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

Nasser Zangiabadi1,2, Hossein Mohtashami1*, Mohammad Shabani3 and Mandana Jafari1
1Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Iran
2Afzal 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 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 intraperitoneal. 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, Tahmassehbad crossing, Ehbe-e-sina St. 76189-13519, 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

Copyright: © 2014 Zangiabadi N, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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, pH 4.5) 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 saline intraperitoneally for 2 weeks and fourth group (Experimental): received 5 ml/kg/day cerebrolysin intraperitoneally 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*45 cm 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 investigation, axons of nerves in sciatic transverse section were investigated in regard to edema and axonophase state (de-myelination 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 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 1, 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).

| Animal group | Body weight (g) | Blood glucose (mg dL^-1) |
|--------------|----------------|-------------------------|
|              | 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.
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-
The effect of DFE on histomorphometric parameters of rat sciatic nerve.

and axon diameter reduction among diabetic rats in two weeks (Table
Cerebrolysin administration caused significant improvement in myelin
showed significant decrease in comparison to the control group.
and axon diameters of nerve fibers in the vehicle and diabetic groups
prevent abnormal cases in a wide extent. Data related to the myelin
in the diabetic and vehicle groups. Treatment with cerebrolysin could
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 neurons
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.
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].

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**

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. Krit J, Padjen AL (2003) Intraxonal recording from large sensory myelinated axons: demonstration of impaired membrane conductances in early experimental diabetes. Diabetologia 46: 213-221.
5. Courtex C, Eschalier A, Lavarenne J (1993) Streptozotocin-induced diabetic rats: behavioural evidence for a model of chronic pain. Pain 53: 81-88.

6. 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.

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

8. 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.

9. Skalska S, KyseloVA, Gajdosikova A, Karasu C, Stefanek M, et al. (2008) Protective effect of stobadine on NCV in streptozotocin-diabetic rats: augmentation by vitamin E. Gen Physiol Biophys 27: 105-114.

10. Affili 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.

11. Zangiabadi N, Asadi-Shekaraee 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.

12. Zangiabadi N, Ahradi MN, Nakhtee 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.

13. Nicolucci A, Carinci F, Cavalleri 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.

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

15. Rockenstein E, Torrance M, Mente A, Amade M, 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.

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

17. Gromova OA, Avdeenko TV, Burtsev EM, Skal’nyÄ, Solov’ov 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.

18. Gromova O, Kudrin A, Kataev S, Mazina S (2003) Zhurnal nevrologii i psikhiatrii imenii SS Korsakova[Ministerstvo zdravooukhranienia i meditsinskoi promyshlennosti Rossiskoi Federatsii, Vserossiiskoe obshchestvo nevrologov i psikhiatov obshchestvo nevrologov i psikhiatov]. Vserossiiskoe obshchestvo nevrologov i psikhiatov 103: 59.

19. Zhvokin IK, Brozhik NS, Krotulik LN (1973) [Use of cerebrolysin in patients with cerebral arteriosclerosis]. Vrach Delo 10: 109-111.

20. Gomazkov OA (2002) [2nd International Symposium. ‘Cerebrolysin: pharmacological effects and role in clinical practice’]. Zh Nevrol Psikhiatr Im S S Korsakova 102: 69-70.

21. Litvinsev SV, ShamraVK, 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 102: 17-21.

23. Ukraiintseva SV, Arbeev KG, Michalsky AJ, Yashin AI (2004) Antilaging 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-gminated brown rice intake on diabetic neuropathy in streptozotocin-induced diabetic rats. Nutr Metab (Lond) 4: 25.

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

29. Sharma HS, Ali SF, Patnaik R, Zimmermann-Meiningen 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-Meiningen 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 1195: 138-148.

31. Liepinsh E, Vilkskerst R, Zvejnieces P, Sulaive B, Skapare N, et al. (2009) Protective effects of the peptidergic drug cerebrolysin on primary cultures of embryonic chick cortical neurons. J Neural Transm 108: 459-473.

32. 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 monocoy-generated reactive nitrogen species. J Clin Invest 105: 1085-1108.

33. Calcutt NA, Mızisin 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.

34. Xia QQ, Peng R, Qi 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.

35. Mızisin AP, Shellton GD, Wagner S, Rushbridge C, Powell HC (1998) Myelin splitting, Schwann cell injury and demyelination in feline diabetic neuropathy. Acta Neuropathol 95: 171-174.

36. Khaira M, Schmelzer JD, Khaira 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.

37. 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: 8-13.

38. Thored P, Avdissson 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.

39. 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.

40. 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.

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

42. González ME, Francis L, Castellano O (1998) Antioxidant systemic 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.

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

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

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