NEUROPROTECTIVE EFFECTS OF AN ERYTHROPOIETIN NASAL FORMULATION ON BRAIN ISCHEMIA. II. A GLOBAL ISCHEMIA MODEL


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Erythropoietin (EPO) is a candidate neuroprotector for the treatment of brain ischemia(1), with well documented effects on animal models of brain ischemia and other brain diseases. A human recombinant EPO, low in sialic acids (EPOhr-bas)(2), avoids its normal stimulation effect on erythropoiesis. On the other hand, in recent years it has been proven that intranasal administration is a rapid and direct way to the brain: the blood-brain barrier is avoided and smaller drug doses become effective(3).

The aim of this work was to study the possible neuroprotective effect of a specific intranasal formulation of EPOhr-bas in a model of global brain ischemia on Mongolian gerbils. Transient global ischemia was achieved by a 10-minute occlusion of the two common carotid arteries. Three groups of animals were established: 6 control gerbils, 11 ischemic treated with the formulation vehicle (IT-V) and 11 ischemic treated with EPOhr-bas (IT-EPO). 30μl of EPOhr-bas or the formulation vehicle were intranasally administered during 4 days. The neurological state and the exploratory behavior were evaluated. The gerbils were perfused with 10% neutral formaldehyde on the 7th day after the operation. Two coronal sections of the brain were taken at +0.1 and -1.6 mm from Bregma, and included in paraffin. 4 μm sections were dyed with Hematoxiline-Eosine and Luxol Fast Blue. A semi-quantitative histological evaluation of the parietal and temporary cortex, hippocampus, as well as thalamic and caudate-putamen nuclei was undertaken.

This intranasal formulation of EPOhr-bas improved the neurological state of the IT-EPO gerbils. The histological analysis evidenced neuroprotection in the CA2, CA3 and CA4 hippocampal regions, severe neuronal necrosis being limited to the CA1 region. On the contrary, the IT-V group showed selective neuronal death and increased microgliae in all hippocampal regions and large infarction areas with abundant macrophages were observed in the caudate-putamen nucleus on both hemispheres (Figure 1).

The previous finding that CA1 neuronal death is enough to develop a cognitive deficit(4) was confirmed by the fact that EPOhr-bas didn't improve the ischemia-induced cognitive impairment (CA1 was the only hippocampal region affected by neuronal loss on the IT-EPO group).

In this global ischemia model the strong structure to function relation was evidenced by a direct correlation between the neurological and histological states. The results of this work suggest that this intranasal formulation of EPOhr-bas reaches the brain and shows neuroprotective effects in a global brain ischemia model.

REFERENCES
Figure 1. Global ischemia induced neuronal loss on all hippocampal regions on vehicle treated animals (B, E, H, K). EPOhr-bas did not protect CA1 (C), but protected CA2, CA3 and CA4 regions (F, I, L). Hematoxiline-Eosine.