The neuroprotective and anti-amnestic effects of derivatives of erythropoietin in experimental ischemic injury of the cerebral cortex

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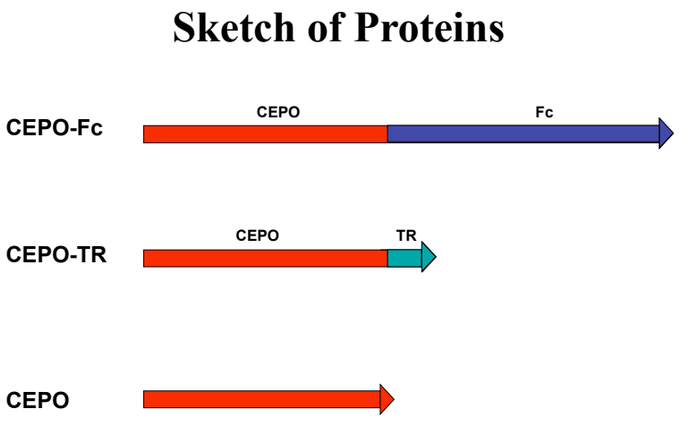
Introduction. Stroke is one of the leading reasons of death rate and proof disability in the developed countries. Stroke development leads to morphological defect of brain structures and functional disturbances. Search of the medical products reducing degree neurodegeneration and improving mnestic functions, broken at a stroke, is an actual medical and socially-significant problem. A photochemical thrombosis of cortex vessels of rat brain - the noninvasive method, allowing to choose the necessary localization of the ischemic infarction. Our previous studies [2] demonstrated that photothrombosis of the cerebral cortex in rats induced formation of local ischemic focus affecting the whole thikness of the cortex, separated from the surrounding undamaged tissue by a clear boundary. We offer model of selective local photochemical ischemic damage of the prefrontal rat brain cortex, using which it is possible to estimate cognitive deficiency and to define morfological volume of damage of a brain.

Additional oligosaccharides, linked to the erythropoietin, prolong its half-life and increase bioactivity in vivo. For this purpose we, for the first time, used TR domain from glycoprotein MUC1, bearing 5 additional sites for O-glycosylation. Our result indicated that ligation of TR-domain to the coding sequence of CEPO did not affect secretion of the chimeric protein into the medium, receptor binding affinity in vitro bioactivity, compared with CEPO wild type. However, both the in vitro potency and half-life in circulation of CEPO bearing TR or Fc fragments were significantly enhanced [3]. By working out neuroprotective preparations the special attention is given to derivatives of carbamylated erythropoietin (CEPO) and CEPO fusion protein containing TR domain from glycoprotein MUC1 (CEPO-TR).

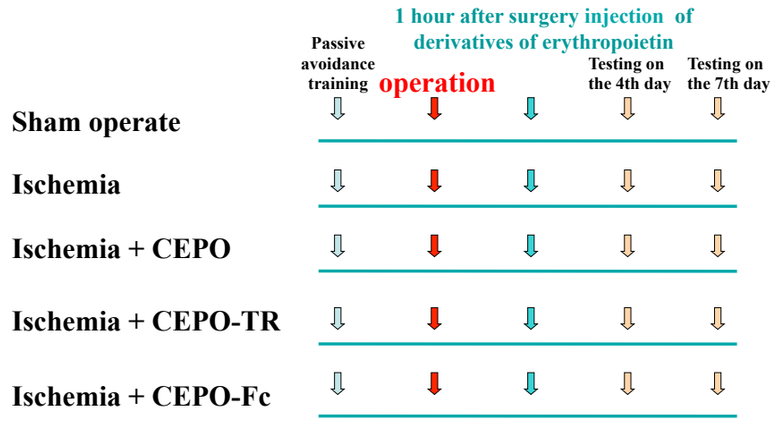
Purpose: ***To compare the neuroprotection of carbamylated erythropoietin (CEPO) and CEPO fusion protein containing TR domain from glycoprotein MUC1 (CEPO-TR), and CEPO fusion protein with modified Fc fragments of IgG1(CEPO-Fc) against ischemic brain injury, including behavioral disturbances, in a bilateral focal ischemic infarction of the rat prefrontal cortex.***

Methods: The carbamylated erythropoietin and erythropoietin derivatives were produced by treatment of purified proteins with potassium cyanate in borate buffer. The resulting carbamylated erythropoietin and derivatives exhibit no erythropoietic activity in UT-7/ CEPOR cell viability assay. Bilateral focal ischemic infarction of the prefrontal cortex (areas Frl and Fr2) was induced by the method [1] of photochemical thrombosis in rats. After passive avoidance training and testing rats were treated, respectively, with following regimens: saline, 50 μg/kg; CEPO, 50 μg/kg; CEPO-TR, 50 μg/kg; CEPO-Fc 50 μg/kg. The substance was injected intraperitoneally in 1h after operation. Neurological deficit scores and infarct volume were assessed at 4 and 7 days after operation. Functional state of CNS was determined by latency passive avoidance response (LPA). Morphometric measurements of the areas and volumes of the ischemic focus on serial slices were carried out on animal brain fixed by plunging into formalin–ethanol–acetic acid mixture (2:7:1). The data were statistically processed using Statistica 6.0 software.



The following sketch shows a schematic view of proteins used for injections

DESIGN OF EXPERIMENT
the effect of derivatives of erythropoietin on passive avoidance in rats with ischemic damage of brain prefrontal cortex



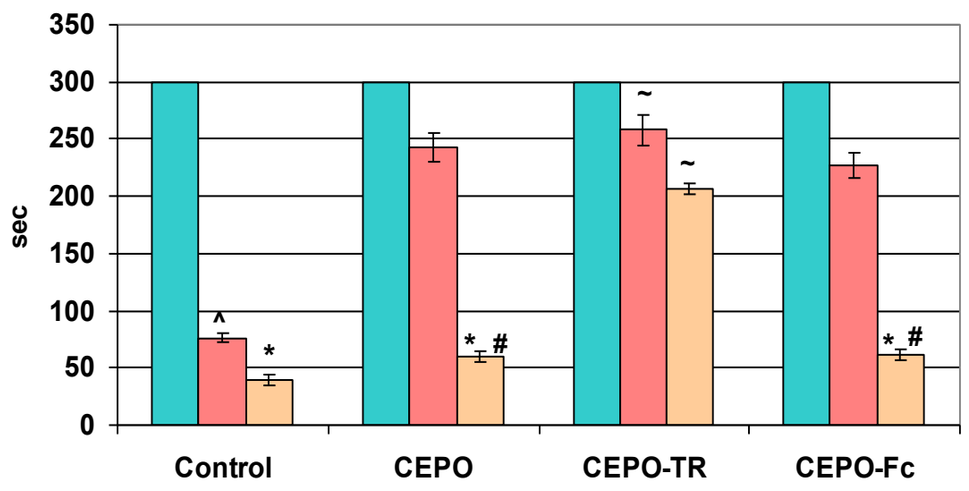


Figure 1: Effect of intraperitoneal administration CEPO, CEPO-TR, CEPO-Fc restoration PA rats after photothrombosis bilateral prefrontal cortex

Blue columns - the latent period before the operation, the red columns - the latent period on the 4th day after operation, the yellow columns - the latent period on the 7th day after operation. Control – photothrombosis + saline.

* P <0,001 compared with LPA (latency period PA) before photothrombosis

# P <0,05 compared with the LPA on the 4th day after operation

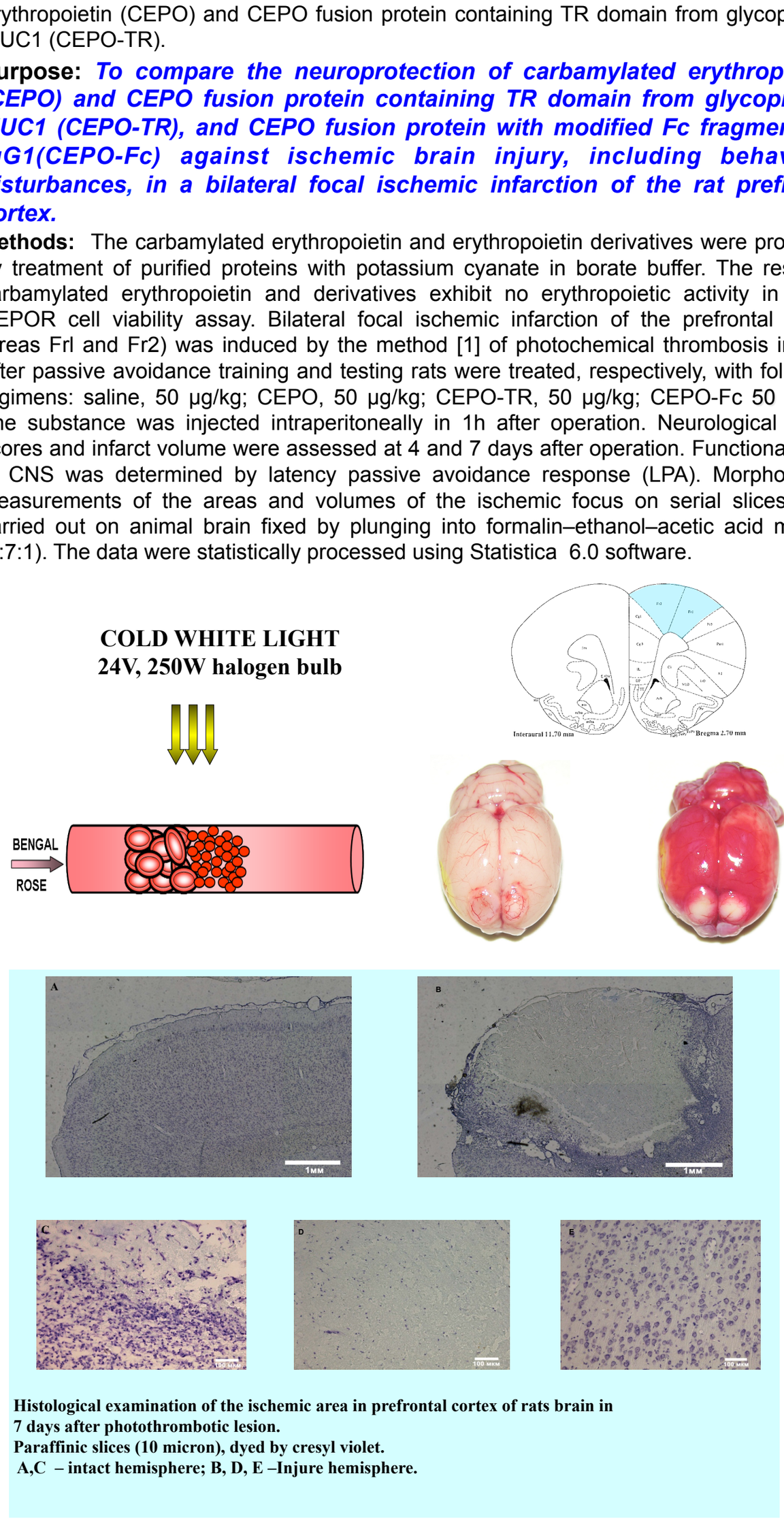
^ P <0,05 compared with LPA before photothrombosis

~ P<0,05 group CEPO-TR as compared with group Control.

Groups	Photothrombosis + saline (0,5 ml)	Photothrombosis +CEPO (50 μg/kg)	Photothrombosis +CEPO-TR (50 μg/kg)	Photothrombosis +CEPO-Fc (50 μg/kg)
The average volume (mm^3) damage in one hemisphere	12,1±1,8	14,4±3,2	8,0±2,1	11,8±1,5
The total volume (mm^3) of brain damage on the rat	24,22±4,7	28,8±7,5	15,8±5,4*	23,5±4,0

Figure 2: Morphometric measurement of the average and total (rat) the volume of the ischemic lesion focus groups

* P <0,05 compared to the group with administration of saline.



Histological examination of the ischemic area in prefrontal cortex of rats brain in 7 days after photothrombotic lesion.
Paraffinic slices (10 micron), dyed by cresyl violet.
A,C – intact hemisphere; B, D, E –Injure hemisphere.

Summary of results: After photothrombosis and injection of saline, the latency of entry into the dark compartment decreased from 300 to 76 sec. Treatment of rats with CEPO, CEPO-TR, and CEPO-Fc after photothrombosis restored latency of PA to 243, 258, 227 sec, respectively, on day 4. Only injection of CEPO-TR retains passive avoidance latency 207 sec on the day 7 (Fig.1). Ischemic damage volume in animals treated with CEPO-TR on 7th day after operation was 15,8± 5,4 against 24,22 ± 4,7 with control injection of saline (P<0,05). This phenomenon on 7th day was observed only with CEPO-TR (Fig.2). Protection efficiency coefficient, calculated from these experiments, represented 34%. Another erythropoietin derivatives did not give statistically significant results.

Conclusion: ***Treatment of rats with CEPO and CEPO fusion proteins after photothrombosis of cortex resulted in retention of passive avoidance response and diminishing of volume of ischemic damage. Those, our study indicates that CEPO-Fc and CEPO-TR display neuroprotection and anti-amnestic activity, while CEPO-TR demonstrates prolongation ability.***

References:

1. Watson BD, Dietrich WD, Busto R. et al. 1985. Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann Neurol 17:497-504.
2. Romanova G., Shakova F., Gudasheva T., Ostrovskaya R. 2002. Bull exp biol med 12: 614-616.
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No potential conflict of interest

P.1.g.031

The neuroprotective and anti-amnestic effects of carbamylated erythropoietin derivates in ischemic brain injury

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Purpose: To compare the neuroprotection of carbamylated erythropoietin (CEPO) and EPO fusion protein containing TR domain from glycoprotein MUC1 (CEPO-TR), and EPO fusion protein with modified Fc fragments of IgG1 (CEPO-Fc) against ischemic brain injury, including behavioral disturbances in a bilateral focal ischemic infarction of the rat prefrontal cortex.

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Summary of results: Additional oligosaccharides, linked to the erythropoietin, prolong its half-life and increase bioactivity in vivo. For this purpose we, for the first time, used TR domain from glycoprotein MUC1, bearing 5 additional sites for O-glycosylation. Our result indicated that ligation of TR-domain to the coding sequence of EPO did not affect secretion of the chimeric protein into the medium, receptor binding affinity in vitro bioactivity, compared with EPO wild type. However, both the in vitro potency and half-life in circulation of EPO bearing TR or Fc fragments were significantly enhanced. After photothrombosis and injection of saline, the latency of entry into the dark compartment decreased from 300s to 76s. Treatment of rats with CEPO, CEPO-TR, and CEPO-Fc after photothrombosis restored latency of PA to 243, 258, 227s, respectively, on day 4. Only injection of CEPO-TR retains passive avoidance latency 207s on the day 7. Ischemic damage volume in animals treated with CEPO-TR on 7th day after operation was 15.8±5.4 against 24.22±4.7 with control injection of saline ($P<0.05$). This phenomenon on 7^{th} day was observed only with CEPO-TR. Protection efficiency coefficient, calculated from these experiments represented 34%. Another erythropoietin derivate did not give statistically significant results.

Conclusion: Treatment of rats with CEPO and CEPO fusion proteins after photothrombosis of cortex resulted in restoration of passive avoidance response and diminishing of volume of ischemic damage. Those, our study indicates that CEPO-Fc and CEPO-TR display neuroprotectionand anti-amnestic activity, while CEPO-TR demonstrates prolongation ability.

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Keywords

Behavioural pharmacology

Cognitive enhancing drugs

Neuroprotection