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Original Article

The Serum Protein Fractions in Streptozotocin (STZ) Administrated Rat Models

Cennet Ragbetli[1], Semiha Dede[2*], Feride Koc[3], Veysel Yuksek[4], Murat Cetin Ragbetli[5]

ABSTRACT

Background: Experimental diabetes can be induced using chemical agents such as streptozotocin **Objective:** This study aimed to investigate the effect of streptozotocin (STZ) which is most important agent to produce experimental diabetic model at two different doses on serum protein fractions in rat models. **Materials and Methods:** Twenty-four male rats that weighed an average of 250 g and were 3–4 months old were used as the experimental models. They were sorted into three groups composed of eight rats each of STZ 55 mg/kg, STZ 65 mg/kg and control. Diabetes was induced by administering STZ 55 mg/kg and 65 mg/kg intraperitoneally. The serum protein fractions were analyzed by cellulose acetate electrophoresis. **Results:** No significant difference was observed between the groups for all fractions except alpha-2 and beta globulins. The alpha-2 and beta globulin levels were significantly higher in the 55 mg/kg group than in the 65 mg/kg STZ and control groups ($p < 0.05$). **Conclusion:** This increase may be due to the involvement of different proteins in the alpha-2 and beta globulin protein fractions.

Key words: electrophoresis, experimental diabetes, rat, serum proteins, STZ

Cennet Ragbetli[1], Semiha Dede[2*], Feride Koc[3], Veysel Yuksek[4], Murat Cetin Ragbetli[5]

[1]Department of Microbiology, Medicine Faculty, Veterinary Medicine School, Van, 65080 TURKEY.
[2]Department of Biochemistry, Veterinary Medicine Faculty, Veterinary Medicine School, Van, 65080 TURKEY.
[3]Department Pharmacology and Toxicology, Veterinary Faculty Erciyes University, Veterinary Medicine School, Biochemistry Department, Van, 65080 Turkey.
[4]Ozalp Vocational High School, YuzuncuYil University, Veterinary Medicine School, Van, 65080 TURKEY.
[5]Department of Histology and Embryology, Medicine Faculty, Veterinary Medicine School, Biochemistry Department, Van, 65080 TURKEY.

Correspondence

Prof. Dr. Semiha Dede, Yuzuncu Yil University, Veterinary Medicine School, Biochemistry Department, Van, 65080 TURKEY.

Tel no: +904322251027/1520,
Fax no: +904322251127,

E-mail: sdede@yyu.edu.tr

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INTRODUCTION

Experimental animal models are used to understand the pathogenesis and complication of diabetes. Experimental diabetes can be induced using chemical agents or viruses or spontaneously acquired. An important agent for experimental diabetes is streptozotocin.[1,2] Streptozotocin (2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose; STZ) is a metabolite of *Streptomyces griseus* and has antibiotic, diabetogenic, antitumoral, and carcinogenic effects. By damaging pancreas beta cells, STZ causes insulin-dependent and -independent diabetes. A single-dose (40–60 mg/kg) intravenous application of STZ to adult mice has been reported to cause insulin-dependent diabetes and the application of a single dose of 100 mg/kg STZ intraperitoneally or intravenously to infant mice was reported to cause insulin-dependent diabetes.[3]

Standard serum protein electrophoresis leads to the identification of five main protein fractions (albumin, alpha-1, alpha-2, beta, and gamma globulins). The serum protein electrophoretic profile was altered in various conditions, such as different infectious diseases, liver disorders, acute inflammatory and proliferative cases, tissue damage such as trauma, and many other physiological disorders. The albumin level is implicated in the transport of various lipophilic compounds including corticoids, pharmaceutical drugs etc. The alpha-globulin fraction is named alpha-2-macroglobulin, and the haptoglobin value increases mainly in trauma. Some alterations in lipoprotein metabolism induce changes in the β-globulin fraction that are useful for the diagnosis of cancers involving B-lymphocytes and can be used as a fraction test in patients undergoing kidney transplantation. However, since many factors are responsible for these changes, it is generally difficult to identify them. Immunoglobulins and C-reactive protein (CRP) migrate in the gamma globulin area.[4-10]

The serum protein electrophoresis test measures specific proteins, and it can be a precursor to focuse new proteins determinations and separation in the variable doses of STZ induced experimental diabetic rats.

MATERIALS AND METHODS

Animals

For this study, 24 male Wistar albino rats weighing 250 g and 3-4 months old were obtained from the Experimental Research Laboratory of Yuzuncu Yil University Medicine Faculty. Rats were accommodated in cages with permanent food and fresh water and 12 h dark/light, and the temperature set at between 18 and 24°C. The experiments were conducted according to ethical guidelines and under the supervision of the Yuzuncu Yil University Local Ethics Committee on Animal Experiments.

Study groups

The 24 animals were randomly divided into three groups. Group 1 was the control group. Eight randomly

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selected rats were separated as the control group. Group 2 received 55 mg/kg single-dose streptozotocin (STZ) (Sigma, USA) in citrate buffer pH 4.5 that was administered intraperitoneally (i.p.). Group 3 received 65 mg/kg single-dose streptozotocin (STZ) (Sigma, USA) in citrate buffer pH 4.5 that was administered i.p.

A single dose of 55 mg/kg and 65 mg/kg STZ was administered to 16 rats; 48 h later, the glucose levels in the blood samples taken from the tail vein were determined using a glucometer. The rats with blood glucose 200 mg/dl and higher were regarded as diabetic and were included in the study.

Biochemical analysis

Blood samples were collected from all groups, and the serum samples was separated by centrifugation at 500 ×g for 10 min. Total protein concentrations were analyzed using the biuret method. The serum protein fractions were separated using the Helena Lab-Titan III® Serum Protein Electrophoresis device (Cat No. 3023), Helena Lab-Titan III Cellulose acetate cards, and Electra HR Buffer (Cat No. 5805) tampon solutions (Helena, Bioscience Europe, UK) and then stained with the Ponceau S Stain solution. The bands obtained after electrophoresis were evaluated for serum protein fractions in Platinum 3.0 program, and the protein concentrations were determined.

Statistical analysis

The data were analyzed with a one-way analysis of variance (ANOVA). Duncan's test was applied for multiple comparisons. Differences were considered significant when the p value was less than 0.05 using SPSS 22.0 statistical software.

RESULTS AND DISCUSSION

The results for the serum samples are summarized in Tables 1 and 2. There was no significant difference between the groups for all fractions except the alpha-2 and beta globulins. The alpha-2 and beta globulin levels were significantly higher in the 55 mg/kg STZ group compared to the 65 mg/kg STZ and control groups ($p<0.05$). There was no significant difference between the groups for all fractions.

The data obtained after the evaluation of the percentage of the serum protein fractions in total protein are summarized in Table 2. Although there were relative differences between the treatment groups, no statistically significant differences were determined.

After analyzing of serum protein fractions in two different dose STZ treated rats, frequencies of serum protein fractions have no statistical differences between controls and study groups. But some

Although Kafa (12) reported that a decrease in the level of total serum proteins was detected in the group administered 50 mg/kg i.p. STZ 21 days after the samples were collected. However, in the present study, as shown in Table 1, it was determined that the total protein levels were not affected after 48 h after STZ was administered. The total serum protein levels were not affected within a short duration after STZ injections.

Albumin in the blood has several functions including protecting the fluid balance between the blood and other tissues.[5,11] In the present study, there was no change in albumin levels after two different doses of STZ were administered. Several studies on albumin levels in diabetes and experimental diabetes found no change.[12,13]

Albumin is one of the most abundant proteins in blood plasma and can be intensely glycated in diabetics.[14] Although albumin levels increase in individuals with type 2 diabetes[15] and in dogs with diabetes mellitus,[16] the albumin levels in experimental diabetic groups significantly decreased ($p<0.05$) compared to the control group during 4 weeks after STZ injection.[9] These results are consistent with data in the literature that albumin levels decrease in diabetes, but STZ injection had no effect on albumin levels in serum samples collected after 48 h.

Serum globulin levels can provide information along with other tests that are important for the determination of liver diseases and increases in acute and chronic liver diseases.[5,11] Alpha-1 globulin levels were determined to be significantly decreased ($p<0.05$) in experimental diabetic groups compared to the control group.[9] However, in the present study, the alpha-1 fraction did not change 48 h after the STZ injection.

It has been reported that alpha-1-antitrypsin, alpha-2-macroglobulin, haptoglobin, and C3 proteins on alpha-1 and alpha-2 bands decreased in cases of severe hunger or progressive chronic diseases, enteropathy, and nephropathies. In cirrhosis, alpha-globulin concentrations increased depending on the increase in serum lipoprotein.[5,11] Alpha-2-macroglobulin, haptoglobin, ceruloplasmin which are among alpha-2 globulins are significantly affected by many metabolic conditions as well as diabetes.[18] The band density representing the alpha-2 fractions did not show any difference in STZ-induced diabetes.[9] It has been reported that alpha-2 macroglobulin and ceruloplasmin (alpha-2 globulins)[19] increased significantly in dogs with diabetes mellitus[17] and diabetics. Sun *et al.*[20] conducted a study to identify the changes in mitochondrial protein expression in diabetic renal parenchyma and to characterize the molecular functions and biological processes in diabetes. The results showed that the two proteins with the most obvious changes in protein expression were identified asalpha-2uglobulin (mature protein, named A2) and its proteolytically modified form (named the A2 fragment). These proteins were found in the mitochondria of male rat renal parenchyma and were down-regulated in diabetic rats simultaneously. Other studies have reported that a significant increase was observed in the levels of ceruloplasmin, an antioxidant in diabetic rats.[21-23] The increase in serum haptoglobin levels in diabetic rats is a parameter indicating the start of virus infections.[24] In this study, the higher alpha-2 globulin levels in the 55 mg/kg STZ group may be due to the dominance of the decrease in haptoglobin, although the ceruloplasmin forming this band increases. STZ induced diabetes may affect production of inflammation, antioxidant or protease systems. To determine which systems are affected, it needs further analysis.

A decrease has been observed in beta and gamma globulins in nephropathies with protein loss.[5,11] In the present study, although the beta globulin fractions were determined to be significantly higher ($p<0.05$) in the 55 mg/kg STZ group than in the control and 65 mg/kg groups, the gamma globulin fraction did not change for all groups. Beta globulins include β-lipoproteins (apoliprotein B), complement proteins (C3, C4), β2-microglobulin, hemopexin and transferrin. It can say that, in the STZ induced experimental diabetes, the production or translation of these proteins are affected, and, they should clarify by high resolution techniques.

Immunoglobulins grouped as gamma globulins are referred as immunoglobulins. In the evaluation of serum protein fractions, the decrease in albumin is balanced with the increase in immunoglobulins in the gamma fraction.[5,11] Gamma globulin has been reported to decrease significantly in dogs with diabetes mellitus.[16] However, high gammaglobulin levels in individuals with type 2 diabetes are an indicator of the risk of diabetes, and immune function or activation may have a role in the development of type 2 diabetes.[24] Gamma globulin levels were determined to be significantly lower ($p<0.05$) in experimental diabetics compared with the control group.[9]

However, the A/G (albumin/globulin) ratio was determined to be significantly increased ($p<0.05$) in the diabetic group compared to other groups.[9] In the present study, there was no difference between the groups for the alpha-1 globulin fraction and the A/G ratio, the albumin and total globulin levels of the groups did not change.

Table 1: The concentrations of serum protein fractions (g/L) in control and STZ treated groups

Groups	Total protein	Albumin	Alpha 1	Alpha 2	Beta	Gamma	A/G
Control group	6.16 ± 0.31	2.98 ± 0.31	0.61 ± 0.07	0.65 ± 0.06[a]	1.49 ± 0.13[a]	0.43 ± 0.09	0.95 ± 0.099
55 mg group	6.84 ± 0.49	2.99 ± 0.21	0.60 ± 0.03	0.95 ± 0.11[b]	1.81 ± 0.08[b]	0.51 ± 0.03	0.86 ± 0.054
65 mg group	6.79 ± 0.31	3.15 ± 0.36	0.65 ± 0.05	0.87 ± 0.03[ab]	1.73 ± 0.07[ab]	0.42 ± 0.03	0.96 ± 0.061

There is a difference of $p<0.05$ between different letters.

Table 2: Frequency (in %) in control and STZ treated groups

Groups	Albumin	Alpha 1	Alpha 2	Beta	Gamma	A/G
Control group	47.86 ± 1.94	10.06 ± 0.67	10.92 ± 0.42	24.06 ± 0.48	7.09 ± 1.07	0.94 ± 0.083
55 mg group	43.41 ± 2.12	8.55 ± 1.05	13.49 ± 1.15	27.06 ± 1.40	7.48 ± 0.40	0.79 ± 0.06
65 mg group	45.92 ± 2.68	9.20 ± 1.75	13.30 ± 0.90	25.15 ± 1.01	6.42 ± 0.78	0.87 ± 0.08

There was no significant difference between groups for all fractions.

In the present study, although there were differences between the treatment groups, there was no statistically significant difference in the percentages of the serum protein fractions in the total protein.

Kaviarasan and Pugalendi[25] reported that diabetes was induced on the 17th day by a single i.p. injection of STZ (50 mg/kg) and the plasma globulin levels and the A/G ratio significantly decreased, whereas total protein and albumin increased. These results supported that the protein and fraction levels depend on the dose when STZ is administered.

In conclusion, there was no significant difference between the groups for all fractions except the alpha-2 and beta globulin concentrations. The alpha-2 and beta globulin levels were significantly high in the 55 mg/kg STZ group 48 h after the STZ injection. This increase may be due to the involvement of different proteins in the alpha-2 and beta globulin protein fractions and should be explored in further studies. The fractions will be determined to have different proteins, an indicator for future study. STZ is most used agent to induce experimental diabetes. The researchers have to know most affecting systems by STZ. The protein fractions including the proteins are important for diabetes pathophysiology. This study is a preliminary investigation to select which proteins are important for diabetes and its complications for future studies and aims to help showing mainly affecting proteins for further studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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