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An In Vivo Study of a Rat Fluid-Percussion-Induced Traumatic Brain Injury Model with [^{11}C]PBR28 and [^{18}F]flumazenil PET Imaging

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RESEARCH QUESTION

To investigate whether the two radioligands, [^{11}C]PBR28 and [^{18}F]flumazenil, are able to accurately quantify in vivo molecular-cellular changes in a rodent TBI-model.

BACKGROUND

Traumatic Brain Injury (TBI):

- Can be defined as external trauma causing brain injury;
- Is a major public health concern in Singapore[1];
  - Trauma is the fifth highest killer in Singapore;
  - TBI contributes to half of trauma-related deaths locally;
  - TBI causes both physical and psychological suffering in survivors.

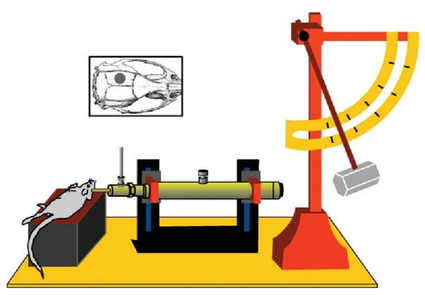
INTRODUCTION

To analyse cellular and molecular alterations of TBI in humans, an LFPI rodent model was developed. LFPI is presently the most well-endorsed model for evaluating mixed focal and diffuse brain injury.

TBI is associated with neuroinflammation and neuronal cell death:

- To quantify these, we examined molecular biomarkers using PET radioligands;
- [^{11}C]PBR28 is a radioligand of the 18 kD translocator protein, which corresponds to the level of neuroinflammation[2];
- [^{18}F]flumazenil is a radioligand for GABAA-benzodiazepine receptors, whose level mirrors cell death[3] .

EXPERIMENTAL DESIGN



Induce mixed focal and diffuse damage with LFP in the ipsilateral cerebral cortex as well as in the hippocampus.

Each rat* was injected with *220 ± 90 µL* [^{11}C]PBR28 over a duration of 20 min, followed by administration of *120 ± 50 µL* [^{18}F]flumazenil over 2.5 h at days 2 and 14 post-operation (post-op).

Use PET imaging to quantify neuroinflammation and loss of neuronal density based on radioactivity of [^{11}C]PBR28 and [^{18}F]flumazenil respectively.

Brains perfused with fixative (10% neutral buffered formalin), extracted for further processing and immunohistochemical analyses with OX-42 primary antibody

** If rats displayed persistent pain or suffering at any time, they were euthanised. Six rats were sacrificed at day 3 and the remaining six rats were sacrificed at day 14 post-FPI.*

RESULTS

- Significant increase in [^{11}C]PBR28 uptake and decrease in [^{18}F]flumazenil uptake of whole brain on Day 14 post-op (Fig. 2);
- Significantly higher [^{11}C]PBR28 uptake in right hemispheres (Fig. 4) compared to the left which was not subject to injury;
- Down-regulation of [^{18}F]flumazenil is not marked and hence not conclusive (Fig. 4);
- Immunohistochemistry data unequivocally support the PET imaging results.

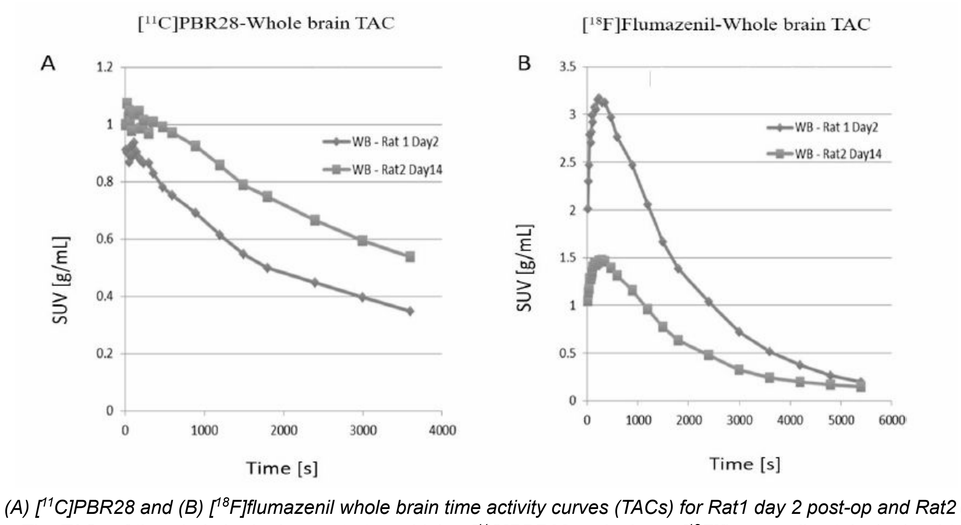


Fig 1: (A) [^{11}C]PBR28 and (B) [^{18}F]flumazenil whole brain time activity curves (TACs) for Rat1 day 2 post-op and Rat2 day 14 post-op. The TACs of the whole brain demonstrates a higher [^{11}C]PBR28 and a lower [^{18}F]flumazenil uptake in day 14 compared to day 2 post-op.

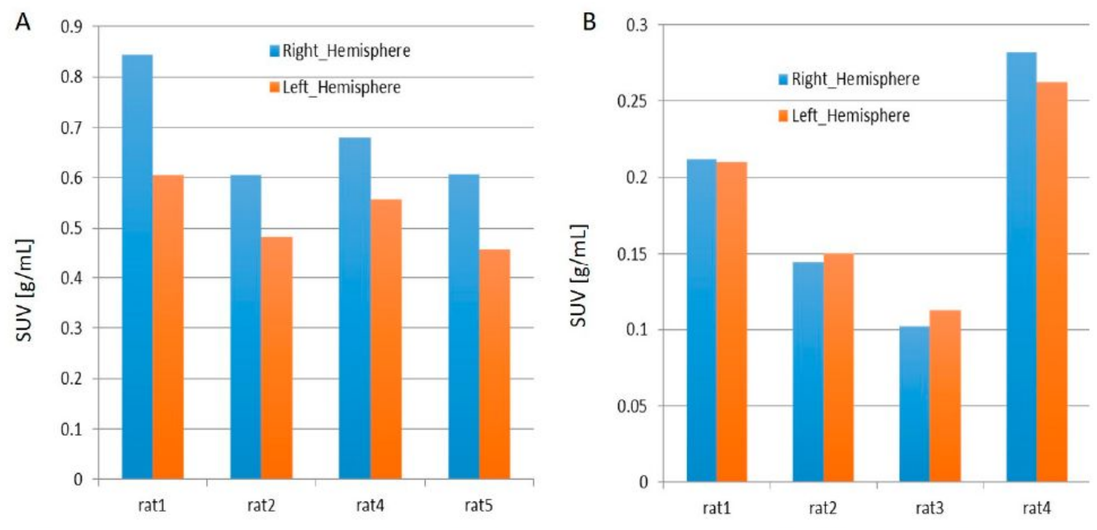


*Fig 2: Average standardized uptake values (SUVs) for the last 30 min frames with (**A**) [^{11}C]PBR28 and (**B**) [^{18}F]flumazenil.*
Fig 2A: For all the rats injected with [^{11}C]PBR28, right hemisphere displayed elevated standardized uptake values (SUVs) than the left, reflecting increased neuroinflammation in TBI.
Fig 2B. 50% of the rats injected with [^{18}F]flumazenil demonstrated an upregulation in right hemisphere compared to left, whereas 50% demonstrated a downregulation in right hemisphere compared to left. Hence, no conclusion can be drawn regarding changes in neuronal density following TBI.

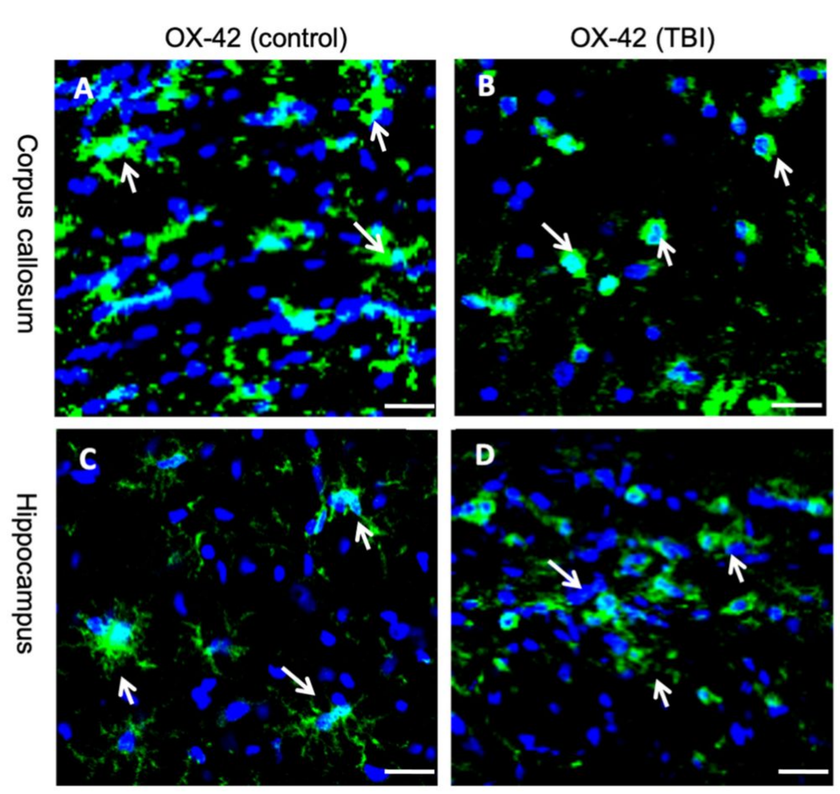


*Fig 3: Immunohistochemistry data for corpus callosum ((**A**)—control, (**B**)—TBI) and hippocampus ((**C**)—control, (**D**)—TBI). The neuroinflammation marker, OX-42, shows a higher binding in the injured right hemisphere.*

DISCUSSION AND CONCLUSION

Main findings include:

- The possibility to quantify regional changes of neuroinflammation in TBI with [^{11}C]PBR28 PET imaging;
- An inconclusive relationship between neuronal density changes and [^{18}F]flumazenil PET imaging.

FUTURE PERSPECTIVE

Further studies can be conducted on neural cell specific radiotracers like:

- [^{18}F]UCB-J, [^{18}F]UCB-H and their associated fluorine-18-labelled derivatives.

This preliminary study can conceivably pave the way for the conjunction of the aforementioned PET radiotracers with future nanomedical therapies for TBI.

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