PET Imaging of Peripheral Benzodiazepine Receptor Standard Uptake Value Increases After Controlled Cortical Impact, a Rodent Model of Traumatic Brain Injury.

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Research

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Abstract

Background: Traumatic brain injury (TBI) disrupts the complex arrangement of neuronal and glial cells. As a result of TBI there is activation of microglia. Activated microglia after injury can be measured *in vivo* by using positron emission topography (PET) ligand peripheral benzodiazepine receptor (PBR28) and their phenotypes (activated vs resting) can be assessed (*ex vivo*) using morphology. This study aims to utilize *in vivo* (PET) and *ex vivo* (morphology) to assess the changes in microglia after a controlled cortical impact (CCI), a rodent model for TBI.

Methods: Male Sprague Dawley rats underwent a sham injury or severe CCI. Microglia activation was assessed 120 hours after the injury by PET/CT imaging using the radioligand [11C] PBR-28. Standardized uptake values (PBR28suv) were calculated over the duration of the scan and mean values were compared. In order to verify *in vivo* results, *ex vivo* morphological analysis [ramified (resting) or amoeboid-shaped (activated)] was performed (dentate gyrus, corpus callosum and thalamus) with the antibody IBA-1. To further conclude that PBR is a marker for activated microglia after CCI, we examined co-staining of PBR with microglia and astrocytes.

Results: *In vivo* and *ex vivo* results were complementary. Injured animals displayed greater PBR28suv when compared to sham animals. Immunohistochemistry demonstrated elevated numbers of activated microglia in the ipsilateral dentate gyrus, corpus callosum and thalami of injured animals compared to sham animals. Additionally, PBR co-stained with microglia and not astrocytes.

Conclusion: CCI, a rodent model of TBI resulted in a significant increase in PBR28suv due to injury. Similarly, morphological analysis demonstrated a significant increase in amoeboid-shaped (activated) microglia. These results serve as a surrogate marker for increased neuroinflammation in the brains of severely injured animals. PBR28suv can serve as an *in vivo* tracking system for monitoring neuroinflammation following TBI and cellular therapies.

Introduction

Traumatic Brain Injury (TBI) causes a prolonged secondary neuroinflammatory response within the central nervous system (CNS) that leads to neurological deficits, both motor and cognitive, beyond that caused by the primary injury [1–3]. Central to the secondary inflammatory response after TBI are the microglia, the macrophages of the CNS [4]. Microglia promote learning dependent synapse formation, axonal regeneration, and removal of defunct axon terminals [5, 6]. Under homeostasis, microglia are highly mobile and provide continuous surveillance of their cellular milieu [7, 8]. These “resting” or ramified microglia possess a distinct morphology – small, relatively stable rod-shaped somata with thin ramified withdrawing processes [8].

After TBI, microglia are activated and undergo considerable remodeling. They retract their processes and typically adopt an amoeboid morphology [2, 7, 9–13]. Once activated, microglia can polarize towards a pro-inflammatory phenotype which can result in chronic neuroinflammation, oxidative stress and

neuronal dysfunction. On the other hand, microglia can also be considered anti-inflammatory and can result in resolution of inflammation, clearance of debris and neural repair. The pro- or anti-inflammatory phenotype is dependent upon the local environment and timing after injury [14].

Another protein that increases in expression after injury is the translocator protein (TSPO), expressed by reactive glia and macrophages [15, 16]. TSPO or peripheral benzodiazepine receptor (PBR) is localized on the outer mitochondrial membranes of astrocytes, microglia and macrophages [17]. TSPO or PBR can be detected in rodents and patients using positron emission topography (PET) ligands. Evidence from human TBI patients using ligand PK11195 (TSPO) suggests that amoeboid microglia/macrophages and/or reactive astrocytes remain present up to 17 years after injury [3].

In order to determine if PET ligand for PBR (PBR28) is indeed a viable marker to measure activated pro-inflammatory microglia, we utilized a multi-pronged *in vivo* (PET) and *ex vivo* (morphology and co-staining) approach. Elucidating the extent to which PBR28suv (*in vivo* analysis) is accompanied by amoeboid-shaped microglia (*ex vivo* analysis) and co-staining of PBR with microglia pre-clinically will allow us to advance the use of this ligand to assess the level of activated microglia after TBI or other CNS related neuroinflammatory diseases in clinic. In addition PPBR28suv can serve as a biomarker for efficacy of cellular therapies that target neuroinflammation/microglia after TBI or stroke [18].

Methods

Adult male Sprague Dawley rats aged six to eight weeks (Harlan/Envigo, Indianapolis, IN, USA) were housed on a twelve-hour light/dark cycle with ad libitum access to food and water. All protocols involving the use of animals were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (HSC-AWC-15-0003).

Controlled cortical impact (CCI) model of traumatic brain injury

Prior to creation of the TBI, each animal went through a pre-operative checklist to maximize survival following the CCI. Each animal was initially anesthetized with 4% isoflurane with a 1:1 N₂O/O₂ mixture in a vented anesthesia chamber. When the animal failed to respond to foot and tail pinch, they were removed from the anesthesia chamber and continually anesthetized with a 2-2.5% isoflurane mixture via facemask. Aseptic surgical technique was used for the surgical procedure. Body temperature was monitored by a rectal thermometer and regulated by the use of a heating pad throughout the operation. Prior to any incision, the subcutaneous tissue was infiltrated with < 0.1 mL/kg of 0.25% Bupivacaine.

The CCI began with a midline skull incision and the right-sided soft tissue was reflected laterally to expose the skull. Unilateral craniectomy was made midway between the bregma and the lambda (3 mm right of midline) with the medial edges adjacent to the midline suture. A single impact was performed

using a sterile impactor tip depending on the degree of injury. The scalp was then stapled closed with sterile wound clips.

In order to assess the spatial and temporal microglia response to injury, the rats were randomly selected to undergo either a sham injury or a right sided CCI (Device: Impact One Stereotaxic Impactor, Leica Microsystems, Buffalo Grove, IL). CCI severity is based on injury depth, and was measured independently by a device attached to the impactor tip (6 mm). The severe injury consisted of a 3.1 mm impact depth at a velocity of 5.6 m/s, dwell time 200 ms. The sham procedure entailed a midline incision and reflection of the soft tissue laterally to expose the skull. A craniectomy was not performed in this animal group. Cole et al. demonstrated that a traditional sham operation that incorporates craniectomy results in profound inflammatory and anatomical damage that may severely confound results of a TBI animal model, therefore it has become standard to not perform a craniectomy for sham procedures (Cole et al., 2011). This methodology has remained standard in our lab, which focuses primarily on rodent TBI models (Liao et al., 2014). Five animals (body weight 225–249 g) were assigned to each injury group.

Synthesis of [^{11}C] PBR-28

The radioligand [^{11}C] PBR-28 was purchased and synthesized from the University of Texas MD Anderson Cancer Center Cyclotron Radiochemistry Facility. In brief, [^{11}C] CO_2 is produced in the cyclotron target by the bombardment with protons on nitrogen gas ($\text{N}_2^+ 1\% \text{O}_2$) mixture via the nuclear reaction $^{14}\text{N}_{(p,\alpha)}^{11}\text{C}$. After irradiation, the [^{11}C] CO_2 from the target is transferred to a processing module where it is first trapped and then converted to [^{11}C] methane. Subsequently, the [^{11}C] methane is reacted to yield [^{11}C] methyl iodide. The [^{11}C] CH_3I is then passed through another column containing silver triflate at elevated temperatures, which in turn produces [^{11}C] methyl triflate. The [^{11}C] methyl triflate is then transferred to a second automated synthesis module where it reacts with the precursor. After the reaction, the solution is injected into a Prep HPLC column where the final product is purified. From this second module, the radiopharmaceutical is passed through a sterilizing filter into a sterile final product vial. A sample from the final product vial is removed and analyzed to ensure the product meets release criteria. Finally, the vial is moved to the radiopharmacy for individual dose dispensing.

PET/CT imaging

Imaging was performed at the MD Anderson Cancer Center for Advanced Biomedical Imaging (CABI)/Small Animal Imaging Facility (SAIF) 120 hours after creation of the injuries. A total of five rats with TBI were used for a dynamic [^{11}C] PBR- PET/CT imaging study and an additional five rats were used as sham-treated controls. Rats were anesthetized using 2% isoflurane via facemask. At the start of the PET scan, the rats were injected with 300 μCi of [^{11}C] PBR-28 in 200- μL of sterile saline via a tail vein catheter. Each rat was imaged with a sixty-minute PET scan and a five-minute CT scan (400 μA , 45 kV, 120 projections) on a Bruker Albira PET/SPECT/CT scanner (Bruker Biospin Corp., Billerica, MA, USA).

Imaging data analysis

The PET data was reconstructed into the following bins: 15 × 20 s, 5 × 60 s, and 4 × 300 s using a maximum likelihood expectation maximization (MLEM) method. Analysis was performed with P_{mod} (PMOD Technologies Ltd., Zürich, Switzerland). Using the skull outline from the CT image, two regions were drawn to cover the two cerebral hemispheres of the brain. The time-activity curve developed was PBR uptake in each region over the period of the PET scan was expressed as Standard Uptake Values (SUV [g/ml]). This allows for body mass and minor variations in injected dose. Scatter, randoms, decay, and attenuation corrections were applied.

The SUV has become widely used in PET imaging analysis, making it an excellent tool for comparing radioligand uptake amongst our three different TBI injury severity groups. The SUV is considered a semi-quantitative analysis defined as the ratio of tracer activity in the tissue of interest divided by normalizing tracer activity (background activity, organ activity, etc) (Thie, 2004). The regions of interest (ROIs) included: 1) the ipsilateral-injured cerebral hemisphere and 2) bilateral cerebral hemispheres to account for the contrecoup injury. Soft tissue uptake of the radioligand [¹¹C] PBR-28 was excluded from analysis, so only intracranial microglia activation was evaluated. The SUV was determined by the ratio of mean intracranial SUV to that of muscle. Contralateral brain hemisphere normalization was not possible, as contrecoup injuries would artificially lower mean SUVs in the severe injury group.

Tissue harvest and immunohistochemistry

After approximately ten half-lives passed for the carbon-11 isotope, the animals were euthanized. The animals first underwent bilateral thoracotomies under isoflurane anesthesia. Then, using a right ventricle puncture technique, the animals were simultaneously exsanguinated and perfused with 20 mL of cold phosphate buffered saline (PBS). Following the PBS infusion, 20 mL of freshly prepared, cold 4% paraformaldehyde (PFA) was instilled to reduce non-specific binding and auto-fluorescence. The brains were carefully removed and post-fixed with 4% PFA for 24 hours while stored at 4°C. The brains were then transferred to a 30% sucrose solution, where they were maintained at 4°C for at least 72 hours and allowed to sink. Brains were then put in a 3% melted agar mold and sectioned into 30-μm-thick slices using a vibrating-blade microtome (Leica Microsystems, Bannockburn, IL).

Brain sections were stained for microglia using a free-floating protocol. The protocol was carried out over several days. On day one, the brain slices were transferred to twelve well plates and washed twice in PBS with 0.01% Triton X-100 (PBST; T-8787; Sigma-Aldrich, St. Louis, MO) for 1 minute. Next, the slices were incubated for 20–30 minutes in PBS with 0.02% Triton X-100 for permeabilization. The slices were then blocked for one hour in blocking buffer consisting of 3% goat serum (no. 005-000-121; Jackson ImmunoResearch Laboratories, West Grove, PA) in PBST. The last step of day one involved incubating the brain slices with the primary antibody IBA-1, which is used to identify microglia/macrophage morphology (rabbit polyclonal primary antibody, 1:500; Wako Chemicals USA, Cat# 019-19741, RRID: AB_2313566) [10–13, 19] The primary antibody was prepared in PBTB (PBST, 2% bovine serum albumin [A9647; Sigma-Aldrich] and 1% goat serum), and sections were incubated overnight at 4°C. The following day, sections were washed with PBST and incubated with a goat anti-rabbit IgG secondary antibody (1:500; red/568;

Molecular Probes (Invitrogen) Cat# A11011, RRID: AB143157) in PBTB for 2 hours at room temperature. The sections were again rinsed four times with PBST, mounted on slides, and cover-slipped with Fluoromount-G (SouthernBiotech) for analysis.

Microglia Morphology Quantification

Ex vivo analysis in CCI and sham was done by quantifying microglia morphology (ipsilateral and contralateral) with photomicrographs of the dentate gyrus (hippocampus), thalamus (lateral and medial) and corpus callosum. Photomicrographs were taken at 20X magnification using a Leica fluorescent microscope Dm4000B LED (<https://www.leica-microsystems.com/products/light-microscopes/p/leica-dm4000-b-led>) and Leica Application Suite V4.12 (<https://www.leica-microsystems.com/products/microscope-software/p/leica-application-suite>). A single slice per animal was examined and the sections of interest were approximately mid-injury (small cavity or bruise left by the impactor tip). Two adjacent 20X pictures of the ipsilateral and contralateral thalami were photomicrographed and cells expressing IBA-1 were quantified. We marked several microglia as landmarks while moving to the adjacent photomicrograph to prevent “double-counting”. IBA-1 labeled cells were further quantified based on the following microglia morphologies: ramified or amoeboid as previously described (Torres-Platas et al., 2014), (Zhang et al., 2008), (Bedi et al., 2013, 2018).

Co-Staining of IBA1/PBR and GFAP/PBR

A single brain section from each animal of each group were co-stained with IBA1 and PBR. In addition, we also co-stained glial fibrillary acidic protein (GFAP) and PBR. Primary antibodies included: Anti-PBR (goat polyclonal primary antibody, 1:50; Abcam Cat# ab118913, RRID: AB_10898989), GFAP (rabbit polyclonal antibody, 1:500; Abcam Cat# ab7260, RRID: AB_305808), and IBA-1 (rabbit polyclonal primary antibody, 1:500; Wako Chemicals USA, Cat# 019-19741, RRID: AB_2313566). Secondary fluorescent antibody products included: Alexa Fluor® 488 (donkey polyclonal secondary antibody to rabbit IgG H&L, 1:500; Abcam Cat# ab150073, RRID: AB_2636877) and Alexa Fluor® 568 (donkey polyclonal secondary antibody to goat IgG H&L, 1:500; Abcam Cat# ab175704). Antibody staining combinations included: IBA1/PBR and GFAP/PBR.

Statistical analysis

All data are expressed as means $\pm$ standard deviation. Statistical analysis was performed with Prism software (version 7.0b; GraphPad Software Inc., La Jolla, CA). *In vivo* SUV were analyzed with Two-way analysis of variance (ANOVA) was used for data comparison between sham vs CCI groups followed by Tukey's multiple comparison tests. *Ex vivo* microglia cell counts were compared using unpaired non parametric Mann-Whitney test. Statistical significance is indicated with * for $p \leq 0.05$, ** indicates statistical significance for $p \leq 0.01$, *** indicates statistical significance $p \leq 0.001$, and **** indicates statistical significance $p \leq 0.0001$.

Results

CCI, a rodent model of TBI increases PBR28suv

Representative PET images are displayed in Fig. 1AB. The time-activity curves of the ipsilateral hemisphere shows that the uptake (SUV) was maximum around 30 s for CCI and 10 s for Sham. When comparing the PBR28suv (ipsilateral) over time, there was a significant increase in SUV in injured rats in comparison to sham (Fig. 1C, $p < 0.0001$).

The time-activity curve of the whole brain shows that the uptake (SUV) was maximum around 30 s for CCI and 10 s for Sham. PBR28suv of total brain of sham animals is significantly lower in than the PBR28suv of CCI animals ($p < 0.0001$, Fig. 1C). From 50 s to 1050s, CCI animals have a higher SUV than sham. [^{11}C]PBR28 uptake is relatively stable over the course of the imaging.

CCI results in an increase in amoeboid-shaped microglia in the ipsilateral corpus callosum

Controlled cortical impact resulted in a significant increase in amoeboid-shaped IBA1 positive cells in the corpus callosum. Specifically, there was an increase in the amoeboid-shaped microglia ipsilateral to the injury (Fig. 2B, $p = 0.029$, $n = 4$, 102 ± 13) when compared to sham ($n = 4$, 3.75 ± 4.2), but not contralateral to the injury [CCI: $n = 5$, 10.8 ± 13 vs Sham: $n = 4$, 5.00 ± 3.9 (Fig. 2B, $p = 0.13$)]. Interestingly, there were no differences in the number of ramified microglia on either the ipsilateral [Fig. 2A, CCI: $n = 5$, 274 ± 90 vs Sham: $n = 4$, 136 ± 12 ($p = 0.11$)] or contralateral hemisphere [Fig. 2B, CCI: $n = 4$, 142 ± 6.8 vs Sham: $n = 4$, 134 ± 18 ($p = 0.34$)].

CCI results in an increase in amoeboid-shaped microglia ipsilateral to the injury in the dentate gyrus

Controlled cortical impact resulted in a significant increase in amoeboid-shaped IBA1 positive cells in the ipsilateral dentate gyrus only [Fig. 3B, CCI: $n = 5$, 91.2 ± 97 vs Sham: $n = 4$, 4.25 ± 2.2 ($p = 0.016$)] There was no increase in the number of amoeboid-shaped IBA1 positive cells contralateral to the injury [Fig. 3B, CCI: $n = 5$, 10.8 ± 13 vs Sham: $n = 4$, 5.00 ± 3.9 ($p = 0.63$)] when compared to sham. There were no differences in the number of ramified microglia in the ipsilateral [Fig. 3A, CCI: $n = 5$, 158 ± 95 vs Sham: $n = 4$, 135 ± 12 ($p = 0.60$)] or contralateral hemisphere [Fig. 3A, CCI: $n = 5$, 143 ± 47 vs Sham: $n = 4$, 134 ± 18 ($p = 0.99$)] when compared to sham.

CCI results in an increase in amoeboid-shaped microglia in the medial ipsilateral thalamus

Controlled cortical impact resulted in a significant increase in amoeboid-shaped IBA1 positive cells in the medial ipsilateral thalamus when compared to sham [Fig. 4B, CCI: $n = 5$, 50.2 ± 21 vs Sham: $n = 4$, 9.00 ± 7.1 ($p = 0.016$)]. There were no differences in the contralateral side when compared to sham [Fig. 4B, CCI: $n = 4$, 8.50 ± 15 vs Sham: $n = 3$, 1.00 ± 0.0 ($p = 0.99$)] Additionally, there were no differences in the number of ramified IBA1 positive cells ipsilaterally [Fig. 4A, CCI: $n = 5$, 38.2 ± 13 vs Sham: $n = 4$, 28.0 ± 5.7 ($p = 0.34$)] and contralaterally [Fig. 4A, CCI: $n = 4$, 21.5 ± 13 vs Sham: $n = 4$, 36.0 ± 8.4 ($p = 0.17$)] when compared to sham.

CCI results in an increase in amoeboid-shaped microglia in the lateral ipsilateral thalamus

Similar to the medial thalamus, CCI resulted in a significant increase in amoeboid-shaped IBA1 positive cells in the lateral ipsilateral thalamus when compared to sham [Fig. 5B, CCI: $n = 5$, 69.6 ± 34 vs Sham: $n = 4$, 7.00 ± 7.2 ($p = 0.016$)]. There were no differences in the contralateral side when compared to sham [Fig. 5B, CCI: $n = 5$, 13.0 ± 14 vs Sham: $n = 4$, 2.00 ± 2.7 ($p = 0.11$)]. Also, there were no differences in the number of ramified microglia on either the ipsilateral [Fig. 5A, CCI: $n = 5$, 35.4 ± 16 vs Sham: $n = 4$, 24.0 ± 7.8 ($p = 0.31$)] or contralateral hemisphere [Fig. 5A, CCI: $n = 5$, 27.8 ± 17 vs Sham: $n = 4$, 22.5 ± 17 ($p = 0.37$)] when compared to sham.

CCI results in PBR co-staining with IBA1 and not GFAP

We stained one section from each animal (ipsilateral) to examine the loci of PBR in astrocytes and microglia. Qualitatively we did not observe any co-staining of GFAP and PBR (Fig. 6A-D), however there was considerable co-staining of PBR and IBA1, specifically in the amoeboid shaped cells (Fig. 6E-H).

Discussion

Our results demonstrate that [^{11}C]PBR28 can quantitatively measure activated microglia *in vivo*. These data are translationally relevant as activated microglia are being explored as therapeutic targets after TBI and stroke [18]. Traumatic brain injury can be studied in laboratory setting via a number of different models which include CCI, weight drop injury (WDI), fluid percussion injury (FPI) and blast-induced TBI (bTBI). We have used CCI (pneumatic and electromagnetic) to induce a reproducible and well-controlled injury [20]. CCI provides control over the depth of injury, velocity, tip size and dwell time of the injury. All these variables can be successfully reproduced in an experimental setting. CCI, with a 3.1 mm depth, 200 ms dwell time and 5.6 m/s velocity resulted in a significant increase in PBR28suv in the ipsilateral, contralateral and bilateral hemispheres (Fig. 1). This increase in uptake is accompanied by significant increases in amoeboid shaped (activated) microglia in the ipsilateral corpus callosum, dentate gyrus, medial thalamus and lateral thalamus (Figs. 2–5), that is specific to microglia (Fig. 6).

In vivo PET imaging allows us to assess the level of activated microglia. TSPO/PBR, an often PET-imaged protein is upregulated after injury [15, 16]. Recent evidence from Pannell et al (2020) demonstrated that TSPO is upregulated in astrocytes and microglia when stimulated with lipopolysaccharide (LPS), and this is reflected with an increase in PET imaging of TSPO ligand ^{18}F -DPA-713 [21]. In addition, they demonstrated TSPO expression was significantly increased in microglia after AdTNF injections [21]. These data indicate that TSPO is a good marker to target in order to observe increases of pro-inflammatory microglia *in vivo*. *Ex vivo*, we have previously utilized morphology to delineate between activated and resting microglia after TBI [10–12, 22]. Microglia are activated after a CNS injury. They retract their processes and adopt an amoeboid morphology [2, 7, 9–12]. Microglia are responsible for clearance of dead cells and other debris, such as dead axons and myelin and, therefore, have a neuroprotective role [23], however, chronic activation of microglia can negatively affect neuronal function and hippocampal dependent behavior [10, 24, 25]. In current experiments, in addition to an increase in PBR28suv, we also found increases in the number of amoeboid shaped in varying regions of

the brain after TBI. Specifically, there was an increase in amoeboid shaped IBA1 positive cells in the ipsilateral corpus callosum (Fig. 2). Previously, case analysis of patients that suffered TBI showed presence of activated microglia with white matter degeneration ranging from months to 47 years. Specifically, there was a reduction in corpus callosum thickness surrounded by activated microglia [26]. We observed an increase the number of amoeboid-shaped microglia in the ipsilateral corpus callosum only (Fig. 2). There was a very modest increase on the contralateral side but it was not significant (Fig. 2). In addition to the ipsilateral corpus callosum, there were significant increases in amoeboid-shaped microglia in the ipsilateral dentate gyrus of the hippocampus (Fig. 3) and the ipsilateral lateral and medial thalamus (Fig. 4,5). Both areas have previously demonstrated increases in amoeboid shaped microglia after CCI [13]. In addition to quantifying amoeboid shaped microglia in the hippocampus, we also qualitatively examined co-staining of PBR with IBA1 or GFAP. This was done in order to determine whether PBR28 is expressed in microglia or astrocytes after CCI. In the CCI group, there was no co-staining of PBR and GFAP (Fig. 6A-D). Interestingly, there was robust co-staining of PBR and IBA1 (Fig. 6E-H), and this was limited amoeboid shaped cells (Fig. 6GH).

Amoeboid shaped microglia have also been observed as early as 7 days and up to 28 days after injury in the ventral posteromedial nucleus of the thalamus using FPI [27]. Studies have linked TBI and increased activity in the ipsilateral auditory thalamus (medial geniculate nucleus) to a fear conditioning paradigm (amygdala) [28]. The dentate gyrus is an area in the hippocampus that is critical for spatial learning after TBI [12]. The lack of increase in the contralateral (Fig. 3) was surprising since previously we had previously observed increases in the contralateral side as well just 24 hours after injury. However those experiments were conducted in mice [13].

Our results indicate that TBI results in an increase in PBR28^{suv}, an indicator of activated microglia. Furthermore, this change observed *in vivo* is corresponded by increases in amoeboid-shaped (activated) microglia in the corpus callosum, dentate gyrus, lateral thalamus and medial thalamus as measured by immunohistochemistry (*ex vivo*). A possible limitation to this study is the reliance of microglia, as it is well understood that the secondary injury from TBI is a result of a complex inflammatory response that involves the activation and proliferation of peripheral immune cells such as T lymphocytes, natural killer cells, neutrophils, etc. These experiments demonstrated the feasibility of utilizing PBR28^{suv} based on injury vs sham in a small cohort of animals, however will require a larger sample size and chronic time points as the next step in exploring this potential diagnostic tool. Traumatic brain injury results in a neuroinflammatory response marked by activated microglia/macrophages. While these cells may help at the site of injury by clearing damaged tissue, their chronic activation contributes to worsening secondary brain injury. PBR28^{suv} has been a useful ligand that allowed visualization of activated microglia *in vivo* among several neurodegenerative diseases [29].

Conclusion

PBR28 may be a promising tracer for monitoring neuroinflammation in TBI. Ideally, longitudinal PET/CT imaging studies would allow clinicians to track the neuroinflammatory response to different therapeutics.

Our study was able to confirm the feasibility of utilizing PET/CT imaging in discriminating between TBI and sham by demonstrating an injury-dependent increase in PBR28suv. In addition, we demonstrated that there is an ipsilateral increase in amoeboid shaped microglia, and that PBR is present in amoeboid-shaped microglia and not astrocytes.

Declarations

Ethics approval and consent to participate:

All protocols involving the use of animals were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (HSC-AWC-15-0003).

Consent for publication:

All authors have consented for publication

Availability of data and material:

All data and material are available.

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Authors' contributions:

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CS Cox Jr: Overall planning and writing of manuscript.

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Figures

Figure 1

Figure 1

CCI results in an increase in the PBR28suv 5 days after injury. PET imaging was done 5 days after CCI in Sham and CCI animals. (A) PET/CT image of a sham animal. Note the lack of red (PBR28) in the Sagittal and Horizontal views (B) PET/CT image of an injured animal 120 hours after a CCI. Note the red (PBR28) in the Sagittal and Horizontal views. Extra-cranial soft tissue radioligand uptake was excluded from analysis. (C) There were significant increases in PBR28suv in the ipsilateral hemisphere from 50s to 1050s. (D) Whole brain PBR28suv also resulted in a significant increase in uptake values. Cross hairs indicate site of injury.

Figure 2

CCI results in an increase in the number of amoeboid shaped IBA1 positive cells in the ipsilateral corpus callosum 5 days after injury. (A) CCI modestly increased the number of ramified cells in comparison to sham (ipsilateral and contralateral) but this was not significant. (B) Ipsilateral to the injury, CCI increase the number of amoeboid shaped IBA1 positive cells in comparison to sham, while there was no change in the contralateral side. (C) Photomicrographs of ramified (Sham) and amoeboid-shaped (CCI) microglia.

Figure 3

Figure 3

CCI results in an increase in the number of amoeboid shaped IBA1 positive cells in the ipsilateral dentate gyrus 5 days after injury. (A) There was no increase due to CCI in the number of ramified cells in comparison to sham (ipsilateral and contralateral). (B) Ipsilateral to the injury, CCI increase the number of amoeboid shaped IBA1 positive cells in comparison to sham, while there was no change in the contralateral side. (C) Photomicrographs of ramified (Sham) and amoeboid-shaped (CCI) microglia.

Figure 4

Figure 4

CCI results in an increase in the number of amoeboid shaped IBA1 positive cells in the ipsilateral medial thalamus 5 days after injury. (A) There was no increase due to CCI in the number of ramified cells in comparison to sham (ipsilateral and contralateral). (B) Ipsilateral to the injury, CCI increase the number of amoeboid shaped IBA1 positive cells in comparison to sham, while there was no change in the contralateral side.

Figure 5

Figure 5

CCI results in an increase in the number of amoeboid shaped IBA1 positive cells in the ipsilateral lateral thalamus 5 days after injury. (A) There was no increase due to CCI in the number of ramified cells in comparison to sham (ipsilateral and contralateral). (B) Ipsilateral to the injury, CCI increase the number of amoeboid shaped IBA1 positive cells in comparison to sham, while there was no change in the contralateral side.

Figure 6

Figure 6

PBR co-stains with microglia and not astrocytes after 5 days after injury. (A-D) Photomicrographs demonstrating the lack of co-staining of PBR and GFAP as examined in the ipsilateral hippocampus. Red is PBR and Green is GFAP. (E-H) Photomicrographs ipsilateral to the injury of PBR and IBA1, there was considerable co-staining of PBR and IBA1, and that was generally localized to amoeboid shaped cells.