Beta blockade in TBI: Dose-dependent reductions in BBB leukocyte mobilization and permeability in vivo

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BACKGROUND: Traumatic brain injury (TBI) is accompanied by a hyperadrenergic catecholamine state that can cause penumbral neuroinflammation. Prospective human studies demonstrate improved TBI survival with beta blockade (bb), although mechanisms remain unclear. We hypothesized that deranged post-TBI penumbral blood brain barrier (BBB) leukocyte mobilization and permeability are improved by bb.

METHODS: CD1 male mice (n = 64) were randomly assigned to severe TBI—controlled cortical impact: 6 m/s velocity, 1 mm depth, 3 mm diameter—or sham craniotomy, and IP injection of either saline or propranolol (1, 2, or 4 mg/kg) every 12 hours for 2 days. At 48 hours, in vivo pial intravital microscopy visualized live endothelial-leukocyte (LEU) interactions and BBB microvascular leakage. Twice daily clinical recovery was assessed by regaining of lost body weight and the Garcia Neurological Test (motor, sensory, reflex, balance assessments). Brain edema was determined by hemispheric wet-to-dry ratios.

RESULTS: Propranolol after TBI reduced both in vivo LEU rolling and BBB permeability in a dose-dependent fashion compared with no treatment ($p < 0.001$). Propranolol reduced cerebral edema ($p < 0.001$) and hastened recovery of lost body weight at 48 hours ($p < 0.01$). Compared with no treatment (14.9 ± 0.2), 24-hour Garcia Neurologic Test scores were improved with 2 (15.8 ± 0.2, $p = 0.02$) and 4 (16.1 ± 0.1, $p = 0.001$) but not with 1 mg/kg propranolol.

CONCLUSION: Propranolol administration reduces post-TBI LEU mobilization and microvascular permeability in the murine penumbral neurovasculature and leads to reduced cerebral edema. This is associated with hastened recovery of post-TBI weight loss and neurologic function with bb treatment. Dose-dependent effects frame a mechanistic relationship between bb and improved human outcomes after TBI. (*J Trauma Acute Care Surg.* 2022;92: 781–791. Copyright © 2022 American Association for the Surgery of Trauma.)

KEY WORDS: Traumatic brain injury; beta blockers; neurovascular inflammation.

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality in young adults, and globally impacts approximately 50 million individuals yearly.[1] Neurological dysfunction in TBI may manifest as motor deficits or pervasive neurocognitive deficiencies with the degree of dysfunction dependent on the extent and tissue volume of injury.[2] With blunt TBI, the initial kinetic energy transfer establishes primary brain injury, a condition that may be exacerbated by secondary brain injuriants.[3] Secondary brain injury affects injured but potentially salvageable cells around zones of severely injured and unsalvageable ones. Secondary brain injuriants are exemplified by hypoxemia, hypercarbia, hyperpyrexia as potentially alterable elements of care. However, other injuriants are not therapeutically manipulable and include the local release of inflammatory mediators, toxic oxygen metabolites, unregulated intracellular calcium, and proteinases that follow for days after injury.[2–5] Secondary brain injury is marked with blood brain barrier (BBB) disruption, leading to capillary leak of fluids and plasma proteins—events that increase cerebral water, intracranial volume and pressure, as well as sympathetic excess.[6–8]

The hypermetabolism of TBI results in an increased cerebral metabolic rate consuming both oxygen and glucose and offering a potential therapeutic target to improve outcomes.[9–12] Indeed, critically ill patients with mild to severe TBI treated with propranolol (a nonselective β1 and β2 receptor antagonist) demonstrate improved survival and shortened recovery times compared with untreated counterparts and those treated with other classes of beta blockers.[7] Accordingly, beta-blockade therapy for those with evidence of adrenergic excess has received varying degrees of support.[13–15] Nonetheless, the mechanism underpinning this effect remains unclear but may reasonably include the host immune response with associated sympathetic and inflammatory cascade modulation. Consequently, we hypothesized that propranolol reduces post-TBI leukocyte mobilization, reduces BBB leakiness and tissue edema, while augmenting neurological recovery.

METHODS

Experimental Design and Study Groups

Procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Thirty-gram (30 g) CD1 adult male mice (Charles River Laboratories, Wilmington, MA) were acclimated in standard housing with water and chow ad libitum, for 5 days to 7 days. Mice underwent controlled cortical impact (CCI) or sham craniotomy, and received twice daily intraperitoneal injections of normal saline solution (0.9% NS; Baxter; Deerfield, IL) or propranolol-hydrochloride (APP Pharmaceuticals LLC; Schaumburg, IL) at either 1 mg/kg, 2 mg/kg, or 4 mg/kg doses starting 1-hour postinjury or sham craniotomy. Dosing regimens were derived from prior dose-response reports focused on post-TBI cerebral perfusion.[7] Sixty-four mice were randomly assigned to one of eight groups (n = 8 for each): (1) CCI (injury [I]) and saline (I + 0); (2) CCI and 1 mg/kg propranolol (I + 1); (3) CCI and 2 mg/kg propranolol (I + 2); (4) CCI and 4 mg/kg propranolol (I + 4); (5) sham craniotomy and normal saline, (S + 0); (6) sham craniotomy and 1 mg/kg propranolol (S + 1); (7) sham craniotomy and 2 mg/kg propranolol (S + 2); (8) sham craniotomy and 4 mg/kg propranolol (S + 4).

Animal Research: Reporting of In Vivo Experiments guidelines were fulfilled in their entirety (Supplemental Digital Content, http://links.lww.com/TA/C297).

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Severe TBI Model

Controlled cortical impact was used to replicate TBI through a well-validated rodent model.[16] Briefly, on Day 1 after acclimation, mice were anesthetized with intraperitoneal ketamine (Hospira, Lake Forest, IL), xylazine (Akorn, Decatur, IL), and acepromazine (Boehringer Ingelheim, St. Joseph, MO) (KXA: 100, 10, 1 mg/kg, respectively) and placed prone in a stereotactic device. After scalp exposure, a left-sided, 4-mm craniotomy was created between bregma and lambda using a dental drill (Henry Schein, Melville, NY) carefully avoiding dural violation. The left parietotemporal cortex was injured using a controlled cortical impactor (CCI) (AMS201; AmScien Instruments, Richmond, VA) which resulted in a reproducible injury correlated to severe TBI (3-mm-diameter impactor tip, impact velocity of 6 m/s, cortical deformation depth of 1 mm).

Pial Intravital Microscopy

Intravital microscopy (IVM) for in vivo assessment of the cerebral pial microcirculation was performed 48 hours post-CCI as described previously.[17] Under KXA anesthesia, the right jugular vein was cannulated, and a second 2.5-mm craniotomy was created anterior to the first and covered with a 5-mm coverslip (Fisher Scientific, Waltham, MA). Secured in the stereotactic device, mice were transferred to an intravital microscope (ECLIPSE FN1; Nikon Instruments, Melville, NY) and received a 50-μL intrajugular injection of 0.3% rhodamine 6G (Sigma-Aldrich, St. Louis, MO) to fluorescently label circulating leukocytes (LEU) and enable visualization at a 590-nm λ epi-illumination emission exposure. Randomly selected areas of nonbranching pial venules measuring 30 μm to 50 μm in diameter were selected for 1-minute digital recordings (QuantEM camera; Photometrics, Tucson, AR). Intravenous bovine fluorescein isothiocyanate (FITC)-labeled albumin (100 mg/kg) (Sigma-Aldrich) was then administered to assess venular permeability in the same penumbral pial venules. The FITC-labeled albumin leakage was observed under a 488-nm fluorescent filter and digitally recorded for 10 seconds.

Offline Quantification of LEU/Endothelial Cell Interactions and Albumin Leakage

Digital data captured at a 590-nm λ exposure were imported into analysis software (NIS-Elements; Nikon Instruments, Melville, NY) and LEU-EC interactions were quantified by a blinded observer using the following parameters: (1) LEU rolling: number of labeled LEU crossing a 100-μm-long venular segment in 60 seconds; (2) LEU adhesion: number of LEU stationary for at least 30 seconds during the same recording period. Fluorescently labeled spherical cells measuring 7 μm to 12 μm were considered LEU, and interactions were reported as the number of cells/100 μm/minute.

Data captured under the 488-nm λ filter were evaluated for fluorescence intensity from FITC-labeled albumin measured in three distinct regions within the vessel (venular intensity [Iv]) and outside the vessel (perivenular intensity [Ip]). The ratio of mean Iv to mean Ip was averaged for each venule to determine the permeability index for the given vessel indicating the degree of vessel macromolecular (albumin) leakage.

Cerebral Hemispheric Water Content

Forty-eight hours after CCI and immediately following IVM, mice were euthanized, and their brains excised and separated into injured (ipsilateral) and uninjured (contralateral) hemispheres. The wet weight (WW) for each hemisphere was immediately assessed. Dry weight (DW) was determined for each sample after 72 hours of dehydration at 70°C. Tissue water content was calculated using the wet-to-dry ratio [% water content = 100 × (WW − DW)/WW].

Body Weight Loss, Neurologic Recovery

As a surrogate of animal recovery, animal body weights (BWs) were obtained just before (W0h) as well as at 12 hours, 24 hours, 36 hours, and 48 hours after CCI with weight loss expressed as a ratio [(W0h − W12h, W24, or W36 hours)/W0h × 100%].

Murine neurologic function was observed at 12 hours, 24 hours, 36 hours, and 48 hours post-TBI and quantified using the modified Garcia Neurologic Test (GNT), which assesses rodent motor, sensory, reflex and balance abilities with 18 points as the maximum achievable score.

Statistical Analysis

All data are presented as means ± SEM. Statistical analyses were performed using SPSS (SPSS, Chicago, IL, 2019). Graphic illustrations were created with Prism (GraphPad Software, San Diego, CA). Intergroup differences were evaluated using the Kruskal-Wallis test; significance was assumed for p less than 0.05.

RESULTS

48-Hour IVM: Leukocyte-Endothelial Cell Interactions and Microvascular Permeability

In vivo evaluation of the pial microcirculation 48-hour postinjury, revealed a dose response reduction of leukocytes mobilized to the penumbral zone of injury (Fig. 1). Specifically, 4 mg/kg propranolol (I + 4, 5.9 ± 0.39 LEUs/100 μm/min) significantly reduced LEU rolling compared with both untreated (I + 0, 23.6 ± 1.0 LEU/min, $p < 0.001$) and 1 mg/kg propranolol-treated (I + 1, 13.0 ± 0.45 LEUs/100 μm/min $p < 0.001$) injured animals. It did not result in significant rolling differences with the 2 mg/kg dose (I + 2, 8.7 ± 0.64 LEUs/100 μm/min, $p = 0.08$). In uninjured sham counterparts, propranolol also reduced LEU rolling compared with the untreated group (S + 0, 10.37 ± 0.67 LEUs/100 μm/min) but only significantly at 4 mg/kg (S + 4, 1.3 ± 0.37 LEUs/100 μm/min, $p < 0.001$). All sham animals demonstrated significant lower LEU rolling compared with untreated injured (I + 0) animals. In injured animals, all propranolol doses returned LEU rolling levels to sham levels. LEU adhesion was infrequent (mean of all groups: 0.17 ± 0.06 LEUs/100 μm/30 seconds), was highest in I + 0 animals (0.38 ± 0.26 LEUs/100 μm/30 seconds), and similar across all injured propranolol treatment groups (I + 4: 0.25 ± 0.16 LEUs/100 μm/30 seconds).

When evaluating pial microcirculation permeability in the same penumbral territories, leakage of the FITC albumin macromolecule occurred most markedly in injured untreated animals (I + 0, 56.8 ± 1.9%) (Fig. 2). When compared this untreated

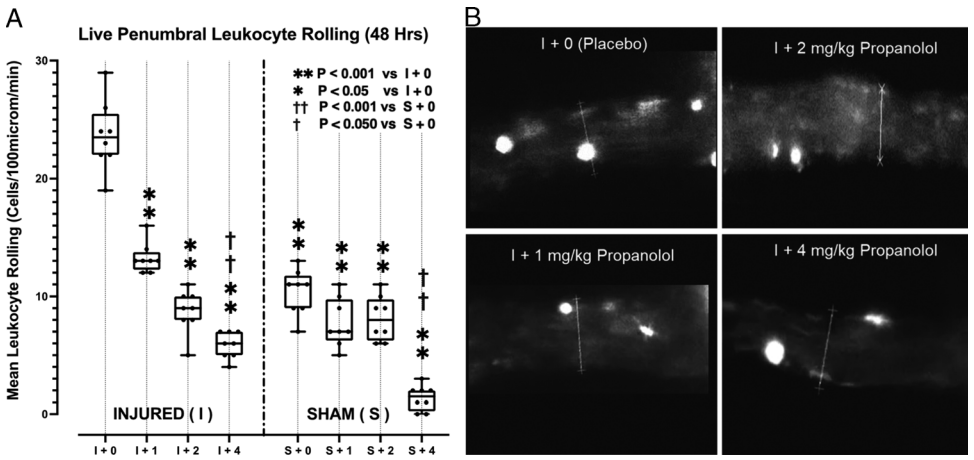

Figure 1. (*A*) In vivo leukocyte rolling on penumbral microcirculatory endothelium 48 hours after TBI. I: injured animals, S: Sham animals, I + 0: injury and NS BID, I + 1: injury and 1 mg/kg propranolol BID, I + 2: injury and 2 mg/kg propranolol BID, I + 4: injury and 4 mg/kg propranolol BID, S + 0: sham and NS BID, S + 1: sham and 1 mg/kg propranolol BID, S + 2: sham and 2 mg/kg propranolol BID, S + 4: sham and 4 mg/kg propranolol BID. (*B*) Representative images taken from footage observed in the live penumbral circulation of injured animals using pial IVM. I: injured animals, I + 0: injury and NS BID, I + 1: injury and 1 mg/kg propranolol BID, I + 2: injury and 2 mg/kg propranolol BID, I + 4: injury and 4 mg/kg propranolol BID.

injured group, propranolol resulted in a dose-dependent reduction in microvascular permeability with 4 mg/kg (39.3 ± 1.2%, $p < 0.001$), 2 mg/kg (45.2 ± 1.2%, $p < 0.001$), and 1 mg/kg (49.8 ± 1.2%, $p < 0.001$).

48-Hour Cerebral Water Content

Comparing wet to dry tissue weights of injured (ipsilateral) and uninjured (contralateral) cerebral hemispheres, animals treated with propranolol demonstrated significant reductions in brain tissue water content, although this did not follow a dose response (Fig. 3).

Among injured animal groups, the mean ipsilateral hemisphere wet-to-dry ratio of untreated animals (78.34 ± 1.2%) was significantly reduced by all propranolol doses equally ($p < 0.001$). In the contralateral (uninjured) hemisphere, propranolol also reduced water content but only significantly at the 4-mg/kg dose (I + 4, 67.9 ± 0.7% vs. untreated I + 0, 75.2 ± 0.6%, $p < 0.001$).

All sham animals demonstrated similar tissue water content (ipsilateral or contralateral hemispheres) regardless of treatment, and levels were significantly lower than I + 0 counterparts (Fig. 3, $p < 0.05$).

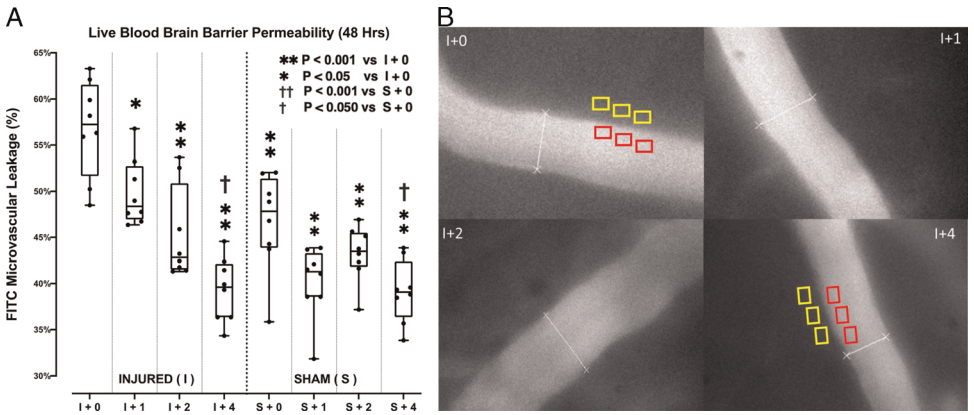

Figure 2. (*A*) In vivo penumbral BBB permeability 48 hours after injury (pial IVM). I: injured animals, S: Sham animals, I + 0: injury and NS BID, I + 1: injury and 1 mg/kg propranolol BID, I + 2: injury and 2 mg/kg propranolol BID, I + 4: injury and 4 mg/kg propranolol BID, S + 0: sham and NS BID, S + 1: sham and 1 mg/kg propranolol BID, S + 2: sham and 2 mg/kg propranolol BID, S + 4: sham and 4 mg/kg propranolol BID. (*B*) Representative images taken from footage observed in the live penumbral circulation of injured animals using pial IVM after FITC albumin injection. Note the first (I + 0) and last (I + 4) panel examples with three yellow (within the vessel, perivenular, Ip) and red (outside the vessel, Iv) distinct regions of equivalent areas used to calculate degree of leakage (degree of whiteness). The ratio of mean Iv to mean Ip was determined for each venule and averaged to determine the permeability index for the given vessel indicating the degree of vessel macromolecular leakage. I: injured animals, I + 0: injury and NS BID, I + 1: injury and 1 mg/kg propranolol BID, I + 2: injury and 2 mg/kg propranolol BID, I + 4: injury and 4 mg/kg propranolol BID.

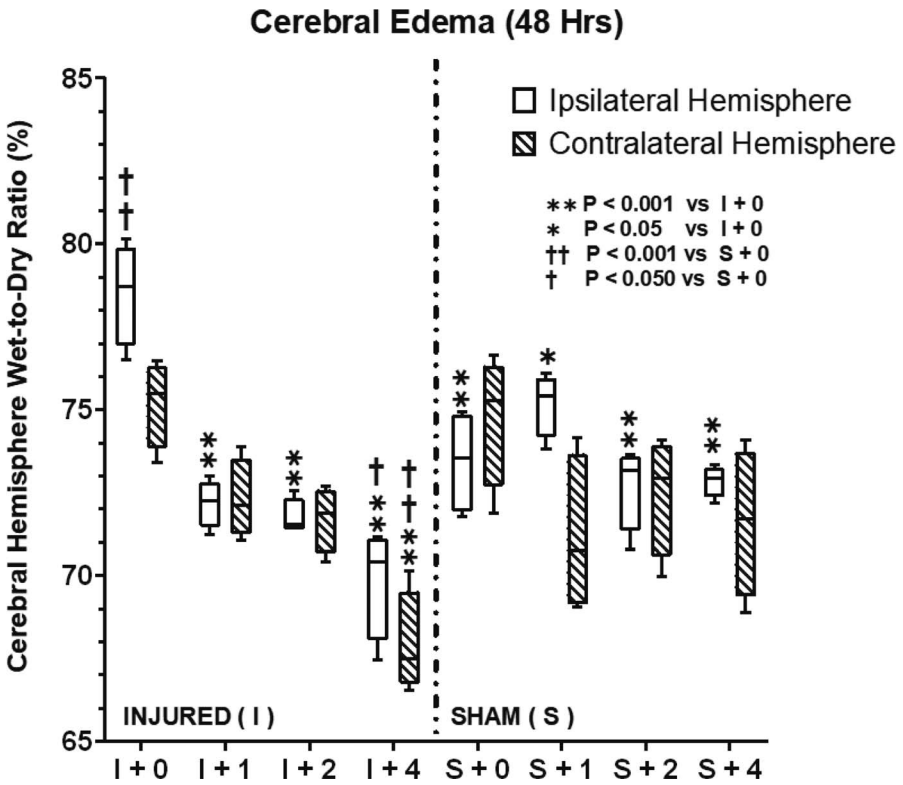

Figure 3. Hemispheric edema (ipsilateral—injured or contralateral—uninjured) in both injured and Sham animals as measured with wet-to-dry ratios. I: injured animals, S: Sham animals, I + 0: injury and NS BID, I + 1: injury and 1 mg/kg propranolol BID, I + 2: injury and 2 mg/kg propranolol BID, I + 4: injury and 4 mg/kg propranolol BID, S + 0: sham and NS BID, S + 1: sham and 1 mg/kg propranolol BID, S + 2: sham and 2 mg/kg propranolol BID, S + 4: sham and 4 mg/kg propranolol BID.

BW Loss and Neurological Function

As a surrogate of animal neurological recovery and resumption of activities of daily living, animal BW and cage activity (GNT) were tracked every 12 hours. Brain injury caused a marked decline in animal BW, which recovered rapidly with propranolol treatment (Fig. 4). Untreated injured (I + 0) animals demonstrated a persistent reduction in BW with the greatest loss observed at 36 hours (−5.8 ± 0.9% preinjury weight). At this time, all injured propranolol-treated mice demonstrated recovery of lost weight, and specifically in the 2 mg/kg propranolol group, animals even demonstrated weight gain as compared with preinjury BW [1 mg/kg (−1.22 ± 0.3%), 2 mg (+0.13 ± 0.4%), and 4 mg/kg (−0.13 ± 0.4%), $p < 0.001$ vs. I + 0]. High-dose propranolol treated mice demonstrated the greatest BW recovery by 48 hours post-TBI (I + 4: +0.9 ± 0.4%) when compared with untreated counterparts (I + 0) that remained well below their preinjury weight (−4.9 ± 0.8%, $p < 0.001$).

Neurological recovery was scored by the Garcia Neurological Test grading animal motor, sensory, reflex and balance abilities (Fig. 5). As compared with untreated injured animals (I + 0, 14.9 ± 0.2), propranolol treatment improved 24-hour post-TBI GNT scores with 1 mg/kg (15.1 ± 0.1, p = ns), 2 mg/kg (15.7 ± 1.6, $p = 0.02$), and 4 mg/kg (16.1 ± 0.1, $p = 0.001$) with only the latter two doses reaching statistical significance. The GNT scores of sham animals were also slightly worse after sham surgery and accompanying anesthesia but recovered similarly quickly, improving with time regardless of propranolol treatment or lack thereof.

DISCUSSION

In the present study, propranolol treatment in a validated murine TBI model was used to elucidate mechanisms underpinning improved neurological outcomes and reduced mortality in TBI survivors receiving beta antagonists. Using a 48-hour study interval, in vivo findings demonstrated that penumbral leukocyte mobilization is blunted in animals receiving increasing doses of propranolol. These findings were flanked by a concurrent dose-related decrease in BBB leakage of albumin. A second validated marker of cerebral edema (wet-to-dry weight ratio) confirmed a reduction in cerebral water content. As a clinical correlate, these changes were accompanied by faster recovery of postinjury weight loss and more rapid normalization of animal diurnal activity (GNT) with propranolol treatment. These combined findings provide a plausible mechanism by which administration of nonselective beta blockade may reduce cerebral inflammation and reduce at least two important aspects of secondary brain injury—leukocyte trafficking and microvascular permeability—that can impede neurologic recovery.

Accelerated endogenous catecholamines after TBI drive hypercatabolism as well as other untoward sequelae of sympathetic excess. The intensity and duration of the postinjury catecholamine surge directly influences cerebral oxygen demand

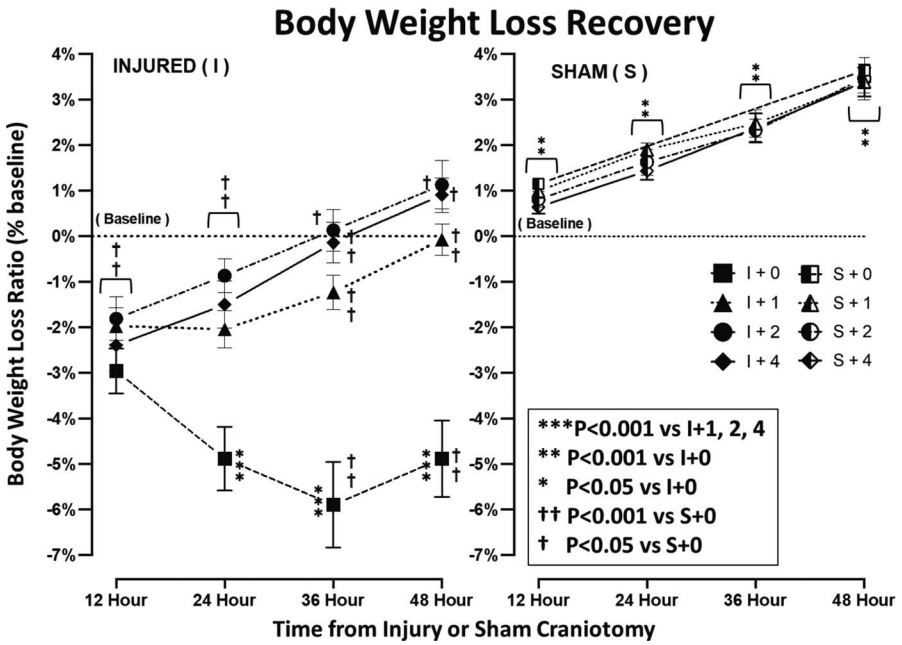

Figure 4. Animal BW loss ratios as compared with baseline preinjury animal weight as determined over the 48-hour study period. I: injured animals, S: Sham animals, I + 0: injury and NS BID, I + 1: injury and 1 mg/kg propranolol BID, I + 2: injury and 2 mg/kg propranolol BID, I + 4: injury and 4 mg/kg propranolol BID, S + 0: sham and NS BID, S + 1: sham and 1 mg/kg propranolol BID, S + 2: sham and 2 mg/kg propranolol BID, S + 4: sham and 4 mg/kg propranolol BID.

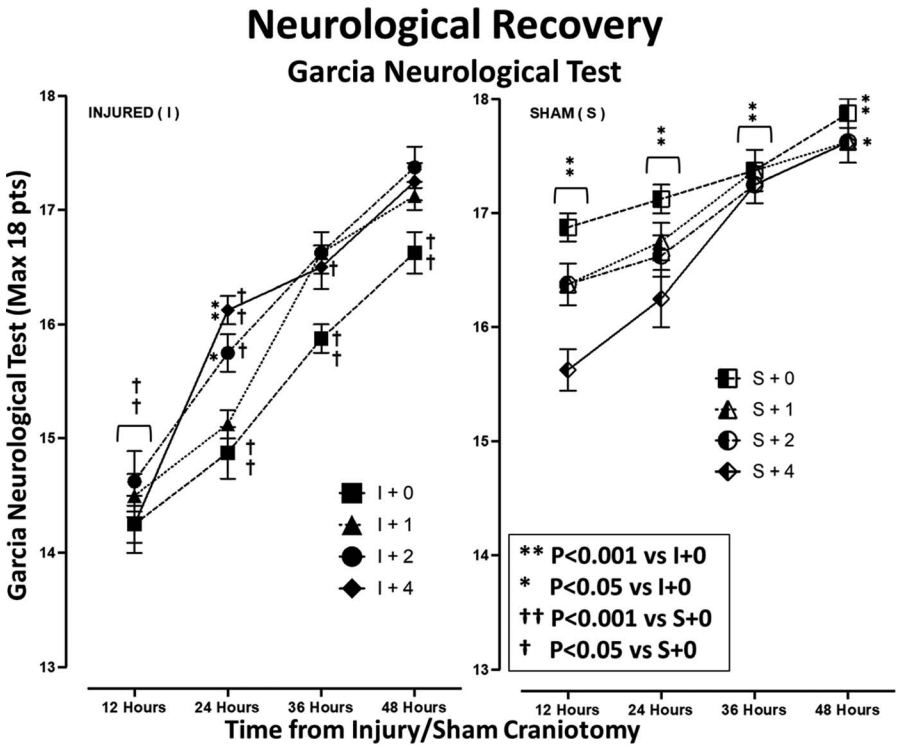

Figure 5. Neurological recovery of animals across the 48-hour study period as measured by the Garcia Neurological Test (max score, 18). I: injured animals, S: Sham animals, I + 0: injury and NS BID, I + 1: injury and 1 mg/kg propranolol BID, I + 2: injury and 2 mg/kg propranolol BID, I + 4: injury and 4 mg/kg propranolol BID, S + 0: sham and NS BID, S + 1: sham and 1 mg/kg propranolol BID, S + 2: sham and 2 mg/kg propranolol BID, S + 4: sham and 4 mg/kg propranolol BID.

and varies proportionally with the degree of activation of the acute inflammatory response.[18] Catecholamines—such as norepinephrine and epinephrine—bound by beta-adrenergic receptors (β_1, β_2, and β_3)—activate adenylate cyclase through a G-protein coupled mechanism.[8] Activation releases cyclic adenosine monophosphate and triggers protein kinase A type I more strongly than type II.[8] Protein kinases are important in regulating cell proliferation and may have important overlap with postinjury apoptosis, disordered autophagy, or mitophagy as influencers of postinjury bioenergetic adaptation to preserve cell viability.[19]

Propranolol's lack of receptor specificity may explain its apparent superiority compared with selective beta blockers as selectivity may obviate the effectiveness of the agent in blunting cerebral inflammation. Persistently elevated plasma and urinary catecholamines post-TBI directly correlate with the severity of subsequent neurologic deficits.[20] Further, elevated intensive care unit admission plasma concentrations of norepinephrine and epinephrine are associated with worse 6-month Glasgow Outcome Scale—Extended scores; mortality correlated with higher epinephrine concentration.[21] In the current study, while not measuring plasma catecholamine levels in treated animals, better outcomes were encountered in groups receiving higher propranolol doses.

Both animal and human data support post-TBI beta blockade. In human patients where propranolol is the most intensively studied agent, beta blockade after TBI reduces both catecholamine concentration and mortality.[9–12] Propranolol is the most lipophyllic of the commercially available beta blockers and demonstrates the greatest partition coefficient across the BBB, therefore, plausibly affecting the local cerebral milieu, as well as peripenumbral cytokine production and release.

In one animal TBI model, propranolol reduced 1 hour grip strength deficits and histological brain edema.[22] The same authors further noted that propranolol treatment increased post-TBI oxygen delivery and cerebral perfusion on both immunohistochemical and micropositron emission tomography analysis. Similar findings have been reported by other groups with beneficial impact on both cerebral blood flow and tissue hypoxia.[5,23] In a murine model similar to ours, propranolol was shown to improve 7-day animal cognition while concurrently reducing histopathological hippocampal CA1 and glial fibrillary acidic protein-positive astrocytes as compared with untreated CCI controls.[24] Relatedly, we also demonstrated that greater doses of propranolol administration significantly and more rapidly hastens injured animals' BW loss recovery and, to a lesser degree, animal GNT scores.

Beta blockade is a systemic intervention that may have an asymmetric impact in the brain after injury. As such, there may be systemic influences that should also be explored to assess their contribution to identified outcomes as beta blockade administration may unequally shift the balance between beta receptor stimulation and antagonism in different compartments. Specifically, β-adrenergic stimulation influences multiple innate immune cell lineages as noted by increased natural killer cell, $CD14^+$ monocyte, T lymphocyte, and B lymphocyte activation.[25] Moreover, β antagonism particularly affects leukocyte function and profoundly affects polymorphonuclear neutrophils (PMNs) by augmenting activation and their release of proteinases and toxic oxygen metabolites. In a rat multitrauma model (lung contusion, hemorrhagic shock), beta blockade administration reduced bone marrow high mobility group box-1, granulocyte-colony stimulating factor, and neutrophil elastase expression compared with untreated but injured counterparts.[26] These data indicate beta blockade administration exerts an early and diffuse effect on interwoven elements essential for a coordinated immune response including hematopoietic progenitor elements. In vitro studies found that beta blockers enhance PMN motility in response to chemoattractants causing dose dependent inhibition of neutrophil CD11b expression, superoxide production and hydrogen peroxide release potentially explaining their observed decreased inflammation and foreshortened recovery time.[27–29] CD11b is a key surface PMN adhesion receptor believed to interact with endothelial counter receptors (ICAM-1, VCAM-1) and cause firm PMN-endothelial adhesion.[30] Polymorphonuclear neutrophil adhesion is preceded by rolling where the activated PMN interacts with activated endothelial cells mediated by Sialyl LewisX (sLeX) overexpression of surface endothelial receptors and surface PMN selectins, primarily L-selectin.[31] Interestingly, surface PMN L-selectin expression is significantly increased by epinephrine exposure, an effect completely blocked by propranolol in a flowcytometric analysis of human monocytes.[32] Consistent with this data, our pial IVM model directly demonstrated in vivo, propranolol dose-related reductions in the penumbral presence of rolling leukocytes.

The effect of beta blockade on EC-PMN interactions is further underpinned by the direct activation of the endothelium by catecholamines, specifically upregulating surface expression of ICAM, VCAM.[33] This well-described activation of endothelium by injury is termed the endotheliopathy of trauma[34,35] and directly involves the sympathetic response in TBI.[36] Further discussion of beta blockade effects on endothelial cells and endotheliopathies is beyond the scope of this study and can be pursued in some excellent reviews of this topic.[34,35]

Microcirculatory host immune stimulation via maladaptive leukocyte responses have been described with systemic inflammation following multiple trauma, pancreatitis, the adult respiratory distress syndrome and postcardiac bypass. In each of these examples, a variety of interlinked elements of the host response is activated by the milieu of proinflammatory cytokines and lymphokines. After TBI, multisystem activation is postulated to occur as a result of β adrenergic stimulation of microglial cells that triggers the release of proinflammatory cytokines—an effect that is abrogated by nonselective β blockade.[37] How exactly the BBB becomes more porous or "leaky" can be related to effects on one or more of the three cell types found at the BBB (endothelial cells, astrocytes and pericytes) and how its breakdown is characterized by a complex interplay of junctional proteins resulting in microvascular leak. Relatedly, proinflammatory cytokine production (IL-6 and TNF-α) is blunted by β antagonism.[38,39] Chemotaxis of migrating leukocytes is also affected by norepineprhine, which decreases production of CCL3, a chemokine that regulates leukocyte recruitment to sites of inflammation, another effect which is abrogated by propranolol in murine injury models.[40–43]

Leukocyte-endothelial overactivation in the microcirculation leads to inadvertent host capillary injury, ultimately causing an inappropriately permeable capillary bed that impedes salt, water and protein retention in the capillary space. Disruption of BBB vascular permeability results in vasogenic edema whereby, in disrupted vessels, cerebral oncotic pressure is reversed by leakage

of macromolecules into the cerebral parenchyma. Cerebral water content expands in the setting of inappropriately high vessel permeability and may collapse parts of the microcirculation. Again, how the BBB breakdown relates to tissue water or edema potentially involves cell death, cellular surface adhesion receptor expression and fluid shifts via endogenous mechanisms and has been studied in increasingly sophisticated in vitro models providing measurements of junctional proteins related to brain tissue viability. Extrinsic compression of fragile capillaries may cause downstream tissue hypoperfusion, tissue dysoxia or hypoxia, secondary brain injury, and neurologic compromise. Beta blockers appear to reverse injury-related microvascular permeability and the resultant tissue edema found after central neuraxial injury, an effect that is not uniformly reported. In a rat model of lateral fluid percussion (LFP) injury, for example, propranolol was not found to reduce brain water content when measuring leakage of the tracer chromium-51–labeled ethylenediamine tetraacetic acid at 24 hours.[44] This observation could have been related to the tracer used instead of albumin, and there may be time-dependent elements of tracer assessment that may impact results. On the other hand, in a rat ischemic stroke model, pretreatment with propranolol reduced cerebral infarct size (histology), edema (wet-to-dry ratio), and cellular apoptosis.[45] Consistent with multicompartment effects, propranolol administration also reduced brain oxidative stress, cerebral inflammation (tissue CRP, free fatty acids, corticosterone), and cytokine (TNF-α, IL-6) production in the same model. Propranolol (2.5 mg/kg) administration reduced injured cerebral tissue water content and improved cognitive function in a weight drop murine model.[22] Similarly, in another murine model using a similar CCI model to ours, 24-hour brain edema (wet-to-dry), 4-day BBB permeability (Alexa Fluor tracer) and microglia activation were all significantly improved by propranolol administration.[46] Our findings parallel the majority of reported studies, and further delineate substantially reduced leukocyte rolling and microvascular permeability in vivo, in the vulnerable penumbra after propranolol administration. Moreover, post mortem injured cerebral hemisphere wet-to-dry ratios confirmed a propranolol-related reduction in cerebral water content. Importantly, the Lund protocol that is used in intensive care units worldwide, currently incorporates beta blockade into human post-TBI care as a key method to decrease vasogenic edema.[47]

While our data link injury, nonselective beta blockade and neurologic recovery with key indicators of inflammation, there are important limitations to this murine model. First, neither plasma nor tissue catecholamine levels were measured. Therefore, aligning the propranolol dose with the endogenous catecholamine concentration cannot occur and would have been optimal to attribute a causative effect on higher propranolol dose-related outcomes. Second, we did not obtain hemodynamic profiles around beta blockade and cannot, therefore, parse the influence of therapy on mean arterial pressure nor cerebral perfusion pressure as we did not assess these parameters. Nonetheless, increasing doses of propranolol would plausibly decrease heart rate and blood pressure and would presumably worsen cerebral perfusion. Instead, propranolol receiving groups fared better in nearly every measured parameter. Third, principally LEU rolling—but not LEU adhesion—was impacted by propranolol. This could be explained by the 48-hour study interval when adhesion is quite rare,[48] likely preventing the capture of any subtle impact of propranolol on LEU adhesion. Fourth, propranolol dosing was preplanned and was not tethered to a specific trigger other than group assignment. While human bedside doses are titrated to symptom abrogation, murine doses were fixed and not tied to a behavior or other metric leading to the potential for excess dosing for that specific animal. Nonetheless, propranolol dosing used was consistent with doses used for hyperadrenergic crisis management after TBI, and outcomes were generally enhanced with higher doses of beta blockade. Fifth, how the BBB was affected by injury in our model—or by propranolol treatment—was not measured. Evaluation of junctional proteins (e.g., ZO-1, Occludin) to determine whether BBB breakdown was present in this model via cell death or by endogenous mechanisms would have determined the exact cause of leakage and increased tissue water. Finally, extrapolating from a small animal study to the human circumstance is problematic. However, the focus of this study was to delineate realistic mechanisms to explain observations from human care that could reasonably explain enhanced survival and recovery.

CONCLUSION

The current study provides mechanistic insights into how post-TBI beta blockade administration may improve outcome. In this rodent model, animals receiving nonselective beta blockade were found to have reduced leukocyte rolling, BBB permeability, and cerebral tissue water. These observations all correlate with improved measures of neurocognitive recovery and weight recovery in a fashion suggestive of dose dependence. Consistent with the systemic—compared with local—administration of a therapeutic agent, beta blockade administration also improved measures of tissue water and microvascular leakage in uninjured tissue and even uninjured animals. These data underscore the importance of tissue injury, including that of sham procedures, on outcome measures in animal models. Enabling targeted beta blocker therapy for specific individuals based on circulating catecholamine concentration may be the next step in providing a precision-medicine approach to improving neurocognitive outcomes.

AUTHORSHIP

A.J.L.: literature search, study design, experimental procedures, data collection, data analysis, data interpretation, writing, critical revision. M.E.: literature search, study design, data interpretation, critical revision. C.J.: data analysis, data interpretation, critical revision. A.G.: study design, experimental procedures, data analysis, data interpretation, critical revision. M. C.C.: data interpretation, critical revision. S.A.: data interpretation, critical revision. M.A.K.: data interpretation, critical revision. L.J.K.: data interpretation, critical revision. D.H.S.: data interpretation, critical revision. J.L.P.: literature search, study design, data collection, data analysis, data interpretation, writing, critical revision.

DISCLOSURE

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DISCUSSION

STEPHEN L. BARNES, M.D. (Columbia, MO): Thank you, Dr. Pritts, Dr. Gurney. I want to thank the association for the opportunity and the privilege of the podium and congratulate the authors on what is clearly a well-designed, expertly executed, and a well-written paper. I have no doubt it's going to contribute more to our understanding of brain injury.

Brain injury is a significant component of what we do globally, with extraordinary costs and societal effects. Anything we can do to improve brain injury outcomes would be of great benefit.

The Center of Brain Injury and Repair at Penn has demonstrated a favorable dose dependent relationship between propranolol and secondary brain injury, specifically in the areas of leucocyte endothelial interactions, microvascular permeability, and functional recovery in a well-established, controlled cortical impact rodent model of brain injury.

I have five questions.

The catecholamine-driven endotheliopathy of trauma upregulates the expression of I-cam and V-cam on the endothelial surface while, at the same time, increases expression of CD-11 and L-seloectins on the now-activated leukocytes, resulting in adhesion and the untoward downstream effects of inflammation in response to injury that happen after that adhesion takes place.

Through intravital microscopy you were able to demonstrate that treatment with propranolol reduced leukocyte rolling in both the injured and the sham groups, but were not able to demonstrate any dose-dependent reduction in adhesion. Can you postulate as to why?

The dose-dependent response seen in leukocyte rolling, microvascular permeability and recovery were not seen in 48 hour cerebral edema. Do you believe this was related to the lack of demonstrated adhesion or do you have other thoughts as to why the end result of that negative inflammatory response tissue edema in our injured patients brains was not dose-dependent?

In higher-dose groups you demonstrated remote effects in uninjured tissue. Beta adrenergic stimulation has significant influence on our inflammatory response and our immune function. How do we balance what you have demonstrated that are clearly positive effects on the injured brain with what could be considered negative consequences in other areas?

So I'm sold. I think patients sustaining TBIs should be on propranolol. What's the dose?

My son is a hell of a football player, plays both ways – fullback and outside linebacker. Should I pretreat him before games with propranolol to reduce his mother's anxiety over brain injury?

And, finally, what is next for the Center of Brain Injury and Repair? Where do we go from here?

It's truly impressive work from a mature lab dedicated to discovery related to injury. I hope your excellent work will continue. Thank you very much.

MARK HOOFNAGLE, M.D. (St. Louis, Missouri): I repeat, again, this is an excellent study. Very interesting. Provides a possible mechanism for an effect that we have now seen in randomized controlled trials by Khallili et al.

And similar to the comment about the dose response effect, what are the corresponding doses being generated by your dosing regimen compared to, say, the 20 milligrams BID dosing that we're now doing clinically to try to decrease mortality and improve outcomes after TBI?

ALFONSO J. LOPEZ, M.D. (Homestead, Florida): Thank you for your questions. About the lack of adhesion differences, it's important to understand that it's probably more important – adhesion is probably more important than rolling.

And while this lab has viewed injury when it comes to muscle, lung, and liver, brain injury is pretty much different compared to these other organs. And sometimes adhesions is non-existent.

The next point for that one will be that I think that we have no difference between the observation time that was conducted for one minute and this is pretty much where ??_____?? and then adhesions are a very rare occurrence.

With respect to not finding differences at 48 hours in edema, I agree, it's less evidence but the mean water content tends to decrease with the increasing doses of propranolol.

Statistically different, I think this was not quite there but will be necessary to increase the number of groups to have a statistical number for a more evident brain water content reduction.

With respect to the possible lowering of the immunity by propranolol, you are right. I was surprised to see a reduction in leukocyte mobilization and blood brain barrier leakage in uninjured animals.

The study is not shown as a purpose for immunity. What we know is that many animals and human studies treated with beta blocker show improvement but have not described an increase in infection or anemia or other effects in the innate immunity.

About the pretreatment of beta blockers, I think it's an excellent point other than to have to look to – we would have to look into pretreatment studies that have shown benefits to protect the effects in the cellular level.

Our study did not evaluate the pretreatment but I believe the verdict is still there to treat high-risk individuals for brain injury.

In terms of the future direction, our group is currently collaborating with the Penn bioengineering department that is creating a, that has created a blast injury model for rodents and currently is looking into beta blockade as an important feature for TBI to treat our troops that are high-risk individuals for traumatic brain injury.

And for the question when it comes to the components of adhere-IV dosing, the study used these doses as comparable of intraperitoneal, the concentration of intraperitoneal injection of propranolol that would reach the brain compared to an IV.

The doses were selected based on a rodent model that found that four milligrams of IP injection were equivalent to one milligram of IV when it comes to brain concentration of propranolol.

And thank you.

