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PREOPERATIVE PHARMACOLOGICAL CONDITIONING INFLUENCES CLINICAL AND MOLECULAR OUTCOME OF ISCHEMIA/REPERFUSION INJURY OF THE SPINAL CORD AFTER THORACOAORTIC CROSSCLAMPING IN MICE.

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1) Background

Patients, undergoing clamping procedure for thoracoabdominal aortic repair, may suffer postoperatively from the consequences of ischemia/reperfusion (I/R) injury, what can cause paraplegia. Studies show a central role of the endoplasmic reticulum (ER) stress as a result from the I/R and the resulting free radicals. **Erythropoietin (Epo)** and it's **carbamylated derivate (cEpo-Fc)** are able to act antioxidative and antiapoptotic. It was shown that Epo and cEpo-Fc can improove the nerological outcome of animals after spinal cord injury. In this study, we examine the effects of pre-operative use of native erythropoietin (EPO) and its derivative cEPO-Fc on paraplegia and ER stress *in vivo*.

2) Method

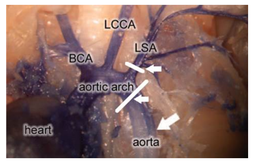
An ischemia of the spinal cord was induced in male mice (C57BL/6J) by clamping the thoracic aorta and the left subclavian artery.

Three studygroups (Epo, cEpo-Fc and control) were observed for different periods of time (6h, 24h, 96h).

The clinical, neurological outcome of the mice was evaluated by using the Basso-Mouse-Scale (BMS).

The spinal cord was stained in Hematoxylin-Eosin (HE) and Luxol-Fast Blue (LFB). Immunohistochemical stainings for ER stress relevant proteins (GRP78 and Caspase 12) were performed as well.

Clamping-time: 7 minutes



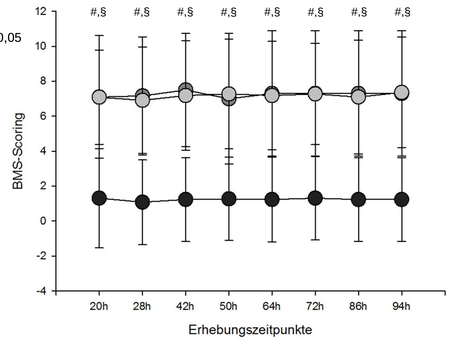
Clamping of the thoracic Aorta and the Art. subclavia sinistra

3) Clinical neurological outcome (BMS)

Animals in the Epo and cEpo-Fc groups (96h observation) show a significant better clinical, neurological outcome than animals in the control group

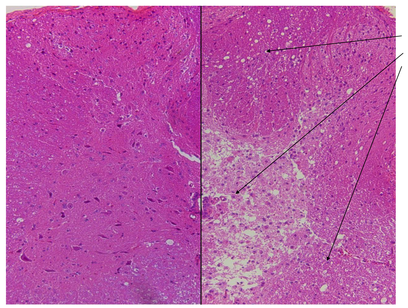
#: Epo vs. control p<0,05
§ : cEpo-Fc vs. control p<0,05

-Control
-Epo
-cEpo-Fc



4) Histological stainings (examples)

4.1) Hematoxylin-Eosin staining and scoring



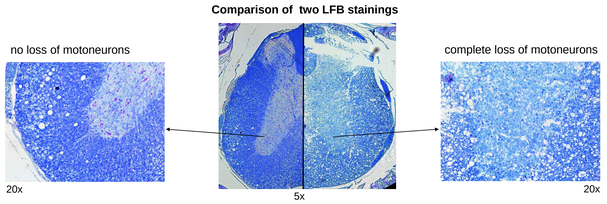
criteria of necrosis:
1) cystic transformation of white matter
2) central necrosis in grey matter
3) loss of motoneurons
4) focal parenchymal necrosis

ranking of criteria:
1) none -> 0 Pkt.
2) slightly -> 1 Pkt.
3) intermediate -> 2 Pkt.
4) severe -> 3 Pkt.

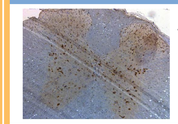
grade of necrosis:
1) no necrosis -> 0 Pkt.
2) light necrosis -> 1-3 Pkt.
3) moderate necrosis -> 4-6 Pkt.
4) severe necrosis -> 7-9 Pkt.
5) most severe necrosis -> 10-12 Pkt.

4.2) Luxol-Fast-Blue staining

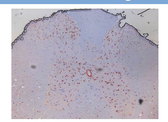
Comparison of two LFB stainings



4.2) Immunohistochemistry



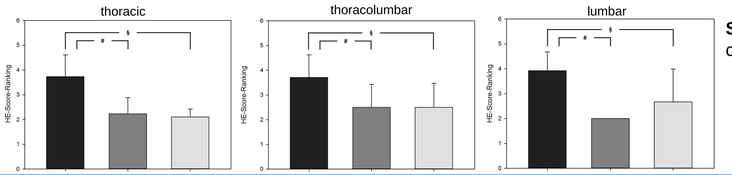
GRP78
- Detection with DAB – KIT
- positive reaction ➡dark brown



Caspase12
- Detection with AEC – KIT
- positive reaction ➡red

5) Results of the Histopathology

5.1) Hematoxylin-Eosin staining (HE)

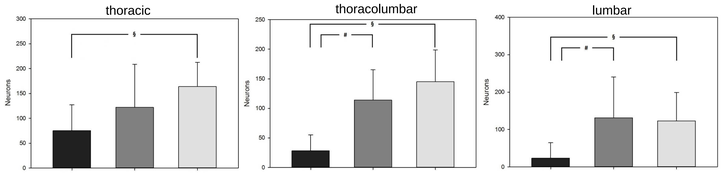


Significantly decreased necrosis in the Epo and cEpo-Fc groups in comparison to the control group.

- Control
- Epo
- cEpo-Fc

#: Epo vs. Control p<0,05
§ : cEpo-Fc vs. Kontrolle p<0,05

5.2) Luxol-Fast-Blue staining (LFB)

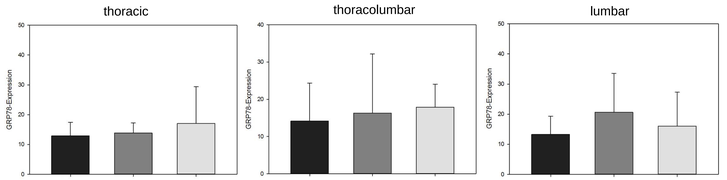


Significantly higher amount of motoneurons in the Epo and cEpo-Fc groups in comparison to the control group.

- Control
- Epo
- cEpo-Fc

#: Epo vs. Control p<0,05
§ : cEpo-Fc vs. Control p<0,05

5.3) Immunohistochemistry GRP78

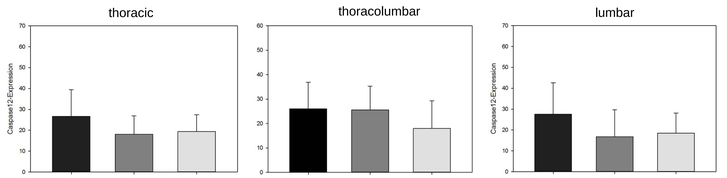


Increased expression of GRP78 in the Epo and cEpo-Fc groups compared to the control group.

- Control
- Epo
- cEpo-Fc

#: Epo vs. Control p<0,05
§ : cEpo-Fc vs. Control p<0,05

5.4) Immunohistochemistry Caspase 12



Decreased expression of Caspase12 in the Epo and cEpo-Fc groups compared to the control group.

- Control
- Epo
- cEpo-Fc

#: Epo vs. Control p<0,05
§ : cEpo-Fc vs. Control p<0,05

6) Conclusion

The results show a significant positive effect of Epo and cEpo-Fc on the clinical neurological and histological outcome of the mice. The increased expression of Caspase12 (control) makes an increased apoptotic rate likely. The increased expression of GRP78 (Epo, cEpo-Fc) makes an increased formation of the UPR likely.

Native Epo and cEpo-Fc can significantly improve the clinical outcome of mice (C57BL/6J). Significant effects are also shown in histological stainings. The molecular mechanisms undlerlying these effects are being further evaluated.

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