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Effects of traumatic brain injury on intestinal contractility

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Abstract

Background—Patients with traumatic brain injury (TBI) often suffer from gastrointestinal dysfunction including intolerance to enteral feedings. However, it is unclear how TBI affects small intestinal contractile activity. The purpose of this study was to determine if TBI affects intestinal smooth muscle function.

Methods—Sprague–Dawley rats were subjected to controlled cortical impact injury (TBI). Sham animals underwent a similar surgery but no injury (SHAM). Animals were sacrificed 1, 3, and 7 days after TBI and intestinal smooth muscle tissue was collected for measurement of contractile activity and transit, NF-κB activity, and cytokine levels. Brains were collected after sacrifice to determine volume loss due to injury.

Key Results—Contractile activity decreased significantly in ileum, but not jejunum, in the TBI group 7 days after injury compared with SHAM. Brain volume loss increased significantly 7 days after injury compared with 3 days and correlated significantly with the contractile activity 1 day after injury. In the intestinal smooth muscle, NF-κB activity increased significantly in the TBI group 3 and 7 days after injury vs SHAM. Wet to dry weight ratio, indicating edema, also increased significantly in the TBI group. Interleukin-1α, -1β, and -17 increased significantly in the TBI group compared with SHAM.

Conclusions & Inferences—Traumatic brain injury causes a delayed but significant decrease in intestinal contractile activity in the ileum leading to delayed transit. The decreased intestinal contractile activity is attributed to secondary inflammatory injury as evidenced by increased NF-κB activity, increased edema, and increased inflammatory cytokines in the intestinal smooth muscle.

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DISCLOSURES

No disclosures.

AUTHOR CONTRIBUTION

AO performed the research and wrote the article; RH performed the research; HX performed the research; DB performed the research; KA performed the research and analyzed data; EJ performed the research and analyzed the data; SB performed the research; CC designed the research study; KU performed the research, designed the research study, analyzed the data and wrote the article.

Keywords

brain injury; inflammation; intestinal contractility

Current estimates suggest that as many as 5.3 million Americans live with long-term disabilities related to traumatic brain injury (TBI). The annual economic cost of TBI as it relates to direct medical and rehabilitation care is estimated at 60 billion dollars annually.¹ According to the Center for Disease Control and Prevention, more than 50 000 Americans died annually from isolated head trauma, affecting mostly the very young (<5 years old) and elderly (>75 years old). In children ranging from 0 to 19 years of age, injury is the leading cause of death with up to 50% of injury-related deaths being associated with TBI.² Despite these impressive statistics, treatment of TBI is limited to controlling intracranial pressure (ICP), optimizing cerebral perfusion pressure (CPP), and minimizing hypoxia to prevent secondary brain injury.³

Traumatic brain injury can lead to several physiologic complications including gastrointestinal dysfunction. Traumatic brain injury patients often have some level of feeding intolerance manifested by vomiting and abdominal distension.^{4,5} A literature review by Krakau showed that a majority of patients with moderate to severe traumatic brain injury have upper gastrointestinal intolerance in the first few weeks after injury.⁴ Feeding intolerance in TBI patients is caused by impaired gastrointestinal motility and reduced gut absorption.⁵ Motility disorders in brain injured patients include gastroesophageal reflux related to reduced tone in the lower esophageal sphincter and delayed gastric emptying.^{6,7} Gastrointestinal dysfunction including motility disorders can lead to increased septic complications in TBI patients.^{8,9} Furthermore, the nutritional status and drug absorption of TBI patients, critical for recovery, is often compromised due to gastrointestinal dysfunction.^{10,11}

Although dysmotility in upper gastrointestinal tract has been documented in both patients and animal models of brain injury, the effects of brain injury on small intestinal motility are not well understood. Wang *et al.* recently showed that TBI slowed intestinal transit¹²; however, the effects of TBI on intestinal smooth muscle contractility are unknown. Inflammation of the mucosal layer of the small intestine leading to increased permeability has been demonstrated suggesting inflammatory damage to the small intestine^{12,13}; however, whether the intestinal smooth muscle layers also incur inflammatory damage after TBI is unknown. The purpose of the study was to determine the effects of TBI on intestinal contractility and motility and the role of inflammation in mediating these effects. In this study, we demonstrate that TBI induced a decrease in both intestinal contractility and transit and an increase in inflammation in the intestinal smooth muscle suggesting that motility is inhibited in the small intestine due to inflammatory damage secondary to the brain injury.

MATERIAL AND METHODS

Animal model of TBI

All procedures were approved by the University of Texas Medical School Institutional Animal Care and Use Committee and are consistent with the NIH 'Guide for the Care and Use of Laboratory Animals'. Male Sprague–Dawley rats weighing between 250 and 350 g were used for all experiments. The scalp was shaved and cleaned with betadine/isopropanol to prevent infection. After infiltration of the surgical site with bupivacaine, a midline incision was made and the soft tissue was reflected to expose the skull. A burr hole 6–7 mm in diameter, 1 mm lateral, and 1 mm posterior to the bregma was created to expose the dura mater through a vertical incision over the cranium. The rats were mounted in the injury

device stereotaxic frame in a prone position. Rats were subjected to unilateral controlled cortical injury (CCI) at a 3.1-mm impact depth and an impact velocity of 5.0 m s^{-1} with a dwell time of 150 ms using a 5 mm blunt tip (eCCI Model 6.3; VCU, Richmond, VA, USA).^{14,15} Sham injured animals underwent surgery to expose the bony skull, but no craniectomy or CCI was performed. After injury, the scalp was close using sterile suture. Animals were monitored daily after surgery. No postsurgery infections occurred. At time of sacrifice, intestines were collected for contractility measurements. The rats were then perfused transcardially with phosphate buffer solution (PBS) followed by 4% paraformaldehyde (PFA). Decapitation/extraction of the brain was carried out and the brain was placed in 15 mL of PFA solution.

Intestinal contractility

Intestinal contractile activity was measured 1, 3 and 7 days after TBI as described previously.¹⁶ Full thickness intestinal strips (~10 mm in length) from ileal or jejunal sections of the small intestine, two from each section, were mounted (in the intestinal longitudinal axis orientation) in 25-mL organ baths filled with Krebs solution (in mmol L^{-1} : 103 NaCl, 4.7 KCl, 2.5 CaCl_2 , 25 NaHCO_3 , 1.1 NaH_2PO_4 , 15 glucose). The solution was buffered with albumin to avoid edema formation during incubation in the tissue chamber and gassed with 5% CO_2 –95% O_2 . Isometric force was monitored by an external force displacement transducer (Experimetria Ltd., Budapest, Hungary) connected to a PowerLab (AD Instruments, Colorado Springs, CO, USA). Each strip was stretched to 0.5 g tension and allowed to equilibrate for 30 min. After equilibration, 10 min of basal contractile activity data were recorded. After recording contractile activity, length of each strip was measured and tissue was removed, dried and weighed. Contractile activity parameters were all calculated over 5 min of recorded data. Total contractile activity was calculated as the area under the curve. Basal tone was defined as the average minimum of the contraction cycle. Amplitude was calculated as average cycle height. All force development was normalized to tissue cross-sectional area. All measurements were performed in duplicate on two separate intestinal strips and averaged.

Intestinal transit

Intestinal transit was measured at 7 days postinjury in a subset of animals. At the time of injury, a silastic catheter for transit measurements was introduced into the proximal duodenum of rats via vertical laparotomy incision. The catheter was then tunneled through the musculature of the left abdominal wall and subcutaneous tissue and externalized behind the neck.

A solution of 70 kD non-absorbable fluorescein isothiocyanate (FITC) Dextran ($150 \mu\text{L}$) was injected into the duodenum via the catheter. Forty-five minutes after administration of FITC Dextran animals were sacrificed. The entire small intestine was removed. The small intestine was divided into 10 equal segments and each segment was flushed with 3 mL of 10% mmol L^{-1} Tris-buffer solution (TBS). Spectrophotometry (490 nmol L^{-1}) was used to determine FITC-Dextran concentrations in each sample and expressed as absorbance units (AU).

Intestinal tissue water

Intestinal samples were weighed immediately after collection. After drying in a 65°C oven, samples were weighed again. Wet to dry weight ratio was calculated as $[(\text{wet weight}) - (\text{dry weight})]/\text{dry weight}$.

Histology and mucosal injury scoring

Ileal and jejunal segments harvested from rats in each of the groups (sham, 1, 3 and 7 days post TBI) were snap frozen at -80°C and stored in 10% formalin until processing. Tissue was embedded in paraffin blocks, sectioned in $7\text{ }\mu\text{m}$ slices, placed on glass slides, and stained with hematoxylin and eosin. Light microscopic evaluation of the tissues was performed in a blinded fashion and scored using a system described by Chiu *et al.*: grade 0 = normal mucosa, grade 1 = subepithelial space developing at the tip of the villus, grade 2 = lifting of the epithelial layer from the lamina propria and moderate extension of the subepithelial space, grade 3 = some denuded tips of the villi and massive lifting of the epithelial layer, grade 4 = dilated and exposed capillaries and denuded villi, and grade 5 = hemorrhage, ulceration, and disintegrated lamina propria.¹⁷

Nuclear factor $-\kappa\text{B}$ assay

Nuclear Factor-kappa B (NF- κB) activation was measured in nuclear extracts using a Transcription Factor Assay Kit (Active Motif, Carlsbad, CA, USA) following manufacturer's directions. Briefly, intestinal smooth muscle nuclear extracts from each group were added to each well of a 96 well plate in which oligonucleotide containing the NF- κB consensus sequence was immobilized. After incubation with lysates, a NF- κB p65 antibody was added followed by incubation with a secondary antibody conjugated to the horse radish peroxidase (HRP) enzyme. A colorimetric HRP substrate was then added. The colorimetric reaction was stopped with oxalic acid. Wells were washed after each incubation period. The plate was read at 450 nm wavelength. A recombinant P65 standard was used to generate a standard curve. Specificity of the assay was confirmed by competition with wild-type or mutant NF- κB consensus site oligonucleotides. Each sample was assayed in duplicate and normalized to total nuclear protein.

Brain cavity volume loss

X-ray-based Computed Tomography (CT) imaging was carried out at the Department of Internal Medicine Preclinical CT Imaging Core Lab at The University of Texas Health Science Center at Houston. The eXplore Locus Ultra Preclinical CT Scanner (GE Healthcare, London, ON, Canada) is a high-resolution cone-beam CT system engineered for small animal imaging. To collect brains, rats were perfused transcardially with PBS followed by 4% PFA. Decapitation/extraction of the brain was carried out, and the brain was placed in 15 mL of PFA solution. The brains were patted dry with gauze and placed on a foam holder to best allow isolation of the brain in the CT image. Each scan was comprised of a 70 kVp, 50 mA, 16 s single rotation producing 1 000 projection views. The scans were reconstructed into volume files made up of 0.154 mm isotropic voxels. Analysis was carried out on Microview Software (ABA 2.2, GE Healthcare). A region grow plugin isolated each brain, based on voxel CT value threshold (-500 HU). This threshold value was selected to incorporate the maximum amount of brain tissue without also incorporating non-brain elements in the image, such as foam and air. An isosurface rendering, also based on a -500 HU value, was generated to produce a three dimensional (3D) image depicting the surface of each brain. Total brain volume was recorded in mm^3 . Brain image sets were analyzed for volume loss using a volume reconstruction program (Simpleware Ltd., Exeter, UK) to create a 3-dimensional mesh of the injured brain. Then, 3D CAD software (SolidWorks, Dassault Systems, Vélizy-Villacoublay, France) was used to measure cavity volume. A surface that matched the contour of brain tissue surrounding the injury site was made, creating a 'cap' over the injury. Extruding a volume across the cavity, then subsequently trimming the volume with the surface of the brain and the 'cap' resulted in a final solid volume that could be measured by the program's 'mass properties' feature.

Cytokine array

A rat cytokine array was utilized to measure cytokine and chemokine levels in the rat ileal smooth muscle (R&D Systems, Minneapolis, MN, USA) following manufacturer's directions. In the cytokine array kit, capture antibodies for the following 29 cytokines and chemokines are immobilized on nitrocellulose membrane: Cytokine-induced neutrophil chemoattractant (CINC)-1, CINC-2 α/β , CINC-3, ciliary neurotrophic factor (CNTF), fractalkine, granulocyte macrophage colony-stimulating factor (GM-CSF), soluble intercellular adhesion molecule (sI-CAM)-1, interferon (IFN)- γ , interleukin (IL)-1 α , IL-1 β , IL-1 receptor agonist (ra), IL-2, IL-3, IL-4, IL-6, IL-10, IL-13, IL-17, interferon gamma-induced protein (IP)-10, lipopolysaccharide-induced CXC chemokine (LIX), L-selectin, monokine induced by interferon-gamma (MIG), macrophage inflammatory protein (MIP-1 α), MIP-3 α , regulated upon activation normal T-cell expressed (RANTES), thymus chemokine, tissue inhibitor of metalloproteinase (TIMP)-1, tumor necrosis factor (TNF)- α , and vascular endothelial growth factor (VEGF).

Cytoplasmic extracts from smooth muscle obtained from the distal small intestine were prepared. Cytoplasmic extracts (200 μ g) were diluted to the required volume and incubated with biotinylated detection antibodies for 1 h at room temperature. After blocking the membrane, the sample/detection antibody mixture was incubated on the membrane overnight at 4 °C. The membrane was then washed followed by incubation with streptavidin-HRP. After incubation with the chemiluminescent reagent, the membrane was exposed to X-ray film and developed. Spots were quantitated using ImageJ.¹⁸

Statistical analysis

Values are expressed as mean $\pm$ SEM. The statistical significance among groups was determined by *t* test or analysis of variance followed by Fisher LSD post hoc analysis where appropriate, and a *P* < 0.05 was considered statistically significant.

RESULTS

Intestinal contractility

Contractile activity was measured in ileal segments of the small intestine from sham and TBI groups at 1, 3 and 7 days postsurgery. As shown in Fig. 1A, contractile activity was significantly decreased in the TBI group 7 days postinjury compared with the sham group (18.47 ± 3.49 vs 28.08 ± 9.57 , *P* < 0.01). There were no significant differences in contraction frequency (Fig. 1B). Average contraction amplitude (Fig. 1C) was significantly decreased 7 days after injury (0.059 ± 0.0083 vs 0.087 ± 0.012 , *P* < 0.05) and basal tone (Fig. 1D) was significantly decreased 3 days after TBI (0.55 ± 0.057 vs 0.74 ± 0.070 , *P* < 0.05).

At 7 days postinjury, contractile activity was measured in both the jejunum and ileum. As shown in Fig. 2A, contractile activity was significantly decreased in the ileum (18.47 ± 1.42 vs 29.44 ± 2.92 , *P* < 0.05) compared with the jejunum in the TBI group, but not in the SHAM group. As expected, frequency of contractions was significantly decreased in ileal segments compared with jejunal segments in the Sham group (0.61 ± 0.11 vs 0.41 ± 0.01 , *P* < 0.01). In contrast, there were no significant differences in contraction frequency in the TBI group (*P* = 0.76; Fig. 2B). Frequency of contraction in the jejunum from the TBI group tended to be lower than in the sham (*P* = 0.09); however, these differences did not reach statistical significance. As shown in Fig. 2C, contraction amplitude was significantly decreased in the ileum compared with the jejunum in the TBI group (0.06 ± 0.01 vs 0.10 ± 0.02 , *P* < 0.05) but not the sham group. No differences were seen in basal tone at 7 days postinjury (Fig. 2D).

Intestinal transit

Intestinal transit was measured in both the TBI and SHAM groups 7 days after injury as shown in Fig. 3. As contractile dysfunction was predominantly limited to the distal small intestine, dye concentrations in the intestine were measured 45 min after dye injection (instead of the standard 30 min) to allow dye to pass into the distal intestine. Although geometric center of dye distribution were not significantly different between the SHAM and TBI groups, total dye in the small intestine was significantly increased in the TBI group compared with the SHAM group indicating less transit of dye into the large intestine in the TBI group (Fig. 3A and B).

Chiu score

Mucosal injury was quantitated using the Chiu scoring system as judged by a blinded investigator. Representative images from 7 days postinjury in sham and TBI groups are shown in Fig. 4A and B. Chiu scores in all samples from 1, 3 and 7 days postinjury were between 0 and 2 indicating very little mucosal damage. Although mucosal damage was detected in several of the intestinal samples from injured animals, the differences between Sham and TBI groups was not significant.

Brain cavity volume loss

Traumatic brain injury brains were scanned using a CT scanner and volume loss due to TBI was calculated. All brains in the TBI group developed some cavity volume loss. Sham brains were not scanned as there was no injury and therefore no cavity volume loss. Representative 3D reconstructions for each time point are shown in Fig. 5A. As shown in Fig. 5B, significantly more volume loss occurred 7 days postinjury compared with 3 days postinjury (0.046 ± 0.0050 vs 0.026 ± 0.0042 , $P < 0.05$, respectively). There was a negative correlation between brain volume loss and intestinal contractile activity 1 day postinjury ($R = 0.83$, $P = 0.004$) but not in the 3 and 7 day postinjury (Fig. 5C).

NF- κ B activity and edema

Nuclear Factor-kappa B activation was measured in nuclear extracts from SHAM and TBI groups 1, 3 and 7 days postinjury. At 3 and 7 days postinjury, NF- κ B activation was significantly higher in the TBI group compared with the respective sham groups as shown in Fig. 6A (TBI vs SHAM, 2.35 ± 0.17 vs 1.73 ± 0.15 and 1.92 ± 0.073 vs 1.65 ± 0.087 , $P < 0.05$).

As shown in Fig. 6B, wet to dry weight ratios indicating the development of intestinal edema measured 7 days after injury were increased significantly in the TBI group compared with the SHAM group (3.56 ± 0.05 vs 3.42 ± 0.05).

Cytokines

A panel of cytokines and chemokines were measured in the ileal smooth muscle in both the SHAM and TBI groups 3 and 7 days after injury. Interleukin-1 α and -1 β were both significantly increased in the TBI group 7 days after injury compared with the SHAM group (SHAM vs TBI, 0.60 ± 0.30 vs 1.38 ± 0.13 and 0.15 ± 0.31 vs 1.08 ± 0.16 , respectively), as shown in Fig. 7A and B. Interleukin-1 α was also significantly increased in the 7 Day TBI group compared with the 3 days TBI group (TBI 7 days vs 3 day, 0.60 ± 0.30 vs 0.50 ± 0.02). Interleukin-17 also increased significantly in the TBI group 7 days after injury compared with the SHAM group (SHAM vs TBI, 1.33 ± 0.54 vs 2.58 ± 0.39) and compared with the 3 day TBI group (TBI 7 days vs 3 day, 2.58 ± 0.39 vs 0.87 ± 0.05 ; Fig. 6C). There were no significant differences in Interleukin-1 α and interleukin-1 β between days 3 and 7 in the SHAM group. There were no significant increases in any inflammatory mediators

measured in the TBI group at 3 days postinjury compared with the SHAM group; however, IL-6 and MIG increased significantly at 7 days in the SHAM group compared with the 3 days time point and compared with the TBI group at 7 days (data not shown). There were no other significant increases in any of the other cytokines and chemokines measured (see list in Methods section).

DISCUSSION

Most studies on gastrointestinal dysfunction after TBI have focused on the upper gastrointestinal tract including delayed gastric emptying and esophageal reflux. In this study, we show that motility in the small intestine is also affected by TBI. Decreased intestinal contractile activity may contribute to the symptoms of vomiting and abdominal distension described in patients after TBI. Our study shows that moderate TBI with no substantial mucosal damage causes a delayed but significant decrease in intestinal contractile activity. Contractile activity was significantly decreased primarily in the ileum due to decreased contraction amplitude. Furthermore, intestinal transit was decreased after TBI. Altered contractile activity and motility in the small intestine was associated with increased inflammation in the intestinal smooth muscle. Interestingly, there was a significant negative correlation of brain volume loss vs contractile activity (i.e. larger cavity volumes correlated with decreased contractile activity) at 1 day postinjury but not at 3 and 7 days suggesting a correlation between decreased contractile activity and increased severity of injury.

Contractile dysfunction occurred predominantly in the ileum; although jejunal contraction frequency was also decreased. The reason for the dysfunction in the distal small intestine is unclear. In humans, the ileum contains a higher density of lymphoid tissue and we speculate that the increased lymphoid tissue in the ileum may leave the distal intestine more vulnerable to inflammatory injury via systemic inflammation; however, it is unclear if this is true in the rat intestine, as lymphoid tissue distribution varies between species and strains.¹⁹ The distal small intestine is also subjected to increased bacterial load. Thus, even slight mucosal damage may cause inflammatory damage to the intestine.

The degree of TBI in our model was relatively moderate. We have shown that this level of injury will result in little or no motor defects and moderately impaired spatio-temporal memory (measured as latency to platform in the Morris water maze).²⁰ Although mucosal injury is a well-documented phenomenon in traumatic brain injury, we detected only minor mucosal damage in several samples and the differences between groups did not reach statistical significance (Fig. 4). It should be noted that the use of Chiu scores to detect mucosal damage is a relatively insensitive method and will only detect mucosal structural damage and not mucosal inflammation. In contrast to our data, published studies have shown that TBI induces mucosal damage.^{13,21} Previous experimental models including a weight drop mechanism for traumatic brain injury may cause a more widespread, severe injury compared to our method of controlled cortical impact.^{12,13,22,23} The difference in the degree of TBI with the weight drop model compared to the controlled cortical injury utilized in this study may explain differences in mucosal injury. Despite the apparent moderate degree of injury with little or no mucosal damage, intestinal contractile activity was decreased.

We hypothesize that the decreased intestinal motility induced by TBI was triggered by inflammation for several reasons. Firstly, we have shown that NF- κ B activation and edema are increased in the intestinal smooth muscle indicating inflammation (Fig. 6). Secondly, we showed that inflammatory mediators including IL-1 α and -1 β and IL-17 are increased in the intestinal smooth muscle of injured animals 7 days after injury at the same time as decreased contractile function (Fig. 7). Finally, intestinal dysfunction was delayed and coincided with

secondary damage at the brain injury site. As shown in Fig. 5, volume loss increased at 7 days postinjury compared with earlier time points and systemic inflammation is one of the key elements involved in this secondary damage after brain injury.²⁴ After a traumatic brain injury, there is neuronal loss due to necrosis and apoptosis in the damaged area. This is followed by a delayed secondary loss of neurons in the area surrounding the injury site due to apoptosis. This secondary phase is thought to be mediated by systemic inflammation due to increased chemokine production in the liver.^{25–28} Systemic inflammation induced by brain injury has been associated with damage to peripheral organs, including the heart, lung, liver, kidneys and gut.^{29–31} In addition, alterations in communication between the brain and the enteric and central nervous systems have been implicated in mediating peripheral organ damage after TBI.²¹ Acute brain injury is thought to induce sympathetic neural stimulation that mediates release of inflammatory cytokines in peripheral organs including the gut.^{32,33} In summary, our data suggest that the decreased intestinal motility after TBI was likely due to systemic inflammation secondary to the brain injury.

The effects of inflammation on intestinal motility are unclear with reports of both hypomotility and hypermotility in response to inflammation. There is some evidence that the type of response to inflammation and immune activation will affect the intestinal response.³⁴ A Th1 immune response with the release of proinflammatory cytokines is associated with decreased motility; whereas a Th2 response is associated with increased intestinal motility.^{35–39} To determine the nature of inflammation in the intestinal smooth muscle after TBI, we measured a panel of pro- and anti-inflammatory cytokines and chemokines. IL-1 α , IL-1 β and IL-17 were all increased in the TBI group compared with the SHAM group. Although the cytokine profile does not indicate any clear changes in TH1 or TH2 responses, the increased IL-1 α , IL-1 β and IL-17 indicate increased inflammation in the intestinal smooth muscle, possibly through a TH17 response. TH17 cells have been implicated in chronic tissue inflammation⁴⁰ and may contribute to the development of intestinal smooth muscle dysfunction after TBI; however, more experiments are needed before any conclusions can be drawn.

Several inflammatory cytokines, including IL-1 β , signal through NF- κ B and are often increased in peripheral organs after a traumatic injury.³² NF- κ B has been shown by our group and other investigators to be associated with decreased intestinal smooth muscle contractility.^{37,41–44} We have previously shown that intestinal edema induces increased NF- κ B activation in the smooth muscle and inhibition of NF- κ B attenuated edema-induced decreases in intestinal contractile activity.⁴⁴ Other investigators have shown that NF- κ B activation by TNF α suppresses cell contractility via induction of ICAM-1.⁴³ In an animal model of Crohn's disease, NF- κ B was shown to decrease colonic circular smooth muscle contractility.³⁷ Treatment of rabbit colonic smooth muscle cells with IL-1 β attenuates acetylcholine-stimulated contraction via NF- κ B mediated up-regulation of RGS4 (Regulator of G-protein signaling 4) expression and down regulation of CPI-17.^{41,42} Thus, NF- κ B is clearly associated with decreased intestinal contractility and may mediate decreased intestinal contractile activity after TBI.

In summary, TBI causes a delayed but significant decrease in intestinal contractile activity predominantly in the distal intestine. The decreased intestinal contractile activity is likely caused by inflammation secondary to the injury. Gastrointestinal motility problems can delay enteral feeding, alter drug absorption, and interfere with treatment of TBI. More studies are needed to understand the mechanism by which TBI induces intestinal contractile dysfunction and the type of inflammatory response evoked by TBI in the intestinal smooth muscle to design optimal treatment strategies to improve intestinal motility.

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Figure 1.

Contractile activity in the ileum 1, 3 and 7 days after SHAM surgery or TBI. (A) Total contractile activity. (B) Frequency of contractions. (C) Contraction amplitude. (D) Basal tone. All data shown as mean with SEM. ($n = 5$ and 7 for SHAM and TBI at 1 day; $n = 5$ and 7 for SHAM and TBI at 3 days; $n = 14$ and 12 for SHAM and TBI at 7 days; *, $P < 0.05$ vs SHAM) (TBI, Traumatic brain injury).

Figure 2.

Contractile activity in the ileum and jejunum 7 days after SHAM surgery or TBI. (A) Total contractile activity. (B) Frequency of contractions. (C) Contraction amplitude. (D) Basal tone. All data shown as mean with SEM. ($n = 14$ and 12 for SHAM and TBI, respectively; *, $P < 0.05$ vs SHAM; +, $P < 0.05$ vs Jejunum) (TBI, Traumatic brain injury).

Figure 3. Intestinal transit was measured in both the TBI and SHAM groups 7 days after injury. (A) Marker concentration in each segment is shown for three animals in the TBI group and four in the SHAM group. (B) Total dye concentration in the small intestine measured 45 min after injection of FITC-Dextran (70 kD) into proximal duodenum. Data are shown as mean + SEM. ($n = 4$ in SHAM group and $n = 3$ in TBI group; *, $P < 0.05$) (TBI, Traumatic brain injury).

Figure 4. Representative hematoxylin and eosin stained sections of ileum from SHAM (A) and TBI (B) groups 7 days after injury. (bar shows 500 μ m; Paraffin embedded, Magnification 10 $\times$).

Figure 5. Brain volume loss after injury in TBI group. (A) Representative 3D images showing cavity loss 1, 3 and 7 days after TBI. (B) Average volume loss from each day (mean with SEM; $n = 7, 12$ and 10 for days 1, 3 and 7, respectively). (C) The negative correlation between volume loss and contractile activity in the ileum 1 day after injury in the TBI group ($R^2 = 0.83$, $P = 0.004$).

Figure 6.

(A) NF-kB DNA binding activity in nuclear lysates from SHAM and TBI groups 1, 3 and 7 days after injury. Means + SEM are shown. ($n = 6$ and 6 for SHAM and TBI for 1 day; $n = 11$ and 16 for SHAM and TBI for 3 day; $n = 11$ and 10 for SHAM and TBI for 7 day; *, $P < 0.05$ vs SHAM) (B) Wet to dry weight ratios indicating interstitial fluid accumulation in SHAM and TBI 7 days after injury. ($n = 8$ and 9 for SHAM and TBI; *, $P < 0.05$ vs SHAM).

Figure 7.

The following cytokine levels in SHAM and TBI groups 3 and 7 days after injury are shown: (A) Interleukin-1 α ; (B) Interleukin -1 β ; (C) Interleukin -17. Twenty-nine different inflammatory mediators were measured using a cytokine antibody array. No significant increases were detected in any other cytokines in the TBI group. Mean + SE are shown. ($n = 3$ per group; *, $P < 0.05$ vs SHAM; +, $P < 0.05$ vs 3 Day).