Published in final edited form as:

Brain Res. 2008 April 18; 1205: 100–107.

Role of Histamine in Brain Protection in Surgical Brain Injury in Mice

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Abstract

Surgical resection of brain tissue is associated with tissue damage at the resection margin. Studies of ischemic brain injury in rodents have shown that administration of L-histidine and thioperamide reduces ischemic tissue loss, in part by inhibition of apoptotic cell death. In this study we tested administration of L-histidine and thioperamide in surgical brain injury in mice. Mice were randomized to one of three groups: Sham surgery (n=18), surgical brain injury without treatment (SBI) (n=33), and surgical brain injury with combined L-histidine and thioperamide treatment (SBI + H) (n=29). Surgical brain injury was induced via right frontal craniotomy with resection of the right frontal lobe. L-histidine (1000 mg / kg) and thioperamide (5 mg / kg) were administered to the SBI + H group immediately following surgical resection. Postoperative assessment included neurobehavioral scores, Evans blue measurement of blood-brain barrier breakdown, brain water content, Nissl histology, and immunohistochemistry for IgG and cleaved caspase 3. Postoperative findings included equivalent neurobehavioral outcomes at 24 and 72 hours in the SBI and SBI + H groups, similar histological outcomes between SBI and SBI + H, and similar qualitative staining for cleaved caspase 3. SBI + H had increased BBB breakdown on Evans blue analysis and a trend towards increased brain edema which was significant at 72 hours. We conclude that combined treatment with L-histidine and thioperamide leads to increased BBB breakdown and brain edema in surgical brain injury.

1. Introduction

Surgical resection of brain tissue is associated with tissue damage at the edge of the resection site due to direct surgical trauma, electrocautery burn, retractor stretch, hemorrhage and edema (Matchett et al., 2006, Jadhav et al., 2007). Tissue at the edge of a resection site demonstrates elements of blood-brain barrier (BBB) breakdown, edema, necrotic and apoptotic cell death (Matchett et al., 2006; Jadhav et al., 2007). In principle drugs or therapies that reduce BBB breakdown, brain edema, necrotic or apoptotic cell death would tend to improve outcomes in surgical brain injury. Such therapies would also limit complications and permit more-aggressive surgical resection in confined spaces. Previous experimental work has shown that

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preservation of the BBB by pharmacologic inhibition of Src tyrosine kinase results in improved outcomes in surgical brain injury (SBI) in rodents (Jadhav et al., 2007).

Histamine is an endogenous neurotransmitter in the brain that is important in a variety of signaling processes (Raber 2007). Histaminergic neurons originating in the posterior hypothalamus project to many areas of the brain and are important in a variety of processes including the release of stress hormones such as vasopressin (Raber 2007). A number of studies have examined the role of histamine in ischemic brain pathology, and many of these studies point to a neuroprotective role for histamine in ischemic pathology (Adachi et al., 2004; Adachi et al., 2005; Tang et al., 2007). In this study we used a model of surgical brain injury in mice to test for the neuroprotective therapeutic potential of histamine activation after surgical brain injury.

Elevated histamine levels in the setting of focal ischemic brain injury have been shown to be neuroprotective for at least 24 hours after ischemia (Adachi et al 2004; Adachi et al., 2005). To achieve elevated brain histamine levels several studies have described administration of L-histidine. L-histidine is converted to histamine in the brain (Adachi et al., 2004; Adachi et al., 2005). Intraperitoneal post-ischemic injection of L-histidine has been shown to be neuroprotective in focal ischemia in a dose-dependent manner from 200 to 1000 mg / kg, with higher doses showing improved neuroprotective effect (Adachi et al., 2005). L-histidine crosses the BBB where it is converted to histamine, and parenteral administration of L-histidine leads directly to elevated brain concentration of histamine (Motoki et al., 2005; Prell et al., 1996). L-histidine is neuroprotective in global ischemia (Adachi et al., 2006), focal cerebral ischemia (Adachi et al., 2004), and *in vitro* (Tang et al., 2007).

Several studies have examined the therapeutic potential of combined L-histidine and thioperamide treatment in ischemic brain pathology (Adachi et al., 2006; Motoki et al., 2005). Thioperamide is a histamine H3 receptor antagonist. Histamine H3 receptors serve a negative-feedback (inhibitory) function endogenously. Blockade of histamine H3 receptors leads to increased synthesis and production of histamine in the brain (Adachi et al., 2005; Arrang et al, 1983). The net effect of combined L-histidine and thioperamide administration is a general increase in the histaminergic state of the brain. Typical dosing regimens describe combined administration of L-histidine (1000 mg / kg) and thioperamide (5 mg / kg) (Motoki et al., 2005). Previous work has shown that combined L-histidine and thioperamide therapy is neuroprotective in global and focal ischemia (Adachi et al., 2006; Motoki et al., 2005). The neuroprotective mechanisms of L-histidine and thioperamide have only been partially explored to date, although an anti-apoptotic mechanism in the setting of ischemia is likely (Adachi et al., 2006; Motoki et al., 2005).

In this study we tested combined treatment with L-histidine (1000 mg / kg) and thioperamide (5 mg / kg) in a model of surgical brain injury (SBI) in mice. The SBI model has been described previously in rats, and for this study we adapted it to mice (Matchett et al., 2006; Jadhav et al., 2007). We hypothesized that combined therapy would improve outcomes in this model by reducing postoperative tissue loss at the periphery of the resection site, possibly by anti-apoptotic mechanisms.

2. Results

2.1 Mortality

Three groups of animals were used: surgical brain injury (SBI, n=33), surgical brain injury with combined L-histidine and thioperamide treatment (SBI + H, n=29) and Sham surgery (Sham, n=18). Four animals died in the SBI group (4 / 33, 12%), 2 animals died in the SBI+H

group (2 / 29, 6.9%), and 1 animal died in the Sham group (1 / 18, 5.6%) prior to planned sacrifice, statistically equivalent on chi-squared analysis.

2.2 Nissl Histology

Gross histological outcomes between the SBI and SBI+H groups were similar (Figure 1A–C). Tissue at the edge of the surgical resection revealed damage consistent with necrotic cell death, tissue breakdown, and general loss of tissue integrity. We observed damage to the contralateral (Left frontal) lobe damage in addition to ipsilateral (right frontal) lobe damage. This spillover damage may relate to electrocautery use during the surgical procedure. Bipolar electrocautery was used at the cut tissue surface, although spillover to the contralateral frontal lobe likely occurred, resulting in damage to the contralateral frontal lobe. We observed a rim of tissue with necrotic-like morphology at the cut tissue edge. This tissue was variable in thickness, perhaps in part due to the electrocautery effect. Immunohistochemical staining for cleaved caspase 3, a marker of apoptosis, demonstrated a variable rim of apoptotic tissue in close proximity to the cut rim of tissue (Figure 3B). Qualitatively the rim of apoptotic tissue was similar between both the SBI and SBI + H groups. The sham group had no evidence of apoptosis.

2.3 Brain Water Content and Blood-brain barrier breakdown

Under the conditions used for this study brain water content was elevated in both hemispheres of the SBI and SBI+H groups following surgery (Figure 2A). This increase in brain edema was statistically significant when compared to the sham group at 24 hours postoperatively. Seventy-two hours after surgery brain edema was persistently elevated in the SBI group, and a trend toward increased brain edema in the SBI+H group became evident (Figure 2A). Brain edema was significantly higher in the SBI+H group in the non-operative (left hemisphere) at 72 hours (Figure 2A). The percent values for brain water content $\pm$ standard error of the mean at 24 hours were as follows: SBI left hemisphere 0.796 ± 0.005 , SBI+H left hemisphere 0.795 ± 0.003 , Sham left hemisphere 0.776 ± 0.004 , SBI right hemisphere 0.805 ± 0.006 , SBI+H right hemisphere 0.802 ± 0.003 , Sham right hemisphere 0.781 ± 0.003 . At 72 hours the values for brain water content were as follows: SBI left hemisphere 0.803 ± 0.004 , SBI+H left hemisphere 0.821 ± 0.007 , SBI right hemisphere 0.825 ± 0.007 , SBI+H right hemisphere 0.836 ± 0.007 .

Blood-brain barrier breakdown was significantly greater in the SBI+H group 24 hours after surgery (Figure 2B). The SBI group also demonstrated a trend towards increased BBB breakdown, although this was not statistically significant compared to the sham group (Figure 2B). Absorbance per mg values for Evans Blue BBB breakdown are presented in mean $\pm$ standard error of the mean. At 24 hours the values for Evans Blue BBB breakdown were as follows: SBI left hemisphere 2.36 ± 0.72 , SBI+H left hemisphere 2.57 ± 0.22 , Sham left hemisphere 2.11 ± 0.4 , SBI right hemisphere 2.03 ± 0.25 , SBI+H right hemisphere 3.26 ± 0.33 , Sham right hemisphere 1.59 ± 0.29 . Qualitative BBB assessment of IgG leak revealed worse IgG leak in the SBI+H group than in the SBI or Sham groups (Figure 3A).

2.4 Neurological Outcomes

Neurological testing included study of spontaneous activity, response to side stroke, vibrissae touch, limb symmetry, lateral turning, climbing, and forelimb walking. This testing revealed decreased performance in both the SBI and SBI+H groups. The difference in outcomes between these two groups was not significantly different at 24 or 72 hours (Figure 2C). There was a trend towards improved neurobehavioral performance in the SBI group compared to the SBI +H group, although this difference was not statistically significant. At 24 hours neurological scores were as follows: SBI 13.0 ± 1.1 , SBI+H 11.6 ± 0.86 , Sham 20.3 ± 0.25 . At 72 hours neurological scores were as follows: SBI 15.1 ± 1.2 , SBI+H 11.0 ± 1.4 .

3. Discussion

In this study we found that combined therapy with L-histidine and thioperamide in a model of surgical brain injury in mice was associated with significantly increased blood-brain barrier breakdown and brain edema 72 hours after surgery. Even though brain edema and BBB breakdown was worse with L-histidine and thioperamide treatment, qualitative histological results and neurological scores were similar between the untreated and treated groups. Neurological scores, while statistically equivalent between the SBI and SBI+H groups, demonstrated a trend toward worse performance in the SBI+H group. It is possible that this trend would become statistically significant with larger groups of animals.

Several factors may explain the lack of a neuroprotective effect of L-histidine and thioperamide treatment in surgical brain injury in mice. Many of the previous experimental studies of L-histidine and thioperamide therapy have described neuroprotection in the setting of ischemic brain injury. For example, the combined L-histidine and thioperamide treatment strategy has been used successfully in previous studies of focal ischemia (Motoki et al., 2005) and global ischemia (Adachi et al., 2006) in rodents. Although ischemic injury is present after surgical brain injury, the ischemic component may play a relatively small role in the pathophysiology of the injury (Matchett et al., 2006; Jadhav et al., 2007). Prior histological study has shown that ischemic injury is relatively small after surgical brain injury and limited to a thin rim of tissue at the cut tissue surface (Matchett et al., 2006; Jadhav et al., 2007). This may have limited potential benefit from L-histidine and thioperamide treatment.

Another reason for the relative lack of neuroprotection from L-histidine and thioperamide therapy may relate to the relative lack of apoptosis in the surgical brain injury model. Previous studies of combined L-histidine and thioperamide have found that the neuroprotective effects of L-histidine and thioperamide in ischemic injury are associated with anti-apoptotic processes (Adachi et al., 2006; Motoki et al., 2005). Although apoptosis was present in the brain after surgical brain injury (Matchett et al., 2006, and Figure 3), apoptosis appears to play a relatively small role in cell death in this model. Blood-brain barrier breakdown and edema appear to play a substantial role in the pathophysiology of the SBI model. L-histidine and thioperamide may have had an anti-apoptotic effect in our study, although this effect was not evident on qualitative examination (Figure 3). Any anti-apoptotic effect may have been dwarfed by the BBB breakdown and brain edema in the treatment group.

It is noteworthy that treatment with L-histidine and thioperamide seemed to contribute to blood-brain barrier dysfunction and brain edema in our study. This finding is consistent with several previous reports. Domer (1983) reported that both low (1.25 $\mu\text{g} / \text{kg}$) and high (5 $\mu\text{g} / \text{kg}$) dose histamine caused increased BBB leakage to small molecules such as sodium pertechnetate in normotensive Wistar rats. Furthermore, Domer *et al.* also found that high-dose histamine caused BBB leakage to large molecules like albumin in spontaneously hypertensive Wistar rats. In our study L-histidine and thioperamide therapy contributed significantly to BBB breakdown and brain edema (Figure 2). It is possible that a lower dose of either L-histidine or thioperamide may have resulted in less BBB breakdown. Furthermore it is also possible that treatment with either L-histidine or thioperamide alone instead of combination treatment may have resulted in less BBB breakdown. Domer (1983) reported that high-dose histamine is associated with significant BBB breakdown and leakage of large molecules. Our treatment protocol may have resulted in similarly elevated histamine levels that resulted in the BBB leakage, thereby obscuring any potential neuroprotective effect.

Additional support for a possible role of histamine in BBB leakage comes from studies of histamine H₂ receptor blockade in the setting of ischemic brain injury. Tosaki (1994) found that ranitidine (a histamine H₂ receptor antagonist) pretreatment prior to bilateral common

carotid artery occlusion in Sprague-Dawley rats results in a dose-dependent *decrease* in brain edema. Likewise, Patanaik (2000) found that histamine H2 receptor blockade was associated with reduced BBB breakdown and reduced brain edema after hyperthermic brain injury in rats. All of this seems to suggest that histaminergic activation may lead to increased susceptibility to BBB breakdown and brain edema, which is consistent with our findings in surgical brain injury.

Whether or not histamine H2 receptor blockade is neuroprotective or neurotoxic outside of effects on the BBB remains an open question. Several reports seem to support contradictory results. Malagelada (2004) found that histamine H2 receptor blockade with ranitidine protects against neural death in an *in vitro* model of oxygenglucose deprivation (OGD) (Malagelada et al., 2004). Mechanisms were not explored in this paper, although the authors suggest that ranitidine may have prevented histamine-mediated release of excitotoxic neurotransmitters. Conversely, several other studies describe a relative neurotoxic effect of histamine H2 receptor blockade. Adachi (2004, 2005) reported that the beneficial effects of L-histidine treatment in focal cerebral ischemia are blocked by ranitidine. Adachi (2002) found that blockade of histamine H2 receptors facilitated ischemia-induced release of dopamine and also aggravated ischemic neuronal damage in forebrain ischemia in gerbils (Adachi et al., 2002). Adachi (2001) found that histamine H2 receptor blockade with ranitidine resulted in increased extracellular glutamate and worse neuronal damage in gerbils with transient forebrain ischemia. Ranitidine also facilitated ischemic depolarization and caused increased depletion of ATP in the treated animals. Both studies suggest mild neurotoxicity from ranitidine. Consistent with these findings, Hamami (2004) found that preischemic administration of histamine resulted in decreased levels of dopamine and glutamate during focal ischemia in rats. Ranitidine partly abolished the improvement caused by histamine. Fugitani (1996) found that histamine given intracerebroventricularly improves delayed CA1 damage after forebrain ischemia in Gerbils. Conversely, administration of cimetidine or ranitidine made neuronal damage worse (Fugitani et al., 1996). From this study, anoxic depolarization was worse in the groups that received cimetidine and ranitidine. Sugimoto (1994) found that histamine depletion with S alpha-fluoromethylhistidine (FMH) results in worse ischemic damage in gerbils with forebrain ischemia. FMH inhibits histamine synthesis from histidine. It is possible that the protective effects of L-histidine and thioperamide may relate to activation of other receptors besides the histamine H2 receptors. Whether or not histamine H2 receptors serve a neuroprotective or neurotoxic effect remains an open question.

The dosing regimen in this study was made with guidance from prior publications. Motoki (2005) found that the optimum dose of L-histidine and thioperamide was 1000 mg / kg and 5 mg / kg, respectively, for alleviation of cerebral infarction in rats. In this study combined L-histidine and thioperamide therapy produced superior results to L-histidine alone. Likewise, Adachi (2006) found that combined treatment with L-histidine and thioperamide was superior to L-histidine alone in global ischemic injury (Adachi et al., 2006).

Mechanisms for neuroprotection related to combined L-histidine and thioperamide have been incompletely explored to date. Some evidence suggests that L-histidine therapy may result in increased intracellular neuroprotective mediators such as phosphorylated ERK (pERK) (Grazia et al., 2003) and phosphorylated AKT (pAKT) (Bongers et al., 2007). Both pERK and pAKT have anti-apoptotic properties *in vivo* and *in vitro*. Curiously thioperamide may counter-act the histamine-induced elevation in pAKT and pERK (Grazia et al., 2003; Bongers et al., 2007). These effects of thioperamide suggest that the drug may have mild pro-apoptotic effects. However, thioperamide may also facilitate GABAergic transmission (GABA is an inhibitory neurotransmitter), and thereby may be neuroprotective by simply reducing brain activity (Dai et al., 2006). The molecular events that occur as a result of combined thioperamide and L-

histidine treatment remain unclear. We did not pursue the molecular mechanisms because of the negative results of this study.

In this study we modified the SBI model slightly to include bipolar electrocautery at the cut tissue edge. Electrocautery is frequently used in human patients, and we used electrocautery in our study to better-simulate an actual neurosurgical resection in human patients. Prior iterations of this model included surgical excision of brain tissue with temporary gauze packing but no electrocautery to achieve hemostasis (Matchett et al., 2006; Jadhav et al., 2007). The effect of bipolar electrocautery was somewhat irregular in terms of tissue damage (Figure 1), and this likely increased the heterogeneity of the histological results. Additionally electrocautery may have exacerbated BBB breakdown and brain edema. These effects will require further study in the future. Based on our study it seems that BBB breakdown and brain edema constitute a major pathophysiological response to surgical brain injury, whereas apoptotic cell death plays a relatively minor role. Future therapies that emphasize protection of the BBB and decreased brain edema may improve outcomes in this model.

We conclude that combined treatment with L-histidine and thioperamide results in worse outcomes after surgical brain injury. Combined treatment with L-histidine and thioperamide is associated with increased BBB breakdown and increased brain edema.

4. Methods

4.1. Surgical brain injury model

In this study we used a variation of the surgical brain injury (SBI) model described in previous reports (Matchett et al., 2006; Jadhav et al., 2007; Lo et al., 2007). The surgical protocol was approved by the Institutional Animal Care and Use Committee (IAUCC) at Loma Linda University. 92 male CD1 mice (Harlan Corporation, Indianapolis, IN) were housed in a climate-controlled environment with strict day / night light cycles prior to surgery. Prior to surgery general anesthesia was induced with Ketamine (80 mg/kg intraperitoneally) and xylazine (10 mg/kg intraperitoneally). Spontaneous ventilation without airway management was permitted by this anesthetic combination. After induction of general anesthesia mice were placed in the prone position in a Benchmark Stereotaxic frame under a surgical operating microscope. Scalp fur and skin were cleaned and prepared in a sterile manner. A No 11 blade was used to incise the skin down to the skull through a single sagittal incision. The periosteum was reflected to expose the right frontal skull. Using the bregma as a landmark, a small square of skull (approx 4 mm × 4 mm) was thinned and removed with a bone drill. The dura was excised with a no. 22 gauge needle. The entire right frontal lobe anterior to the bregma was excised by sharp dissection and electrocautery. The resection was carried down to the skull base. Preliminary studies were conducted to demonstrate the consistency of the size of resection based on the weight of the removed specimen. Once the brain tissue was excised, intraoperative packing and saline irrigation along with brief (~1 second) bipolar electrocautery application to the cut surfaces was used to control bleeding. Hemostasis was confirmed by close observation after removal of packing. After hemostasis was assured the skin was closed with 3-0 silk suture (Ethicon Inc, Cornelia, GA, USA). Sham surgery included general anesthesia, skin incision, and craniotomy but no dural incisions. Preliminary studies were conducted to assure consistency of the resected brain weight (Matchett et al., 2006).

4.2. Treatment Methods

Immediately following the conclusion of surgery but before the end of general anesthesia L-histidine (1000 mg / kg IP, Sigma-Aldrich, St. Louis, MO) and thioperamide (5 mg / kg IP, Sigma-Aldrich, St. Louis, MO) were administered to the treatment group (SBI + H). The non-treatment group (SBI) received an intraperitoneal injection of the carrier solutions (5 mL / kg

of 5% DMSO in 0.9% Normal Saline). Postoperatively all animals received an injection of 0.9% normal saline (30 mL / kg) to prevent postoperative dehydration. Similar combined dosing regimens have been described in previous studies (Adachi et al., 2006; Motoki et al., 2005).

4.3. Histology and Immunohistochemistry

Mice were euthanized under anesthesia by transcardiac perfusion of phosphate buffered saline (PBS, Sigma-Aldrich, St. Louis, MO) and 4% phosphate buffered formalin (Fisher Scientific, Pittsburgh, PA). Brain tissue was extracted whole and allowed to fix in 4% phosphate buffered formalin for at least 7 days. Brains were subsequently dehydrated through a series of alcohols and placed in 4% phosphate buffered formalin. Sagittal slices were taken and placed on slides (Richard Allan Scientific, Kalamazoo, MI). Nissl histology and immunohistochemical / fluorescent studies of formalin-fixed, paraffin embedded tissue followed standard protocols (Matchett et al., 2007). Prior to immunohistochemical and immunofluorescent studies antigen retrieval was undertaken by microwave irradiation in 0.1 M sodium citrate, pH 6, for 10 minutes. The commercially available ABC kit (Santa Cruz Biotech, Santa Cruz, CA) was used for IgG staining (1:200, biotin conjugated goat anti mouse IgG, sc-2039, Santa Cruz Biotech). Standard immunofluorescent protocols were followed for Cleaved Caspase 3 staining (1:100, rabbit anti cleaved caspase 3, #9661, Cell Signaling Technology, Boston MA) along with appropriate secondary antibody (Jackson Immuno Research, West Grove, PA).

4.4. Brain Water Content

Mice were euthanized under anesthesia and brains were removed and separated into separate hemispheres and sub-regions. Samples were immediately weighed on a high-precision balance. Brain samples were then dried for 48 hours at 105 C and then weighed again. Brain water content for each hemisphere was calculated using the formula $([\text{wet weight} - \text{dry weight}] / \text{wet weight}) \times 100\%$ (Matsuo et al., 2001; Shimamura et al., 2006). At 24 hours, SBI n=12, SBI + H n=14, Sham n=9. At 72 hours, SBI n=8 and SBI + H n=6.

4.5. Evans Blue Blood-Brain Barrier Assessment

BBB permeability was measured by the previously reported technique of measurement of Evans blue dye extravasation (Saria et al., 1983; Shimamura et al., 2006). Under general anesthesia Evans blue dye (4% 2.5 mL / kg) was injected intravenously and allowed to circulate for 1 hour. This was followed by perfusion with PBS (20 mL) via the aorta. The brains were subsequently removed and divided into hemispheres. Samples were homogenized and protein was precipitated and quantified spectrophotometrically (Genesis 10uv; Thermo Fisher Scientific, Waltham, MA). For SBI n=7, for SBI + H n=5, for Sham n=6.

4.6. Neurobehavioral Testing

A modified 21-point Garcia scoring system (Garcia et al., 1995, Shimamura et al., 2006B) was used to assess neurological function in the mice 24 and 72 hours after surgery. The scoring system was applied to mice by a blinded observer. Sensorimotor testing was graded on a scale from 0–3 in seven areas: spontaneous activity, side stroking response, vibrissae response, limb symmetry when suspended by tail, lateral turning when suspended by tail, symmetry of walking on forelimbs when partially suspended by tail, climbing ability/response. Neurological scores were assigned as follows: 0, complete deficit; 1 definite deficit with some function; 2, mild deficit or decreased response; 3 no evidence of deficit/symmetrical responses. The theoretical maximum score on this scale is 21 points. At 24 hours SBI n=19, SBI + H n=19, and Sham n=14. At 72 hours SBI n=8, SBI + H n=6.

4.7. Statistical Analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM). All statistical analysis was performed using a commercially available computer program (Sigma Stat, SyStat Software, Richmond, CA). Where applicable $p < 0.05$ was considered statistically significant. Analysis of variance (ANOVA) was used to compare the three groups at 24 and 72 hours for the brain water content and Evan's blue studies (Sham values at 24 hours were used for the ANOVA calculation at 72 hours where applicable). A Chi-squared test was used for analysis of mortality.

Acknowledgements

This study was partially supported by NIH NS052492 to JT and by NS53407 to JHZ.

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Figure 1.

A) Sagittal view of whole brain (left) and Nissl-stained (right) specimens from the surgical brain injury model in mice on postoperative day 1. The model entails right frontal lobectomy via craniotomy. Scale bar = 1mm. **B)** Nissl photomicrographs of tissue at the periphery of the resection site, as indicated by the box. Tissue breakdown with sloughing of tissue at the resection site is evident. Scale bar = 100 micrometers. **C)** Nissl photomicrographs of indicated regions at high power. Scale bar = 100 micrometers (large images). Scale bar = 50 micrometers (inset images).

Figure 2.

A) Brain Edema. Brain water content was found to be elevated at 24 hours in the ipsilateral and contralateral hemispheres after surgical brain injury. At 72 hours after surgical brain injury, treatment with L-histidine and thioperamide was associated with significantly higher brain water content in the contralateral hemisphere. * = significant difference compared to the Sham group, # = significant difference compared to the SBI group, comparisons made with ANOVA, $p < 0.05$. **B)** Blood-brain barrier breakdown. Blood-brain barrier breakdown was highest in the ipsilateral hemisphere in the L-histidine and thioperamide treatment group (SBI + H). * = significant difference compared to the Sham group, # = significant difference compared to the SBI group, comparisons made with ANOVA, $p < 0.05$. **C)** Neurological Scores. Neurological scoring by blinded investigator revealed persistent deficits in both the SBI and SBI + H groups at 24 and 72 hours after surgery. * = significant difference compared to the Sham group, comparisons made with ANOVA, $p < 0.05$.

Figure 3.

A) IgG Stain. Immunohistochemical stain for IgG demonstrated qualitatively greater leak of IgG (brown) in the SBI+H group. IgG leak was also present in the SBI group. No IgG leak was detected in the Sham group. **B).** Cleaved Caspase 3. Stain for Cleaved Caspase 3 demonstrated positive cells in both the SBI and SBI + H groups, qualitatively equivalent. Sham group had no evidence of apoptosis.