Final Report

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Date: 30/08/2022

Title of Project: The effect of Toxoplasma Gondii infection on common acquired brain insults

Summary:
Traumatic brain injury (TBI) and stroke are common acquired brain injuries that lack an intervention that effectively mitigates brain damage and improves functional outcomes in patients. One of the reasons why there is still no treatment for these conditions is because previous studies have failed to account for how factors, such as Toxoplasma gondii (T. gondii) infection, can alter pathobiology which would significantly alter appropriate treatment strategies. T. gondii is a parasite that invades and resides in the central nervous system. T. gondii is carried by 30-50% of the human population globally, and infection rates are upwards of 80% in developing countries. Therefore, many people who sustain a brain injury may have been infected with T. gondii at the time of injury. A hallmark feature of T. gondii infection is the presence of parasitic cysts in neurons, which can result in neuropathophysiological features such as neuroinflammation and excitotoxicity. It is therefore possible that the presence of T. gondii infection can alter the pathobiology of brain injuries, contribute to the brain damage in TBI and stroke, and affect the treatment strategy required for such patients.

The aims of this project are to examine how T. gondii infection modifies TBI/stroke pathobiology and recovery in rodents. It is hypothesized that T. gondii infection will exacerbate neuropathophysiological mechanisms in rodents that given an acquired brain injury, and there will be worse brain damage and functional outcomes in these animals. Although the project has been delayed due to the impact of the COVID-19, as well as limited funding, we were able to complete a substantial study which examined the effects of T. gondii infection on TBI in mice, while leave the stroke element as unanswered questions.

At 6 weeks of age, male and female mice were randomly allocated to receive either a single intraperitoneal (i.p.) injection of T. gondii tachyzoites or vehicle (VEH) only. The mortality rate from the T. gondii infection was 13.8%. Mice were allowed 6-weeks after injection for a chronic T. gondii infection to establish, and at this point, received either a TBI that was induced via the controlled cortical impact (CCI) model or a sham injury. Therefore, this study comprised of four experimental groups per sex: SHAM + VEH; SHAM + T. gondii; TBI + VEH; and TBI + T. gondii. Some mice were euthanized at 2 hours (n = 5-6/group/sex), 24 hours (n = 5-6/group/sex) and 7

days (n = 5-6/group/sex) for RT-qPCR gene expression analyses. The rest of mice (n = 10-12/group/sex) were given a 3.5-month recovery period and underwent behavioural testing. Following that, prior to euthanasia at 18 weeks post-injury, mice were administered 40 mg/kg PTZ i.p. and video-recorded for a period of up to 30 minutes for evaluation of seizure susceptibility using a modified Racine scale.

We found that there were synergistic effects between T. gondii and TBI at 24 hours post-injury (7 weeks post-infection), that the T. gondii infection amplified the neuroinflammation induced by the TBI (Figure 1). Specifically, T. gondii + TBI mice had significantly increased cortical mRNA levels of microglia marker (A-B), cytokine receptor expressed on microglia (C), inflammatory cytokines CCL2 (D), CXCL10 (E) and TNF-α (data not shown, $p<0.05$), as well as the activation of inflammasome (F). We also found that TBI and T. gondii effects were evident on neuroinflammatory and immune cell markers at 2 hours post-injury and 7 days post-injury, respectively (data not shown). In addition, at 24 hours, TBI + T. gondii resulted in increased neuronal damage, evident by significantly decreased MAP2 compared to the T. gondii + SHAM and VEH + TBI mice (data not shown, $p<0.05$). We also found that TBI + T. gondii exacerbated excitotoxicity, as GLT-1 was decreased compared to T. gondii + SHAM and VEH + TBI mice (data not shown, $p<0.05$). Our findings suggested that a history of T. gondii infection could worsen neuroinflammation, excitotoxicity, and neuronal damage, during the acute stage of TBI.

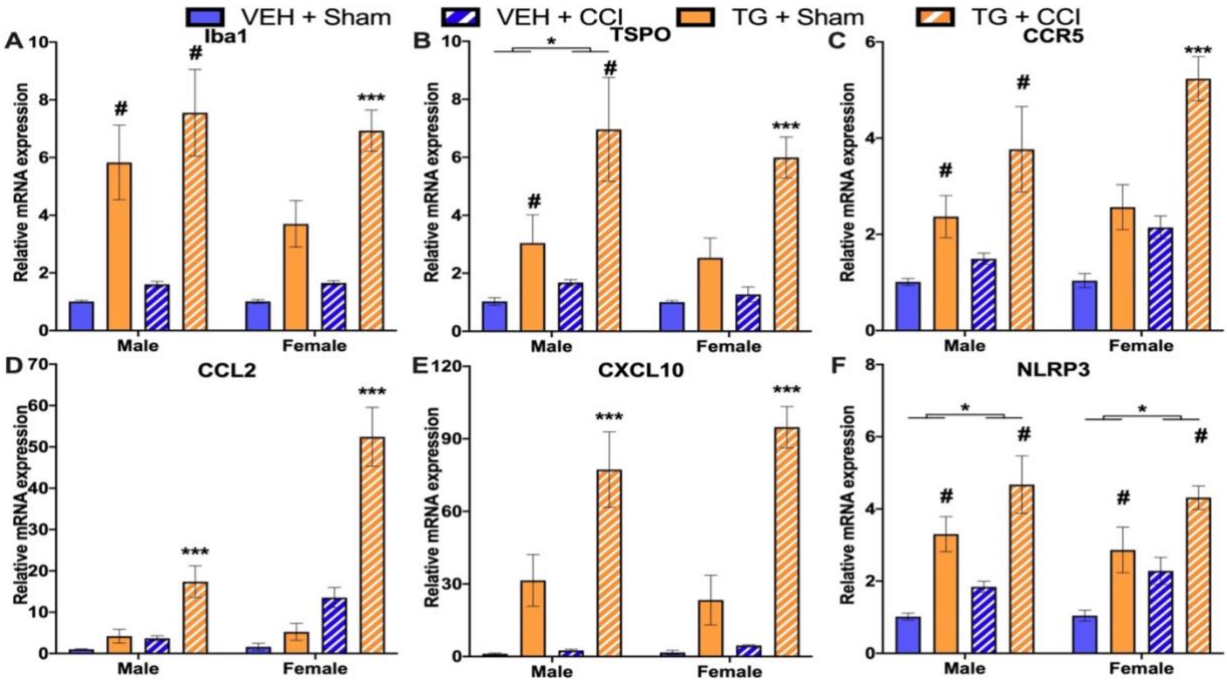

Figure 1. Brain mRNA levels of neuroinflammatory markers at 24h after TBI (7 weeks post-infection). N = 5-6/sex/group; TG = T. gondii, VEH = vehicle; CCI = controlled cortical impact, TBI model; *** > than all groups, # TG > VEH, * TBI > sham, $p < 0.05$; Error bar = SEM.

The mechanisms underlying the development of post-traumatic epilepsy (PTE) currently remain unclear. To test whether the effects of excessive neuroinflammation, excitotoxicity, and neuronal damage induced by T. gondii + TBI may manifest chronically and impact seizure susceptibility in the long-term, mice underwent seizure susceptibility at 3.5 months after TBI. We found that a pre-existing T. gondii infection is an important modifier of seizure susceptibility independent of TBI in mice (data not shown). We also performed a battery of behavioural testing at 3.5 months after the TBI to examine motor ability, sociability, anxiety, depression, and cognition in these mice, and the data analysis is still on-going. MRI brain structure analysis and immunohistochemistry analysis of brain samples from these mice (18 weeks post-injury) is also on-going.