The effect of acetylsalicylic acid on vasopressin, serum insulin levels, insulin resistance, and biochemical parameters in rats induced with experimental diabetes type 2

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Background/aim: Acetylsalicylic acid (ASA) treatment in diabetic patients is very important owing to the increasing hyperactivity of thrombocytes and atherosclerosis. In several investigations, it was reported that diabetes caused increased coronary artery disease, cerebrovascular disease, and death. In this study, we aimed to investigate the effect of ASA on osmoregulation, glycemic control, and some biochemical parameters in rats induced with experimental diabetes type 2.

Materials and methods: Twenty-four rats were randomly divided in four groups: control (I), ASA control (II), diabetic (III), and ASA diabetic (IV). Diabetes was induced by streptozotocin treatment (30 mg/kg, twice, intraperitoneal injection) in obese rats. ASA (150 mg/kg body weight, orally) was administered for 5 weeks in the ASA control and ASA diabetic groups. Serum electrolytes, creatinine, albumin, and total protein levels were analyzed with an autoanalyzer. Arginine vasopressin (AVP) and insulin were analyzed by ELISA techniques.

Results: At the end of the study ASA treatments had decreased the fasting blood glucose levels but had interestingly increased the serum AVP levels in diabetics rats.

Conclusion: AVP levels were increased 2-fold by ASA treatment in diabetic rats. For the first time in this study, the hypoglycemic effect of ASA was attributed to an increase in blood volume by AVP levels. This explanation may be a new approach to the literature on this topic.

Key words: Type 2 diabetes, arginine vasopressin, insulin resistance, osmoregulation

1. Introduction

Diabetes mellitus is characterized by chronic hyperglycemia developed as a result of disorders in carbohydrate, fat, and protein metabolisms related to disorders in insulin secretion, insulin action, or both (1).

Type 2 diabetes is the most common form of diabetes. Its main disorders are characterized by insulin secretion and activity. Type 2 diabetic patients usually have an excess of insulin and insulin resistance rather than insulin deficiency (2).

Morbidity and mortality rates of cardiovascular disease in diabetic patients are high, and cardiovascular complications form the most extensive area in the cost tables caused by diabetes complications (3). The risk of cardiovascular diseases for type 2 diabetics is 2.32 times higher than that of nondiabetics (4). Additionally, there is a link between cardiovascular diseases and glucose levels in diabetics. A 1% increase in HbA1c levels doubles the risk of cardiovascular diseases (5).

Inflammation plays a key role in the development of atherosclerosis (6). It was also suggested that type 2 diabetes has an effective role in atherosclerosis with its NOS, NO, redox stress, and inflammatory components (7).

Today it is clearly known that there are close relations between diabetes, inflammation, atherosclerosis, and thrombosis (8). In the global treatment strategies for type 2 diabetes, acetylsalicylic acid (ASA) is used as a therapeutic agent and its use is recommended (9). Therefore, ASA treatment for type 2 diabetics in order to minimize the risk of cardiovascular diseases is essential.

Arginine vasopressin (AVP) is produced in the supraoptic and paraventricular cores found in the hypothalamus and is transferred to the posterior pituitary gland via the supraoptic hypophyseal channel and stored...
there. It is released by exocytosis in response to osmotic and nonosmotic stimuli (10).

In this study, it was aimed to investigate the effect of ASA on osmoregulation, glycemic control, and some biochemical parameters in rats induced with experimental diabetes type 2.

2. Materials and methods

2.1. Animals

The 24 male Wistar rats of 6–8 weeks old (140–200 g) used in this study were obtained from the Süleyman Demirel University Experimental Animal Research Center (Isparta, Turkey). Care and feeding of rats was carried out at 21 ± 2 °C ambient temperature and 55%–60% relative humidity, with a 12/12-h light/dark cycle. High-energy normal feed and daily fresh water supply was provided continuously according to the groups. Rats were randomly divided in four groups: control (I), ASA control (II), diabetic (III), and ASA diabetic (IV).

2.2. Preparation of a high-fat diet

In order to cause experimental type 2 diabetes, insulin resistance should be developed in diabetic rats. High-energy diets are used for this purpose. However, the high-energy feed used for this purpose has not been found from the manufacturers in Turkey. Therefore, studies on type 2 diabetes formation with streptozotocin (STZ) injection following a high-energy diet were reviewed and the feeds used, energy contents, and foods that increase the energy contents were determined. The high-fat diet (HFD) administered to test animals in order to create obesity or insulin resistance varies depending on the study and the fat content changed between 22% and 60% (11–13). HFD content was prepared according to the average feed contents in studies using a standard diet and is given in Table 1.

2.3. Formation of insulin resistance in rats

Because the fastest weight gain in HFD-applied groups started at the 5th week and continued until the rats were 20 weeks old, the study was initiated with rats of 6–8 weeks old (14). Rats were brought to the Afyon Kocatepe University Experimental Animal Research and Application Center and were adapted to the environment by feeding with standard pellet feed for 1 week. Rats, except the control and ASA control groups, were administered the HFD for 6 weeks and the changes in weights of the rats in the control and HFD-applied groups were monitored weekly.

At the end of the HFD administration period (6 weeks), an insulin tolerance test (ITT) was performed on all rats and the rats with insulin resistance were determined. As explained in the literature, HFD-administered rats that had a weight gain of 5% compared to control group rats and additionally developed insulin resistance were regarded as obese (15), and type 2 diabetes formation was carried out with STZ injection.

2.4. Formation of type 2 diabetes model

In the 8th week of the study, obese rats were starved for 12 h and fasting blood sugar (FBS) levels were measured (Accu-Check Go, Bayer, Germany) in order to compare the blood glucose levels after STZ injection. Following ketamine (65 mg/kg) and xylazine (7 mg/kg) anesthesia, rats were injected with STZ dissolved in citrate buffer (pH 4.5) with a 30 mg/kg dosage twice in weekly intervals with i.p. injection. FBS levels of the rats were measured one week after the last injection and the rats with levels higher than 300 mg/dL FBS were regarded as having type 2 diabetes (16). Following the development of obesity, the rats in the type 2 diabetes model injected with a low dose (30 mg/kg) of double STZ injections (i.p.) were divided into groups for the following phases of the study.

2.5. Insulin tolerance test

Insulin solutions prepared for the rats were administered at a dose of 0.50 IU/kg by i.p. injection, and the plasma glucose levels before the injection (minute 0) and at minutes 15, 30, and 60 following the injection were measured and insulin resistance was determined.

Table 1. The content of the high-fat diet and standard diet.

<table>
<thead>
<tr>
<th></th>
<th>Standard pellet feed ingredients</th>
<th>High-energy (fatty) feed ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat ratio (%)</td>
<td>4.1</td>
<td>57.3</td>
</tr>
<tr>
<td>Protein ratio (%)</td>
<td>17</td>
<td>13.6</td>
</tr>
<tr>
<td>Carbohydrate ratio (%)</td>
<td>76.4</td>
<td>30.1</td>
</tr>
<tr>
<td>Other substances (%)</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Energy level (cal/kg)</td>
<td>2600</td>
<td>4930</td>
</tr>
</tbody>
</table>
and hepatic glycogenolysis. In this manner, the peak in
the antihyperglycemic activity of insulin is considered to
occur at the 30th minute and 30-min data were used for
the ITT (18), and data obtained at the end of this time were
used for the statistical analyses.

2.6. Analysis of the biochemical parameters
The rats were sacrificed under ketamine and xylazine
anesthesia and intracardiac blood was taken with 10 mL
syringes. The blood samples were centrifuged for 5 min
at 4000 rpm and serum samples were obtained. Sodium,
potassium, chloride, creatinine, total protein, and albumin
values of serum samples as biochemical parameters were
analyzed at the Afyon Kocatepe University Ahmet Necdet
Sezer Research and Training Hospital in the biochemistry
laboratories using a Roche Cobas C501 autoanalyzer.

2.7. Determination of serum insulin and vasopressin lev-
sels by ELISA technique
Both insulin and vasopressin were analyzed from blood
samples. Analyses were carried out by ELISA techniques
using commercial kits for insulin (EMD Millipore
Corporation, USA) and vasopressin (Bachem, Switzerland)
determinations by ELISA reader (Trinity Biotech, Ireland).

2.8. Assessment of beta cell function (HOMA-β: Homeo-
static Model Assessment, Beta Cell Function)
With the blood glucose and insulin levels obtained at the
end of the study, HOMA-β values were calculated with the
following formula:

\[ \text{HOMA-β} = 20 \times \text{fasting insulin level} \text{ (mU/L)} / \text{fasting glucose level} \text{ (mmol/L)} - 3.5 \]

(19).

2.9. Assessment of insulin resistance (HOMA-IR: Ho-
meostasis Model Assessment, Insulin Resistance)
With the plasma glucose and insulin levels obtained at the
end of the study, HOMA-IR values were calculated with the
following formula:

\[ \text{HOMA-IR} = 20 \times \text{fasting insulin level} \text{ (mU/L)} / \text{fasting glucose level} \text{ (mmol/L)} / 22.5 \]

(19).

3. Results
3.1. Effects of ASA treatment on blood biochemical pa-
rameters in type 2 diabetic rats
Serum sodium, chloride, potassium, creatinine, albumin,
and total protein levels are shown in Table 2. According
to these data, a significant difference was found in serum
sodium levels between the ASA-applied diabetics group
and non-ASA-applied diabetics group (Figures 1A–1F; Table 2).

It was determined that serum sodium levels decreased
following the ASA administration. Serum potassium levels
between the ASA-applied diabetics group and other groups
were statistically significant; in the ASA-applied diabetics
group serum potassium levels increased (Figure 1).

Regarding the serum chloride levels, no significant
difference was found between the diabetic group and ASA
diabetic group, or between the control and ASA control
group. However, the difference in serum chloride levels
between the nondiabetic group (control and ASA control)
and diabetic group (diabetic and ASA diabetic) was
statistically significant and serum chloride levels in the
diabetic group were lower (Figure 1; Table 2).

Serum creatinine levels in ASA-applied diabetic
groups were lower than those of non-ASA-applied
diabetic groups (Table 2). Serum albumin levels were also
significantly lower in the ASA diabetic group than in other
groups, as was the case for all biochemical parameters
except serum potassium (Table 2). As a result of serum
total protein analyses, it was found that the control group
had significantly higher total protein values than the other
groups.

3.2. Serum arginine vasopressin (antidiuretic hormone)
levels
Vasopressin levels in the control group, ASA control
group, diabetic group, and ASA diabetic group were
2.33 ng/mL, 14.38 ng/mL, 37.03 ng/mL, and 70.4 ng/
ml, respectively. Statistically significant differences were

Table 2. Changes in biochemical parameters.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ASA control</th>
<th>Diabetic</th>
<th>ASA diabetic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mEq/L)</td>
<td>141.17 ± 0.65b</td>
<td>142.60 ± 0.68b</td>
<td>140.50 ± 0.85b</td>
<td>133.5 ± 0.50a</td>
<td>0.001</td>
</tr>
<tr>
<td>Postassium (mEq/L)</td>
<td>5.18 ± 0.24a</td>
<td>5.46 ± 0.29a</td>
<td>5.28 ± 0.15a</td>
<td>6.28 ± 0.03b</td>
<td>0.003</td>
</tr>
<tr>
<td>Chlorine (mEq/L)</td>
<td>100.27 ± 0.92a</td>
<td>100.64 ± 0.80b</td>
<td>96.95 ± 1.27a</td>
<td>95.82 ± 1.18a</td>
<td>0.011</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.39 ± 0.03b</td>
<td>0.35 ± 0.02ab</td>
<td>0.45 ± 0.01c</td>
<td>0.33 ± 0.01a</td>
<td>0.001</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.45 ± 0.01b</td>
<td>3.47 ± 0.06b</td>
<td>3.77 ± 0.06c</td>
<td>3.18 ± 0.09c</td>
<td>0.001</td>
</tr>
<tr>
<td>T. protein (g/dL)</td>
<td>6.22 ± 0.043b</td>
<td>5.47 ± 0.15a</td>
<td>5.63 ± 0.06c</td>
<td>5.46 ± 0.07a</td>
<td>0.001</td>
</tr>
</tbody>
</table>

a, b, c: The differences between values with different superscripted letters in the same line are statistically significant (P < 0.005).
determined between all groups except the control group and ASA control group (Table 3).

3.3. Serum insulin levels
There was no significant difference in insulin levels between any groups.

3.4. HOMA-β and HOMA-IR results
According to HOMA-β data that show the beta cell functionality, the differences between control groups and diabetic groups were statistically significant. In this manner, beta cell functionality of the diabetic groups was lower than that of control groups. Additionally, statistically nonsignificant decreases were observed in beta cell functionality in both control groups and diabetic groups with ASA administration (Table 3).

Regarding the HOMA-IR results that show the insulin resistance coefficients, similar to the HOMA-β results, statistically significant differences were determined between control groups and diabetic groups. In other words, the insulin resistance coefficient was found significantly higher in diabetic groups than in control groups. Additionally, statistically insignificant decreases were observed in insulin resistance coefficients in both control groups and diabetic groups with ASA administration (Table 3).

FBS findings show that the differences between diabetic groups and nondiabetic groups were not significant (Table 3). As a result of ASA administration, a significant decrease was found in the FBS levels in the ASA diabetic group.
compared to the diabetic group. The FBS levels decreased directly proportional to the insulin resistance coefficients (Table 3).

3.5. Insulin tolerance test results
Very low or no decreases in FBS levels show that insulin resistance was established and that the diabetes model was type II (16,17). In many studies that applied the ITT, it was reported that the glucose-lowering effect of insulin lasted until the 30th minute; however, the plasma glucose level started to increase after the 30th minute due to gluconeogenesis and hepatic glycogenolysis. In this manner, the peak in the antihyperglycemic activity of insulin is considered to occur at the 30th minute and 30-min data were used for ITT (18). In this manner, 30-min data obtained during ITT studies were used for the statistical analyses.

According to the 30-min data, insulin sensitivity in the HFD group (diabetic group) was found to be higher than that in the control group, meaning that insulin resistance had developed.

4. Discussion
The main objective of diabetes treatment is to reach normal blood glucose levels, eliminate the symptoms, and prevent or delay the complications. In this study, the effect of ASA on osmoregulation and glycemic control in terms of diabetes has been discussed, considering the relations between ASA, obesity, insulin resistance, diabetes, cardiovascular disease, and AVP secretion.

In the literature, it was reported that salicylates decreased the FBS by 13% (P < 0.002) and HbA1c by 17% (20). In another study, it was reported that salicylates decreased the HbA1c levels and therefore might be a new way of treatment for glycemic control in type II diabetics; however, it was emphasized that further studies are required for renal and cardiac safety (21). Parallel to the literature results mentioned above, in this study, it was found that glycemic states were suppressed in ASA diabetics by making significant differences in FBS with ASA administration between the diabetic group and ASA diabetic group.

It was shown that AVP was synthesized 70% more in diabetic rats compared to nondiabetic rats (22). Similarly, it was reported that AVP levels were higher in diabetics than in nondiabetics (23). In this manner, AVP results obtained in this study were found to be parallel to the data reported in the literature.

The reason for higher AVP synthesis in diabetics compared to nondiabetics is still unknown. However, it was suggested that this increase is associated with the regulation of the osmotic balance (24). Examining the control and ASA control groups and the diabetic and ASA diabetic groups in this study, it was found that ASA application contributed to the increase of AVP levels and this increase was statistically significant between ASA diabetic and diabetic groups.

It was shown that potent inhibition of AVP activity occurred due to the increase in PGE₂ production, and this also caused nephrogenic diabetes insipidus (NDI) (25). It was also found that as a result of the PGE₂ synthesis inhibition caused by using NSAIDs such as indomethacin, polyuria caused by NDI was decreased (26). In light of these two mutually supportive studies, it is understood that PGE₂ is an inhibitor of AVP. In the literature, it was also clearly reported that PG synthesis was inhibited by ASA by inhibiting the COX pathway (27). In light of this information, it can be concluded that ASA has a positive effect on AVP secretion by inhibiting the AVP inhibitor PGE₂.

With this study, a new approach is presented for the subject. In diabetic groups, it was found that AVP decreased significantly with ASA administration and this indicates that blood glucose levels were relatively increased.

Table 3. Serum arginine vasopressin, insulin, and FBS levels, and results of insulin resistance and beta cell function.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ASA Control</th>
<th>Diabetic</th>
<th>ASA Diabetic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasopressin (ng/mL)</td>
<td>2.33 ± 0.44a</td>
<td>14.38 ± 2.95a</td>
<td>37.03 ± 7.11b</td>
<td>70.40 ± 3.86c</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>0.328 ± 0.059a</td>
<td>0.242 ± 0.022c</td>
<td>0.369 ± 0.048a</td>
<td>0.253 ± 0.024a</td>
<td>0.157</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>0.075 ± 0.014a</td>
<td>0.060 ± 0.004a</td>
<td>0.023 ± 0.002b</td>
<td>0.017 ± 0.002b</td>
<td>0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.333 ± 0.245a</td>
<td>0.929 ± 0.144a</td>
<td>5.323 ± 0.778b</td>
<td>4.113 ± 0.437b</td>
<td>0.001</td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>90.83 ± 2.86a</td>
<td>85.00 ± 6.70a</td>
<td>405.33 ± 17.25b</td>
<td>341.00 ± 27.22c</td>
<td>0.001</td>
</tr>
</tbody>
</table>

a, b, c: The differences between values with different superscripted letters in the same line are statistically significant (P < 0.005). FBS: Fasting blood sugar. HOMA-β: Homeostasis Model Assessment, Beta Cell Function. HOMA-IR: Homeostasis Model Assessment, Insulin Resistance.
as a result of high AVP concentrations of hypoglycemic activity caused by ASA. Additionally, the low results of other biochemical parameters including chloride, albumin, total protein, and creatine, similar to the blood glucose results found in the ASA-administered diabetic group, indicate that high AVP levels were dilutional.

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References