Angiotensin Converting Enzyme Activity in the Serum, Lung, Liver and Kidney in Streptozotocin-Induced Diabetic Rats and Diabetic Nephropathy

**Abstract:** To clarify the relationship between the alterations of the levels of angiotensin converting enzyme (ACE) and diabetic nephropathy, ACE activity in the lung, liver, kidney and serum were investigated in streptozotocin (STZ)-induced diabetic rats.

The levels of serum ACE activity unchanged 3 days post STZ treatment but it was significantly an increase 12 and 30 days post STZ treatment in diabetic rats (p<0.001). Lung ACE activity was significantly increased at 12 and 30 days post STZ Treatment in diabetic rats (P<0.05 and P<0.005). There was not showed an increase in the liver ACE activity at 3 and 12 days in diabetic rats such it was significantly elevated at 30 day in diabetic rats (P<0.05). Kidney ACE activity was significantly elevated at 12 day post STZ treatment in diabetic rats, whereas were not occurred significantly an increase at 3 and 30 days in diabetic rats (p<0.05).

Histopathologic examination of the kidneys showed that thickening of the glomerular basement membrane, glomerular sclerosis, tubular dilatation and “Capsular Cap” occurred in the kidneys of STZ-induced-diabetic rats at 12 and 30 days.

In summary, it can be considered that high serum, lung and kidney ACE activity levels were related to with the complications in diabetic rats.

**Key Words:** Angiotensin Converting Enzyme, Streptozotocin-Diabetes, Nephropathy.

Introduction

Diabetes mellitus is associated with an increased of hypertension and renal failure (1,2). Many abnormalities of diabetic state promote microvascular complications, including glomerulopathy. Prominent among these are early hemodynamic maladaptations (systemic and glomerular capillary hypertension), which have been implicated as major risk factors for eventual diabetic glomerulopathy and alterations of renin angiotensin system (RA) (3). Hormonal modulation of these hemodynamic changes in diabetes is complex (3).

Many investigators reported that changes in the RA system and ACE activity may be linked to the onset of microvascular in diabetes mellitus (4). Angiotensin converting enzyme (ACE, EC. 3.4.15.1) is synthesized by endothelial and epithelial cells of many tissues including lung, kidney, heart and blood vessels (5). Although the enzyme bound to pulmonary vascular bed is considered to be responsible for conversion of circulating A I to A II, the presence of ACE can be easily detected in human or animal serum (6). ACE is a large acidic glycoprotein metalloenzyme composed of a single polypeptide chain that contains zinc molecule in a 1:1 molar ratio (6,7). The carboxy terminal dipeptide His9-Leu 10 is then cleaved from A I by ACE to form the active hormone, A II (7). A II is known as a potent vasoconstrictor in human body. Increase in angiotensin II level may result in the elevation of systolic and diastolic pressures (8).

The reasons of development of nephropathy in diabetics may be intrarenal or nonintrarenal (8). Although, the reasons of nephropathy in diabetics nephropathy are very complicated, the main factor is increasement of peripheral vascular resistant. In these cases; RA system can not work due to the barrier present between juxtaglomerular apparatus and the glomerular arterioles (9,10). The role of renin and aldosterone is to prevent hypertension getting worse through decreasing the high levels of the blood potassium, (11,12).
Increased vascular ACE concentration may contribute to vascular hypertrophy and diabetic vasculopathy by increased local synthesis of angiotensin II (13). Changes in serum levels of ACE have been associated with many pathologic conditions of the lung and the liver. In addition, it was reported by Liebermann et all. That level of ACE was elevated in diabetic patients and rats (14). Use of ACE inhibitors and metabolic control of diabetes will likely alter the prognosis of patients with diabetic nephropathy substantially (10).

The present study was designed to clarify the direct effect of diabetes on serum and tissue (lung, liver and kidney) ACE activity in STZ-induced diabetic rats (15).

Material and Method

Forty six male wistar strain rats (8-10 weeks and 180-220 gr. weights) were used in these experiments. Diabetic rats were randomly divided into three groups (Day 3, Day 12 and Day 30). Similarly, the control rats were divided into three groups. Rats were permitted rat chow water ad libitum.

Induction and evaluation of diabetes

Experimental diabetes was induced by injecting intraperitoneal a single dose freshly prepared STZ (60 mg/kg) in 0.1 M citrate Phosphate buffer (pH=4.5). Control rats received an equal volume/kg body weight of buffer (16). STZ injected animals were considered diabetic, if urinary glucose levels and tail vein glucose levels of rats exceeds 250 mg/dl (16,17)

In study days (3,12 and 30 days) rats were lightly ether anesthetized and laparotomized. The blood samples of diabetic and control rats were obtained by cardiac puncture. Blood samples were centrifuged 15 min at 3000 rpm and serum were obtained. After blood sampling the kidney, the lung and the liver were removed from each animal, washed in saline and frozen at -20˚C to be stored for later use.

Biochemical Studies

Serum glucose levels were measured at the end of each experiment using a spectrophotometric glucose oxidase method with Technicon RA-XT autoanalyzer. Serum was stored at-20 C˚ until assayed for glucose.

Measurement of Serum and Tissue ACE Activity

A commercial kit for spectrophotometric determination of ACE, based on the method of Holmquist et all. (21) was a gift from Sigma Diagnostics. (St. Louis, MO, 63103 USA). The package included procedural inserts, lyophilized reagent containing the substrate 2-furanoacryloyl-L-phenylalaninyl-glycylglycine (FAPPG), normal and above controls, and a calibrator. Reconstituted reagent was claimed to have 0.5 mmol/L of FAPPG and a pH=8.2 ± 0.1. All analyses were determined at 37 C˚ over an incubation time of 5 min using the 340/380 nm filter of a spectrophotometer (Schimadzu UV-1201).

Histopathologic Studies

In histopathologic studies, tissue was fixed 10 % buffered formalin and embedded in paraffin. Three micron sections were stained hematoxylin and eosin for pathologic examination.

The homogenates of the lung, the liver and the kidney were prepared in 10 mmol/L sodium phosphate buffer (pH: 6) using a Potter Elvenjem homogenizer at+4 C˚. Homogenates were centrifuged at+4 C˚ for 20 min at 10 000 rpm. The supernatant was used for the measurement of ACE. Protein was determined by the method of Lowry et all. (18-21).

Statistical Analysis

Results were reported as mean ± standard error the mean. Differences in our findings between groups were quantitated using a Mann Whitney U test, 2 tailed, with a 95 confidence interval (22,23).

Results

Levels of blood glucose

Diabetic rats showed a marked rise in blood glucose levels. Levels of blood glucose in STZ-induced diabetic rats was significantly elevated compared with the controls in 3, 12 and 30 days post STZ treatment (P<0.001) (Table 1).

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>Diabetes mellitus</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>136±3,8</td>
<td>398±8,2***</td>
</tr>
<tr>
<td>(n:8)</td>
<td></td>
<td>(n:7)</td>
</tr>
<tr>
<td>12</td>
<td>138±4,7</td>
<td>488±11,7***</td>
</tr>
<tr>
<td>(n:8)</td>
<td></td>
<td>(n:7)</td>
</tr>
<tr>
<td>30</td>
<td>140±3,8</td>
<td>658±29,6***</td>
</tr>
<tr>
<td>(n:8)</td>
<td></td>
<td>(n:7)</td>
</tr>
</tbody>
</table>

Table 1. Serum glucose levels 3-30 days post STZ treatment. (Mann-Whitney U Test)

***P<0.001
Levels of serum ACE activity

ACE activity was measured in serum 3-30 days post STZ treatment. Serum ACE activity were unchanged 3 days post STZ treatment. This indicates STZ has no direct immediate effect on ACE activity. Serum ACE activity measured 12 days and 30 days post STZ treatment, was showed an increase in diabetic rats relative to control groups (P<0.001) (Table 2, Fig. 1).

The lung ACE activity in STZ-induced rats was significantly difference elevated compared with to control at 12 day (P<0.05), whereas on significantly difference in the lung ACE activity at 3 day in diabetic rats (P<0.05). Lung ACE activity was decreased at 30 day in diabetic rats (Table 3, Fig. 2).

The liver ACE activity was not significantly differences at 3 and 12 days in diabetic rats but conversely it was significantly elevated compared with to control at 30 days (P>0.05) (Table 4, Fig. 3).

As shown Table 5, the ACE activity in the kidney was increased partially at 12 days (P>0.05), whereas no significant changes in the ACE activity of kidney at 3 and 30 days in STZ-induced diabetic rats (Table 5, Fig. 4).

Levels of blood urea and creatinine in STZ-induced diabetic rats were showed a significantly increase at 12 days 30 days (p<0.005), whereas no significant changes were found in 3 days. The blood electrolytes changed lightly, too. As shown Table 6; the level of sodium and potassium increased significantly at 12 days (P<0.005), while the levels of sodium and potassium reduced in 30 days post STZ treatment and it was statistically significantly (P<0.005).

Table 2. Serum ACE activity levels control and STZ-induced diabetic rats at 3-30 days. (Mann-Whitney U Test)

<table>
<thead>
<tr>
<th>Days</th>
<th>Serum Angiotensin Converting Enzyme (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>64.9±1.01</td>
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<tr>
<td>(n:8)</td>
<td>(n:8)</td>
</tr>
</tbody>
</table>

Table 6. Serum ACE activity levels control and STZ-induced diabetic rats at 3-30 days. (Mann-Whitney U Test)

<table>
<thead>
<tr>
<th>Days</th>
<th>Sodium</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>140.00</td>
<td>120.00</td>
</tr>
</tbody>
</table>

**P<0.005
Table 3. The level of ACE activity of lung tissue. (Mann-Whitney U Test)

<table>
<thead>
<tr>
<th>Days</th>
<th>3</th>
<th>12</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung ACE Activity Levels (nmol HA / mg protein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>11.28±0.61</td>
<td>11.82±2.18</td>
<td>10.92±1.65</td>
</tr>
<tr>
<td></td>
<td>(n:8)</td>
<td>(n:8)</td>
<td>(n:8)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>11.85±0.58</td>
<td>12.80±1.27*</td>
<td>8.37±0.79**</td>
</tr>
<tr>
<td></td>
<td>(n:8)</td>
<td>(n:7)</td>
<td>(n:7)</td>
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</tbody>
</table>

*P<0.05, **P<0.005

Table 4. The liver ACE activity in control and STZ-induced diabetic rats at 3-30 days. (Mann-Whitney U Test)

<table>
<thead>
<tr>
<th>Days</th>
<th>3</th>
<th>12</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver ACE Activity Level (nmol HA/ mg protein)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.20±0.45</td>
<td>7.33±0.62</td>
<td>7.39±0.37</td>
</tr>
<tr>
<td></td>
<td>(n:8)</td>
<td>(n:8)</td>
<td>(n:8)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7.20±0.65</td>
<td>6.48±1.46</td>
<td>5.73±1.85*</td>
</tr>
<tr>
<td></td>
<td>(n:8)</td>
<td>(n:7)</td>
<td>(n:7)</td>
</tr>
</tbody>
</table>

*P<0.05

Table 5. The kidney ACE activity in control and STZ-induced diabetic rats at 3-30 days. (Mann-Whitney U Test)

<table>
<thead>
<tr>
<th>Days</th>
<th>3</th>
<th>12</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>8.12±2.1</td>
<td>8.25±0.94</td>
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<tr>
<td></td>
<td></td>
<td>(n:8)</td>
<td>(n:8)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8.25±0.37</td>
<td>9.11±2.35*</td>
<td>7.95±1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n:8)</td>
<td>(n:7)</td>
</tr>
</tbody>
</table>

*P<0.05

Histopathologic Results

The renal and pancreatic structural changes caused by diabetes mellitus are examined at light microscopic level using several histochemical techniques. In STZ-Induced diabetic rats showed that langerhans islets in pancreatic gland were occurred damage, including acini degradation and polymorfonuclear leucocyte (PNL) in pancreatic endocrine gland in STZ-induced diabetic rats at 3 days compared with to control. In addition, especially pancreatic islet cells showed that were occurred damage and decreasing in number (Fig. 5). These findings continued more clear at 12 and 30 days in diabetic rats and evidence PNL and partially fibrosis were increased in diabetic rats.

The renal structural changes were not showed at 3 day in diabetic rats. Renal structural changes at 12 days post STZ treatment and this continued for 30 day post STZ tretament. These data indicate that STZ treatment does not induce a direct effect. The main structural changes in kidney of diabetic rats, include proliferation of intraglomerular mesengial cell and here and there PNL infiltration, intersititial nephritis and focal glomerulosclerosis (Fig. 6,7,8). In addition, it was showed that capsular drops “Strongly Eozinophilic Deposits” were in Bowman capsule of kidney (Fig. 9).

Discussion

Diabetes mellitus is mainly a multisystemic disease that it may cause many acute and chronically complications (1,2).

Brownlee and Serami reported that the metabolic alterations and cellular disfunctions in diabetes mellitus may be involved in the development of vascular disease (24). Diabetes is associated with alterations in

Table 6. Some biochemical parameters changes in control and STZ-induced diabetic rats 3-30 days. (Mann-Whitney U Test)

<table>
<thead>
<tr>
<th>Days</th>
<th>Urea mg/dl</th>
<th>Creatinine mg/dl</th>
<th>Sodium meq/L</th>
<th>Potassium meq/L</th>
<th>Calcium mg/dl</th>
<th>Phosphor mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>52.1±2.53</td>
<td>1.05±0.11</td>
<td>142.3±2.6</td>
<td>5.41±0.47</td>
<td>9.88±0.21</td>
<td>6.03±0.55</td>
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<tr>
<td>Diabetes mellitus</td>
<td>52.5±2.7</td>
<td>1.15±0.11</td>
<td>142.6±1.9</td>
<td>5.7±0.31</td>
<td>9.86±0.28</td>
<td>6.08±0.46</td>
</tr>
<tr>
<td></td>
<td>52.4±2.06</td>
<td>11.5±0.15</td>
<td>141.8±2.3</td>
<td>6.12±0.32</td>
<td>9.91±0.32</td>
<td>5.58±0.47</td>
</tr>
<tr>
<td></td>
<td>55.3±3.77</td>
<td>1.2±0.21</td>
<td>143.6±1.76</td>
<td>5.57±0.53</td>
<td>8.88±0.89</td>
<td>6.76±0.52</td>
</tr>
<tr>
<td></td>
<td>100.1±7.14*</td>
<td>2.4±0.58**</td>
<td>145±1.34*</td>
<td>6.5±0.56**</td>
<td>10.92±0.30**</td>
<td>7.31±1.17**</td>
</tr>
<tr>
<td></td>
<td>107.1±2.64**</td>
<td>1.9±0.26**</td>
<td>138.5±1.51*</td>
<td>5.07±0.28**</td>
<td>8.62±0.62**</td>
<td>3.85±0.38**</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.005
Acini degradation, decreasing of number and damage of Langerhans islet cells and polymorphonuclear infiltration in pancreatic gland in STZ-induced diabetic rats at 3 day (HE*200).

Figure 6. The thickening of the glomerular basement membrane, tubular dilatation and proliferation of intraglomerular mesangial cell in kidney in STZ-induced diabetic rats at 12 days (HE*200).

Figure 7. Interstitial nephritis and increasing of mononuclear fagosyt in the kidney of STZ-induced diabetic rats at 30 days (HE*200).

Figure 8. Focal glomerulosclerosis in the kidney of STZ-induced diabetic rats at 30 days (HE*200).

Figure 9. The strongly eosinophilic deposits 'Capsular Drops' in the Bowman capsule and partially tubular necrosis in the kidney of STZ-induced diabetic rats at 30 days (HE*400).

glomerular filtration rate, kidney mass and renal function which may elevate blood pressure through altering endogenous fluid and electrolyte balance (25). RA system has long been known as a circulating hormonal system that controls blood pressure and electrolyte homeostasis. It is now known that components of the RAS are also present in specific tissues, such as the walls of blood vessel, and the heart, lung, liver, kidney, brain, pituitary gland, ovary, testes, uterus, chorion-amnion, placenta, gut and salivary glands. Angiotensin produced locally has an autocrine/paracrine effect on angiotensin-mediated functions in these individual tissues (5,9,26).

In the STZ diabetes model that we chose, the mechanisms of the acceptance of STZ resembles glucose and it is directly and selectively introduced into B-cell (27,28).
In our studies, serum glucose levels were 398±8.2 mg/dl at 3 day post STZ treatment and it were 490±11.75 mg/dl and 663±31 mg/dl at 12 and 30 days post STZ treatment.

Many pathologic changes occurred in pancreatic tissue in STZ-induced diabetic rats at 3 day. Main structural changes, include PNL infiltration, aciner degradation and langerhans islets damage and decrease and decrease of number in STZ-induced diabetic rats. These findings continued for 12 and 30 days post STZ treatment.

Many investigators have reported increased serum ACE activity and tissue ACE activity in diabetic humans. This increasing of ACE activity may be contribute to occurs the complications of diabetes mellitus (14,29). ACE catalyzes the conversion of A I to A II. Since diabetes increases serum ACE activity, serum A II levels may be elevated resulting in a pressure response. Diabetes may produce increased A II levels due to enhanced ACE activity. On other hand, levels may remain unchanged if degradation of A II is increased (16).

Serum ACE activity levels were measured sequence 65.4±0.85 U/L at 3 day, 101.2±2.63 U/L at 12 day 120.1±1.62 U/L at 30 days post STZ treatment in induced diabetic rats. We observed an increase statistically significantly in the serum ACE activity levels at 12 and 30 post STZ treatment in diabetic rats (P<0.005), whereas no significant changes in the serum activity were found compared with to controls (P>0.05).

Schartner et all. have reported increased serum ACE activity in diabetic humans (30). Moreover, Funakawa et all. have reported that serum ACE activity levels were elevated significantly compared with the controls and it were related with hyertension and vascular complications (16). Erman et all. have reported that serum ACE activity was significantly inerased in the diabetic rats 14 days after induction of diabetes and reduced insulin treatment (15,16,32).

The activity of ACE was found to be elevated in DM rats as observed by Lieberman et al and the elevated levels of ACE activity in DM may come from diffuse vascular damage as mentioned by Lieberman et all. (14). Therefore it is excepted that depressed kallikrein generation may result in decrease release of the renin, which is liberated from kinogen by kallikrein and / or the liberated kinin may be rapidly inactived by ACE in diabetes mellitus. These data are explain high level of ACE activity in DM (14,33).

In our studies, we determined that the lung ACE activity was showed a significantly increase in STZ-induced diabetic rats at 12 day (P<0.05) and 30 days (P<0.005) compared with the controls. Huskic et al have reported that STZ induced-diabetes in the rats cause significant increase in ACE activity was significantly elevated at 7 and 14 days post STZ treatment in STZ-induced diabetic rats (15), but some studies reported that the lung ACE activities were not elevated in diabetic rats (16).

The liver ACE activity not changed in STZ induced diabetic rats at 3 and 12 days post STZ treatment, but it was significantly a rise diabetic rats at 30 days (P<0.05). This increasing of ACE activity may be not probably due elevated high glucose levels and diabetes.

Kohler and associate reported that renal renin levels in diabetic rats diminished prior the development of hypertension (35). Recent studies reported that ACE activity in the renal medulla was decreased but it was significantly a rise of the ACE activity in renal cortex in diabetic rats (18). In other study, Maeda et all. have reported that ACE activities in the serum and urine ACE were significantly elevated in diabetic rats and treatment of diabetic rats with insulin to achieve near euglysemia completely prevented these alterations in ACE activity (36). In our studies, kidney ACE activity increased significantly at 12 days in diabetic rats (P<0.05), whereas no significant changes in the activity were found compared with the control rats in induced diabetic rats at 12 and 30 days post STZ treatment.

The blood electrolytes were decreased in STZ induced diabetic rats sodium and potassium levels were significantly decreased in 12 and 30 days to compared with the control groups in STZ induced diabetic rats (10).

Specific pathologic lesions in kidney of STZ-induced-diabetic rats occurred at 12 and 30 days, whereas no significant pathologic changes in kidney of STZ-induce diabetic rats were found in 3 days post STZ treatment. Main structural changes in kidney were focal glomerulosclerosis, increasing of glomerular mesengial cells and interstitial fibrosis in STZ-induced diabetic rats.

In this study, it is indicated that the diabetic nephropathy changes observed in histopathologic examination such as the infiltration of PNLs and the proliferation of mesengial cells around the juxtaglomerular
apparatus are the main reasons for the reduction in renin and angiotensin production in this area. Therefore, ACE activity in the kidney, which was elevated at 12 days, may be indicative for the increased requirement of A II due to pathologic kidney changes occurred in diabetes mellitus induced by STZ.

The results showed that STZ-induced diabetes rats cause significant increase in ACE activity in serum and lung at 12 and 30 days. On other hand, the kidney changes due to occurred diabetes mellitus.

Further investigations should be required to clarify the role of ACE activity of serum and variable tissue (lung, liver and kidney) in diabetes mellitus.

References


