, 20% sugar, 2% cholesterol, 1% sodium cholate and 67% common diet). At the 4th week, from 8 of the 52 rats treated with uncommon diet and the 8 rats treated with common diet (during the whole experiment), 1 ml of blood was exsanguinated (after the rats fasted for 10–12 hours) from the veins at the tip of tail to detect blood sugar and insulin. After insulin resistance, 7 of the 52 rats (treated with uncommon diet) were grouped into control group1 (con1) and treated with some sodium citrate buffer (pH = 4.5) (I.P). The other 45 rats (uncommon group2) were treated with STZ (25 $mgkg^{-1}bw^{-1}$, dissolved in sodium citrate buffer pH = 4.5 I.P). 48 hours later, all the 52 rats were fasted for 10 hours and the 45 rats were examined for blood sugar by using the blood from tail vein but con1 rats were not examined. Blood sugar went up at different rates and all the treatments were done once a week as above for 4 weeks. Those rats with blood sugar $\geq$16.7 mmol/L were acted as type 2 diabetic models. 36 rats that met the above standard, together with the ones in con1 were treated with uncommon diet for 20 weeks. At the end of the 28th week, the 36 diabetic rats were anesthetized with 1% vinbarbital sodium (20 $mgkg^{-1}bw^{-1}$ I.P) and $-dp/dt_{max}$ value was detected by a cannula through right carotid artery. Of the 36 rats, all the living 24 with the values of $-dp/dt_{max} \leq 5250$ mmHg/s developed into diabetic cardiomyopathy and were grouped as follows: 7 were grouped into control group2 (con2) treated with double-distilled H2O (I.P, Q.D); other 8 were grouped into experimental group1 (exp1) treated with CGP42112A (dissolved by the solution made with tri-distilled H2O and 30% acetic acid, pH = 6.0, 4 $\mu gkg^{-1}bw^{-1}$ I.P, Q.D) and another 9 were grouped into experimental group2 (exp2) treated with taurine (dissolved by distilled H2O 20 $mgkg^{-1}bw^{-1}$I.P, Q.D). The treatments above lasted 4 weeks.

2.3. Collecting and treating of tissue specimens and detection of statistic data

At the 32nd week (from the purchasing date on), rats were anesthetized with 1% vinbarbital

sodium (20 $mgkg^{-1}bw^{-1}$ I.P) and then the values of $-dp/dt_{max}$ or $+dp/dt_{max}$ (the maximal velocity decreasing/increasing rate per second in left ventricle within the period of isovolumic relaxation/contraction) were detected by cannula through the right carotid artery. Ejection fraction (EF) was measured through echocardiogram. Isolated rat hearts were washed with cold sodium solution and then dried with filter paper. Heart weight (HW) was measured. The heart specimens were labeled and frozen in liquid nitrogen and stored at $-70^{\circ}C$ for later use.

2.4. Making tissue paraffin-section and detecting AT2 and Bcl-2 by immnohistochemical staining

Tissue paraffin-sections were made as usual, AT2 and Bcl-2 expression was detected according to *Histostain TM-plus kits* and then the expression of AT2 and Bcl-2 in cardiomyocytes was analyzed through a High Resolving Power and Color Medical Image Analysis System (HMIAS-2000).

2.5. Detecting of cardiomyocyte apoptosis according to in situ Apoptosis Detection Kit

Cardiomyocyte apoptosis was detected with the method of terminal-deoxynucltidyl transferase mediated nick end labeling (TUNEL). the mean cardiomyocyte apoptosis index (CAI = stained cell number/total cell number$\times$100%) on the every stained slice in 5 visual fields could be calculated under a light microscope with amplification of 400 times.

2.6. Detecting the values of mRNA of AT2 and Bcl-2 in cardiomyocytes with RT-PCR

The total RNA of cardiomyocytes was isolated with TRIzol reagent. Reverse transcription took place in a mixture of a final volume (20 µl). For amplification of AT2 receptor cDNA, sense primer was 5′-TGAGTCCGCATTTAACTGC-3′, antisense primer was 5′-ACCACTGAGCATATTTCTCAGG-3′, and product length was 596 bp. For amplification of Bcl-2 receptor cDNA, sense primer was 5′-CTCGTCGTCTACCGTCGCGACTTTG-3′, antisense primer was 5′-CAGATGCCGGTTCAGGTACTCAGTC-3′, and product length was 193 bp. GAPDH (glyceraldehydes-3-phosphate dehydrogenase) was used as the internal standard, whose sense primer was 5′-TGAAGGTCGGTGTGAACGGATTTGGC-3′, the antisense primer was 5′-CATGTAGGCCATGAGGTCCACCAC-3′, and product length was 933 bp. cDNA was denatured at 94°C, then annealed at 53°C. After RT- PCR products above were amplified for 30 circles at 72°C, they were separated on a 3.5% polyacylamide gel, stained with ethidium bromide, and the fluorescence bands under ultraviolet were analyzed through Complete Gel Documentation & Analysis System.

2.7. Detecting the protein expression of AT2 and Bcl-2 (by Western Blot)

0.2 g tissues were weighed and put into a glass homogenizer with 0.2 ml cell lyase, and then the tissues were crushed. Tissue suspension was put into 1.5 ml eppendorf tube and was centrifugated at 12000 r/min at 4°C for 20 min. The supernatants were collected to detect protein concentration, then 20 µl sample was taken out and put into another tube, and 20 μl 2$\times$SDS loading buffer was added to the tube. The mixture was boiled for 5 min in water put on ice for the preparation of electrophoresis. Gel was prepared, electrophosis was run and protein was blotted onto a membrane (PVDF). The primary antibody of AT_2 was added to the membrane and they were mixed well. They were incubated at 4°C overnight and then the membrane was washed, incubated with anti-rabbit horseradish peroxidase-conjugated second antibody for 1 hour at room temperature, and washed extensively. An enhanced chemiluminescence system was used as the detection method. Blots were washed and subjected to autoradiography. The detecting process of Bcl-2 or fl-actin protein was the same as AT_2.β-actin was used as an internal standard. Then the blots were scanned into a computer and then relative data were analyzed through Complete Gel Documentation & Analysis System.

2.8. Statistical analysis

Results were recorded as mean ± SEM and analyzed through spss 11.5 software. The significance of the differences between groups was determined by independent sample-T-test. Values of $p < 0.05$ were considered to be significant.

3. Results

3.1. Values of insulin and sugar in common/uncommon groups before/after STZ injection

Before STZ injection (at the 4th week), the common group ($n = 8$) vs. the uncommon group1 ($n = 52$): values of blood sugar (4.6 ± 0.8 vs. 4.8 ± 0.6 mmol/L, $p > 0.05$). Values of blood insulin (18.5 ± 2.4 vs. 34.8 ± 3.6 mU/L, $p < 0.01$). There were no differences in blood sugar between the two groups, but the level of blood insulin from rats treated with uncommon diet was much higher than that of rats treated with common diet, which suggested the appearance of insulin resistance. At the 8th week, uncommon group2 ($n = 45$) vs. con1 ($n = 7$): The values of blood sugar (19.4 ± 2.8 vs. 4.9 ± 1.2 mmol/L, $p < 0.01$); the values of blood insulin (19.5 ± 3.6 vs. 35.6 ± 7.3 mmol/L, $p < 0.01$). In the uncommon group2, 36 rats with their blood sugar ≥ 16.7 mmol/L were acted as type 2 diabetic models.

3.2. Values of HW

Table 1 showed that the values of HW in exp1, exp2 and con2 were higher than those in con1, that the values of

Table 1. *Changes of $-dp/dt_{max}$ (mmHg/s), $+dp/dt_{max}$ (mmHg/s), EF(%), HW(g), CAI(%) and AT2 and Bcl-2 protein in all the groups.*

Items	EF (%)	HW (g)	CAI (%)	Bcl-2 protein (ODTI)	AT2 protein (ODTI)
con1 ($n = 7$)	$62 \pm 9^{\#}$	0.84 ± 0.13	9.85 ± 4.80	$318 \pm 112^{\#}$	13.43 ± 11.46
con2 ($n = 7$)	$59 \pm 7^{\#}$	$1.10 \pm 0.05^{*}$♀	$28.85 \pm 5.6^{*}$	$108 \pm 44^{*\#}$♀	$173.00 \pm 84.16^{*}$
exp1 ($n = 8$)	40 ± 7	$1.27 \pm 0.19^{*}$♀	$40.00 \pm 11.8^{*}$	$40 \pm 32^{*}$♀	$305.00 \pm 138.96^{*}$
exp2 ($n = 9$)	$56 \pm 10^{\#}$	$0.90 \pm 0.07^{*}$	$19.10 \pm 3.8^{*}$	$200 \pm 58^{*\#}$	$89.11 \pm 65.12^{*}$

Annotate: EF (%) was short for ejection fraction; CAI, for cardiomyocyte apoptosis index; HW, for heart weight. Protein (ODTI) indicated the values of AT2 or Bcl-2 protein total integral optical density (ODTI), i.e. interest gene (OD)/ internal standard gene (OD) by immnohistochemical staining. $^{*}p < 0.01$ vs con1, $^{\#}p < 0.01$ vs exp1 and ♀ $p < 0.01$ vs exp2.

HW were higher in exp1 than those in epx2 or con2, and that the values of HW were higher in con2 than those in exp2, respectively, $p < 0.01$. During the later period of diabetes, diabetic rats lost a lot of body weight, so HW not HWI (heart weight·mg/body weight·g) could embody the hypertrophy of cardiomyocytes well.

3.3. Values of EF by echocardiogram

The values of EF in exp1 were the lowest in all groups (exp1 vs. other groups, respectively, $p < 0.01$) and there were no significant differences between the other three groups ($p > 0.05$), which suggest that systolic dysfunction had taken place in exp1, but not in the other three groups (Table 1).

3.4. Values of CAI, AT2 and Bcl-2 protein expression by immnohistochemical staining.

Table 1 indicates that the values of CAI were the lowest in con1 but the highest in exp1 and that the values were much higher in con2 or exp2 than those in con1, respectively ($p < 0.01$). Also, the values of AT2 total integral optical density (ODTI) were higher in exp1,exp2 or con2 than those in con1, higher in exp1 than in exp2 or con2 and higher in con2 than in exp2, respectively ($p < 0.01$). The values of Bcl-2 total integral optical density (ODTI) in exp1, exp2 or con2 were less than those in con1, less in exp1 than in exp2 or con2 and less in con2 than in exp2, respectively ($p < 0.01$). Relative pictures are shown in Figure 4. These are photos under the light microscope with amplification of 400 times.

3.5. Values of $-dp/dt_{max}$ and $+dp/dt_{max}$ by cannula through right carotid artery

Figure 1 showed that the values of $-dp/dt_{max}$ in exp1(3887 ± 78 mmHg/s), exp2 (5427 ± 59 mmHg/s) or con2 (4520 ± 43 mmHg/s) were lower than those in con1(6449 ± 166 mmHg/s), that the values of $-dp/dt_{max}$ in exp1 were lower than those in con2 or in exp2 and that the values of $-dp/dt_{max}$ in con2 were lower than those in exp2, respectively, $p < 0.01$. The values of $+dp/dt_{max}$ in exp1 (5325 ± 450 mmHg/s) were the lowest among all the groups (exp1 vs. the other groups, respectively, $p < 0.01$) and there were no significant differences between the other three groups ($p > 0.05$).

3.6. Relative values of AT2 and Bcl-2 mRNA by RT-PCR

Figure 2 indicates that the ratios (×100%) of values (OD) of AT2 mRNA to GAPDH (internal standard gene) in exp1, exp2 or con2 were higher than those in con1, higher in exp1 than those in exp2 or con2 and higher in con2 than those in exp2, respectively ($p < 0.01$). It also displayed that the ratios (×100%) of values (OD) of Bcl-2 mRNA by RT-PCR to ones of GAPDH in exp1, exp2 or con2 were less than those in con1, less in exp1 than those in exp2 or con2 and less in con2 than those in exp2, respectively ($p < 0.01$).

3.7. Relative values of AT2 and Bcl-2 protein by Western Blot

Figure 3 shows that the ratios of AT_2 protein to β-actin in exp1,exp2 or con2 were higher than those in con1, respectively ($p < 0.01$), higher in exp1 than those in exp2 or con2, and higher in con2 than those in exp2, respectively ($p < 0.01$). The ratios of Bcl-2 protein to β-actin in exp1,exp2 or con2 were less than those in con1, less in exp1 than those in exp2 or con2, and less in con2 than those in exp2, respectively ($p < 0.01$).

4. Discussion

In this study, we have found that taurine may protect diabetic rats from cardiomyopathy amongst others by downregulating AT2 and that it may inhibit apoptosis of cardiac myocyte in diabetic rats.

4.1. Establishment of type2 diabetes and diabetic cardiomyopathy in rats

There are several models of cardiomyopathy in type 2 diabetes in rats, such as the Goto-Kakizaki (GK) rat, the ZDF/Gmi-fa/fa rat, the male obese Zucker diabetic fatty rat and the Cohen diabetic rat, which all are useful experimental models of type 2 diabetes. As these were all unavailable, we established our experimental model of type 2 diabetes in the following way:

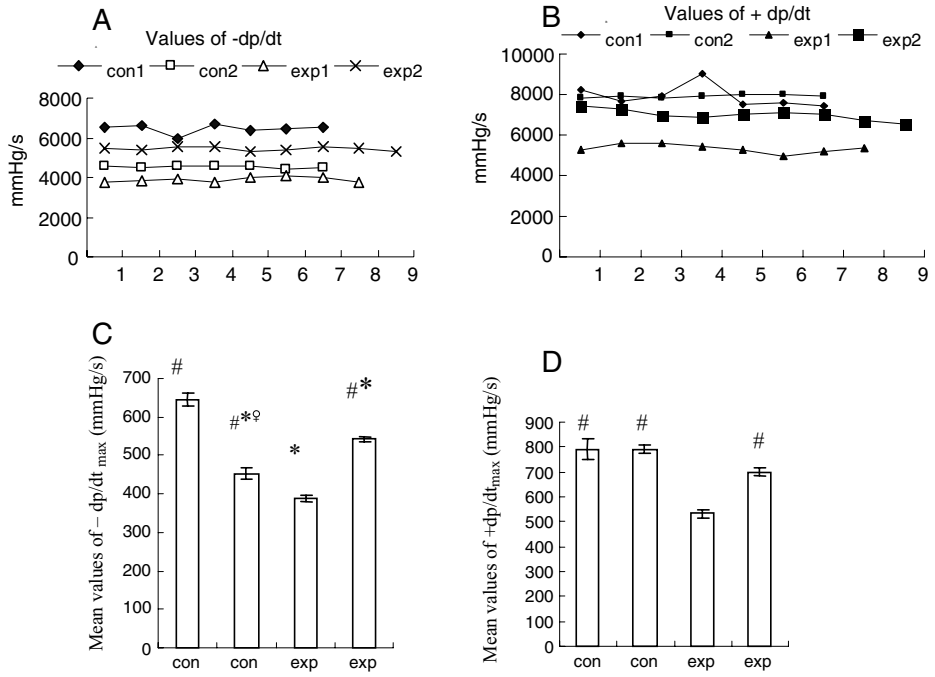

Fig. 1. *$-dp/dt_{max}$ (mmHg/s) or $+dp/dt_{max}$ (mmHg/s) indicates the maximal rate of pressure decrease or increase per second in left ventricle within the period of isovolumic relaxation/contraction, respectively;* A, B *shows the values of $-dp/dt_{max}$ or $+dp/dt_{max}$, respectively, in all the groups;* C *shows the values (mean ± SEM)of $-dp/dt_{max}$. $^{*}p < 0.01$ vs con1, $^{\#}p < 0.01$ vs exp1 and $^{\varphi}p < 0.01$ vs exp2.* D *shows the values (mean ± SEM) of $+dp/dt_{max}$. $^{\#}p < 0.01$ vs exp1.*

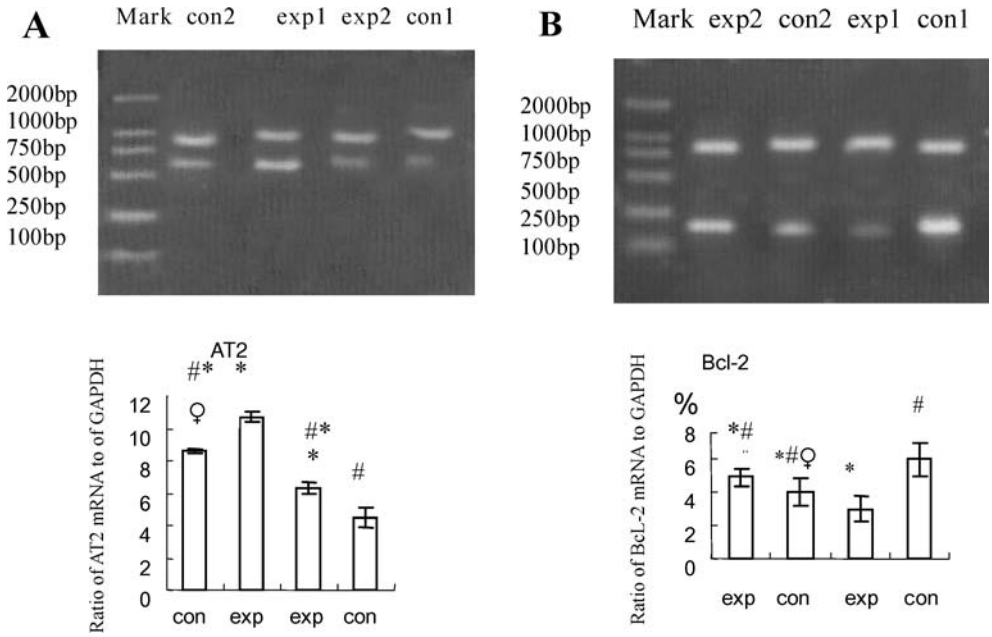

Fig. 2. *Expression of mRNA by RT-PCR. GAPDH was used as the internal standard and its product length was 933 bp. Mark was DNA ladder. A shows the values of AT2 mRNA in all the groups and the ratios (%) of AT2 mRNA to GAPDH. Product length was 596 bp. $^{*}p < 0.01$ vs con1, $^{\#}p < 0.01$ vs exp1 and $^{\varphi}p < 0.01$ vs exp2.* B *shows the values of Bcl-2 mRNA in all the groups and the ratios (%) of Bcl-2 mRNA to GAPDH. Product length was 193 bp. $^{*}p < 0.01$ vs con1, $^{\#}p < 0.01$ vs exp1 and $^{\varphi}p < 0.01$ vs exp2.*

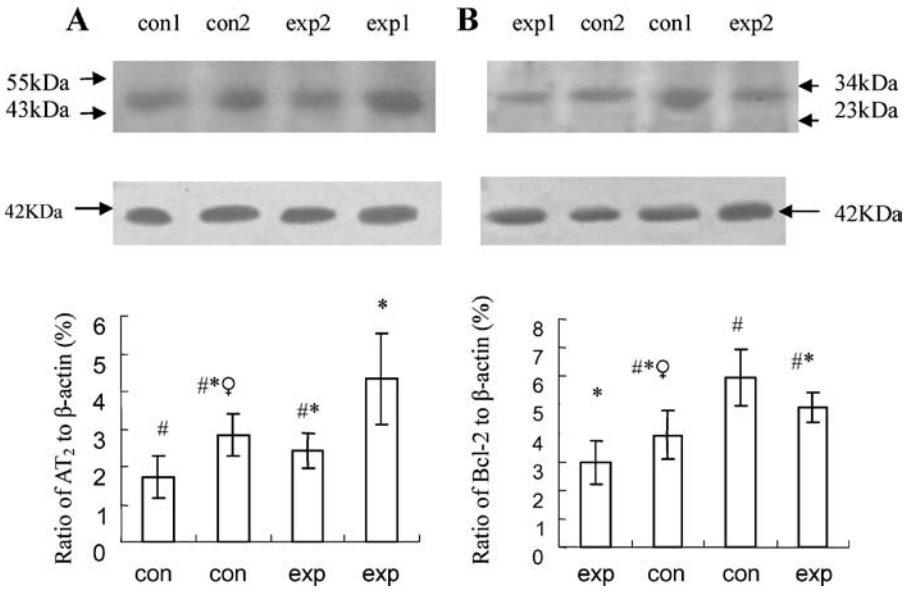

Fig. 3. *Protein expression by western blot. β-actin protein was used as reference protein (M.W: 42 KDa). A shows the AT2 protein expression and the ratios of AT2 to fl-actin. M.W: 50 Kda. *p < 0.01 vs con1, #p < 0.01 vs exp1 and p < 0.01 vs exp2. B shows the Bcl-2 protein expression and the ratios (%) of Bcl-2 to β-actin. M.W: 28 Kda. *p < 0.01 vs con1, #p < 0.01 vs exp1 and ♀ p < 0.01 vs exp2.*

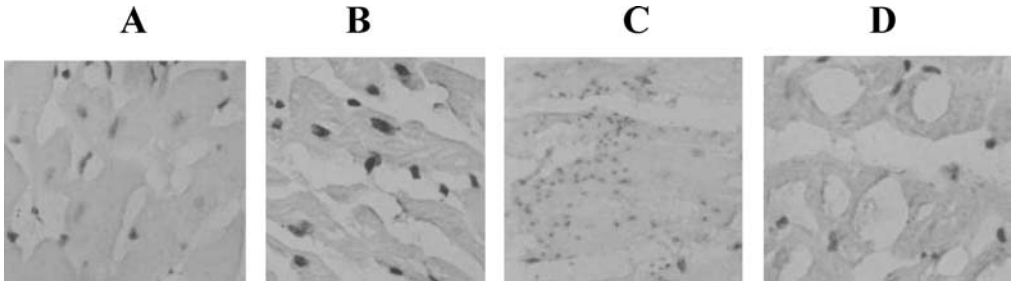

Fig. 4. *A: exp2 (taurine used). All apoptosis nuclei took on yellow brown with the number of apoptosis cell decreasing significantly, whereas normal nuclei were blue. B: exp1 (AT2 agonist used). All the apoptosis nuclei took on yellow brown with the number of apoptosis cells increasing largely, whereas normal nuclei were blue. C: con2 (diabetic cardiomyopathy without drugs interference) and yellow brown parts present AT2 expression (increasing largely). D: exp2 and yellow brown parts present Bcl-2 expression (increased significantly).*

female Wistar rats were treated with high sugar/lipid diet for one month to induce insulin resistance. Then STZ was administered with small amounts per time intraperitoneally. This was repeated several times to reduce the function of pancreatic β-cells step by step in order to induce type 2 diabetes. We used the female model, as they grow more slowly than the male model. We used the standard for type 2 diabetic diagnoses in rats as follows: based on insulin resistance, a fasting blood sugar $\geq$16.7 mmol/L. Some reports indicate alteration of STZ-induced diabetic cardiomyopathic ultrastructure to take place from 8–12 weeks [9–11]. Cardiac function is altered in 6–14weeks [12], which seconded our observation. We determined the presence of type 2 diabetic cardiomyopathy by detecting cardiac function, accepting a level of $-dp/dt_{\max} \leq$ 5250 mmHg/s as the existence of cardiomyocyte hypertrophy.

4.2. Diabetic Cardiomyopathy may increase AT2 expression and AT2 agonists may make diabetic rats susceptible to cardiomyopathy

Our experiment certifies that the AT2 expression is less in the adult rat cardiomyocyte, while diabetes or diabetic cardiomyopathy leads to a higher rate of expression of AT2 receptors. However, there are a lot of differences in AT2 functions in cardiomyocytes. Some experts argue that the effect of AT2 receptors in cardiomyocytes is opposed to that of AT1 in cardiomyocytes, namely, AT2 counters AT1 effects of promoting cardiomyocyte hypertrophy and inhibiting its apoptosis. In contrast, Ichihara et al. suggest that the two receptors get a stimulus through the same signal transduction pathway, lead to cardiomyocyte hypertrophy and inhibit their apoptosis in common [13]. Matsumoto et al. put forward that the effect of AT2 on cardiomyocytes depends on the heart's pathologic status, on

clinical stages, i.e., context-specific [14]. The significantly increasing weight of diabetic rat hearts certifies the existence of cardiac myotrophy. By the administrating CGP42112A, our experiment firstly discovered that AT2 expression increased concomitantly with a decrease in Bcl-2 expression in diabetic cardiomyocytes. Also, in rats with diabetes at the 32nd week, in exp1 (AT2 agonist-CGP42112A administered), the values of EF and $+dp/dt_{\max}$ were much less than those in the other three groups. In contrast, there were no significant differences in these variables between the other three groups. This suggests that on the one hand a not too long duration of diabetes leads to cardiomyocyte hypertrophy only, whereas on the other hand the AT2 agonist contributes to cardiomyocyte damage and did lead to cardiac systolic dysfunction. Consequently, high AT2 expression contributes to apoptosis of cardiomyocytes in diabetic rats, which is an important factor leading to diabetic cardiomyopathy. Furthermore, AT2 inhibiting the expression of Bcl-2 (an apoptosis-inhibiting protein) may be one of the mechanisms by which high AT2 expression accelerates the apoptosis in diabetic rat cardiomyocytes. The latter is consistent with the findings of Horiuchi et al. in PC12W cells (rat pheochromocytoma cell line) [15]. In conclusion, the rationale of AT2 promoting apoptosis of diabetic cardiomyocytes may be as follows: AT2 receptor stimulation inhibits NGF (nerve growth factor)-mediated Bcl-2 phosphorylation by inhibiting ERK (extracellular signal-regulated kinase) activity, leading to induction of apoptosis. Of course, many other mechanisms by which AT2 promotes apoptosis, except through Bcl-2 inhibition pathway, have to be explored.

4.3. Taurine may protect diabetic rats from cardiomyopathy also by downragulating AT2

Taurine might protect diabetic rats in many ways. Taurine abounds in cardiomyocytes, regulates endocardiomyocyte calcium homeostasis and stabilizes the biomembrane [16]. Taurine protects pancreatic cells and enforces the insulin endocrine [17]. Taurine deficiency could result in disordered contractile filaments and losses of myofibrillar bundles in rat heart [18]. Changes in the size of the intracellular taurine pool appear to modulate calcium transport in sarcoplasmic reticulum (SR) through two mechanisms. First, millimolar concentrations of taurine can directly promote release of calcium from Ca^{2+}-loaded junctional SR vesicles. Second, taurine serves as an inhibitor of SR phospholipid methyltransferase, an enzyme that appears to be responsible for methionine-mediated loss in Ca^{2+}-induced Ca^{2+} release activity and promotion of Ca^{2+}-independent Ca^{2+} release. The data imply that modulation of the intracellular taurine pool may affect cellular calcium homeostasis and myocardial contractile function. This may be important in development of the cardiomyopathy linked to taurine deficiency [19]. Taurine is a semi-essential amino acid, and its deficiency is involved in cardiac degenerations. One of the mechanisms by which taurine exerts beneficial effects in diabetes is that taurine deficiency may alter the endocrine pancreas "fetal programming," increasing the risk of insulin resistance in adult life [20]. A taurine sodium symport is activated to cause efflux of sodium and taurine when either rises above their physiological level [21]. A rise in intracellular sodium during periods of exposure to calcium free media would seem to be the critical step that predisposes the mammalian heart to the damaging effects of the calcium paradox. During calcium depletion, heart cells use this energy to efflux taurine and sodium via a taurine/sodium symport and therefore protect against the calcium paradox [22]. In diabetes, the high extracellular levels of glucose disturb the cellular osmoregulation and sorbitol is formed intracellularly due to the intracellular polyol pathway, which is suspected to be one of the key processes in the development of diabetic late complications and associated cellular dysfunctions. Intracellular accumulation of sorbitol is most likely to cause depletion of other intracellular compounds including osmolytes such as myo-inositol and taurine. When considering the clinical complications in diabetes, several links can be established between altered taurine metabolism and the development of cellular dysfunctions in diabetes which cause the clinical complications observed in diabetes, e.g. cardiomyopathy, platelet aggregation, endothelial dysfunction and atherosclerosis [7]. Taurine, an amino acid that exhibits anti-angiotensin II and osmoregulatory activity, is found in very high concentration in the heart. When the intracellular content of taurine is dramatically reduced, the heart develops contractile defects and undergoes an eccentric form of hypertrophy. The development of myocyte hypertrophy has been largely attributed to angiotensin II, whose growth properties are antagonized by taurine. Angiotensin II promotes the translocation of protein kinase C (PKC) epsilon and PKCdelta, the expression of Bax, and the activation of c-Jun N-terminal kinase (JNK), whose effects are greater in the taurine-deficient cell. Because PKC and JNK affect the expression and phosphorylation state of certain Bcl-2 family members, they appear to contribute to the potentiation of angiotensin II-induced apoptosis by taurine deficiency [8]. The mechanisms of taurine antagonizing the myocyte hypertrophy due to angiotensin II and the interaction between AT2 receptors and angiotensin II related to cardiomyopathy, however, are still unknown. Our study showed that exp2 (taurine administered) had the lowest AT2 expression and the least cardiomyocyte apoptosis among all the groups with cardiomyopathy and that taurine protects diabetic rats from developing cardiomyopathy by downregulating AT2 receptors.

4.4. Limitations of this study

During the later period of diabetes, diabetic rats lost a lot of body weight. As this, by measuring the HWI

(heart weight·mg/body weight·g) could lead to overestimation of cardiomyocyte hypertrophy, we didn't record the body weight of rats. Also, we have to realize that extensive DNA fragmentation occurring in late stages of necrosis, may lead to some false positive results if the TUNEL measurement is used. We do believe, however, that in our experiments cardiomyopathy was not so advanced that such stages of necrosis would have occurred.

5. Conclusion and anticipation

The study firstly certifies the fact that taurine inhibits the apoptosis of coardiomyocytes by downregulating AT2 and protects them in STZ-induced diabetic rats. We believe, with forward investigation of AT2 and taurine effect on cardiomyocytes, that cardiovascular treatment is supposed to have a bright tomorrow.

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References

1. Rubler S, Dlugash J, Yuceoglu YZ, et al. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol* 1972;30(6):595–602.
2. Ziegelhoffer A, Bundgaard H, Ravingerova T, et al. Diabetes and semi-starvation-induced changes in metabolism and regulation of Na, K-ATPase in rat heart. *Diabetes Nutr Metab* 2003;16(4):222–231.
3. Bidasee KR, Zhang Y, Shao CH, et al. Diabetes increases formation of advanced glycation end products on sarco (endo) plasmic Ca^{2+}-ATPase. Diabetes 2004;53:463–473.
4. Shan YX, Yang TL, Mestril R, et al. Hsp10 and Hsp60 suppress ubiquitination of insulin-like growth factor-1 receptor and augment insulin-like growth factor-1 receptor signaling in cardiac muscle: implications on decreased myocardial protection in diabetic cardiomyopathy. *J Biol Chem.* 2003;278(46):45492–8.
5. Chen G, Lin LX, Zhuang WT, Yao J, et al. Expression of energy metabolism-related genes in myocardial tissues of rats with diabetic cardiomyopathy. *Di Yi Jun Yi Da Xue Xue Bao*. 2002 Nov;22(11):1009–1010.
6. Choi KM, Zhong Y, Hoit BD, et al. Defective intracellar Ca (2+) signaling contributes to cardiomyopathy in type 1 diabetic rats. *Am J Physiol Heart Circ Physiol* 2002;283(4):1398–408.
7. Hansen SH, The role of taurine in diabetes and the development of diabetic complications. *Diabetes Metab Res Rev* 2001;17(5):330–346.
8. Schaffer S, Solodushko V, Pastukh V, et al. Possible cause of taurine-deficient cardiomyopathy: potentiation of angiotensin II Action. *J Cardiovascular Pharmacology* 2003;41(5):751–9.
9. Thompson EW. Structural manifestations of diabetic cardiomyopathy in the rat and its reversal by insulin treatment. *Am J Anat* 1988;182(3):270–282.
10. Zhu XX, Zhou XP, Zhong XL, et al. Streptozocin induced cardiomyopathy in diabetic rats. *Chin Med J (Engl)* 1993;106(6):463–646.
11. Fein FS, Komstein LB, Strobecket JE, et al. Altered myocardial mechanics in diabetic rats. *Circ Res* 1980;47:922–933.
12. Ni LS, Zheng JC, Wang DW, et al. The primary study on the mRNA expression of angiotensin II type2 receptor and cytocrine in kidney of the diabetic rats. *Zhongguo Zhong Zhong Xi Yi Jie He Za Zhi* 2004;23(1):54–55.
13. Ichihara S, Senbonmatsu T, Edward P, et al. Angiotensin II type2 receptor is essential for left ventricular hypertrophy and cardiac fibrosis in chronic angiotensin II induced hypertension. *Circulation* 2001;104:346–351.
14. Matsumoto T, Ozono R, Oshima T, et al. Type 2 angiotensin II receptor is down regulated in cardiomyocytes of patients with heart failure. *Cardiovasc Res* 2000;46(1):73–81.
15. Horiuchi M, Akishita M, Victor J, et al. Recent progress in angiotensin II type2 receptor research in the cardiovascular system. *Hypertension* 1999;33:613–621.
16. Koyman I. The protective effect of taurine on the biomembrane against the damage produced by the oxygen radical. *Adv ex Med Bio* 1992;315:355.
17. Tokunaga H, Yoneda Y, Kuriyama K. Streptozotocin-induced elevation of pancreatic taurine contact and suppressive effect of taurine on insulin secretion. *Eur J Pharmacol* 1983;87(2-3):237–243.
18. N Lake. Loss of cardiac myofibrils: Mechanism of contractile deficits induced by taurine deficiency. *Am J Physiol* 1993;264(4 Pt 2):H1323–H1326.
19. S Punna, C Ballard, T Hamaguchi, et al. Effect of taurine and methionine on sarcoplasmic reticular Ca^{2+} transport and phospholipid methyltransferase activity. *J Cardiovasc Pharmacol* 1994;24(2):286–292.
20. F Franconi, MA Di Leo, F Bennardini, et al. Is taurine beneficial in reducing risk factors for diabetes mellitus? *Neurochem Res* 2004;29(1):143–150.
21. MS Suleiman, GC Rodrigo, RA Chapman. Interdependence of intracellular taurine andsodium in guinea pig heart. *Cardiovasc Res* 1992;26(9):897–905.
22. MS Suleiman. New concepts in the cardioprotective action of magnesium and taurine during the calcium paradox and ischaemia of the heart. *Magnes Res* 1994;7(3/4):295–312.