Increased Intestinal Permeability in Rats Subjected to Traumatic Frontal Lobe Percussion Brain Injury

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Background: **Dysfunction of the gastrointestinal tract is a common occurrence after traumatic brain injury (TBI). We hypothesized that increased intestinal permeability may result from a precisely controlled percussion injury to the exposed brains of anesthetized rats and that such an effect could be assessed in vitro using excised intestinal mucosae mounted in Ussing chambers.**

Methods: **After craniotomy over the left medial prefrontal cortex on anesthetized rats, neurotrauma was produced using a pneumatically driven impactor on the exposed brain. Control rats were subjected to identical procedures but did not receive an impact. Muscle-stripped rat intestinal ileal and colonic segments were mounted in Ussing chambers within 30 minutes of death. Transepithelial electrical resistance (TEER) and the apparent permeability coefficient (Papp) of [^{14}C]-mannitol were recorded from intestinal tissue for 120 minutes. Histopathologic analysis was also performed to determine any gross morphologic changes in the intestine.**

Results: **Ileal and colonic mucosae showed no differences in TEER in ileum or colon of TBI rats compared with controls. The Papp of mannitol was significantly increased in ilea from rats previously exposed to TBI compared with controls. Histologic analysis showed gross changes to 50% of the ileal but not the colonic sections from TBI rats.**

Conclusion: **TBI results in significantly reduced ileal barrier function, most likely mediated by open tight junctions. For patients with acute head injury, this may have implications for subsequent oral absorption of nutrients. Systemic delivery of luminal endotoxins may contribute to multiple organ failure.**

Key Words: **Traumatic brain injury, Intestinal permeability, Gut-brain axis.**

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In established diseases of the inflamed bowel (e.g., Crohn's disease and ulcerative colitis), studies have demonstrated evidence of enhanced paracellular absorption in vivo[1] and in vitro.[2] Such permeability defects in inflammatory bowel disease patients are thought to contribute to the damaging influence of luminal contents including pathogenic and commensal bacteria.[3] Other examples of increased colonic permeability have also been described in intestinal mucosae from rats exposed to acute stress in response to elevated corticosteroids.[4,5] Furthermore, Saunders et al.[6] showed that isolated mucosae from rats subjected to short-term restraint or after exposure to cold also displayed jejunal barrier dysfunction, as indicated by increased conductance and permeability to the paracellular flux markers, mannitol, and ^{51}Cr-EDTA. Although these stressors impacted on intestinal permeability, they are not traumatic in nature.

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A significant proportion of road traffic crash victims comprise traumatic brain injury (TBI) fatalities and it is the major cause of death in people under the age of 19 in the industrialized world.[7,8] Dysfunction of the gastrointestinal tract (GIT) is a common occurrence after TBI.[9,10] Up to 50% of road traffic crash victims with acute TBI display intolerance to enteral feedings, which has been correlated with the severity of the brain injury.[11] Cross-talk between the central nervous system (CNS) and the small intestine is thought to contribute to pathogenesis.[12] The CNS communicates with the gut via afferent and efferent extrinsic nerves or connections to enteric nerves in the myenteric plexus of the GIT.[13] It is proposed that TBI sets off a sequence of local and systemic inflammatory and immune-amplified events, which may lead to multiple organ dysfunction syndrome (MODS). Several studies on TBI-related GIT dysfunction have concentrated on physiologic aspects on the GIT and have reported increased bleeding,[14] prolonged gastric emptying,[15] and proximal intestinal obstruction caused by duodenum compression.[16]

In response to TBI in rats, modulation of intestinal function may in part be explained by alterations in plasma and jejunal levels of humoral factors or of enteric peptide neurotransmitters, including vasoactive intestinal peptide, cholecystokinin and calcitonin-gene-related peptide.[17] In addition, up-regulated expression of ICAM-1 binding to the jejunum as well as increased nuclear factor kappa B (NF-κB) expression was detected in rats subsequent to TBI[18]. Increases in intestinal permeability may permit absorption of bacteria-derived lipo-polysaccharide (LPS)-endotoxin, which in turn may lead

to over-production of local inflammatory mediators, tissue destruction and sepsis.[19] Hang et al. recently demonstrated compromised intestinal histology at 72 hours post-TBI in rats and this was accompanied by increased intestinal permeability in vivo.[20] Furthermore, data denoting altered intestinal permeability has also been reported in patients with acute brain injury.[21]

Regional intestinal permeability data are lacking and there is considerable debate over the quantitative significance of the effect, whether it pertains in vitro, and how early it is apparent. Consequently, the purpose of this study was therefore to examine the effects of an acute severe head trauma or TBI on the paracellular permeability, electrophysiologic parameters, and histologic responses of both muscle-stripped ileal and colonic mucosae isolated from anesthetized rats subjected to a TBI 6 hours previously. The majority of brain injuries in humans are directed toward the forehead, analogous to the medial prefrontal cortex (mPFC) in the rat. Therefore, a precise TBI was delivered to the left mPFC of anesthetized rats in an attempt to part-replicate some of the damage that may be seen in humans.

MATERIALS AND METHODS

Animals

Twelve age-matched male Sprague Dawley rats weighing 300 to 350 g were purchased from Harlan Laboratories (UK). Animals were housed under controlled environmental conditions regarding temperature and humidity with a 12-hour light or dark cycle. They had free access to tap water and standard laboratory chow. Rats were maintained in accordance with National Institute of Health guidelines for the care and use of laboratory rats.

Rat Model of TBI

The rats were randomly divided into either TBI (n = 6) or control (n = 6) groups. After anesthesia by isoflurane inhalation (4%–2% in air at a rate of 400 mL/min), the animals were placed in a stereotaxic frame (David Kopf Instruments) and the head adjusted until the skull between the bregma and lambda was level. Using sterile techniques, a sagittal incision of the scalp was made along the midline from the level of the eyes to the occipital protuberance, exposing the frontal bones. In accordance with previously published methods,[22] a 4-mm diameter craniotomy was drilled through the skull over the mPFC, exposing the dura. A cortical contusion injury was performed on the left mPFC using a pneumatically driven vertical impactor. The device, consisting of a pneumatic cylinder mounted on an adjustable crossbar, was positioned above the left mPFC to provide a single impact by a 3.5-mm rounded impactor tip. Air pressure was set at 4 bar, and the depth of penetration determined by zeroing the piston to the cortical surface, withdrawing it, and then lowering it to the required impact deformation. The impact was of 1.2 m/s velocity producing a 2.62-mm deformation. Anesthesia was maintained for a further 5 hours 40 minutes before euthanasia by transcardial perfusion. Body temperature was continuously monitored and maintained at 37.5°C using a temperature controlled heating pad (CMA 150 Carnegie Medicine, Sweden). Control rats were treated exactly the same in all respects apart from not receiving an impact to the exposed dura. All live animal procedures were approved by the University College Dublin Animal Research Ethics Committee and performed under license number B100/3366 from the Irish Department of Health and Children. The current study therefore used postmortem intestinal tissue from animals that were undergoing TBI as part of a separate CNS project.

Rat Epithelial Ileal and Colonic Function: Ussing Chamber Studies

Rat ileal and colonic segments were immediately removed postmortem and placed in oxygenated Krebs-Henseleit (KH) buffer. They were opened along the mesenteric border, rinsed free of luminal contents and stripped of longitudinal and circular muscle layers and myenteric plexus. Two adjacent pieces of terminal ileum or of distal colon were mounted in Ussing chambers (World Precision Instruments, UK) with an exposed window surface area of 0.63 cm^2 in accordance with previous descriptions.[23] Epithelial sheets were bathed bilaterally with 5 mL of oxygenated KH and maintained at 37°C by a circulating water bath at a pH of 7.4. The buffer contained: NaCl (118 mM), KCl (4.7 mM), 1.2 KH_2PO_4 (1.2 mM), $MgSO_4 \cdot 7H_2O$ (1.2 mM), glucose (11.1 mM), $NaHCO_3$ (25 mM), and $CaCl_2 \cdot 2H_2O$ (2.5 mM). The chambers contained 3% agar bridges in 3 mol/L KCl connected to Ag and AgCl voltage and current electrodes. These were used to monitor the potential difference (PD) across the tissue and to supply the required short circuit current (Isc) to maintain zero PD via an automated voltage clamp system (EVC-4000, World Precision Instruments, UK). The responses were recorded using a MacLab analog-digital recorder (AD Instruments, UK). Baseline PD (mV) and Isc ($\mu A/cm^2$) values were recorded 15 minutes after the tissues were mounted. Isc was recorded continuously for the duration of the experiment as an indicator of net active ion transport, except for brief interruptions for reading of the PD. The transepithelial electrical resistance (TEER, Ω cm^2) of the tissue, an indicator of ion permeability and tissue viability, was calculated according to Ohm's law every 20 minutes for 2 hours.

Forskolin (10 μmol/L) (Sigma, UK), an activator of adenylate cyclase and a potent agonist of electrogenic chloride secretion in intestinal tissues,[24] was added to the serosal side of tissues at the end of the experiment to analyze electrogenic ion-transporting capacity, as normally reflected by an increase in Isc. Changes in Isc (ΔIsc) were also induced by serosal addition of veratridine (10 μmol/L) (Tocris Biosciences, UK). Veratridine opens Na^+ channels and prevents their inactivation, thus leading to prolonged membrane depolarization and an increase in tetrodotoxin-sensitive electrogenic ion secretion in intestinal tissue.[25]

Mucosal-to-serosal permeability of paracellular flux probes was determined by measuring the transepithelial flux

of ^{14}C-mannitol (Amersham Biosciences, UK). About 1 μCi of ^{14}C-mannitol was added to the mucosal side and allowed to equilibrate for 1 minute before baseline mucosal (100 μL) and serosal (500 μL) samples were taken. Serosal samples (500 μL) were taken every 20 minutes for 2 hours and replaced with nonradioactive buffer. A final mucosal sample (100 μL) was taken at the end of the 2-hour experiment. Radioactivity was measured using a liquid scintillation counter (Packard Tricarb 2900 TR). Fluxes of mannitol were calculated using the apparent permeability coefficient (Papp) equation and expressed as cm/s.[26]

Histopathology

Brain histopathologic analysis was performed after intracardial infusion of 10% paraformaldehyde immediately after impact. In addition, after removal of the ileum and colon, a piece of each was preserved in nutrient-buffered 10% formalin for histopathology. Longitudinal sections (7-μm thick) of paraffin-embedded mucosae were cut with a microtome, stained with hematoxylin and eosin (H & E) and examined under a light microscope. Samples were randomly allocated, coded, and examined blindly. Note that unlike grade scaling for mouse models of inflammatory bowel disease, there is no official scale to assess changes in this rodent model, so an arbitrary one was assigned by the pathologist based on simple measures of mild or significant changes.

Plasma Levels of Endotoxin

Heparinized blood samples for measurement of plasma endotoxin were taken immediately before euthanasia. Plasma samples were assayed for endotoxin content by the chromogenic limulus amebocyte lysate test,[27] according to manufacturer's instructions (Cambrex Bio Science, Belgium).

Statistical Analysis

Statistical analysis was performed using a one-tailed Wilcoxon test. Results are given as mean $\pm$ SEM. $p < 0.05$ was considered significant.

RESULTS

Rat Brain Pathologic Findings After TBI

Controlled severe TBI to the rat left mPFC resulted in fragmentation of submeningeal cortical parenchyma of the superficial cortex. Diffuse parenchymal pallor (edema) and multiple foci of hemorrhage were also noted in this region. These changes were accompanied by meningeal rupture and hemorrhage (Fig. 1A and B).

Epithelial Electrophysiology of Intestinal Mucosae From TBI Rats

Baseline TEER, PD, and Isc values were stable and similar in ileal or colonic mucosae of TBI rats compared with their respective ileal and colonic controls (Table 1). There were no statistical differences in these parameters during the 2-hour time period in the ilea or colonic mucosae of the TBI rats compared with controls (data not shown).

TBI induced a statistically significant increase ($p < 0.05$) in the absorptive Papp of ^{14}C-mannitol across the ileum of TBI rats compared with controls (Fig. 2). The mean ileal TBI Papp value was $1.5 \pm 0.3 \times 10^{-6}$ cm/s (n = 6) and the control ileal value was $9.2 \pm 0.6 \times 10^{-7}$cm/s (n = 6), a 1.6-fold increase in permeability. In contrast, there was no difference in the Papp of ^{14}C-mannitol in the colon of the TBI animals ($2.6 \pm 0.4 \times 10^{-7}$ cm/s, n = 7) compared with controls ($3.0 \pm 0.4 \times 10^{-7}$ cm/s, n = 6).

There was a statistically significant 30% reduction ($p < 0.05$) in the Isc response to veratridine in ileal tissue after a TBI compared with ileal controls. Although there was a slight

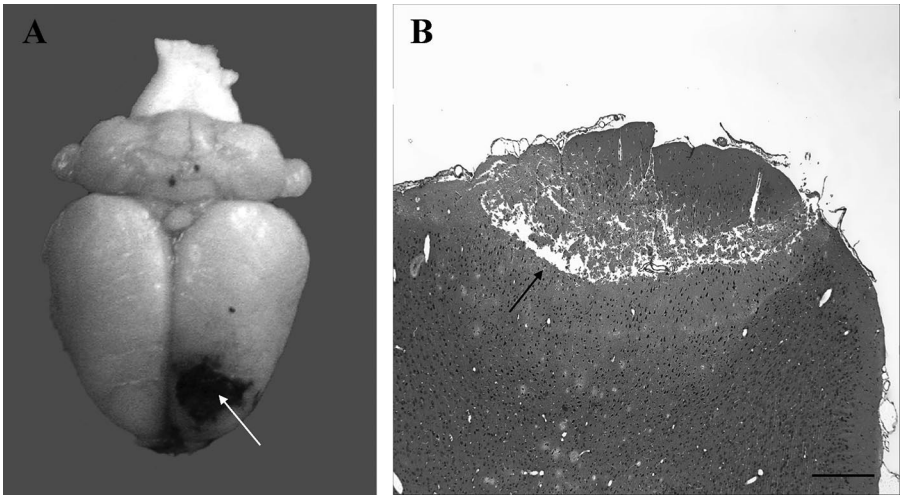

Fig. 1. *(*A*) Contusion after a TBI to the left medial prefrontal cortex of an isoflurane-anesthetized rat after severe TBI (*arrow*). (*B*) Sectioned H & E-stained brain showing deformed structure separating from main brain tissue (*arrow*). Note the diffuse edema and foci of hemorrhage. Horizontal bar = 250 μm.*

Table 1 Baseline TEER, PD, and Isc Responses in Rat Ileal and Colonic Mucosae After a TBI Compared With Controls

	TEER (Ωcm^2)		PD (mV)		Isc ($\mu A/cm^2$)	
	Ileum	Colon	Ileum	Colon	Ileum	Colon
Control (n = 6)	64.0 ± 6.8	84.0 ± 19.2	−2.2 ± 1.1	−7.9 ± 2.2	49.1 ± 4.0	90.5 ± 12.8
TBI (n = 6)	61.6 ± 6.1	56.3 ± 13.8	−1.8 ± 1.0	−6.6 ± 1.9	46.2 ± 3.5	115.9 ± 14.0

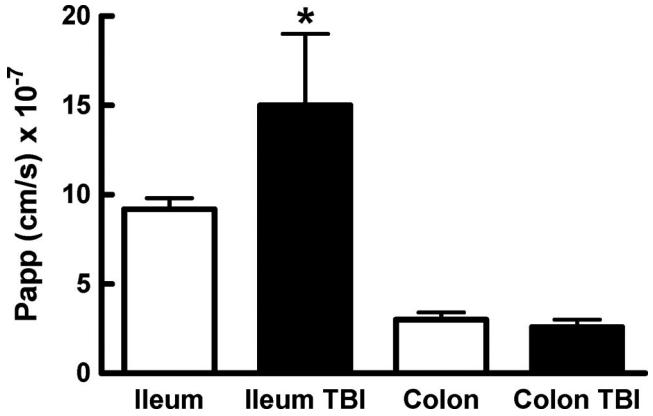

Fig. 2. *Papp values of ^{14}C-mannitol in the mucosal-to-serosal direction across ileal and colonic tissue. N = 6 in each group.* *p < *0.05, for ilea after TBI with respect to ileal controls.*

reduction in the forskolin-stimulated Isc responses in ileal mucosae of TBI animals compared with ileal controls, this was not significant. Furthermore, there was no difference in either the veratridine or forskolin stimulated-Isc responses in the colonic mucosae from the TBI rats compared with colonic controls (Table 2).

Histopathology of Ileal and Colonic Mucosae From TBI and Untreated Rats

Histologic analysis of control ileal segments sampled at the time of death revealed an intact villous and crypt epithelium with finger-like and tongue-shaped villi, whereas minimal lymphocyte and plasma cell infiltration was noted in the lamina propria. There was no evidence of edema and there was no significant vascular congestion of villous tips (Fig. 3A). In the TBI group, however, three out of six ileal samples displayed distorted villous structures that appeared to fold over onto one another (Fig. 3B). This was accompanied by mild edema of the lamina propria. A fourth sample from the TBI group displayed features of villous distortion and mild lamina propria edema in one section, whereas a second section appeared to be more like controls. The fifth and sixth sections showed no distortion of villous architecture or evidence of lamina propria edema. All sections in the TBI ileal group had intact villous and crypt epithelium and vascular

Table 2 Changes in Isc (ΔIsc, μA) Responses to Direct and Indirect Secretagogue Stimulation in Rat Ileum and Colon Mucosae After a TBI

	Veratridine		Forskolin	
	Ileum	Colon	Ileum	Colon
Controls (n = 6)	39.0 ± 3.0	84.1 ± 14.9	76.6 ± 8.4	104.7 ± 20.3
TBI (n = 8)	27.6 ± 4.4*	74.2 ± 5.2	68.1 ± 8.0	87.4 ± 19.1

Values given are net changes in Isc. The concentrations of both agents were 10 μM, added to the basolateral side.
* $p < 0.05$. N = 4–6 in each group.

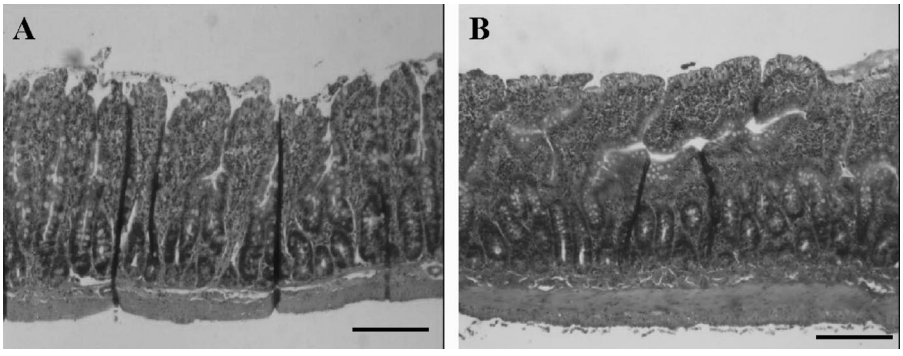

Fig. 3. *H & E staining of rat ileum (original magnification 10×) immediately after euthanasia. (*A*) Controls showing no significant morphologic abnormality. (*B*) TBI showing evidence of diffuse edema and elongation of villi, which appear to flop over onto one another (*arrows*). Horizontal bar = 100 μm.*

Table 3 Grading of Intestinal Histopathology in Rat Ileum and Colon Mucosae After a TBI

	TBI Ileum (n = 6)						TBI Colon (n = 6)						Control Ileum (n = 5)					Control Colon (n = 5)				
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	1	2	3	4	5
Villous distortion	++	++	++	+	−	−							−	−	−	−	−					
Lamina propria oedema	++	++	++	+	−	−	+	+	+	−	−		−	−	−	−	−	−	−	−	−	−
Lamina propria plasma or lymphocyte cell infiltrations	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vascular congestion villous tips	+	+	−	−	−	−							−	−	−	−	−					

Code for arbitrary scale: +++ significant changes; + mild changes; − no change in basal parameter; Blank boxes for colonic mucosae have no villi, so no data can be provided.

congestion of villous tips only occurred in two out of six samples. There was no significant pathological finding in colonic tissue from either controls or TBI groups, although mild edema was noted in the lamina propria in three out of six of the TBI-derived samples. Table 3 summarizes the data from all the mucosae examined. Ileal mucosae with villous distortion were also the ones that had villous tip congestion.

Blood Analysis

There was no difference in the plasma endotoxin levels during the 6-hour time period between the TBI rats and controls. Values in all groups were within the normal plasma range for rat serum endotoxin levels (0.3–0.5 EU/mL).

DISCUSSION

TBI leads to acute disturbances in autonomic nervous system activity resulting in inflammatory responses, metabolic and immune alterations, and intolerance to enteric feeding.[9] TBI-associated dysfunction of the GIT, manifesting in symptoms including stress ulcer, gastrointestinal bleeding,[14] and motility dysfunction,[16] has been widely reported. Sepsis-induced MODS, the leading cause of mortality after TBI, is thought to be associated with increased gut permeability because of derangement of epithelial tight junctions.[28] There is some suggestion that bacterial translocation via the leaky bowel may play a subsequent role in the dissemination of MODS through increased production of local inflammatory mediators.[29] Another possibility is that hemorrhagic shock can cause intestinal permeability changes,[30] perhaps indirectly via a drop in blood pressure. Although we did not measure blood pressure, blood loss was minimal in both treatment groups and, in addition, the intestinal effects were only seen in the TBI group. In the present in vitro study, direct evidence is presented to suggest that increased ileal permeability accompanied by histologic changes follow a specific mPFC-induced TBI performed under anesthesia within a few hours.

Basal electrophysiologic parameters, PD and Isc were not different in the TBI ileal and colonic groups compared with matched controls, suggesting that TBI does not affect electrogenic ion secretion across the ileum or colon. In addition, the epithelial barrier to passive movement of ions, as reflected by electrical resistance measurements was not different in either the ilea or colon of TBI rats compared with controls, indicated by the finding that the TEER values in the ileal and colonic mucosae were similar between matched groups. The relationship between TEER and flux of paracellular markers is complex in epithelial tissues that are regarded from an electrical standpoint as moderately leaky, since a significant portion of overall TEER is contributed by transcellular resistors. Although there is evidence of an inverse relationship between TEER and paracellular flux of small molecular weight tracers in electrically tight epithelia (e.g., Caco-2 monolayers),[31] other studies have shown that TEER and paracellular fluxes across electrically leakier epithelia are not always inversely related.[32] Direct measurement of flux remains the definitive marker of permeability.

In rat intestinal mucosae, stimulated Isc is largely accounted for by the electrogenic chloride secretion.[33] The Isc response to forskolin in TBI ileum was unimpaired, most likely because it directly activates adenylate cyclase. In contrast, the magnitude of the Isc response to veratridine was reduced by 30% in TBI ilea versus controls suggesting partial impairment of neural responsiveness in the small intestine, since at least part of the veratridine-stimulated Isc is mediated through neuronally released neurotransmitter release.[25] Overall, the Isc data suggest that TBI animals retain the capacity for basal and cyclic-AMP electrogenic transepithelial secretion of chloride across intestinal tissue up to 6 hours after the impact.

The increased Papp of the paracellular marker, mannitol, across ileal but not colonic mucosae of TBI rats compared with controls suggests that TBI results in a reduction of the intestinal barrier to passive movement of small hydrophilic substances. The basal Papp values of mannitol across control tissues were similar to previous reports for rat ileal[34] and colonic mucosae.[35] Although increased fluxes of paracellular markers are an accepted surrogate for pharmacologically induced epithelial tight junction openings, as indicated by confocal microscopy techniques in rat ileum,[36] the histologic methods used in this study were designed only to assess gross changes in mucosal structure. A 1.6-fold increase in mannitol

flux across the ilea of TBI rats is similar to increases seen in partially compromised Caco-2 monolayers,[26] yet it is a much lower increase than the 20- to 30-fold value detected in circumstances where epithelial integrity and viability is destroyed, for example in the presence of high concentrations of excipients and solvents.[37] In ileal tissue from 50% of the TBI rats, there was lamina propria edema with dilation of lymphatics coupled with prominent mononuclear cell infiltration of lamina propria and a widening and stunting of villous structures. Importantly, this phenotype was not seen in colonic mucosae from TBI rats, indicating that it is not simply a generalized multiple organ failure at the time points examined. Taken together, ilea from TBI rats display a significant increase in paracellular permeability accompanied by a compromised structure evident in a proportion of tissues.

A recent in vivo study on the jejunum of TBI rats also demonstrated increased permeability to a paracellular flux marker, which was accompanied by overt damage to the mucosal architecture.[20] Using probes in the gut lumen as an indirect assay of intestinal permeability, significant increases in urinary lactulose: mannitol ratios were detected at 12 hours after TBI, whereas the current in vitro study using a direct flux assay suggests that increased permeability to a paracellular marker can be detected in ileum in vitro as early as 6 hours. Hang et al.[20] also found maximal histologic damage in the rat's small intestine between 24 hours and 72 hours after TBI. Similar to the current study, loss of epithelia from villous tips was apparent at 3 hours. Taken together, permeability increases to mannitol in the ileum are likely to precede anticipated intestinal damage. When overt damage becomes apparent, pathogen absorption and sepsis may result. In TBI rats from another study, addition of enriched immunonutrients to an early enteral feeding protocol appeared to protect the mucosa against TBI-induced atrophy.[38] In sum, it seems that differences in intestinal permeability and histopathological results between research groups, although broadly consistent, may be because of different TBI protocols, as well as the likelihood of more significant effects being seen at later time points after TBI.

Analysis of the plasma from the TBI rats in the current study showed no differences in endotoxin levels compared with controls; one interpretation is that the sampling point was considerably earlier than in previous reports.[20] In conclusion, TBI may lead to an initial increase in ileal permeability to paracellular flux markers within 6 hours. Permeability changes most likely precede anticipated gross damage to the epithelium at later time points. This cascade of functional and structural changes may partially explain intolerance to enteral feeding that is commonly observed in patients after a TBI and furthermore, it may pave the way for bacteria to cross the small intestine leading to complications such as sepsis and multiple organ failure. If this is the case, then early intervention to close tight junctions using pharmacological approaches[39] may restore normal function.

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Editorial Comment

Alterations in intestinal permeability following traumatic brain injury (TBI) may play causal roles in predisposition to sepsis and multiple organ dysfunction syndrome. This was the overarching hypothesis that Feighery et al. proposed to test *in vivo* and *in vitro* following pneumatic percussion injury to the frontal lobes in rats.[1] This hypothesis was based on earlier findings suggesting increased permeability after acute and chronic stress, as well as the detrimental effects of increased intestinal permeability resulting in enhanced transepithelial transfer of pathogens and toxins to the systemic circulation and enhanced risk of multiple organ dysfunction syndrome.[2,3]

The authors report no significant differences in transepithelial electrical resistance (TEER), short circuit current (ISC), and potential difference (PD) between ileal and colonic measurements in TBI or control rats. However, the absorptive apparent permeability coefficient (PAPP) measured through C-14 mannitol labeling after TBI was noted to be increased 1.6 fold in ileal measurements but not in colonic measurements. Similarly, Na channel activity was significantly decreased in ileal measurements following TBI; a trend was similarly noted for chloride following forskolin administration, however, no such changes were noted for colonic mucosae. Ileal histopathology in a subset of TBI animals demonstrated distorted villous structures and edema, whereas no such changes were noted for colonic samples. There were no noted differences in systemic endotoxin levels.

This study describes the findings of increased permeability of large molecules in the ileum with some associated ion channel dysfunction and 50% incidence of intestinal edema 6 hours after TBI. These findings could have been much more robust if additional time points were utilized, however, it appears that the study was compromised because it was added on to a previous TBI injury study, with limited potential for additional time points. Hang et al. obtained similar measurements over a longer time course and noted an earlier start and later progression of both permeability as well as histopathological alterations in the gut.[4]

The electrophysiological recordings do lend strength to the experiments, but a single time point waters down the results, as it is unclear whether (or not) the findings are reflective of the overall state, progressive or transient. Additional delayed time points to demonstrate the duration of findings; their progression through modalities such as macromolecular permeability, ion channel fluxes, ionic permeability, and histopathologic changes in both ileal and colonic environments; and concurrent changes in plasma endotoxin or bacterial levels would have significantly strengthened the merits of the presented arguments. Overall, this study clearly demonstrates both *in vitro* and *in vivo* changes in intestinal structure and function that appear to arise from TBI. Further efforts to delineate precise temporal progression and molecular triggers may help us devise interventions to enhance

nutritional support and prevent and interrupt the development of multiple organ dysfunction syndrome and sepsis in patients with TBI.

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