Comparison of Three Novel Neuroprotective Therapies for Traumatic Brain Injury Using a CCI Model in Rats.

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INTRODUCTION

Traumatic brain injury (TBI) is a major cause of death and disability. There is currently no effective treatment for TBI, and survivors suffer from a variety of long-lasting health consequences. Pioglitazone, Amantadine and Glibenclamide have showed promising results in improving locomotor, cognition, and/or histology outcomes in different preclinical models of TBI. In this study, we compared the efficacy of these 3 compounds, concurrently, in a well-established model of TBI in male Sprague Dawley rats.

METHODS

Adult male Sprague Dawley rats (230-260 grams, Charles River) were used in this studies. Parasagittal Controlled Cortical Impact (CCI) was produced by an electronic cortical contusion device (Custom Design & Fabrication, Inc Richmond, VA). Rats were anesthetized with isoflurane (4-5% for induction, 2-3% for maintenance), and mounted on a stereotaxic frame. Under aseptic conditions, a 6-mm diameter trephine drill was used to open the skull centered approximately 4 mm lateral to the sagittal suture, mid-way between bregma and lambda. CCI brain injuries were produced with a 5-mm-diameter rounded brass impactor attached to a computer-controlled piston propelled electronically with following parameters: velocity=2.5 m/s; depth=3 mm; duration=100 ms. The animals were allowed to recover in a warmed recovery chamber and appropriate post-operative care was taken, including fluids (Lactate Rnger, 6cc, SC) and analgesics (buprenorphine 0.05mg/Kg, SC for 2 days). Immediately after CCI, animals received either: Pioglitazone (20mg/kg, PO, for 14 days); Glibenclamide (initial dose of 10µg/Kg, IP. Followed by 0.2µg/hour using a SC minipump); Amantadine (20mg/Kg, IP, for 14 days) or Saline (5ml/Kg, IP, for 14 days). Locomotor changes were evaluated at 3, 5 and 7 days after TBI using the Beam-Balance test, and at 7 and 22 days after TBI using the Paw-Placement preference test and Gait analysis. Cognition changes were evaluated starting at day 14 after TBI using the Morris Water Maze test. Finally, lesion size volume and inflammatory markers were evaluated in brain tissue samples.

RESULTS

Pioglitazone improves locomotor performance evaluate with the Beam Balance Test

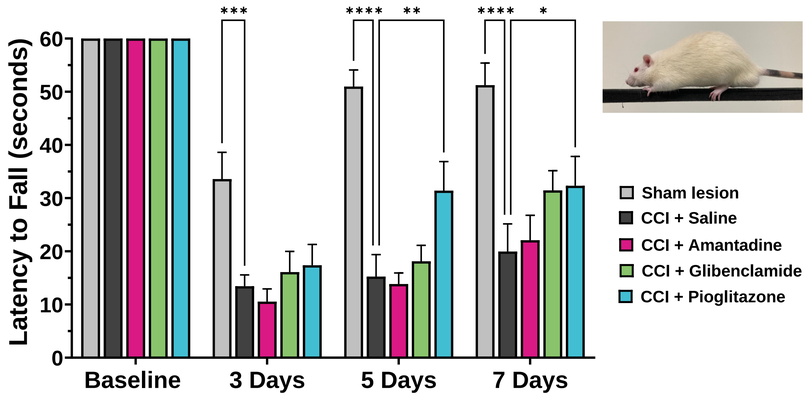

Figure 1: At baseline, all animals were able to maintain their balance on the beam for at least 60 seconds. After surgery, CCI-lesioned animals presented decrease balance ability which lasted until Day 7. Sham-lesioned animals, were able to maintain their balance on the beam significantly longer (more than 50 seconds in average at Day 5 and Day 7 days after TBI) than CCI-lesioned animals. Animals treated with Pioglitazone were able to maintain their balance for significantly longer periods compared to animals treated with Saline at Day 5 and Day 7 after TBI. N=14-16/group; Two-Way ANOVA test +Dunnet's post-hoc.

Pioglitazone improves locomotor performance evaluate with the Paw Preference test (Cylinder Test)

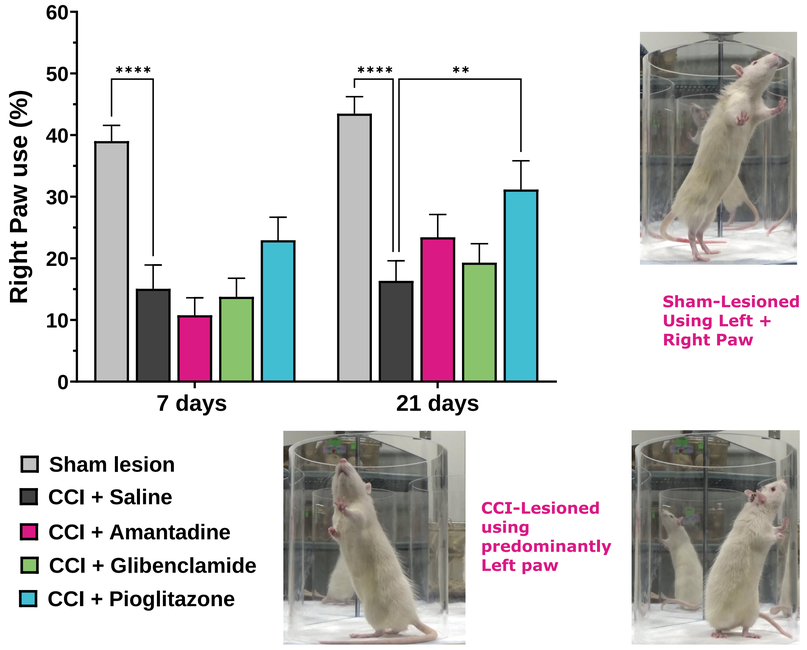

Figure 2: Naïve animals use their right front paw approximately 45-52% of the time to explore a cylinder while rearing. Sham-lesioned rats still use their right paw for exploring the cylinder around 40% of the time at 7 days after surgery. Meanwhile, CCI-Saline rats use their right paw only around 12% of the time. At 21 days after CCI, Sham-lesioned animals showed a paw preference similar to baselines while CCI-saline rats still have decrease right paw preference (~15%). Animals treated with Pioglitazone showed increase right paw preference starting at 7 days, this difference is significantly higher than Saline treated group at day 21 after CCI, showing improve locomotion.

No significant effect of Neuroprotective Therapies on Cognition evaluated by the Morris Water maze test

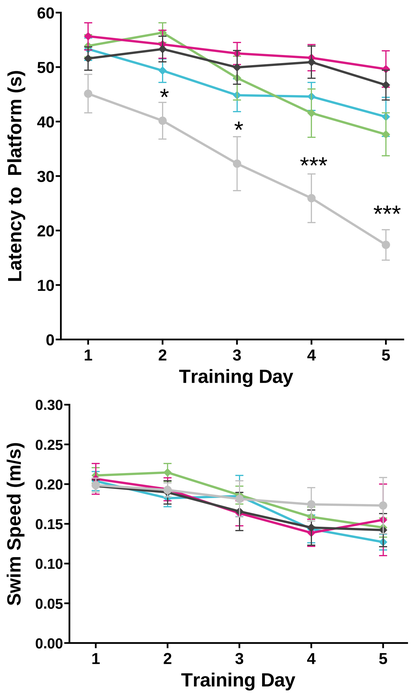

Figure 3: Two weeks after injury, rat were trained consecutively for 5 days to locate the escape platform in the water maze. A shorter escape latency period indicates improved spatial learning and memory of the platform's location.
Sham-Lesioned group had significant shorter escape latency compared to CCI-Saline group from Day 2 to Day 5. No significant difference between CCI-Vehicle and treated animals was noted. Swing speed is similar for Sham and CCI rats, showing equivalent swim ability

Neuroprotective Therapies do not have a significant effect on the MWM Probe trial

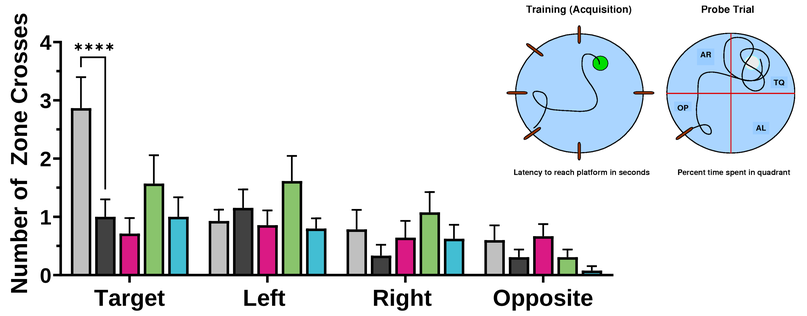

Figure 8: After 5 days of training in the MWM, the escape platform was removed from the pool. The time spent in each quadrant and the number of entries into the target zone were recorded. Sham animals showed a prefers to entry int the Target quadrant.. No significant differences were found between CCI-Saline and CCI-treated groups

Pioglitazone and Glibenclamide reduce lesion volume but do not affect microgliossis

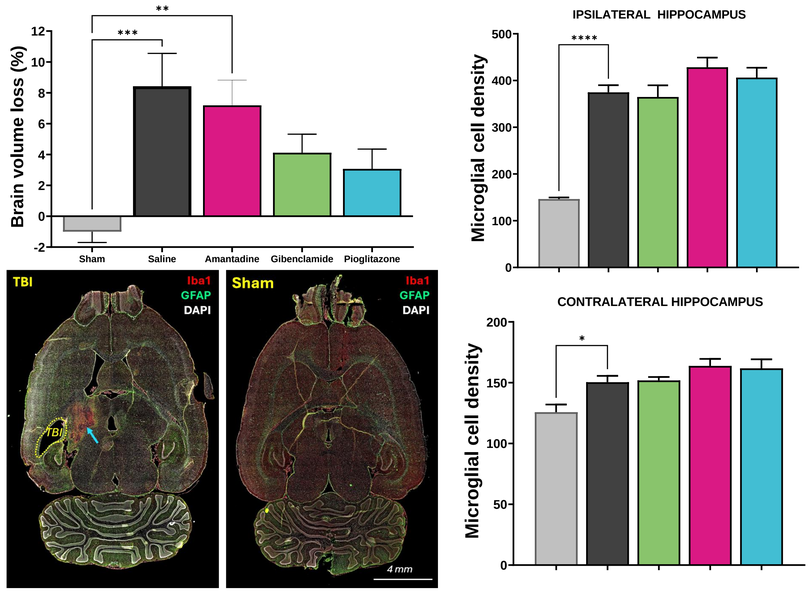

Figure 9: Upper left: Lesion size was estimated as a percent of the contralateral hemisphere. Glibenclamide and Pioglitazone rescued some brain volume. A significant different volume compared to sham was detected for Amantadine but not for Gliblenclamide and Pioglitazone. **Lower left:** Astroglia **(GFAP)** and microglia **(Iba1)** labeling counterstained for nuclei (DAPI, white) in a CCI (left) and a Sham lesioned (right): TBI (yellow dotted line) can lead to widespread pathology as neuroinflammatory response in deeper brain regions like the hippocampus (as indicated by blue arrow). **Right:** Microgliosis, measured by Iba1 cell density in the hippocampus ipsi and contralaterally after CCI. Note a highly significant microglial cytosis in the hippocampus that is even significant in the contralateral hemisphere. Treatments were unable to alleviate the microglial cytosis.

SUMMARY

Three neuroprotective Therapies were tested in a CCI model of TBI. Rats receiving Pioglitazone (FDA approved for diabetes) presented significant improvement in locomotor performance (Beam-Balance test and paw preference test). Animals treated with Glibenclamide and Amantadine did not show any significant improvement in behavior outcomes. Although Pioglitazone and Glibenclamide rescue some lesion volume, no changes in hippocampus inflammation were detected.