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Effects of curcumin administration on Nesfatin-1 levels in blood, brain and fat tissues of diabetic rats

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Abstract. – OBJECTIVE: We evaluated the efficacy of curcumin administration on blood glucose levels and its relationship with nesfatin-1 levels in blood brain and adipose tissue of streptozotocin-induced diabetic rats.

MATERIALS AND METHODS: A total of 28 male rats were divided into four groups: control group, type 2 diabetes mellitus (DM) group, control plus curcumin group and type 2DM plus curcumin group. After fifteen days, blood samples were collected from sacrificed rats. Nesftain-1 levels were analysed from blood, brain, and fat tissues of rats in all groups.

RESULTS: Nesfatin-1 level was found to be significantly lower in blood, brain and fat tissues of type 2 DM rats compared to the control group. A significant decrease in fasting blood glucose levels was observed in the curcumin administration group compared to type 2 DM group. Improvement of fasting blood glucose level was accompanied by improvement of nesfatin-1 levels in blood, brain, and fat tissues.

CONCLUSIONS: As expected, curcumin administration caused significant improvement in fasting blood glucose levels. However, for the first time, we found marked improvements in nesfatin-1 levels in blood, brain, and fat tissues of type 2 DM rats. Thus, considering the crucial role of nesfatin-1 in regulation of glucose metabolism, it is logical to expect an interactive relationship between curcumin and nesfatin-1.

Key Words:
Nesfatin-1, Curcumin, Diabetes, Streptozotocin, Rat.

Introduction

In today's world, diabetes, especially type 2 diabetes mellitus (Type 2 DM), is one of the most commonly observed serious metabolic diseases[1] characterized by impairments of blood glucose homeostasis resulting from defects in insulin action and/or production[2]. The identification of an anorexigenic neuropeptide originating from nucleobindin-2 and termed nesfatin-1[3] increased expectations in the struggle with development and treatment of diabetes. Nesfatin-1 may have a vital role in regulation of energy balance via inhibiting food intake[4,5], glucose homeostasis[6], insulin sensitivity[7] and insulin release in beta cells[8]. However, studies evaluating nesfatin-1 levels in response to type 2 DM resulted in controversial conclusions, with increases[9] or decreases[10] in nesfatin-1 levels.

Interestingly, some natural plants may also be used for the supportive treatment of diabetic patients[11]. Curcumin extract obtained from turmeric was shown to display antidiabetic effects[12]. Curcumin is a therapeutic agent used effectively for the amelioration of diabetes and its complication[13].

Despite many studies performed to evaluate curcumin and diabetes or diabetes and nesfatin-1 relationships, there is no satisfactory information concerning the effect of curcumin application on diabetes and nesaftin-1 relationships. Another important issue is evaluating the status of nesfatin-1 activity in different parts of the body, especially brain, adipose tissues and blood under type 2 DM conditions.

This study aimed to evaluate the effectiveness of curcumin extract administration on the relationship between diabetes and nesfatin-1 in brain tissue, adipose tissue and blood.

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Materials and Methods

This experimental study was carried out in the Experimental Research and Application Center of Van Yuzuncu Yil University. All stages of the study were designed and performed according to the guidelines of the Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University (Protocol No: 2018/10 Date: 10/25/2018).

A total of twenty-eight healthy male Wistar Albino rats (8-10 weeks old), weighing 200-250 g were obtained from the Experimental Animal Research Center of Van Yuzuncu Yil University. The rats were separated and placed in four stainless steel cages, seven rats per cage, and exposed to twelve-hour dark/light cycle. The rats were allowed free access to pellet feed (Purina, Istanbul, Turkey) containing 21% crude protein and daily drinking water *ad libitum* in optimal environmental conditions (22±2°C and 60±5% humidity).

This study consisted of four different experimental groups (n=7 for each group).

Group I (Control group): the rats in this group received 0.5 ml/kg physiologic saline orally for 15 days.

Group II (Diabetes group): in fasting state, a single dose of 45 mg/kg streptozotocin (STZ) (Sigma-Aldrich Chemie Gmbh, Munich, Germany) was injected intraperitoneally to each rat in this group. Then, blood-glucose levels were measured with glycometry (IME-DC, Hof, Germany) from the tail veins over 12 hours of fasting after 72 hours. Rats with blood glucose levels above 250 mg/dl were considered to be diabetic[14,15].

Group III (Curcumin group): Curcumin (Alfa Aesar, Ward Hill, MA, USA), was administered orally once daily for 15 days at a dose of 10 mg/kg[14]. Curcumin is insoluble in water due to the lipophilic polyphenol action; thus, curcumin was dissolved in dimethoxy sulfoxide (DMSO). Curcumin was freshly prepared every day for 15 days, with amounts calculated according to the number of rats in the groups at the determined doses of 1 cc per animal. The animals were given curcumin by intra-gastric gavage.

Group IV (Diabetes + Curcumin group): STZ (Sigma-Aldrich Chemie Gmbh, Munich, Germany) was administered as 45 mg/kg single dose i.p. Starting 72 hr after this administration, 10 mg/kg curcumin (Alfa Aesar, Ward Hill, MA, USA) (dissolved in DMSO) was given orally once a day for 15 days.

Sample Collection

At the end of experiment, after the cardiac blood samples were obtained under ketamine (Ketasol, Ricterphar Up, Vienna, Austria) and bacillazine (Rompin, Istanbul, Turkey) anaesthesia, all rats were sacrificed. Blood samples were centrifuged at 4500 rpm for a period of 10 min. The serums were separated and used to measure the levels of nesfatin-1 hormones.

Enzyme Linked Immunosorbent Assay for Detecting of Nesfatin-1

The kit is a sandwich enzyme immunoassay for the *in vitro* quantitative measurement of nesfatin-1 (SunRed. Biological Technology Co., Ltd., Shanghai, China, Catalog No: 201-12-4341). Each well of the supplied microtiter plate is pre-coated with a target specific capture antibody. Standards and samples are added to the wells and the target antigen binds to the NES-1 antibody labelled with biotin. Afterwards, a streptavidin-HRP conjugate, chromogen A and B substrate, and a stop solution is added to terminate colour development reactions and then the optical density of the well is measured at a wavelength of 450 nm ± 2 nm using automated optical densitometry (intra-assay CV<10%; inter-assay CV<12%). Each sample was run in duplicate, and the mean value was used for analysis. This assay has high sensitivity and excellent specificity for detection of human nesfatin-1. No significant cross-reactivity or interference between human nesfatin-1 and analogues was observed.

Statistical Analysis

The Kolmogorov–Smirnov test was used for the distribution of data. Since the data showed normal distribution, significant differences between the groups for the same parameter were determined by Kruskal-Wallis analysis from the nonparametric tests. Post-hoc multiple comparison tests were used to determine the different groups. The results are expressed as mean±standard deviation, and the statistical significance limit was taken as $p<0.05$.

Results

The mean (±SD) weights of rats were measured at the beginning and at the end of the study in the control (C), diabetes (DM), curcumin (Cur) and diabetes plus curcumin (DM+Cur) groups (Table I). In addition, the response of fasting blood glu-

Table I. Body weight and fasting blood glucose levels at the onset of study and at the end of the study for the control (C), diabetic (DM), curcumin (Cur) and diabetes plus curcumin (DM+Cur) groups.

Parameters	Basal	Last
Body Weight (C) (g)	230 ± 5	235 ± 10NS
Fasting Blood Glucose (C) (mg/dL)	98.5 ± 8.6	103.5 ± 5.8 NS
Body Weight (DM) (g)	230 ± 10	226 ± 8 NS
Fasting Blood Glucose (DM) (mg/dL)	273.0 ± 61.3	326.7 ± 58.3 NS
Body Weight (Cur) (g)	222 ± 19	229 ± 18 NS
Fasting Blood Glucose (Cur) (mg/dL)	104.2±4.3	90.0 ± 4.7*
Body Weight (DM + Cur) (g)	225 ± 8	227 ± 6 NS
Fasting Blood Glucose (DM + Cur) (mg/dL)	307.0 ± 56.5	203.1 ± 38.4*

cose levels for each study group are presented in Table I. There were systematically higher blood glucose levels in DM and DM+Cur groups at the beginning of the study (Table I). However, curcumin administration caused a significant decrease in blood glucose levels in DM+Cur group and also in the Cur group compared to their baseline levels (Table I).

There were significant differences in serum nesfatin-1 levels in DM, Cur and DM+Cur groups compared to the C group (Figure 1). Significantly low levels of nesfatin-1 were observed in DM (22%) and DM+Cur (10%) groups compared to C group at the end of the study (p<0.05) (Figure 1). In contrast, a significantly higher nesfin-1 level was observed in Cur group (13%) compared to C at the end of the study (p<0.05) (Figure 1). Curcumin administration caused significant increases in nesfatin-1 levels (15%) in diabetic rats (p<0.05) (Figure 1).

Nesfatin-1 levels were found to be significantly lower in brain tissue of the DM group (1.845±0.1 ng/mL) but higher in the Cur group (2.737±0.09 ng/mL) compared to C group (2.266±0.07 ng/mL) (p<0.05). In addition, nesfatin-1 levels in brain tissue of DM+Cur group were found to be higher (2.202±0.2 ng/mL) than the DM group (p<0.05).

Analysis of nesfatin-1 in fat tissue of rats also revealed various responses for each group. Nesfatin-1 levels decreased in the DM group (1.484±0.07 ng/mL) but increased in the Cur group (2.043±0.07 ng/mL) compared to C group (1.843±0.07 ng/mL) (p<0.05). Curcumin administration caused significant increases in nesfatin-1 levels in fat tissue of DM rats (DM+Cur) (1.699±0.09 ng/mL) compared to DM group (p<0.05).

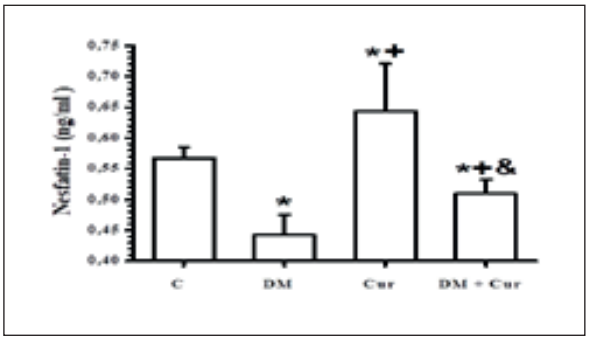

Figure 1. Mean (±SD) values of nesfatin-1 for the control (C), diabetic (DM), curcumin (Cur) and diabetes plus curcumin (DM+Cur) groups. *Reflects significance compared to control. +Reflects significance compared to diabetic group. &Reflects significance compared to Curcumin group.

Discussion

We aimed to evaluate the efficacy of curcumin administration on fasting blood glucose and its interaction with nesfatin-1 levels in blood, brain tissue, and fat tissue of rats with type 2 DM induced by streptozotocin.

Nesfatin-1 was first identified as a hypothalamic molecule that has significant functions for the regulation of food intake with leptin independent mechanisms[3]. The regulatory role of nesfatin-1 in glucose homeostasis and energy expenditure was shown[16]. Antihyperglycemic effects of nesfatin-1 under the condition of impaired glucose metabolism were also documented[6]. The relationships between blood nesfatin-1 levels and type 2 DM were examined in many studies, but the conclusions are contradictory[17]. In the present study, the observation of lower levels of serum nesfatin-1 was associated with findings from some previous human studies[10,18,19]. However, the opposite conclusion of increased nesfatin-1 levels was suggested in type 2 DM patients[9,20] and streptozotocin-induced type 2 DM mice[21] compared to controls.

The distribution of nesfatin-1 in various tissues in the body was shown[22]. Nesfatin-1 was widely expressed in many parts of the brain, especially related to the energy regulatory section[3,23]. In addition, peripherally produced nesfatin-1 including in adipose tissue was also proven[24]. We have observed variations of nesfatin-1 levels in brain tissue, fat tissue and blood in the control group, i.e. highest in brain tissue and lowest in blood serum. The evaluation of the serum, brain and fat tissue levels of nesfatin-1 in type 2 DM rats showed that nesfatin-1 levels were significantly lower in the type 2 DM group compared with the levels in the control group. Interestingly, we observed an improvement in nesfatin-1 levels in blood, brain and fat tissues of diabetic rats administered curcumin compared to the type 2 DM group. Higher fasting blood glucose levels were accompanied by lower nesfatin-1 levels in diabetic rats compared to controls. In the literature, current findings of studies suggest beneficial actions of peripheral nesfatin-1 in regulation of glucose levels[6,24-27]. However, we have found marked decrease in nesfatin-1 levels in brain and fat tissues in addition to serum levels in DM group.

Curcumin has attracted attention in clinical medicine as a potential therapeutic agent against a number of human diseases[13,28,29]. The beneficial effects of curcumin administration on homeostasis of blood glucose level were shown in many studies[30-32]. In this study, we also found significant improvement (approximately 33% decrease) in fasting blood glucose levels after curcumin administration in the diabetic group. Importantly, we showed that curcumin administration caused significant increases in serum nesfatin-1 levels in both diabetic and control plus curcumin groups. In the literature, the beneficial effects of curcumin administration in patients with diabetes have been shown in many studies[33]. The mechanisms by which curcumin administration may decrease fasting blood glucose levels are still not clear. There are many explanations for this fasting blood glucose reducing effect in DM, including increased antioxidant enzyme activity, suppressed tumor necrosis factor and interleukin-1 levels, lipid peroxidation, and increases in insulin concentration[34-36].

Curcumin is frequently used as adjuvant in the treatment of dysglycaemia and obesity-related metabolic disorders[37,38]. Nesfatin-1 has gained increased attention in clinical medicine as an effective therapeutic agent for diabetes obesity and also some other metabolic disorders[39,40].

Conclusions

Our findings revealed substantial rise of nesfatin-1 levels in serum, brain tissue and fat tissue among DM+Cur rats as compared to DM rats, as also associated with significant improvement of fasting blood glucose levels for the first time. Curcumin has long been known as a potential agent used for type 2 DM that end up with straighten in blood glucose levels. However, specifying the improved fasting blood glucose levels following curcumin administration and relationships with increased nesfatin-1 levels could widen the scientific standpoint against many metabolic diseases. Importantly, further studies are required to achieve information about interaction between curcumin administration and metabolic/endocrine effects of nesfatin-1 and its relationships with leptin, ghrelin Glut 4 and other substances regulating energy balance in-patients with metabolic disorders.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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