Journal of Diabetes and Metabolism
ISSN: 2155-6156

Zangiabadi et al., J Diabetes Metab 2014, 5:4
DOI: 10.4172/2155-6156.1000355

Research Article Open Access

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

Nasser Zangiabadi[1,2], Hossein Mohtashami[1]*, Mohammad Shabani[1] and Mandana Jafari[1]

[1]*Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Iran*
[2]*Afzal Research Center, Iran*

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 conductionvelocity decreases. The aim of this study was to investigate the effect of cerebrolysinon 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} cerebrolysinand 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], aldosereductase 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 pediatrics 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.

***Corresponding author:** Hossein Mohtashami, Researcher in Kerman Neuroscience Research center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Tahmassebabad crossroad, Ebne-sina St. 76198-13159, Iran, Tel: +98-341-226-4198; E-mail: mohtashamihossein@yahoo.com

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

Citation: Zangiabadi N, Mohtashami H, Shabani M, Jafari M (2014) Neuroprotective Effect of Cerebrolysin on Diabetic Neuropathy: A Study on Male Rats. J Diabetes Metab 5: 357 doi:10.4172/2155-6156.1000357

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/darkcycle 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^{-1} saline intraperitoneally for 2 weeks and fourth group (Experimental): received 5 ml/kgday^{-1}cerebrolysinintraperitoneally for two weeks. This dose of cerebrolysin has been identified as neuroprotective inducing dose in nerve injuries [29,30].

Tail flick test

Tail flick is one of the standard tests for measuring the rate of analgesia. In this test, thermal light with the intensity of 5 was directed on the distal part of the animal's tail by Tail flick instrument (made by Spanish Lsi LETICA, model LE7406) and tail flick latency was measured. In order to prevent tissue damage, light was directed for a maximum time of 10 seconds. For each animal, tail flick latency was measured three times with 5 minutes intervals and mean of them was reported as tail flick latency [31].

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*45cm box. At the end of 6th 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, using 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 stimulated points (mm) by 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 bouin's fixative [34]. Then a section of sciatic nerve (1 cm) was removed and kept in bouin'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)(x400) [35].

In this evaluation, axons of nerves in sciatic transversesection were investigated in regard to edema andaxoplasm 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-parametric Kruskal Wallis was applied.

Results

Metabolic parameters

In all diabetic groups, mean plasma concentration in the 8^{th} week was 280% more than that in the control group. All diabetic rats showed high blood sugar and weight gain disorder in the 8^{th} week after STZ injection. As it has been presented in Table1, weight of diabetic animals compared to the non-diabetic control group had significantly decreased in the 8^{th} 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).

Animal group	Body weight (g)		Blood glucose (mg dL̄1)	
	Before STZ injection	End experiment	Before STZ injection	End experiment
Control (8)	243/25 ± 30/36 *	279/62 ± 28/91	147/37 ± 13/05	161/12 ± 28/36
Sham (8)	255/42 ± 17/01	230/85 ± 22/95	454/28 ± 73/61	463/71 ± 62/27 × × ××
Vehicle (8)	235/80 ± 23/74	204/00 ± 26/19	503/50 ± 39/28	520/50 ± 53/29 × × × ×
Cerebrolysin (8)	255/22 ± 24/68	208/33 ± 20/40	523/83 ± 34/60	533/16 ± 66.51 × × ××

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.

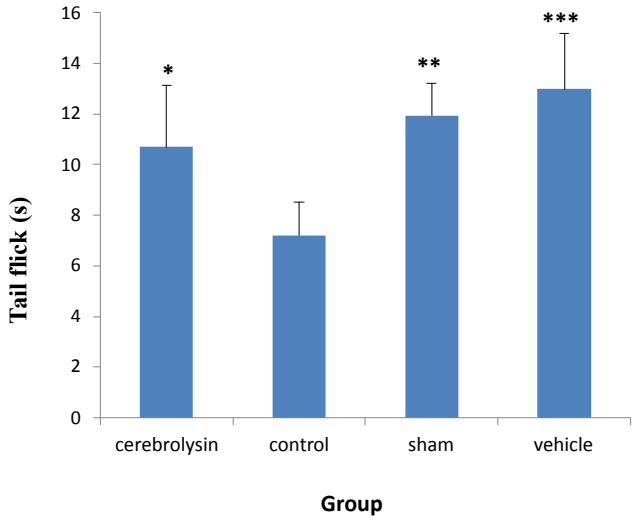

Figure 1: Effect of cerebrolysin (5 mg kg day^{-1}, ip, for 2 weeks) on the pain threshold values in streptozotocin-injected diabetic rats subjected to tail flick. *P<0.05, **P<0.01 and ***P<0.001 as compared to control group. There are no significance effects between cerebrolysin-treated and diabetic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

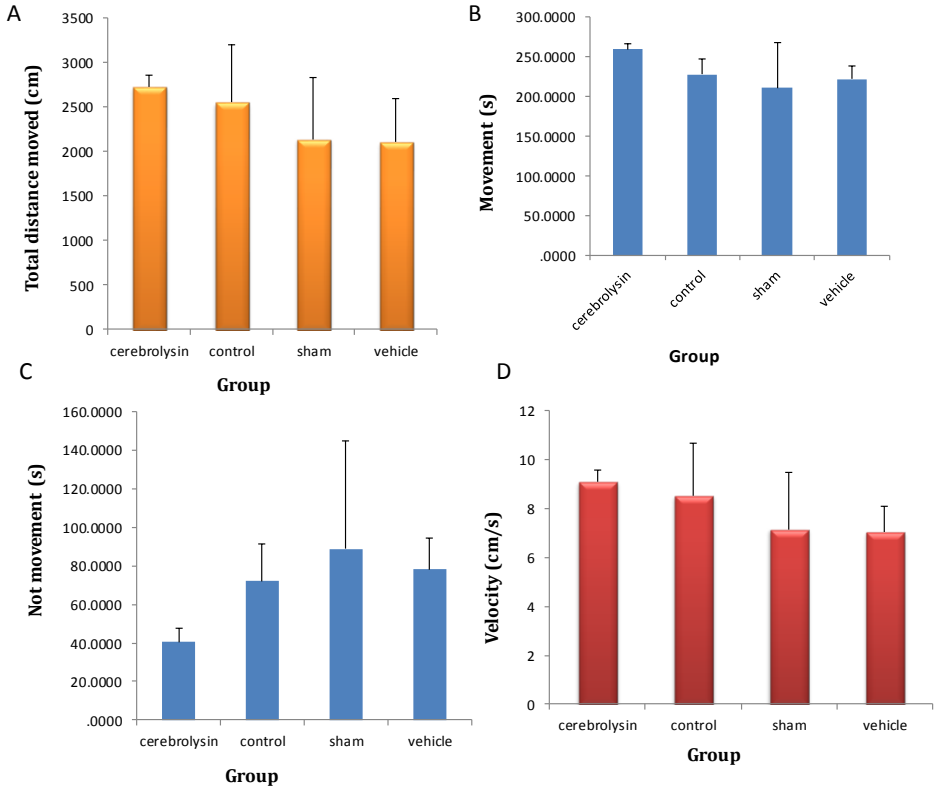

Figure 2: Effect of cerebrolysin on explorative behavior of rats in open field test. (A) Total distance moved , (B) movement (C) not movement and (D) velocity. Data are the Mean ± SEM. (n=8 rats in each group).

The 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-

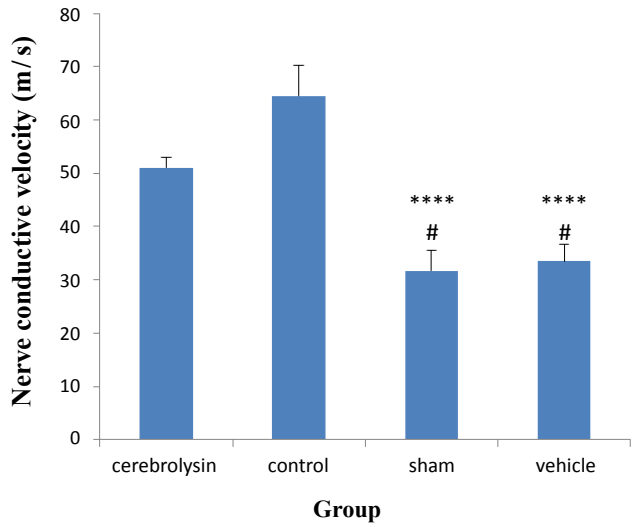

Figure 3: Effect of cerebrolysin on motor nerve conductive velocity (MNCV) in rats. ****P<0.0001 as compared to control group; #P<0.05 as compared to cerebrolysin-treated group. Data are the Mean ± SEM. (n=8 rats in each group).

Groups	MSD (µm)	AD (µm)	MMFD (µm)	N
Control	7.96 ± 0.98	6.53 ± 0.932	14.50 ± 0.778	6
Cerebrolysin	6.74 ± 0.735	6.63 ± 0.742	13.38 ± 0.918	6
Sham	4.59 ± 0.453	5.00 ± 0.446	9.59 ± 0.657	6
Vehicle	4.71 ± 0.247	4.98 ± 0.485	9.70 ± 0.296	6

N: Number of animals; MMFD: Mean-Myelinated Fiber Diameter; AD: Axon Diameter; MSD: Myelin Sheath Diameter. Data are presented as Mean ± SEM.

Table 2: The effect of DFE on histomorphometric parameters of rat sciatic nerve.

received group (p=0.012 and p=0.024 respectively). While There are no significance effects between cerebrolysin-treated and control groups (p=0.107). This finding shows significant efficacy of cerebrolysin in improving nerve function in the cerebrolysin-received group compared to vehicle and diabetic groups (Figure 3).

The effect of cerebrolysin on morphological alterations of nerve myelin

Microscopic investigations showed normal structure and morphology of myelin in the control group, but in the diabetic and vehicle groups, edema and myelin sheath splitting were observed. Increase in the number of fibers with abnormal myelin was observed in the diabetic and vehicle groups. Treatment with cerebrolysin could prevent abnormal cases in a wide extent. Data related to the myelin and axon diameters of nerve fibers in the vehicle and diabetic groups showed significant decrease in comparison to the control group. Cerebrolysin administration caused significant improvement in myelin and axon diameter reduction among diabetic rats in two weeks (Table 2 and Figure 4).

Discussion

In several in vivo and *in vitro* studies, neuroprotective and neurotrophic effects of cerebrolysin have been reported. Cerebrolysin has caused improvement in cell oxidative stress during cerebral ischemia in animals.

Considering the identified properties of cerebrolysin as a neuropeptide anti-inflammatory mixture [14] and also previous related studies, the probable mechanism of cerebrolysin effect on diabetic neuropathy can be explained as follow.

Since ischemia is associated with decrease of blood flow followed by oxygen and food shortage and consequently stopping of energy production in tissue vessels, it has a significant role in producing and extension of pathologic changes in various neuropathies including peripheral neuropathies especially in sciatic nerve. These pathologic alterations in nerves are related to the degeneration of fibers and edema. In ischemia phase, blood flow stopping and oxygen shortage result in anaerobic metabolism, energy loss, ATP decrease and accumulation of hypoxanthine in ischemic cells. Lack of energy affects ATPase ion pump of cell membrane and causes sodium, calcium and water accumulation and consequently cell edema [37].

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].

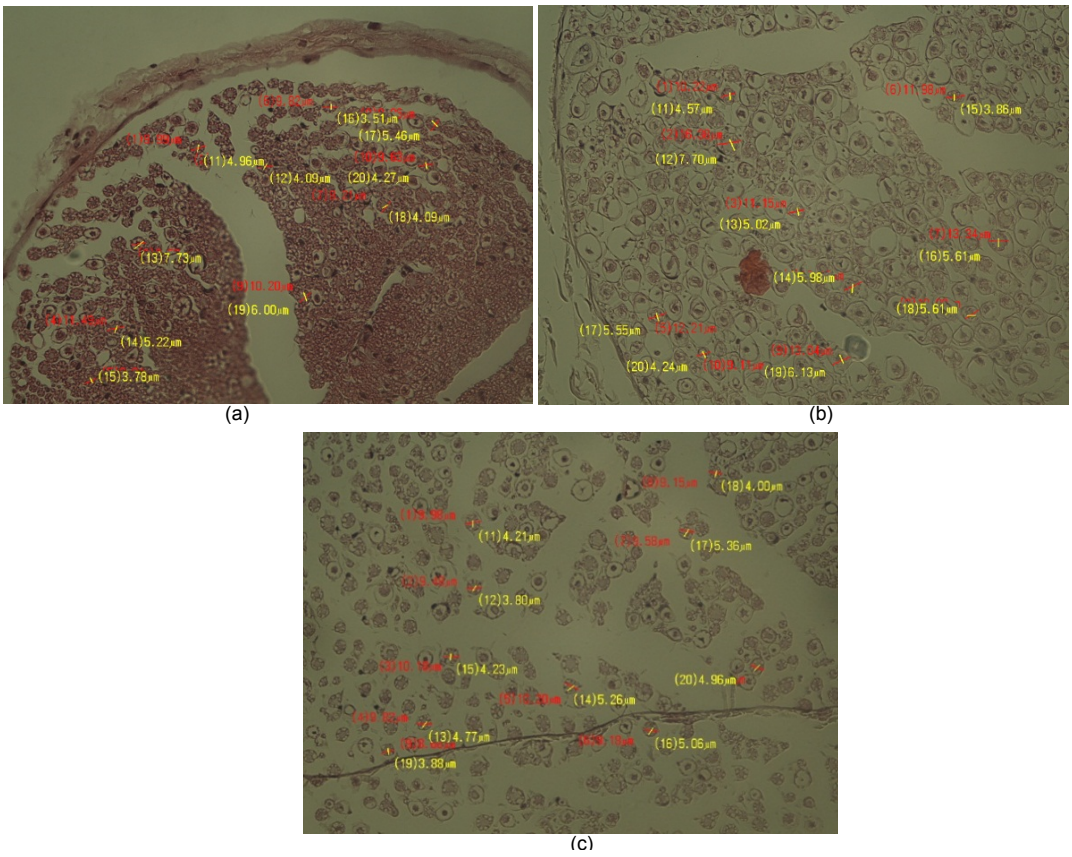

Figure 4: Light micrograph of transverse semi-thin sections of rat sciatic nerves. (A): Control group, myelinated nerve fibers are in normal structure and morphology. (B): Diabetic group, nerves fibers show some abnormalities such as myelin splitting (black arrow), and Edema (E). (C): The cerebrolysin-treated group, the proportion of nerve fibers with abnormalities was reduced. ×1000.

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

1. Belchetz P, Hammond PJ (2003) Mosby's color atlas and text of diabetes and endocrinology: Mosby Edinburgh.
2. Lederman RJ (2012) Bradley's Neurology in Clinical Practice. JAMA 308: 1694-a.
3. Kriz J, Padjen AL (2003) Intra-axonal recording from large sensory myelinated axons: demonstration of impaired membrane conductances in early experimental diabetes. Diabetologia 46: 213-221.

4. Courteix C, Eschalier A, Lavarenne J (1993) Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain. Pain 53: 81-88.

5. Boulton AJ, Vinik AI, Arezzo JC, Bril V, Feldman EL, et al. (2005) Diabetic neuropathies: a statement by the American Diabetes Association. Diabetes Care 28: 956-962.

6. Tanenberg RJ (2009) Diabetic peripheral neuropathy: Painful or painless. Hospital Physician: 1-8.

7. Tanenberg R, Schumer M, Greene D, Pfeifer M (2001) Neuropathic problems of the lower extremities in diabetic patients. Bowker J, Pfeifer M Levin and O'Neal's. The diabetic foot Ed Mosby, St Louis: 33-64.

8. Skalska S, Kyselova Z, Gajdosikova A, Karasu C, Stefek M, et al. (2008) Protective effect of stobadine on NCV in streptozotocin-diabetic rats: augmentation by vitamin E. Gen Physiol Biophys 27: 106-114.

9. Afifi NM (2013) Neuroprotective effect of melatonin in a rat model of streptozotocin-induced diabetic neuropathy: Light and electron microscopic study. Egyptian Journal of Histology 36: 321-335.

10. Zangiabadi N, Asadi-Shekaari M, Sheibani V, Jafari M, Shabani M, et al. (2011) Date fruit extract is a neuroprotective agent in diabetic peripheral neuropathy in streptozotocin-induced diabetic rats: a multimodal analysis. Oxidative medicine and cellular longevity.

11. Zangiabadi N, Ahrari MN, Nakhaee N (2007) The Effect of Omega-3 Fatty Acids on Nerve Conduction Velocity (NCV) and F-wave Latency in Patients with Diabetic polyneuropathy. American Journal of Pharmacology & Toxicology 2: 1.

12. Nicolucci A, Carinci F, Cavaliere D, Scorpiglione N, Belfiglio M, et al. (1996) A meta-analysis of trials on aldose reductase inhibitors in diabetic peripheral neuropathy. The Italian Study Group. The St. Vincent Declaration. Diabet Med 13: 1017-1026.

13. Zangiabadi N, Shafiee K, Alavi KH, Assadi AR, Damavandi M (2012) Atorvastatin treatment improves diabetic polyneuropathy electrophysiological changes in non-insulin dependent diabetic patients: a double blind, randomized clinical trial. Minerva Endocrinol 37: 195-200.

14. Rockenstein E, Torrance M, Mante M, Adame A, Paulino A, et al. (2006) Cerebrolysin decreases amyloid-beta production by regulating amyloid protein precursor maturation in a transgenic model of Alzheimer's disease. J Neurosci Res 83: 1252-1261.

15. Hartbauer M, Hutter-Paier B, Skofitsch G, Windisch M (2001) Antiapoptotic effects of the peptidergic drug cerebrolysin on primary cultures of embryonic chick cortical neurons. J Neural Transm 108: 459-473.

16. Gromova OA, Avdeenko TV, Burtsev EM, Skal'nyÄ AV, Solov'ev OI (1998) [Effects of cerebrolysin on the oxidant homeostasis, the content of microelements and electrolytes in children with minimal brain dysfunction]. Zh Nevrol Psikhiatr Im S S Korsakova 98: 27-30.

17. Liu W, Leng H, Zhu Z, Chen G (2001) [Analysis of the content of ten kinds of metal elements in cerebrolysin by atomic absorption spectrophotometry]. Guang Pu Xue Yu Guang Pu Fen Xi 21: 397-399.

18. Gromova O, Kudrin A, Kataev S, Mazina S (2003) Zhurnal nevrologii i psikhiatrii imeni SS Korsakova/Ministerstvo zdravookhraneniia i meditsinskoi promyshlennosti Rossiiskoi Federatsii, Vserossiiskoe obshchestvo nevrologov [i] Vserossiiskoe obshchestvo psikhiatrov 103: 59.

19. Zhovnir IK, Brozhik NS, Krotiuk LN (1973) [Use of cerebrolysin in patients with cerebral arteriosclerosis]. Vrach Delo 11: 109-111.

20. Gomazkov OA (2002) [4th International Symposium. "Cerebrolysin: pharmacological effects and role in clinical practice"]. Zh Nevrol Psikhiatr Im S S Korsakova 102: 69-70.

21. Litvintsev SV, ShamreÄ VK, Reznik AM, Arbuzov AL (2002) [Perspectives on the treatment of organic mental disorders by the use of nootropic agents]. Voen Med Zh 323: 59-62.

22. Gomazkov OA (2002) [Apoptosis in neuronal structures and the role of neurotrophic growth factors. Biochemical mechanisms of brain derived peptide preparations]. Zh Nevrol Psikhiatr Im S S Korsakova : 17-21.

23. Ukraintseva SV, Arbeev KG, Michalsky AI, Yashin AI (2004) Antiaging treatments have been legally prescribed for approximately thirty years. Ann N Y Acad Sci 1019: 64-69.

24. Chukanova EI (2005) [The effect of cerebrolysin on the clinical symptoms and the course of ischemic encephalopathy]. Zh Nevrol Psikhiatr Im S S Korsakova 105: 42-45.

25. Gothe M (1974) [Therapy with Cerebrolysin, a parenterally administered organ hydrolysate]. Z Allgemeinmed 50: 588-589.

26. Babenkova IV, Teselkin IuO, Makashova NV, Guseva MR (1999) [Antioxidative activity of histochrome and some other drugs used in ophthalmology]. Vestn Oftalmol 115: 22-24.

27. Usuki S, Ito Y, Morikawa K, Kise M, Ariga T, et al. (2007) Effect of pre-germinated brown rice intake on diabetic neuropathy in streptozotocin-induced diabetic rats. Nutr Metab (Lond) 4: 25.

28. Sigaudo-Roussel D, Fromy B, Saumet JL (2007) Diabetic neuropathy in animal models. Drug Discovery Today: Disease Models. 4: 39-44.

29. Sharma HS, Ali SF, Patnaik R, Zimmermann-Meinzingen S, Sharma A, et al. (2011) Cerebrolysin Attenuates Heat Shock Protein (HSP 72 KD) expression in the rat spinal cord following morphine dependence and withdrawal: possible new therapy for pain management. Current neuropharmacology 9: 223.

30. Sharma HS, Muresanu D, Sharma A, Zimmermann-Meinzingen S (2010) Cerebrolysin treatment attenuates heat shock protein overexpression in the brain following heat stress: an experimental study using immunohistochemistry at light and electron microscopy in the rat. Ann N Y Acad Sci 1199: 138-148.

31. Liepinsh E, Vilskersts R, Zvejniece L, Svalbe B, Skapare E, et al. (2009) Protective effects of mildronate in an experimental model of type 2 diabetes in Goto-Kakizaki rats. Br J Pharmacol 157: 1549-1556.

32. Shabani M, Hosseinmardi N, Haghani M, Shaibani V, Janahmadi M (2011) Maternal exposure to the CB1 cannabinoid agonist WIN 55212-2 produces robust changes in motor function and intrinsic electrophysiological properties of cerebellar Purkinje neurons in rat offspring. Neuroscience 172: 139-152.

33. Podrez EA, Febbraio M, Sheibani N, Schmitt D, Silverstein RL, et al. (2000) Macrophage scavenger receptor CD36 is the major receptor for LDL modified by monocyte-generated reactive nitrogen species. J Clin Invest 105: 1095-1108.

34. Calcutt NA, Mizisin AP, Yaksh TL (1993) Impaired induction of vasoactive intestinal polypeptide after sciatic nerve injury in the streptozotocin-diabetic rat. J Neurol Sci 119: 154-161.

35. Xia CQ, Peng R, Qiu Y, Annamalai M, Gordon D, et al. (2007) Transfusion of apoptotic beta-cells induces immune tolerance to beta-cell antigens and prevents type 1 diabetes in NOD mice. Diabetes 56: 2116-2123.

36. Mizisin AP, Shelton GD, Wagner S, Rusbridge C, Powell HC (1998) Myelin splitting, Schwann cell injury and demyelination in feline diabetic neuropathy. Acta Neuropathol 95: 171-174.

37. Kihara M, Schmelzer JD, Kihara Y, Smithson IL, Low PA (1996) Efficacy of limb cooling on the salvage of peripheral nerve from ischemic fiber degeneration. Muscle Nerve 19: 203-209.

38. Gusev EI, Burd GS, Gekht AB, Skvortsova VI, Bogomolova MA, et al. (1994) [The clinico-neurophysiological study of the effect of cerebrolysin on brain function in the acute and early recovery periods of hemispheric ischemic stroke]. Zh Nevrol Psikhiatr Im S S Korsakova 94: 9-13.

39. Thored P, Arvidsson A, Cacci E, Ahlenius H, Kallur T, et al. (2006) Persistent production of neurons from adult brain stem cells during recovery after stroke. Stem Cells 24: 739-747.

40. Muresanu DF, Buzoianu A, Florian SI, von Wild T (2012) Towards a roadmap in brain protection and recovery. J Cell Mol Med 16: 2861-2871.

41. Zhang L, Chopp M, Meier DH, Winter S, Wang L, et al. (2013) Sonic hedgehog signaling pathway mediates cerebrolysin-improved neurological function after stroke. Stroke 44: 1965-1972.

42. Masliah E, Díez-Tejedor E (2012) The pharmacology of neurotrophic treatment with Cerebrolysin: brain protection and repair to counteract pathologies of acute and chronic neurological disorders. Drugs Today (Barc) 48: 3-24.

43. González ME, Francis L, Castellano O (1998) Antioxidant systemic effect of short-term Cerebrolysin administration. J Neural Transm: 333-341.

44. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414: 813-820.

45. Forbes JM, Coughlan MT, Cooper ME (2008) Oxidative stress as a major culprit in kidney disease in diabetes. Diabetes 57: 1446-1454.

46. Watson CP, Moulin D, Watt-Watson J, Gordon A, Eisenhoffer J (2003) Controlled-release oxycodone relieves neuropathic pain: a randomized controlled trial in painful diabetic neuropathy. Pain 105: 71-78.