Evaluation of the combined therapeutic effect of methylprednisolone and cerebrolysin in traumatic medullar lesion

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Abstract

Objectives: The pharmacological effects of Methylprednisolone and Cerebrolysin have been extensively debated, but from our knowledge there are no studies to evaluate the association of these two drugs in spinal cord injury (SCI).

Methods: Twenty-four Wistar rats underwent traumatic spinal cord injury by using clip-compression model. The animals were divided into four groups: group I received Methylprednisolone (MP); group II was injected with Cerebrolysin (C); group III received Methylprednisolone together with Cerebrolysin (MP+C); in the control group we have performed only decompression. The motor recovery of the animals was evaluated using the Ferguson et al. modification of the BBB scale. After ten days the rats were sacrificed.

Results: The study demonstrated that the MP + C group presented the most notable recovery of the motor function, but no statistically significant ($p>0,05$). The first and the second group also presented better results than the fourth group, but the enhanced recovery of those group relative to control group was not statistically significant ($p>0,05$)

Conclusion: The combination of MP and Cerebrolysin in experimental conditions seems to have promising results, but more experimental and clinical studies are necessary to evaluate the real benefit for SCI patients.

Keywords: Cerebrolysin, Methylprednisolone, motor recovery, spinal cord injury.

Introduction

Spinal cord injury (SCI) is a devastating disease with a high morbi-mortality even in specialized centers. The personal, familial and social implications of an injured patient are hardly quantifiable. There is a lack of evidence regarding this pathology in Romania, but according to literature data the incidence fluctuates between 10.4 and

83.0 per million inhabitants per year, from this one-third of the patients are reported to have tetraplegia and 50% have complete lesions at the mean age of 33.

In the Department of Neurosurgery of the Cluj County Emergency the incidence of SCI operated cases have increased from 45 cases/year in 2000 to 159 in 2009 due to the dramatically growth of the number of traffic accidents, thus increasing the addressability of our department in respect to the nationwide programme for preventing spinal deformity after spine injury.

Contrary to his important impact, the therapeutic armamentarium is very limited and there is no effective treatment to diminish the damage or to promote functional recovery.

If the rationale of early surgery is decompression, in order to theoretically limit the secondary injury by diminishing the local ischemia, and stabilization in order to prevent subsequent lesion by an unstable spine, for secondary injury there are no available clinical proven therapies, in spite of numerous tested drugs in vitro and in vivo conditions.

In theory it is possible that at the time of the surgery to add some factors that could block the secondary injury's cascade or could promote neuronal or axonal recovery at the site of lesion.

The great problem is to find the proper factors and the proper way of releasing at the site of the injury. For that reason we designed an experimental study in order to find some substances and the proper way of administration to improve the neurological evolution of this disabling disease.

Material and method

Twenty-four albino Wistar rats (weight approximately 180-250g) were used during the study. The rats were anesthetized with Ketamine 75-100 mg/kg, Xylazine 10mg/kg, intraperitoneally. A longitudinal incision was made on the midline of the back, and the paravertebral muscles were dissected to expose vertebrae T8 to T10. A three level (T8-T10) laminectomy was performed to expose the spinal cord with the intact dura matter. In the absence of a standard impactor, the spinal cord injury was produced by applying a Yasargil temporary clip 50 grams pressure force for 1 minute, the produced lesions mimicking compression- luxation injury. The layers were closed with 3/0 silk.

Study groups

Immediately after SCI the rats were randomly grouped in 4 study groups of 6 rats each either : Group MP – single dose intrathecal administration of 30mg/kg metilprednisolon 10 minutes after spinal cord injury; Group C- daily intraperitoneal administration of 2,5ml/kg of Cerebrolysine for 10 days; Group MP+C - single dose intratecal administration of 30mg/kg at 10 minutes after spinal cord injury and 2,5ml/kg daily, for ten days, i.p.; Control Group – decompression only (T8-T10 laminectomy has a decompressive role in condition of spinal cord injury). Ten days after the injury the rats were anesthetized (60 mg/kg intraperitoneally) and then decapitated. The spinal cords were rapidly removed and preserved in 10% neutral formaldehyde solution.

Histopathologic examination

Serial sections were taken from 2 mm caudal and rostral to and from the epicenter, tissue sections being stained with hematoxylin-eosin before light microscope examination.

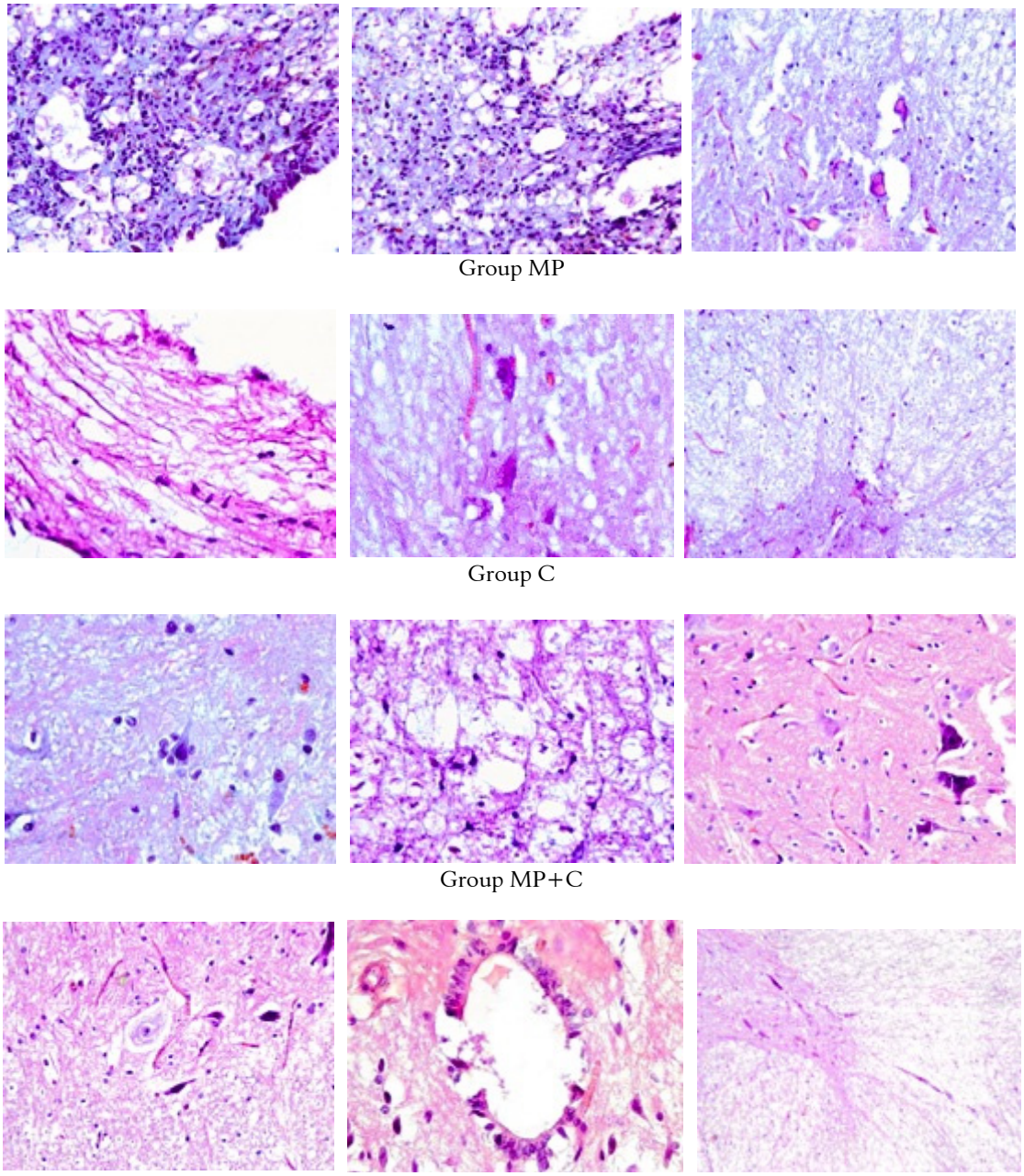


Figure 1 There are no clear differences between the four groups concerning the pathological aspects in H –E staining below 40 x magnifications. In all cases it can be observed a large vacuolization, disorganization of white mater; the nuclei are hipercromatic with nucleollyses. At the level of a dorsal root there are large demielinisations

Neurological evaluation

For the motor evaluation of the Wistar rats we used the BBB Locomotor Rating Scale modified by Ferguson et al.

TABLE 1

Ferguson et al. Modification of the BBB Locomotor Rating Scale

0	No observable hind limb (HL) movement
1	Slight movement of one or two joints, usually the hip and/or knee
2	Extensive movement of one or two joints or Extensive movement of one joint *and* slight movement of one other joint or Slight movement of all three joints
3	Slight movement of two joints *and* extensive movement of the third
4	Extensive movement of two joints *and* slight movement of the third
5	Extensive movement of all three joints of the HL
6	Sweeping with no weight support or Plantar placement of the paw with no weight support
7	Plantar placement of the paw with weight support in stance only (i.e., when stationary) or Occasional, frequent, or consistent weight supported dorsal stepping and no plantar stepping
8	Occasional weight supported plantar steps, no forelimb (FL)-HL coordination
9	Frequent to consistent weight supported plantar steps *and* no FL-HL coordination
10	Frequent to consistent weight supported plantar steps *and* occasional FL-HL coordination
11	Frequent to consistent weight supported plantar steps *and* frequent FL-HL coordination
12	Consistent weight supported plantar steps, consistent FL-HL coordination or Frequent plantar stepping, consistent FL-HL coordination, and occasional dorsal stepping

Definitions:

Slight: partial joint movement through less than half the range of joint motion.

Extensive: movement through more than half of the range of joint motion.

Sweeping: rhythmic movement of HL in which all three joints are extended, then fully flex and extend again; animal is usually side lying, the plantar surface of paw may or may not contact the ground; no weight support across the HL is evident.

No weight support: no contraction of the extensor muscles of the HL during plantar placement of the paw; or no elevation of the hindquarter.

Weight support: contraction of the extensor muscles of the HL during plantar placement of the paw; or elevation of the hindquarter.

Plantar stepping: the paw is in plantar contact with weight support then the HL is advanced forward and plantar contact with weight support is reestablished.

Dorsal stepping: weight is supported thorough the dorsal surface of the paw at some point in the step cycle.

FL-HL coordination: for every FL step an HL step is taken and the HLs alternate.

Occasional: less than or equal to half;

Frequent: more than half but not always: 51–94%

Consistent: nearly always or always; 95–100%

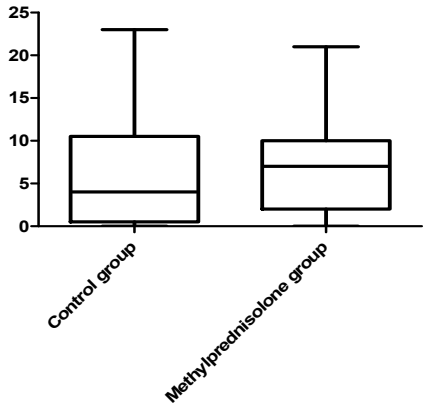
The data were statistically analyzed with T test and One Way ANOVA methods. For the quantitative variables this analysis was done by observing the minimum and maximum values and calculating their means, standard deviations and medians. Absolute and percentage frequencies were also calculated.

Results

There were no statistically significant differences between control group and the

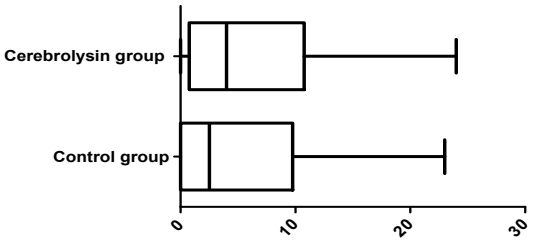
one who received Methylprednisolone (MP) regarding motor recovery. (p=0,89) (Figure 2).

The same lack of significance was observed between control group and the group that received Cerebrolysin (C) (p= 0,79) (Figure 3).



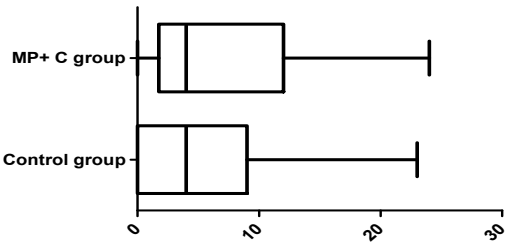
Unpaired t test	
P value	0.8951
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.1339 df=16

Figure 2 Motor recovery in the control group compared to the group that received Methylpredinisolone (MP)



Unpaired t test	
P value	0.7918
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.2679 df=18

Figure 3 Motor recovery in the control group compared to the group that received Cerebrolysin (C)



Unpaired t test	
P value	0.6844
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.4108 df=27

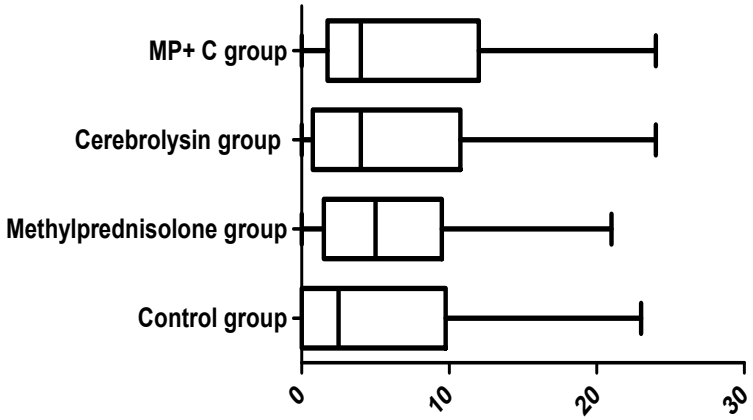
Figure 4 Motor recovery in the control group compared to the group that received Methylprednisolone and Cerebrolysin

Average values of scores obtained by rats in the control group are not significantly higher than that the values obtained by the rats which received the combination of Methylprednisolone (MP) and Cerebrolysin (C) (Figure 4).

One way ANOVA methods showed no statistically significant differences between different groups in terms of motor recovery in rats. (Figure 4). The MP and C group presented functional results that were better than those of the control group but no statistically significant (p> 0,05).

Discussion

Several experimental models have been described for producing a spinal cord injury. Experimental evaluations of the effectiveness of a drug for animals with a spinal cord injury require the production of a standardized injury that has a motor function response as similar as possible among many animals. We choose to use the clip-compression model because it allows a precise monitoring of the force (50g) and duration of compression (1 minute). It is also a more subtle technique.



Bartlett's test for equal variances					
Bartlett's statistic (corrected)	0.3263				
P value	0.9550				
P value summary	ns				
Do the variances differ signif. (P < 0.05)	No				
ANOVA Table	SS	df	MS		
Treatment (between columns)	12.60	3	4.200		
Residual (within columns)	1921	36	53.37		
Total	1934	39			
Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Control group vs Methylprednisolone group	-0.4000	0.1224	No	ns	-9.522 to 8.722
Control group vs Cerebrolysin group	-0.9000	0.2755	No	ns	-10.02 to 8.222
Control group vs MP+ C group	-1.500	0.4591	No	ns	-10.62 to 7.622
Methylprednisolone group vs Cerebrolysin group	-0.5000	0.1530	No	ns	-9.622 to 8.622
Methylprednisolone group vs MP+ C group	-1.100	0.3367	No	ns	-10.22 to 8.022
Cerebrolysin group vs MP+ C group	-0.6000	0.1836	No	ns	-9.722 to 8.522

Figure 5 Comparative analysis of the results obtained from the four groups

The ideal tool for evaluation of motor function should be sensitive, easy to use, and able of showing small changes. Today, the evaluation method that comes closest to those purposes is the BBB Scale. Because the discontinuous nature of the lower portion of the scale presents a problem for both parametric and nonparametric statistical [1] we used a modification of the scale after Ferguson et al.

In our study we sacrificed animals ten days after the spinal cord trauma as the purpose was to analyze the functional recovery during the acute phase. We choose to use Methylprednisolone because it provides clinical benefits and also improvement in neurological function as demonstrated in several studies [4,12].

Cerebrolysin contains a mixture of neurotrophic peptide and it was proven to be a useful treatment for enhancing neurological recovery after stroke [13].

The rationale for our experimental study was to add neuroprotective factors with multiple mechanisms of action on both local and general modes in order to augment the neuroprotective effect

Methylprednisolone is a glucocorticoid with potent anti-inflammatory properties. This group of drugs induced synthesis and release of many anti-inflammatory peptides. Among them, lipocortins inhibit calcium activated phospholipase activity by binding to membrane phospholipid substrates. Methylprednisolone is not only an anti-inflammatory drug but also a potent immunosuppressive. This drug inhibits phospholipase A2 activity, alters neuronal excitability and improves post-traumatic spinal cord blood flow.

Yoon et all showed that Methylprednisolone has a very short therapeutic window and the best results are obtained with a dose of 30 mg/kg applied within the first 30 minutes after contusion [7]. Based on that study, we administrated 30 mg/kg Methylprednisolone 10 minutes after the injury.

High dose of Methylprednisolone causes adverse side effects including pneumonia, wound infections and acute corticosteroid myopathy accompanied by only modest improvements in neurological recovery [8, 17]. There is a need for localized delivery of MP to the lesion site to minimize the systemic delivery related side effects. To achieve this local delivery, we injected the drug into the spinal cord, at the site of injury.

The glucocorticoid steroids were widely used in the clinical treatment of spinal cord trauma by the middle of the 60's and throughout the 1970. The rationale mechanism for their use was centered on the expectation that they would reduce the spinal cord edema. This was based upon the remarkable reduction of peritumoral brain edema

The first National Acute Spinal Cord Study (NASCIS 1) that began in 1979 wanted to compare high and low dose of Methylprednisolone in spinal cord injury. The trial did not involve a placebo group. The study was published in 1985 and found no significant difference between high and low dose of Methylprednisolone started within 48 hours after spinal cord injury. There also was a suggestion that the 10-days high-dose regimen increased the risk of infections. The study raised question at that time about the efficiency of glucocorticoids and brought to the forefront the teory of free radicals.

In 1990 the second National Spinal Cord Injury Study (NASCIS 2) showed a significant improvement in motor and sensory recover in patients treated with high dose of Methylprednisolone within 8 hours after spinal cord injury. However, when started more than 8 hours after the injury the drug was proven not only ineffective but deleterious. This study suggests that a therapeutic time window exists in spinal cord injury.

Weaver et all demonstrated that the non-selective and enduring effects of immunosuppressive therapy with Methylprednisolone not only fail to improve neurological outcomes in rats with spinal cord injury, but also can block the beneficial actions of selective therapies (anti-CD11d mAb) [25].

Cerebrolysin is a neurotrophic peptidergic mixture with antioxidant

properties. The drug has been studied since the early 1970's. In stroke and neurodegenerative diseases, double-blind placebo controlled trials have reported sustained improvements and slowing down of progressive memory loss, cognition impairment, mood changes, and motor and sensory symptoms

Several studies have suggested that Cerebrolysin has neurotrophic and neuroprotective effect in vitro and in vivo. In animals, following cerebral ischemia, Cerebrolysin has been shown to ameliorate the effect of oxidative cell stress [9]. The inhibitory effect of the drug on calpain has been demonstrated on a molecular level [26]. Cerebrolysin reduces apoptosis triggered by growth factor withdraw and induces neuritis outgrowth in cultivated neurons [20].

Tatebayashi et all demonstrated that Cerebrolysin enhanced neurogenesis in the dentate gyrus of adult animals, which correlates with improved spatial memory performance in these animals [24]. After bilateral artery occlusion in rats, Cerebrolysin reduces mortality of the animals with about 50 %, and also reduces the infarct size as well as the loss of MAP-2 immunoreactivity in a middle cerebral artery occlusion model [22, 23]. In humans the drug was safely used for the treatment of several conditions [3, 16, 18].

Haninec, et al. reported that insulin-like growth factor I (IGF-I) and Cerebrolysin enhances survival of motoneurons after ventral root avulsion [18]. The drugs were effective when given intrathecally to the spinal cord. In 2005, Bul'on, et al published a study that compared the effects of cytoflavin and Cerebrolysin in rats after spinal cord compression injury [5]. The neuroprotective effects of cytoflavin were greater than of Cerebrolysin's.

In 1999, Lombardi, et al [14] demonstrated that applying Cerebrolysin to astrocytes and microglia cultures of rats, prevented microglial activation after LPS activation and reduced interleukin-1b expression. Mallory, et al. [15] reported that when the peptide mixture was applied to the human teratocarcinoma cell line (NT2) Cerebrolysin markedly increased expression of synaptic-associated proteins, suggesting that it has synaptotrophic effects mediated through regulation of APP expression. Alvarez, et al. showed that this drug reduced microglial activation both in vitro and in vivo [2].

In 2002 Guttmann et al. demonstrated that Cerebrolysin protects cortical neurons cultures of the chickens from cell death caused by a wide variety of factors, including glutamate, iodoacetate, and ionomycin; they proposed that Cerebrolysin stabilizes calcium ionic homeostasis [10]. Safarova, et al. [19] showed that Cerebrolysin improved survival of PC12 cells in serum-free medium. They obtained a decrease of apoptosis from 32% to 10%. In 2005, Schauer, et al. found that a single addition of Cerebrolysin to culture medium resulted in significant protection of tissue cultures against ischemia and hypoxia for up to 2 weeks. The treatment has the same beneficial effects even if it is delayed for as long as 96 hours [21].

Our study has some weakness that could affect the results. Firstly the traumatic model is not largely accepted, the produced lesions being possibly too severe, and as a consequence, irreversible. Secondly the local administration of Methyprednisolone could be harmful per se, adding possible new lesions, in the absence of a special

device for microinjection. More than that it is uncertain if the injectable form we used is compatible with a local administration. On the other hand it is known that Cerebrolysin is a dose dependent drug.

In spite of these criticisms, from our knowledge this is the first experimental study of a combination between MP and Cerebrolysin in SCI. Their mechanism of action is not completely understood, but there are experimental data demonstrating an apoptotic inhibition on the line of calpains. At least for MP there are experimental data proving the favourable action on topic application [6]

More experimental data are necessary in order to demonstrate the synergic action of MP and Cerebrolysin, the optimal interval for drug administration and the optimal dose of the drugs taking into account that at a dose of 30mg/kg MP have many deleterious effects. Cerebrolysin is a dose dependent drug and until now there are no studies regarding the local application of this drug.

Finally, taking in consideration that in control group, the simple laminectomy group, the results were no significantly different that in those medically treated, the role of urgent decompressive laminectomy cannot be underestimated.

Conclusion

We found that Methylprednisolone and Cerebrolysin didn't significantly enhanced neurological recovery in rats with severe clip-compression models of spinal cord injury, administrated alone or in combination from a statistic point of view.

Methylprednisolone is the first drug shown to improve recovery in human spinal cord injury and remains the only form of treatment shown in a phase 3 trial to have efficiency in managing this injury. Therefore, it remains the standard therapy to which all further treatments should be compared.

Some clinical evidence suggest that Cerebrolysin may be beneficial for many neurological conditions, including extrapyramidal hyperkinesis associated to narcoleptic therapy, acute and chronic stroke, brain trauma, organic mental disorders, ischemic encephalopathy, diabetic neuropathy, Rett syndrome, vascular dementia, multiple sclerosis, anti-aging and other neurodegenerative disorders. Little data is available concerning the effect of Cerebrolysin on spinal cord injury. More studies are needed to ascertain the benefits of the drug for both acute and chronic spinal cord injuries.

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References

1. Adam Ferguson, Michelle A. Hook, Guadalupe Garcia, Jacqueline C. Bresnahan, Michael S. Beattie, James W Grau, A Simple Post Hoc Transformation that Improves the Metric Properties of the BBB Scale for Rats with Moderate to Severe Spinal Cord Injury, Journal of Neurotrauma, Vol 21, No 11, 2004, 1601-1613

2. Alvarez XA, Lombardi VR, Fernandez-Novoa L, Garcia M, Sampedro C, Cagiao A, Cacabelos R and Windisch M (2000). Cerebrolysin reduces microglial activation in vivo and in vitro: a potential mechanism of neuroprotection. J Neural Transm Suppl. 59:281-92.
3. Bae, C. -Y., Cho, C. -Y., Cho, K., Oh, B. H., Choi, G. K., Lee, H. S., et al., (2000). A double-blind, placebo-controlled, multicenter study of Cerebrolysin for Alzheimer's disease, J Am Ger Soc 48, 1566-1571.
4. Bracken MB. Pharmacological interventions for acute spinal cord injury. Cochrane Database Syst Rev 2: CD001046, 2000.
5. Bul'on VV, Kuznetsova NN, Selina EN, Kovalenko AL, Alekseeva LE and Sapronov NS (2005). Neuroprotective effect of cytoflavin during compression injury of the spinal cord. Bull Exp Biol Med. 139:394- 6.
6. Chen-Guang Yu, Aashish Joshi, James W. Geddes : Intraspinal Mdl28170 Microinjection Improves Functional and Pathological Outcome Following Spinal Cord Injury ;Journal of Neurotrauma 25:833–840 (July 2008)
7. Do Heum Yoon, Young Soo Kim, Wise Young, Yonsei Therapeutic time window for Methylprednisolone in spinal cord injured rats, Medical Journal, Vol 40, No 4, pp 313-6.
8. Gerndt SJ, Rodriguez JL, Pawlik JW, Taheri PA, Wahl WL, Micheals AJ, et al. Consequences of high-dose steroid therapy for acute spinal cord injury. J Trauma. 1997 Feb;42(2):279–284.
9. Gonzalez, M. E., Francis, L. & Castellano, O. (1998). Antioxidant systemic effect of short-term Cerebrolysin administration, J Neural Transm 53, 333-341.
10.Gutmann B, Hutter-Paier B, Skofitsch G, Windisch M and Gmeinbauer R (2002). In vitro models of brain ischemia: the peptidergic drug cerebrolysin protects cultured chick cortical neurons from cell death. Neurotox Res. 4:59-65.
11.Haninec P, Houst'ava L, Stejskal L and Dubovy P (2003). Rescue of rat spinal motoneurons from avulsion-induced cell death by intrathecal administration of IGF-I and Cerebrolysin. Ann Anat. 185:233-8.
12.Hurlbert RJ. The role steroids in acute spinal cord injury: an evidence-based analysis. Spine. 2001;26:39-46.
13.JingMei Rena, Dana Sietsmaa, Shumei Qiua, Herbert Moesslerb and Seth P. Finklestein Cerebrolysin enhances functional recovery following focal cerebral infarction in rats; Restorative Neurology and Neuroscience 25 (2007) 25–31 25.
14.Lombardi VR, Windisch M, Garcia M and Cacabelos R (1999). Effects of Cerebrolysin on in vitro primary microglial and astrocyte rat cell cultures. Methods Find Exp Clin Pharmacol. 21:331-8.
15.Mallory M, Honer W, Hsu L, Johnson R, Rockenstein E and Masliah E (1999). In vitro synaptotrophic effects of Cerebrolysin in NT2N cells. Acta Neuropathol (Berl). 97:437-46
16.Panisset, M., Gauthier, S., Moessler, H., Windisch, M. & Group, C. S. (2002). Cerebrolysin in Alzheimer's Disease: A randomized, double-blind, placebo-controlled trial with a neurotrophic agent, J Neural Transm 109, 1089-1104.
17.Qian T, Guo X, Levi AD, Vanni S, Shebert RT, Sipski ML. High-dose methylprednisolone may cause myopathy in acute spinal cord injury patients. Spinal Cord. 2005 Apr;43(4):199–203. 320, 1999.
18.Ruether, E., Husmann, R., Kinzler, E., Diabl, E., Lingler, E., Spatt, J., et al. (2001). A 28 week, double-blind, placebo-controlled study with Cerebrolysin in patients with mild to moderate Alzheimer's disease, Int Clin Pschopharm 16, 253-263.
19.Safarova ER, Shram SI, Grivennikov IA and Myasoedov NF (2002). Trophic effects of nootropic peptide preparations cerebrolysin and semax on cultured rat pheochromocytoma. Bull Exp Biol Med. 133:401-3.
20.Satou, T., Itoh, T., Tamai, Y., Ohde, H., Anderson, A. J.&Hashimoto, S. (2000). Neurotrophic effects of FPF-1070 (Cerebrolysin) on cultured neurons from chicken embryo dorsal root ganglia, ciliary ganglia, and sympathetic trunks, J. Neural Transm. 107, 1253-1262.
21.Schauer E, Wronski R, Patockova J, Moessler H, Doppler E, Hutter- Paier B and Windisch M (2005). Neuroprotection of Cerebrolysin in tissue culture models of brain ischemia: post lesion application indicates a wide therapeutic window. J Neural Transm
22.Schwab, M., Antonow-Schlorke, I., Zwiener, U. & Bauer, R. (1998). Brain derived peptides reduce the size of cerebral infarction and loss of MAP-2 immunoreactivity after focal ischaemia in rats, J Neural Transm 53, 299-311.
23.Schwab, M., Schaller, R., Bauer, R. & Zweiner, U. (1997). Morphofunctional effects of moderate forebrain ischaemia combined with short-term hypoxia in rats – protective effects of Cerebrolysin, Exp Toxic Pathol 49, 29-37.
24.Tatebayashi, Y., Lee, M. H., Li, L., Iqbal, K. & Grundke-Iqbal, I. (2003). The dentate gyrus neurogenesis. A therapeutic target for Alzheimer's disease, Acta Neuropathol 2003, 225-232.
25.Weaver LC, Gris D, Saville LR, Oatway MA, Chen Y, Marsh DR, Hamilton EF, Dekaban GA, Methylprednisolone causes minimal improvement after spinal spinal cord injury in rats, contrasting with benefits on an anti-integrin treatment, J Neurotrauma, 2005, Dec; 22 (12) 1375-87
26.Wronski, R., Tompa, B., Hutter-Paier, B., Crailsheim, K., Friedrich, B. & Windisch, M. (2000). Inhibitory effect of a brain derived peptide preparation on the intracellular calcium Ca++- dependent protease, calpain, J Neural Transm 107, 145-157.