

Effect of cerebrolysin on dopaminergic neurodegeneration of rat with oxidative stress induced by 3-nitropropionic acid

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The study tested the hypothesis that cerebrolysin protects the brain from free radicals in rats treated with 3-nitropropionic acid (3-NPA). To address this hypothesis, the levels of dopamine (DA) and some oxidative stress biomarkers were measured after administration of 3-NPA. Young male Fischer rats were treated for three days with cerebrolysin, 3-NPA or both substances. Their brains were extracted, and DA, lipid peroxidation (LP), glutathione (GSH), calcium, and H₂O₂ were measured using validated methods. In the cortex, hemispheres and cerebellum/medulla oblongata of the group treated with cerebrolysin and 3-NPA, the levels of DA and LP decreased. In addition, calcium and H₂O₂ levels decreased in the hemispheres of the same group, while GSH increased in cortex. The increased dopamine metabolism due to the administration of cerebrolysin led to increased formation of radical species and oxidative stress, especially when free radicals were generated by 3-NPA.

Keywords: cerebrolysin, dopamine, 3-nitropropionic acid, oxidative stress

Studies of neurological disorders suggest that excitotoxicity induces a huge increase in intracellular Ca²⁺ concentrations and an increase in the production of free radicals *via* lethal pathways (1). This damage is the primary event in 3-nitropropionic acid (3-NPA) toxicity, which induces neurodegeneration in female Wistar rats, and its systemic administration to rats is used as an important model of Huntington's disease (2). Cerebrolysin is a drug that consists of small brain-derived peptides, which are similar or identical to those produced endogenously. Its content of 25 % of low-molecule peptides allows it to partially cross the blood-brain barrier (3). Cerebrolysin improves synaptic plasticity and behavioral performance and has been successfully studied in animal models, in which it was proved to substantially decrease oxidative stress (4), which particularly affects the central nervous system. However, its mechanism of action is not completely clear. The above findings suggest that cerebrolysin may have therapeutic efficacy. As free radicals are known to damage

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cell components, mainly plasma membrane lipids (5), the central nervous system is particularly susceptible to their effects.

Based on the above, the purpose of the present study is to compare the protective effect of cerebrolysin alone with that of cerebrolysin combined with 3-nitropropionic acid on the levels of dopamine (DA) and of selected oxidative stress biomarkers in important brain regions in young rats.

EXPERIMENTAL

Animal management and care were conducted in accordance with the international guidelines on animal care and Mexican Guidelines ZOO-062. Approval of the institutional ethical committee was obtained.

Twenty-four young male Fischer rats (80 g, 45 days old, were deployed for the study and were procured from the certified Bioterium of the Metropolitan University of Mexico City, Mexico, with a balanced food (Rodent diet 5001) and drinking water *ad libitum*. The animals were divided into 4 groups ($n = 6$) and were treated as follows: control group (NaCl 0.9 %), cerebrolysin group (2.5 g kg⁻¹ b.m.), cerebrolysin (2.5 g kg⁻¹ b.m.) + 3-nitropropionic acid group (20 mg kg⁻¹ b.m.) and 3-nitropropionic acid group (20 mg kg⁻¹ b.m.). All substances were administered intraperitoneally every 24 hours for 3 consecutive days. Thirty minutes after the final drug administration, the animals were sacrificed using a guillotine and their brains were immersed in saline solution (NaCl 0.9 %) at 4 °C.

Blood was obtained immediately after sacrifice and evaluated for glucose and hemoglobin levels. Brain region dissections were carried out for the cortex, hemispheres, and cerebellum combined with the medulla oblongata. Dissected brain regions were stored in 5 volumes of 0.05 mol L⁻¹ Tris-HCl, pH 7.4, and evaluated for ATPase, lipid peroxidation and H₂O₂ levels. An aliquot from each region was homogenized in 0.1 mol L⁻¹ perchloric acid (50:50, V/V) to evaluate the concentrations of reduced glutathione (GSH), dopamine, and calcium.

Measurement of blood glucose was performed in all groups of animals at the moment of sacrifice in non-anticoagulant blood using Accu-Chek Active (Roche Mannheim, Germany) glucose meters (6).

The DA levels were quantified in the supernatant of tissue homogenized in HClO₄ after centrifugation at 9,000 rpm for 10 min in a microcentrifuge (Mikro 12-42, Hettich Zentrifugen, Germany) using the method reported by Calderon *et al.* (7).

GSH levels were measured in the perchloric acid supernatant of the homogenized tissue, obtained after centrifuging at 9,000 rpm for 5 min using a method modified after Hisin and Hilf (8).

TBARS determination was performed using modified method of Gutteridge and Halliwell (5). To measure calcium, the supernatant from brain homogenates of all animal groups was evaluated using the Ca-Color Arsenazo III AA direct colorimetric method produced by Wiener Laboratory (Rosario, Argentina).

The ATPase activity was assayed according to the method proposed by Calderón-Guzmán *et al.* (9). Supernatant absorbance was quantified at 660 nm (Helios-α UNICAM spectrophotometer, UK).

Determination of H₂O₂ was performed using a modified method from Asru (10).

Statistics

Kruskal-Wallis and ANOVA tests were used with their corresponding contrasts and previous variance homogeneity comparison. Statistical significance was defined at $p < 0.05$. JMP Statistical Discovery software of the SAS version 8.0.0 was used.

RESULTS AND DISCUSSION

Hemoglobin level showed a non-significant decrease in animal groups treated with cerebrolysin plus 3-NPA or with 3-NPA alone but these groups showed a statistically significant increase in the glucose level ($p < 0.05$).

Dopamine levels in brain regions of young rats treated with cerebrolysin and 3-nitropropionic acid decreased significantly ($p < 0.03$) in the cortex (Table II), hemispheres (Table III) and cerebellum/medulla oblongata (Table IV) compared to the control or cerebrolysin group.

In animals treated with cerebrolysin alone or cerebrolysin plus 3-NPA, lipid peroxidation in the cortex decreased significantly ($p < 0.02$, Table II) compared to the control. In animals treated with cerebrolysin and 3-NPA, calcium and H_2O_2 levels decreased significantly ($p < 0.001$) in hemisphere regions (Table III) compared with to the control group.

The GSH concentration increased in cortex regions in animals treated with cerebrolysin plus 3-NPA because of the production of reactive oxygen species is the primary event in 3-NPA toxicity (11). ATPase activity, which is dependent on calcium and magnesium, increased in the cortex and hemisphere regions of the animals that received cerebrolysin alone or in combination with 3-NPA, whereas it decreased in animals treated with 3-NPA alone. Increased Ca^{2+} , Mg^{2+} – ATPase activity protects against substances that induce brain injury by inhibiting free radical production, regulating calcium-dependent processes, and supporting the antioxidant redox system.

DA signaling and distribution are primarily modulated by the DA transporter (DAT) and the vesicular monoamine transporter (VMAT)-2, which transport DA into presynaptic terminals and synaptic vesicles. These transporters are regulated by complex processes

Table I. Glucose and hemoglobin in the blood of young rats treated with cerebrolysin and 3-nitropropionic acid

Group	Glucose (mg per 100 mL)	Hemoglobin (g per 100 mL)
Control (NaCl 0.9 %)	126.3 ± 2.2 ^a	22.2 ± 4.7
Cerebrolysin	124.0 ± 4.7 ^b	20.6 ± 3.6
Cerebrolysin + 3-NPA	149.3 ± 7.8 ^{a,b}	17.2 ± 4.2
3-NPA	146.00 ± 3.0 ^{a,b}	26.14 ± 3.1

Applied doses: cerebrolysin (2.5 g kg⁻¹), 3-nitropropionic acid (3-NPA) (20 mg kg⁻¹).

Mean value ± SD, $n = 6$.

^{a,b} Statistically significant differences at $p < 0.05$.

Table II. Dopamine levels and biomarkers of oxidative stress in the cortex regions of young rats treated with cerebrolysin and 3-NPA

Group	DA ($\mu\text{mol g}^{-1}$) ^a	TBARS (μmol malondial- dehyde g^{-1}) ^a	Calcium (mg per 100 mL)	H ₂ O ₂ ($\mu\text{mol g}^{-1}$) ^a	GSH (nmol g^{-1}) ^a	ATPase ($\mu\text{mol Pi}$ $\text{g}^{-1} \text{min}^{-1}$) ^a
Control	2.20 ± 0.93 ^b	9.58 ± 1.6 ^b	3.98 ± 0.13	0.12 ± 0.03	362.3 ± 98 ^b	161.7 ± 54.0 ^b
Cerebrolysin	3.36 ± 0.38 ^{b,c}	7.84 ± 1.0 ^b	4.09 ± 0.41	0.14 ± 0.02	332.9 ± 128 ^c	235.2 ± 75.5 ^{b,c}
Cerebrolysin + 3-NPA	2.43 ± 0.51 ^c	7.99 ± 1.2 ^b	4.25 ± 0.30	0.13 ± 0.03	441.05 ± 74 ^{b,c,d}	244.1 ± 35.9 ^{b,d}
3-NPA	2.65 ± 0.47 ^{b,c}	8.05 ± 0.7 ^b	4.30 ± 1.42	0.08 ± 0.02	214.47 ± 74 ^{b,c,d}	95.9 ± 53.7 ^{b,c,d}

DA – dopamine, TBARS – thiobarbituric acid reactive substances, GSH – glutathione, 3-NPA – 3-nitropropionic acid
Applied doses: cerebrolysin (2.5 g kg⁻¹), 3-nitropropionic acid (3-NPA) (20 mg kg⁻¹).

Mean value ± SD, *n* = 6.

^a Wet mass (tissue) basis.

^{b,c,d} Statistically significant differences at *p* < 0.05 within a parameter.

such as phosphorylation and protein-protein interactions. Thus, the peptide-containing drug cerebrolysin may act in this complex process (12) offering a therapeutic treatment of DA-related disorders.

Since dopamine levels increased in the cortex and hemisphere regions of animals that received cerebrolysin alone, it may be assumed that cerebrolysin is a novel dopamine agonist. The decrease of lipid peroxidation in the cortex regions of animals that received cere-

Table III. Dopamine levels and biomarkers of oxidative stress in hemispheres regions of young rats treated with cerebrolysin and 3-nitropropionic acid

Group	DA ($\mu\text{mol g}^{-1}$) ^a	TBARS (μmol malondial- dehyde g^{-1}) ^a	Calcium (mg per 100 mL)	H ₂ O ₂ ($\mu\text{mol g}^{-1}$) ^a	GSH (nmol g^{-1}) ^a	ATPase ($\mu\text{mol Pi}$ $\text{g}^{-1} \text{min}^{-1}$) ^a
Control	3.34 ± 0.85	11.8 ± 0.9	4.13 ± 0.14 ^b	0.20 ± 0.02 ^b	419.8 ± 124	253.5 ± 104 ^b
Cerebrolysin	4.27 ± 1.08 ^b	13.05 ± 1.9	4.06 ± 0.16 ^c	0.19 ± 0.02 ^c	369.0 ± 126	603.8 ± 193 ^{b,c}
Cerebrolysin + 3-NPA	3.29 ± 0.86 ^b	12.04 ± 2.0	3.79 ± 0.07 ^{b,c,d}	0.17 ± 0.02 ^{b,c,d}	346.0 ± 57	379.4 ± 80 ^{b,c,d}
3-NPA	3.88 ± 0.88	11.95 ± 2.2	4.75 ± 1.0 ^{b,c,d}	0.15 ± 0.10 ^{b,c,d}	296.9 ± 80	166.8 ± 46 ^{b,c,d}

For symbols see Table II.

Applied doses: cerebrolysin (2.5 g kg⁻¹), 3-nitropropionic acid (20 mg kg⁻¹). Mean value ± SD, *n* = 6.

^a Wet mass (tissue) basis.

^{b,c,d} Statistically significant differences at *p* < 0.05 within a parameter.

Table IV. Dopamine levels and biomarkers of oxidative stress in cerebellum/medulla oblongata regions of young rats treated with cerebrolysin and 3-nitropropionic acid

Group	DA ($\mu\text{mol g}^{-1}$) ^a	TBARS (μmol malondial- dehyde g^{-1}) ^a	Calcium (mg per 100 mL)	H ₂ O ₂ ($\mu\text{mol g}^{-1}$) ^a	GSH (nmol g^{-1}) ^a	ATPase ($\mu\text{mol Pi}$ $\text{g}^{-1} \text{min}^{-1}$) ^a
Control	3.04 ± 0.46 ^b	15.5 ± 2.7	4.26 ± 0.10	0.13 ± 0.01	221.0 ± 73	381.2 ± 111
Cerebrolysin	2.78 ± 0.41 ^c	16.68 ± 2.5	4.29 ± 0.12	0.14 ± 0.02	198.0 ± 76	458.3 ± 192
Cerebrolysin + 3-NPA	2.31 ± 0.55 ^{b,c}	14.23 ± 2.3	4.22 ± 0.09	0.13 ± 0.02	191.0 ± 55	416.3 ± 142
3-NPA	3.40 ± 1.87 ^c	13.64 ± 2.1	4.30 ± 0.98	0.12 ± 0.01	197.0 ± 66	401.7 ± 48.3

For symbols see Table II.

Applied doses: cerebrolysin (2.5 g kg^{-1}), 3-nitropropionic acid (20 mg kg^{-1}).

Mean value ± SD, $n = 6$.

^a Wet mass (tissue) basis.

^{b,c} Statistically significant differences at $p < 0.05$ within DA values.

brolysin coincides with the findings of Patocková *et al.* (13). Besides, the increase of dopamine in the same groups suggests that DA may play a significant role in regulating the response to oxidative stress (14).

CONCLUSIONS

The increased dopamine metabolism due to the administration of cerebrolysin led to increased formation of radical species and oxidative stress, especially when free radicals were generated by 3-NPA. However, we recommend further studies to be conducted to investigate the possible beneficial effect of cerebrolysin on dopamine levels in other animal models.

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