Neuroprotective Effect of Cerebrolysin on Diabetic Neuropathy: A Study on Male Rats

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Abstract

Objective: Diabetes mellitus with 10% prevalence in human population leads to disorders of peripheral nervous system in many affected patients. It causes various polyneuropathies in which nerve conduction velocity decreases. The aim of this study was to investigate the effect of cerebrolysin on the treatment of neural injuries resulted from hyperglycemia.

Method: Diabetes was induced in male rats weighing 250 ± 25 gr by intraperitoneal injection of 65 mg/kg streptozocin (STZ). Six weeks after STZ injection and appearance of neuropathy in diabetic rats, animals were divided into four groups: experimental, vehicle, diabetic and control. The experimental and vehicle groups received respectively single dose of 5 mg/kg day-1 cerebrolysin and saline intraperitoneally for two weeks. At the end, in order to find the efficacy of cerebrolysin, all groups underwent behavioral and electrophysiological tests as well as histological investigation.

Results: Metabolic parameters in different groups showed inefficacy of cerebrolysin in the treatment of metabolic disorders of diabetes. However, electrophysiological investigations showed efficacy of cerebrolysin in the treatment of diabetic neuropathy in rats. Moreover, investigation on morphologic structure of sciatic nerve was evident of the return of axon degenerative changes and myelin splitting in nerve fibers in cerebrolysin-received group. The results of behavioral studies showed increase in recovery in cerebrolysin group.

Conclusion: According to the results, treatment of diabetic neuropathy with daily injection of 5 mg/kg cerebrolysin for two weeks improves rats' condition.

Keywords: Diabetes mellitus; Ischemia; Antioxidant; Anti-inflammatory; Neuropathy; Cerebrolysin; NCV

Introduction

Diabetes mellitus is a disorder recognized with increase of blood sugar level. Impaired insulin release or failure to respond to insulin or both is the cause of this disorder. Chronic hyperglycemia leads to the dysfunction of several organs especially eye, kidney, heart and vessels [1]. Peripheral neuropathy is one of the common complications of diabetes which in turn increases the risk of other diabetes complications such as foot ulcers and amputation [1]. Almost more than half of diabetic patients suffer from different forms of neuropathy after passing 1-2 decades of their disease [2,3]. In animal models of streptozotocin-induced diabetes, this time has decreased to at least two weeks [4]. Peripheral diabetic neuropathy is the result of several factors [5-7] and its probable mechanisms include glycosylation of neural proteins, microangiopathy, neuronal antibodies and ischemia resulted from basement membrane thickening of the vasa nervorum. Abnormalities of polyol pathway and defects of protein kinase C metabolism which cause nerve demyelinating have also been described in diabetic peripheral neuropathy [6]. Based on these mechanisms of injury, various prevention and treatment strategies have been already suggested and are under investigation. For instance, the effects of several antioxidants such as vitamin E [8], melatonin [9] and date extract [10], fatty acid contained diets like Omega 3 [11], aldose reductase inhibitors [12] and also statins compounds such as atorvastatin [13] have been investigated, but none of them have already been approved by FDA. At present, there is no definite treatment for this complication.

Cerebrolysin is a neuropeptide anti-inflammatory mixture isolated from pig brain tissue [14]. It is a neurotrophic peptidregic mixture resulted from enzymatic breakdown of free-lipid porcine brain proteins. It contained 25% low molecular weight peptides (<10 KDA) and 75% free amino acids depending on free nitrogen content [15]. Cerebrolysin contains relatively high concentrations of magnesium, potassium, phosphorus, selenium [16] and also other elements [17,18].

Cerebrolysin was first used in 1973 [19] as a hydrolysate in patients with cerebral arteriosclerosis. It has been suggested for several types of nerve degeneration disorders [20-22], organic mental disorders [21], multiple sclerosis [22], anti-aging [23] and ischemic encephalopathy [24]. This medicine has also been applied in the treatment of pediatric cerebral paralysis, elderly patients and some other conditions [25]. It has been recognized in a comparative study that antioxidant properties of cerebrolysin is approximately 300 times less than that of trolox (vitamin E) [26].

In regard to the positive effects of cerebrolysin on neurotic disorders reported in previous studies, the present study was designed to investigate cerebrolysin as an effective mixture in the process of ischemia and improvement of diabetic neuropathy.

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Received February 03, 2014; Accepted March 22, 2014; Published March 27, 2014


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Material and Methods

Animals

In the present study, male NMRI rats weighing 250 ± 25 gr kept in the animal house of Kerman Neuroscience Research Center were used. Animals were kept at 25 ± 1°C, 12/12 h light/dark cycle and free access to the same food and water. The study protocol was approved by the animal ethics committee of this institution (Code:EC/KNRC/88-15).

Diabetes induction

Rats were divided into four groups: First group consisted of control animals. The second, third and fourth groups consisted of diabetic animals (at least 8 rats in every group).

In order to induce diabetes, streptozotocin purchased from Sigma Company (65 mg/kg in 100 mmol/L sodium citrate buffer, pH4.5) was intraperitoneally injected into at least 24 rats [27]. One week after STZ injection, animals with fasting blood sugar higher than 200 mg/dl were selected for experiments. Six weeks after the injection of STZ and appearance of neuropathy in diabetic animals [27,28], First group (Control): received nothing, second group (Diabetic): received nothing, third group (Vehicle): received 5 ml/kg/day xylazine solution. The environment temperature was kept at 25 ± 1°C and the relative humidity was 75 ± 10%.

Open field test

Open field test was used in order to survey the effect of diabetic neuropathy on exploratory behavior of diabetic rats and the probable protective effect of Cerebrolysin. Exploratory behavior was investigated by a video tracking system (TSE) in a 45° × 45° × 45 cm box. At the end of 6h week after diabetes induction, animals were placed in the center of the arena and their exploratory behavior including horizontal, central and peripheral paved distances were measured for 5 minutes. The duration of staying in the center and peripheral parts as well as the velocity of movement were investigated [32].

Electrophysiological evaluation

Six weeks after the initiation of hyperglycemia, animals were anesthetized with intraperitoneal injection of 50/20 mg/kg ketamine+ xylazine solution. The environment temperature was kept at 25 ± 1°C during all phases of experiments. After shaving back of animal's leg, a small incision was made in the right sciatic notch and ankle. Then, by bi-polar electrodes, the proximal part of sciatic nerve at the sciatic notch and the distal part at the ankle were stimulated and motor neuron conduct velocity (MNCV) of sciatic-tibial motor nerve was recorded (powerlab/ML856; AD Instruments, Sydney, NSW, Australia). Immediately after stimulation, action potential of the first interosseous muscle of back paw was recorded by mono-polar electrodes. The obtained records are biphasic responses with one primary m wave appeared due to the stimulation of motor fibers. Motor nerve conduct velocity (m/s) is calculated through dividing the distance between two stimulation points (mm) with the time difference of two stimulations [10,33].

Histopathological evaluation

After the experiments related to NCV, animals were anesthetized with 400 mg/kg chloral hydrate and following cardiac perfusion by using saline and bouni's fixative [34]. Then a section of sciatic nerve (1 cm) was removed and kept in bouni's fixative. Forty eight hours after remaining in fixative, it underwent tissue processing phases and was embedded in paraffin. Then 4 μm sections were prepared and stained with hematoxylin-eosin for surveying by light microscope (Motic Images China e-kup Co, Ltd)s (s400) [35].

In this evaluation, axons of nerves in sciatictransverse section were investigated in regard to edema and axoplasm state (demyelination and remyelination) [36].

Statistical analysis

Parametric paired t-test was used for comparison of coupled primary and secondary variables and in order to compare quantitative variables among groups ANOVA was applied. Tukey test was used in the case of significant difference and in the case of rejection of null hypothesis, non-parametic Kruskal Wallis was applied.

Results

Metabolic parameters

In all diabetic groups, mean plasma concentration in the 8th week was 280% more than that in the control group. All diabetic rats showed high blood sugar and weight gain disorder in the 8th week after STZ injection. As it has been presented in Table I, weight of diabetic animals compared to the non-diabetic control group had significantly decreased in the 8th week (Table 1).

The effect of cerebrolysin on tail flick test

Diabetic neuropathy caused significant increase in reaction to pain and tail flick latency time in diabetic, vehicle and cerebrolysin groups compared to the control group, but diabetic and vehicle groups showed no significant difference with cerebrolysin-received group in this regard (Figure 1).

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Body weight (g)</th>
<th>Blood glucose (mg dL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before STZ injection</td>
<td>End experiment</td>
</tr>
<tr>
<td>Control (8)</td>
<td>243/25 ± 30/36 *</td>
<td>279/62 ± 28/91</td>
</tr>
<tr>
<td>Sham (8)</td>
<td>255/42 ± 17/01</td>
<td>230/85 ± 22/95</td>
</tr>
<tr>
<td>Vehicle (8)</td>
<td>235/80 ± 23/74</td>
<td>204/00 ± 26/19</td>
</tr>
<tr>
<td>Cerebrolysin (8)</td>
<td>255/22 ± 24/68</td>
<td>208/33 ± 20/40</td>
</tr>
</tbody>
</table>

Data are the Mean ± SEM (n = 8), * p<0.0001, compared with the control group.

Table 1: Body weight and blood glucose levels of all groups.
The results of open field test

The analysis of data related to “total distance moved”, “mobility”, “immobility” and “velocity” in open field test showed no significant difference among studied groups (Figure 2).

Nerve Conduct Velocity

In regard to mean NCV, vehicle and diabetic groups showed significant difference with the control group (p=0.000 and p=0.000 respectively), and also they had significant difference with cerebrolysin-

Figure 1: Effect of cerebrolysin (5 mg kg day⁻¹, ip, for 2 weeks) on the pain threshold values in streptozotocin-injected diabetic rats subjected to tail flick. *P<0.05, **P<0.01 and ***P<0.001 as compared to control group. There are no significance effects between cerebrolysin-treated and diabetic groups. Values are expressed as mean ± SEM. (n=8 rats in each group).

Figure 2: Effect of cerebrolysin on explorative behavior of rats in open field test. (A) Total distance moved, (B) movement (C) not movement and (D) velocity. Data are the Mean ± SEM. (n=8 rats in each group).
The effect of DFE on histomorphometric parameters of rat sciatic nerve.

Table 2:

<table>
<thead>
<tr>
<th>Group</th>
<th>MSD (μm)</th>
<th>AD (μm)</th>
<th>MMFD (μm)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.96 ± 0.98</td>
<td>6.53 ± 0.932</td>
<td>14.50 ± 0.778</td>
<td>6</td>
</tr>
<tr>
<td>Cerebrolysin</td>
<td>6.74 ± 0.736</td>
<td>6.63 ± 0.742</td>
<td>13.38 ± 0.918</td>
<td>6</td>
</tr>
<tr>
<td>Sham</td>
<td>4.59 ± 0.453</td>
<td>5.00 ± 0.446</td>
<td>9.59 ± 0.657</td>
<td>6</td>
</tr>
<tr>
<td>Vehicle</td>
<td>4.71 ± 0.247</td>
<td>4.98 ± 0.485</td>
<td>9.70 ± 0.296</td>
<td>6</td>
</tr>
</tbody>
</table>

N: Number of animals; MMFD: Mean-Myelinated Fiber Diameter; AD: Axon Diameter; MSD: Myelin Sheath Diameter. Data are presented as Mean ± SEM. (n=8 rats in each group).

Gusev et al. [38], treated 30 patients with severe ischemic strokes by administering 10, 20 and 30 mg/day cerebrolysin for 10 days and have reported improvement in patients with moderate disease in comparison to their control group. Indeed, cerebrolysin improves motor activities and EEG signals in rats with moderate ischemia in anterior portion of brain. Also cerebrolysin has high neurotrophic property due to having very useful compounds such as 25% low density proteins (KDA<10), 75% free amino acids [15], high concentrations of magnesium, potassium, phosphor and selenium [16] and also some other elements [18,38]. This medicine, through providing the nerve cell with these elements, helps both cell metabolism process and remyelination.

Neuroplasticity involves the activation of existing but silent connections, synaptogenesis, dendritic arborization and new nervous cell production [17]. Neuroplasticity can be enhanced by administration of neurotrophic factors [39]. Due to its unique composition consisting of active fragments of neurotrophic factors, Cerebrolysin is able to confer neuroprotection and to stimulate neuroplasticity, thereby enhancing the neurorecovery process [40,41].

The other mechanism for explaining cerebrolysin effect is its antioxidant property [26,42]. In the process of diabetic neuropathy, nerve cells and vessels’ membranes are not dependent to insulin for transferring glucose and in diabetes disorder great amount of glucose enter cells. In nerve cells, glucose changes to sorbitol by aldose reductase enzyme and sorbitol accumulation increases free radicals such as hydroxyl-super oxide and hydrogen peroxide and eventually causes cell damage. Based on this mechanism of injury, different prevention and treatment approaches are under investigation [43,44]. As it was mentioned in the introduction, anti-oxidant property of cerebrolysin is 300 times less compared to vitamin E [26]. Therefore, it seems that cerebrolysin with its minor anti-oxidant property could remove free radicals to some extent and caused improvement of diabetic neuropathy.

Diabetic neuropathy in its early stages is associated with increase of nerve fiber activity and disorder of normal sensitivity of peripheral nervous system to injuries and painful stimulators resulted from diabetic hyperalgesia [33,45]. However, after passing early stage the sensitivity of peripheral nerves decreases and caused various range of analgesia.

According to the obtained results in the present study, cerebrolysin (5 ml/kg/day, ip) can exert positive effects within two weeks in the treatment and decreasing the physiological symptoms of diabetic neuropathy in male rats. In the present study, response time to thermal pain in tail flick test showed significant increase in the diabetic group in comparison to the control group that is due to diabetic analgesia.

Cerebrolysin could not significantly reduce this analgesia [46].
In open field test, it was seen that although cerebrolysin cannot exert significant improvement in behavioral variables in comparison to diabetic controls, in some extent it can improve (even though non-significantly) diabetic neuropathy.

Mean NCV in the diabetic group showed 50% reduction in comparison to the control group that shows high neuropathy percentage in diabetic rats. Indeed, it was observed that intraperitoneal injection of cerebrolysin can significantly cause improvement of NCV in neuropathy-induced male rats.

The presence of abnormal fibers in sciatic nerve that showed degenerative changes of axon and myelin splitting was one of the other symptoms of STZ-induced diabetic rats. In fact, one of the main reasons of nerve activity reduction in the process of diabetic neuropathy disorder, is morphological changes occurred due to nerve metabolic disturbances. In the present study, we observed the efficacy of cerebrolysin in improving morphological injuries of sciatic nerve myelin in rats with diabetic neuropathy. Morphological observations showed remyelination after two weeks of treatment with cerebrolysin. Axon diameter (AD), myelin sheath diameter (MSD) and mean myelinated fiber diameter (MMFD) showed absence of any significant difference between cerebrolysin-received rats and controls; however, means of all these indices were higher in the control group as compared with the cerebrolysin-received group. Means of AD, MSD and MMFD in vehicle and diabetic groups had significant decrease in comparison to the control group. This finding shows the efficacy of cerebrolysin in the improvement of degenerated axons of nerve fibers or remyelination in STZ-induced diabetic rats.

As we mentioned above, our study showed that cerebrolysin (5 ml/kg/day, ip) can exert positive effects within two weeks in the treatment of diabetic neuropathy in male rats. We also expect to have more significant effect of cerebrolysin at a different dose level or duration. Therefore complementary studies should be done to reveal the optimum dose and duration.

Conclusion

It was observed in the present study that intraperitoneal injection of cerebrolysin is effective in the treatment of diabetic neuropathy and can improve the function of peripheral nerves.

Acknowledgment

The authors would like to thank Kerman Neuroscience Research Center for financial support of this study and some students of Azad Islamic University, Arsanjan Branch for their cooperation.

References


