The effect of minocycline on the ischemic area and improved motor function in cortical model of photo thrombotic ischemic stroke in rats

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Introduction: Cellular survival and restoration of correct circulation are the goals of ischemic stroke therapy because about 87% of all strokes are ischemic. A well-designed model will facilitate future research on new possibilities of treatment of ischemic stroke and allows animals to participate in behavioral tests.
Minocycline, by launching plethora of neuroprotective mechanisms may be beneficial as the treatment which has been confirmed in many research models of acute brain damage.

Aims: The first aim of our research was to refinement, test and select the most favourable parameters in model of photothrombotic ischemic stroke in rat. The second goal was to testing the effect of minocycline on state of penumbra the level of inflammation in the ischemic stroke area of with evaluation whether regulation of HuR, HSP70 and TNFalpha proteins is involved in this treatment. We have also performed a number of behavioral tests, helpfull in evaluation.

Methods: Phototrombotic ischemia was produced in 84 male Long-Evans rats by evoking the clotting of blood inside the cerebral vessels of the rat, through activation of Bengali rose (tetracholor-tetraiodofluorescein) with the light of 3200 K Energy. Bengal rose (Sigma-Aldrich) was administered (solution 20mg/1 ml PBS in dose of 1 ml / kg b.w. by intravenous injection into the tail vein.

All procedures were performed in accordance to the EU animal protection law and were approved by the Local Animal Research Ethics Committee.

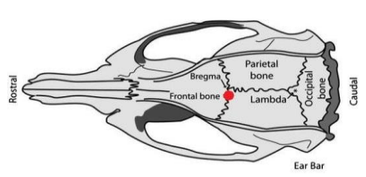
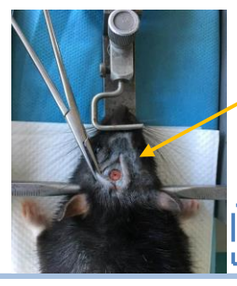


Fig.1. The graphic shows the radiation locations on the Long-Evans rat skull. Ischemic target site.



We use a special mask to limit the area of exposure

Fig.2. Photo of the mask used during the exposure (foto.K.Pawletko)

We tested different time windows: 12h,24h,48h and 7 days after stroke induction. Half of the experimental groups received an intravenous dose of minocycline (1 mg / 1 kg b.w /1ml solution/ Sigma- Aldrich). A dose of minocycline was given 10 minutes after induction of ischemic stroke. All animals form „minocycline groups", receive only one dose of minocycline after the stroke.

CatWalk XT, Grip Strength-test and elevated runway tests were performed. These functional tests were applied before and after ischemic stroke. After a specified period (24h, 48h, 7 days) from the onset of stroke, the animals were euthanized, and the brains of the animals were taken for biochemical tests (Cresyl-violet stained, WesternBlotting and Immunohistochemistry stained).

Results: We present the results of the analysis the elevated runway tests and changes in TNFalpha protein level. In the groups without minocycline, we observed a tendency for the time required to passage the beam to be longer compared to the group treated with minocycline. The passage time on day 6 in um-groups was similar to the value of day 1. There is less slippage in the treatment group compared to the group without treatment (test U Manna Whitneya, test Shapiro-Wilka, ANOVA Friedmana).

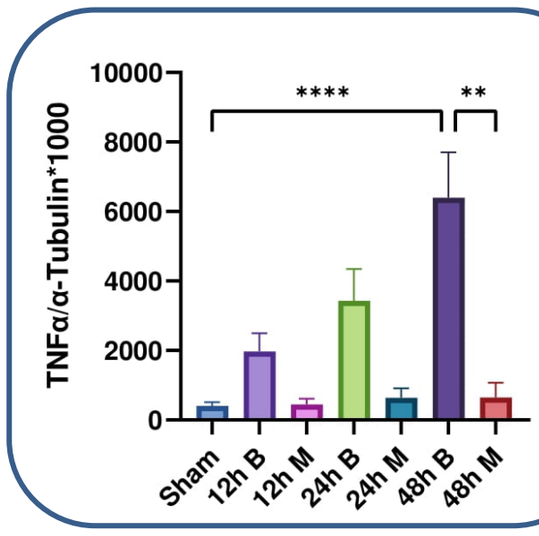


Fig.3. WesternBlotting results: there was a significant effect of minocycline treatment on all mino-groups. TNFalpha bands were normalized to tubiline.
For all panels n = 5 per data group; **p < 0.01, **** p < 0.0001 compared with Sham (one-way ANOVA).

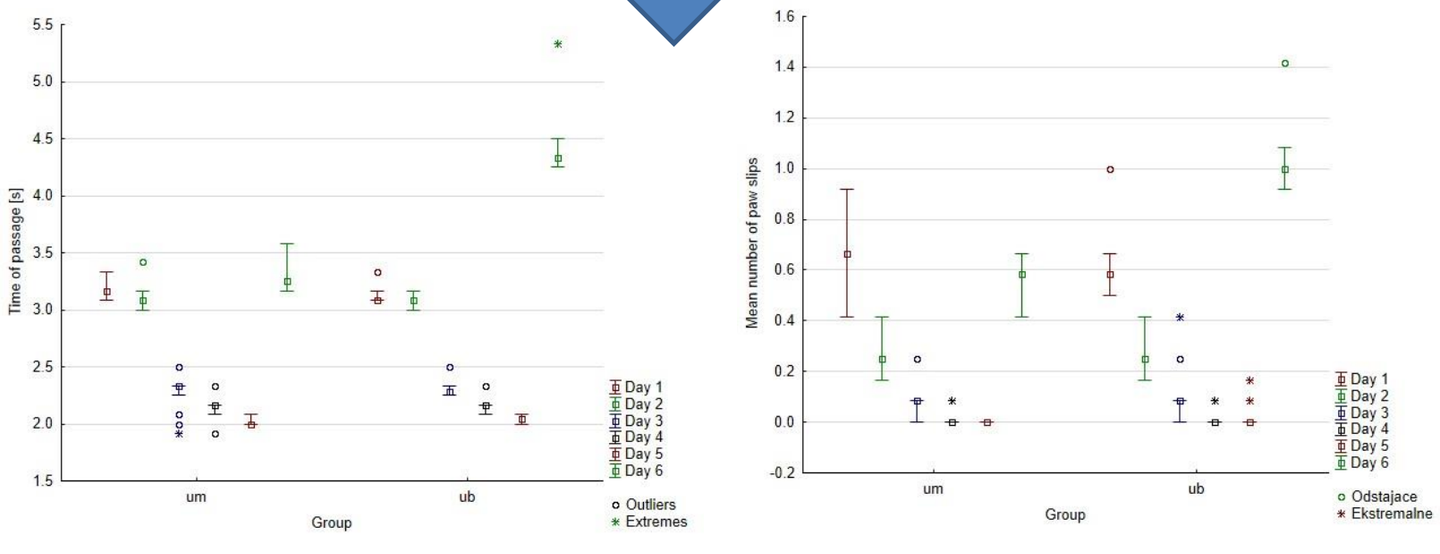


Fig.4 Effect of minocycline treatment on recovery of neurological function after ischemic stroke.

um- groups with treatment with minocycline
ub - groups without treatment

Conclusion:

- Rats in which the Bengal rose were administered via the tail vein can freely participate in behavioral tests (no side effects).
- Minocycline improves motor function and general condition in ischemic rats.
- Minocycline lowered TNF- alpha levels especially in the acute post-stroke phase.