Final Report (A262-76756, PI: Valadka)

A. Specific Aims:

The central aim of this proposal is to evaluate the efficacy of mannitol and hypertonic saline for treating high intracranial pressure following traumatic brain injury in rat models.

Aim 1: **To compare effects of mannitol and HTS at different concentrations on ICP, brain water content, inflammation, and early neuronal cell death immediately after experimental TBI.**
In this aim, we will assess the efficacy of mannitol and HTS at different concentrations that used in clinic in control ICP. In addition, we will measure acute outcomes parameters include mean arterial and central venous pressures, serum sodium and osmolality, brain water content, cell death and early inflammatory cell response.

Aim 2: **To compare neuroprotective properties of mannitol and different concentrations of HTS by determining functional recoveries and brain tissue preservation after experimental TBI.**
In this aim, we will use a battery of behavior testing methods to assess functional recovery of injured animals receiving osmotic treatment. We will also assess cortical contusion volume, survival neuronal cell numbers in the hippocampus, cortical white matter integrity, and glial cell response using histological method.

B: Summary of Results

Aim 1 study. In this study we examined the treatment effect of mannitol and HTS at the acute stage following a severe TBI. Male Sprague-Dawley rats at the age of approximately 4 months old were subjected to a lateral fluid percussive injury at the level of 2.25 ±0.05ATM. Immediately following TBI, intracranial pressure (ICP) was monitored via a solid intracerebral ICP probe. Blood pressure was also monitored via a pressure monitor placed in the femoral artery. At 15 min post-TBI, 1ml of 20% mannitol, 3.6% HTS, 7.2% HTS or 0.9% saline was infused into the femoral vein via a catheter. ICP and blood pressure were monitored continuously for 4 hours. Blood samples were taken every 30 min for blood gas monitoring. After 4 hrs monitoring, animals were sacrificed. Half of the animals in each group were used for brain edema assessment, half of the animals were perfused for histology to assess brain tissue damage and acute inflammatory cell response. The finding for the acute stage study is as followed.

1. Osmotic treatment with mannitol or HTS significantly lowers TBI-elevated ICP. Our injury model at the 2.25 ±0.05ATM induces a moderate to severe injury, with an elevated ICP above 20mmHg. In the injured animals with vehicle (0.9% saline), the elevated ICP persisted throughout the 4-hr monitoring period. In animals which received single dose of 20% mannitol, 3.6% HTS, or 7.2% HTS, TBI-elevated ICP was significantly reduced with time (Fig. 1).

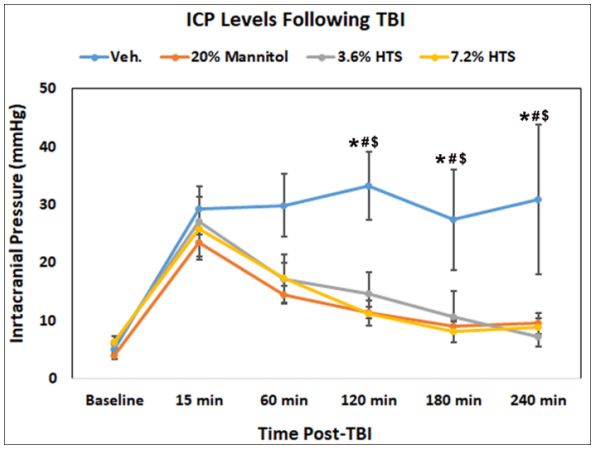

Fig. 1. Changes in ICP level following administration of hyper-osmolar agents after severe TBI. In all injured groups, a surge of ICP above 20mmHg was observed following TBI. During the 4-hr monitoring period, a persistent ICP increase was observed in the vehicle group. Animals treated with 3.6% HTS, 7.2% HTS or 20% mannitol all showed a gradual decrease in ICP with equal effectiveness, and were significant than the vehicle group at 2-4hr monitoring period (*$p<0.05$, vehicle group vs. 20% Mannitol group; #$p<0.05$, vehicle group vs. 3.6% HTS group; \$$p<0.05$, vehicle group vs. 7.6% HTS group. n = 5-7/group.

2. Osmotic treatment with mannitol or HTS did not significantly change mean arterial pressure, metabolic parameters in the blood. Our blood pressure monitoring and blood gas analysis results confirmed that the

osmotic treatment either with 20% mannitol of HTS did not affect the mean arterial pressure (Fig. 2) and blood glucose or lactate level. There was an increase of blood sodium level in 7.2% HTS treatment group as expected. Blood creatinine level was lowered in the 7.2% HTS group at the 4-hour time as compared to the vehicle treated group (Fig. 3).

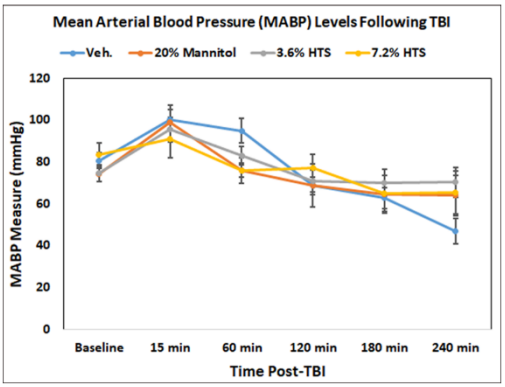

Fig. 2. Changes of mean arterial blood pressure (MABP) following a severe TBI. Following TBI, in all injured groups, a transient increase in blood pressure was observed with the peak at 15 min, then with a gradual decrease throughout the 4-hr period. The vehicle group had a constant decrease during the 4 hrs recording time while the treatment groups remained around baseline level; however, no statistical significance was found.

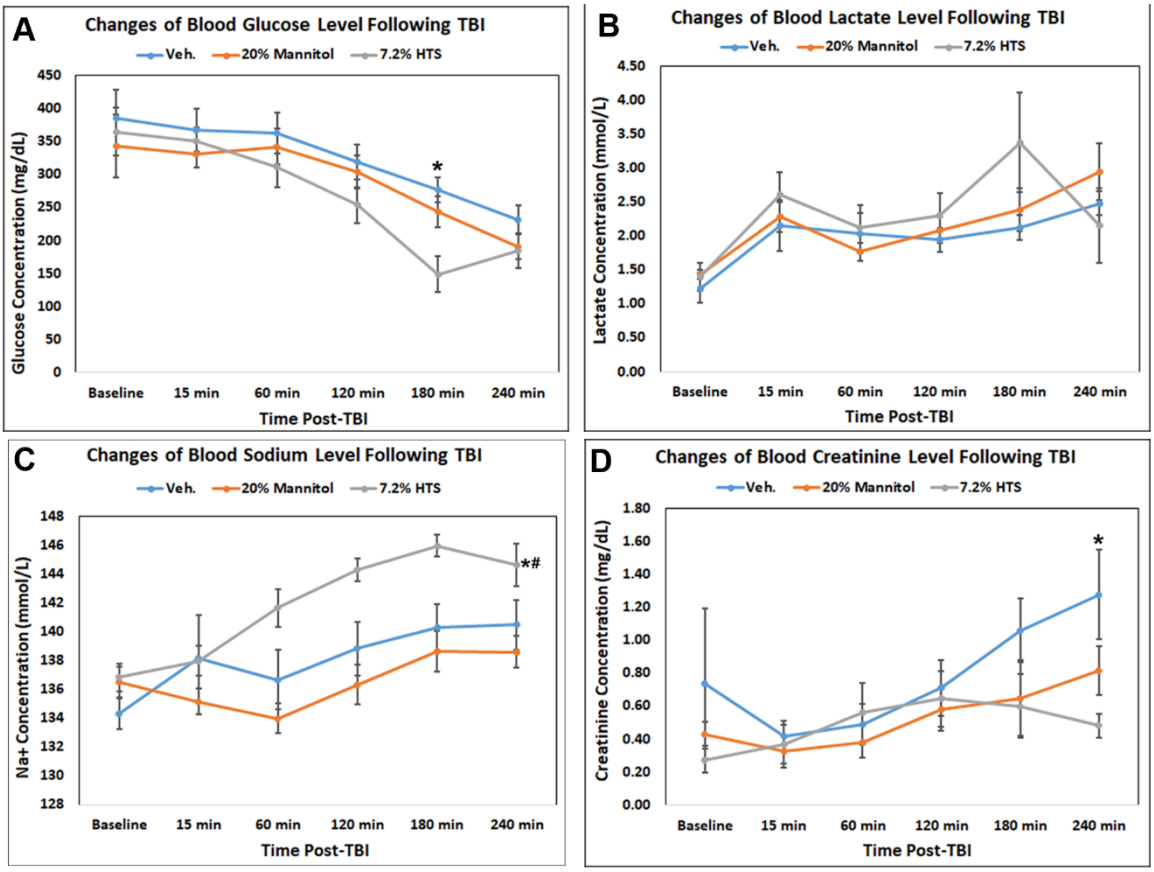

Fig. 3. A). Changes of blood glucose level following a severe TBI. Blood glucose level was consistent in all groups in the first 1hr following TBI, then a gradual decrease was observed and was most significant in the 7.2% HTS treated group at the 3hr time point (*$p<0.05$, vehicle vs. 7.2% HTS). No significant differences was found between vehicle and mannitol groups. **B).** Changes in blood lactate level following a severe TBI. Following TBI, an increase of blood lactate level was observed in all injured groups. The 7.2% HTS treated group was particularly higher at the 3-hr time point compared to other groups. No statistically significant difference was found between groups. **C).** Changes of blood sodium level following a severe TBI. Blood gas data were compared in animals received vehicle, 7.2% HTS or 20% Mannitol infusion. Blood sodium level was gradually elevated in 7.2% HTS treated group and was significant during 1-4hr period in comparison to both vehicle and mannitol treated groups (*$p<0.05$, vs. vehicle; #$p<0.05$, vs. mannitol). **D).** Changes of blood creatinine level following a severe TBI. Blood creatinine was slightly increased after TBI in the first 2-hr monitoring period in all groups. A consistent increase was observed in the vehicle group and was significant than the 7.2% HTS at the 4-hr time point (*$p<0.05$). N=5-7/group.

3. Osmotic treatment with mannitol or HTS significantly reduced acute brain edema following the severe TBI in a rat TBI model. TBI induces brain edema that contributes to ICP elevation. To assess if osmotic treatment in reducing ICP is partially due to resolving of acute brain edema, we measured the brain water content after the 4-hr monitoring. We found that animals which received either 20% mannitol, 3.6% HTS or 7.2% HTS had significant reduction in brain water content (Fig. 4).

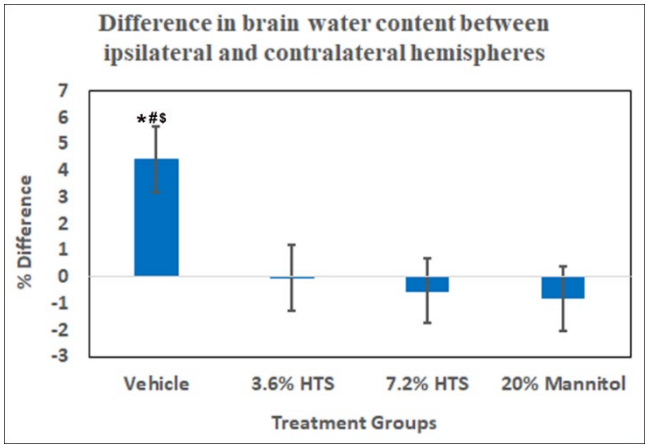

Fig. 4. Percent change in brain water content. The brain water content was assessed at the end of 4hr monitoring period. The water content of each hemisphere was calculated as following: water content = (wet weight – dry weight)/dry weight. The percent change in water in the ipsilateral hemisphere compared to the contralateral side was then calculated as follows: % change in water content = [(ipsilateral water content – contralateral water content)/contralateral water content] x 100. We found that all three treated group had significant less water content compared to the vehicle group with 20% mannitol most effective at reducing water content in the brain (*p<0.05, vehicle group vs. 20% Mannitol group; #p<0.05, vehicle vs. 3.6% HTS group; $p<0.05, vehicle group vs. 7.6% HTS group. n = 4/group).

4. Mannitol but not HTS reduce the number of inflammatory cells in the brain at the acute stage following TBI. To assess whether osmotic treatment has effect on acute Neuroinflammation, we assessed the number of MHC class II antigen presenting cells (OX6 staining) in the brain and quantified the number of cells in the injured cortex and hippocampus. We found that the OX6+ cells were significantly lower in the injured cortex in 20% mannitol treated group (Fig. 5.). Due to extensive diffuse tissue damage with the injury level, the lesion volume and cell death assessment was not successful.

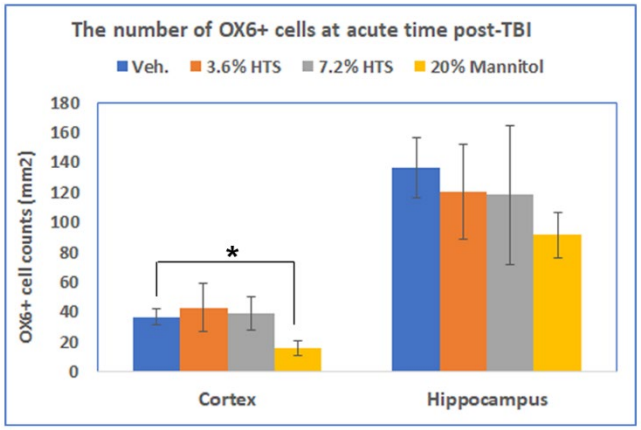

Fig. 5. Quantification of the number of OX6+ cells in the brain. In the ipsilateral cortex, animals treated with 20% mannitol has significantly lower number of OX6+ cells compared to the vehicle treated group (*p<0.05). No group difference was found in the ipsilateral hippocampus.

Aim 2 study: This aim examined whether decrease of ICP with hyperosmotic treatment can promote functional recovery following a severe TBI. In this study injured animals at 15 min post-TBI, received 1ml of 20% mannitol, 3.6% HTS, 7.2% HTS or 0.9% saline infusion into the tail vein. Animals were not monitored for ICP or blood gas as the surgical procedures were invasive preventing animal survival. Following injury, all animals plus a group of non-injured sham control were tested in motor function at 1, 2, 3, 7 and 14 days post-injury in the beam walking tests. Animals were also tested in Morris water maze to assess cognitive functions. We found better motor functional recovery in injured animals with 7.2% HTS treatment (Fig. 6), whereas in MWM test, no significant cognitive functional recovery was found in all injured groups in both the latency test (learning function) and probe trial (memory function) (Fig. 7 and 8).

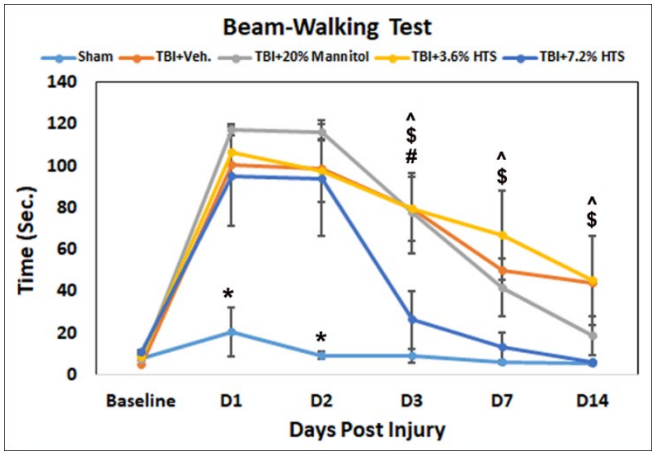

Fig. 6. Motor functional performance- beam walking test. For chronic study, the motor function was assessed in beam-walking test at -1, 1, 2, 3, 7 and 14 days post injury. At the 1 and 2 days post injury, all injured groups showed significant deficits compared to sham (*p<0.01). At D3, vehicle, 3.6% and mannitol groups showed significant deficit than the sham whereas the 7.2% HTS group showed recovery. At day 7 and 14, persistent deficit was found in vehicle and 3.6% HTS groups compared sham (^p<0.05, vehicle vs. sham; $p<0.05, 3.6% HTS vs. sham; #p<0.05, mannitol vs. sham).

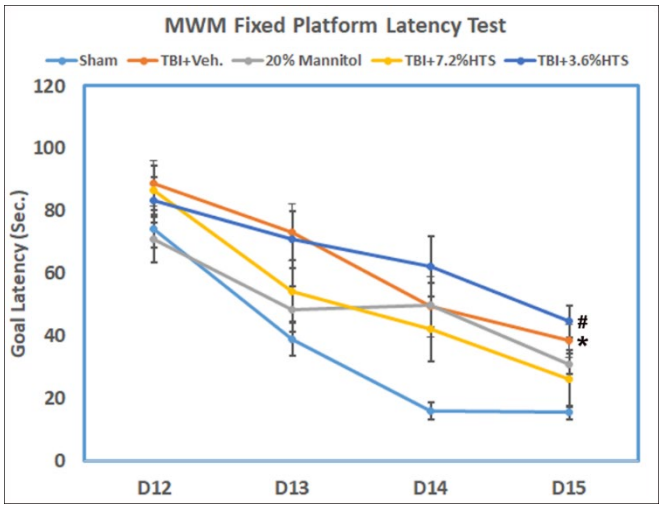

Fig. 7. Cognitive functional assessment-Morris Water Maze probe trial. MWM probe trial was performed at day 16 with 24 hrs delay after the last latency test. Significant difference was noted between the sham and injured groups indicating memory deficits in all injured groups. No difference was found between all injured groups (n=8-10/group).

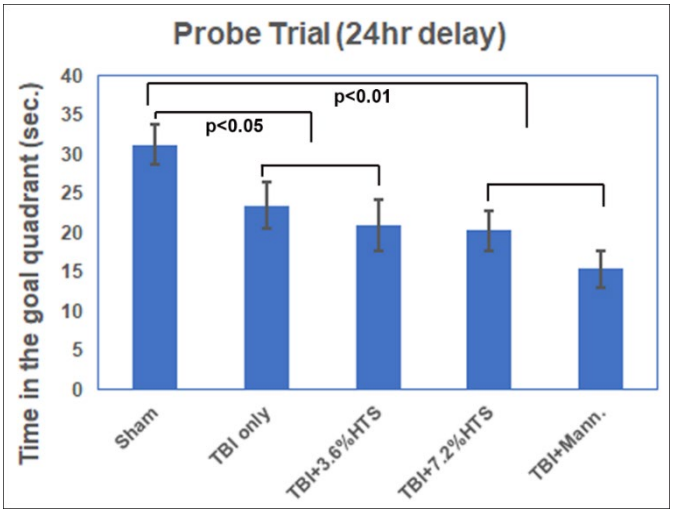

Fig. 8. Cognitive functional assessment-Morris Water Maze goal latency test. MWM goal latency was tested on 12-15 days post-injury. In goal latency test, the time spent to reach the goal platform decreased during the 4 days period in all groups. All injured groups spent longer time to locate the platform compared to sham group indicating cognitive deficits. No difference was found between the injured groups. However, statistically significant difference was only found in vehicle and 3.6% HTS treated groups when compared to sham (*p<0.05, vehicle vs. sham; #p<3.6% HTS vs. sham). Animals received 7.2% HTS or mannitol showed no significant difference in comparison to sham indicating a certain degree of improvement (n=8-10/group).

In summary: Our study has demonstrated that hyperosmolar agents are effective in reducing ICP and brain edema at the acute stage following a severe TBI in rodents. Among physiological measurement, 7.2% HTS treatment increases blood sodium level, temporarily reduces blood glucose level. Other physiological parameters are not significantly affected with HTS or mannitol treatment. At chronic stage, among agents tested, 7.2% hypertonic saline is more effective than 20% mannitol and 3.6% HTS in improving motor functional recovery. For cognitive function, both 20% mannitol and 7.2% HTS treated animals had slightly better performance learning. However, memory function as tested by MWM probe trial was not improved. This

could due to the severe functional deficits observed in our model. Our data suggest that osmotic treatment can reduce TBI-elevated ICP, thus reduce the post-TBI mortality. However, the increased survival does not translate to better functional recovery at recovery stage.

Presentation

1. Zachary Kiernan, Andrew Rolfe, Alex Valadka, Dong Sun (2019). A comparative study of therapeutic effects of mannitol and hypertonic saline for severe traumatic brain injury in rats. A poster presentation was presented at the 2019 Capital Beltway Neurotrauma Symposium held at NIH, Bethesda in March 5th, 2019.
2. Zachary Kiernan, Andrew Rolfe, Timothy Keoprasert, Alex Valadka, Dong Sun (2019). A comparative study of therapeutic effects of mannitol and hypertonic saline for severe traumatic brain injury in rats. Poster presentation. National Neurotrauma Society Symposium. Pittsburg, PA, July, 2019.

Publication

1. Zachary Kiernan, Andrew Rolfe, Alex Valadka, Dong Sun (2019). A comparative study of therapeutic effects of mannitol and hypertonic saline for severe traumatic brain injury in rats. J. Neurotrauma Abstracts.
2. Zachary Kiernan, Andrew Rolfe, Timothy Keoprasert, Alex Valadka, Dong Sun (under preparation). Acute and long-term therapeutic effect of osmotic therapy for severe traumatic brain injury in rats.