



Sustained Tau Phosphorylation and Microglial Activation Following Repetitive Traumatic Brain Injury

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Abstract

Edited by: Slavica Hristomanova-Mitkovska
Citation: Siahaan AMP, Indharty RS, Chrestella J, Sadewo W, Tandean S, Syarifah S. Sustained Tau Phosphorylation and Microglial Activation Following Repetitive Traumatic Brain Injury. Open Access Maced J Med Sci. 2020 Nov 06; 8(A):1-4.
<https://doi.org/10.3889/oamjms.2020.5471>

Keywords: Activated microglia; Repetitive traumatic brain injury; Phosphorylated tau
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Received: 23-Sep-2020
Revised: 23-Oct-2020
Accepted: 26-Oct-2020

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Funding: This study was supported by the 2020-2021 Fund Directorate of Research and Community Service, Ministry of Education Republic of Indonesia.
Competing Interests: The authors have declared that no competing interests exist.
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BACKGROUND: Repetitive traumatic brain injury (TBI), even without acute sequela, can induce a delayed neurodegenerative with overexpression of phosphorylated tau (p-tau) as hallmark, caused by chronic inflammation mediated in part by microglial activation.

AIM: The aim of this study was to examine the dynamics of p-tau accumulation and microglial activation following repetitive TBI.

MATERIALS AND METHODS: Thirty Sprague-Dawley rats were randomized into a sham control group and two treatment groups receiving three successive closed-skull impacts (TBI model) from a 40-g mass dropped from a 1-m height on alternating days (days 0, 1, 3, and 7). The first treatment group was sacrificed on the last day of trauma and the second treatment group after 7 days of no trauma. The expression level of p-tau was evaluated by AT-8 antibody immunostaining and microglial activation by anti-CD-68 immunostaining.

RESULTS: Immunoexpression of AT-8 was significantly elevated 7 days after TBI compared to the last day of trauma and compared to the sham control group, while CD-68 expression was significantly higher than sham controls on the last day of trauma and remained elevated for 7 days without trauma.

CONCLUSION: The study showed that brain trauma can induce p-tau overexpression and microglial activation that is sustained during the non-trauma period.

Introduction

Traumatic brain injury (TBI) is a well-known risk factor for neurodegenerative disease independent of age. In a large-scale study involving 44,925 patients with a history of TBI, Wang *et al.* (2012) reported a 60% increase in dementia incidence [1]. The same finding was reported by Barnes *et al.* (2014) in a study of 188,674 veterans, where TBI increased dementia incidence by up to 60% [2].

Accumulation of phosphorylated tau (p-tau) is thought to be the main driver of neurodegeneration following TBI [3], [4]. Repetitive TBI can induce a constellation of clinical manifestations such as cognitive deficits, mood instability, and behavioral abnormalities associated with the accumulation of p-tau and TDP-43 [5], [6]. This degenerative changed, namely Chronic Traumatic Encephalopathy, has gained much

attention recently due to reports that some relatively young American football players have developed these clinical manifestations and that brain pathology at autopsy has found changes consistent with early-onset dementia [7].

Under physiological conditions, tau protein is crucial for maintaining microtubule stability [8]. Repeated TBI may activate protein tau kinases, leading to the accumulation of p-tau [9], and microglial activation is one potential cause of tau kinase activation [10]. There are two potential causes of progressive neurodegenerative disease following TBI. First, microglial activation may sustain chronic neuroinflammation; indeed, activated microglia have been detected even years following TBI [11]. Second, p-tau is a prion that can be propagated into surrounding cells [12]. Based on these pathogenic mechanisms, the aim of this study was to explore the dynamics of p-tau and activated microglia after a rest period with no trauma following repetitive TBI.

Materials and Methods

Animal model and experimental groups

Thirty Sprague–Dawley rats (6–8 weeks old and weighing 300–350 g) were randomized into three groups, a sham control group and two treatment groups. The animals were maintained in a temperature-controlled room at 22–24°C under a 12 h–12 h dark–light cycle with *ad libitum* access to food and water. Animals were housed under these conditions for 7 days for adaptation before experiments and following TBI.

For induction of TBI, a 40-g mass was dropped onto the cranial vertex from a 1-m height. To prevent skull fracture, a metal helmet of 2.5-cm diameter was placed on the vertex. Each treatment group rat was subjected to 3 impacts on alternating days (days 0, 1, 3, and 7) with rest periods on days 2, 4, 5, and 6. No anesthesia was used in this protocol [13]. The first trauma group was sacrificed on day 7 soon after the last (12th) impact, while the second trauma group was sacrificed 7 days after the last impact without intervening treatment or TBI. This procedure was approved by the Medical Research Ethics Committee of the Medical Faculty, Universitas Sumatera Utara (Medan, Indonesia).

Brain isolation

Rats were anesthetized with ether and decapitated. The skull was cut from the foramen magnum to the anterior pole on the left and right sides and the olfactory bulb discarded. The remaining whole brain was fixed in 10% formalin buffer (10%).

Immunohistochemistry

p-tau was detected using the AT-8 antibody (Thermo Fisher) and activated microglial using anti-CD-68 (Thermo Fisher). Briefly, brain tissue was embedded in paraffin and cut into 4- μ m thick sections, including the cortex under the vertex. For examination of immunoreactivity, cells were counted in 20 randomly chosen fields at 400 \times magnification.

Statistical Analysis

All results are expressed as mean \pm SEM. Group means were compared by analysis of variance with Tukey's post tests for pair-wise comparisons or by independent samples t-test. $p < 0.05$ (two-tailed) was considered statistically significant.

Results

Repetitive traumatic brain injuries caused sustained microglial activation

Trauma Group 1 exhibited an increased number of CD-68+ cells and greater immunostaining intensity compared to the sham control group (Figure 1a and 1b). After 7 days with no TBI, CD-68 expression was still higher in trauma Group 2 than the sham control group, although the staining intensity was reduced compared to immediately after TBI. However, the number of CD-68+ cells did not differ between trauma Groups 1 and 2 (Figure 1b and 1c).

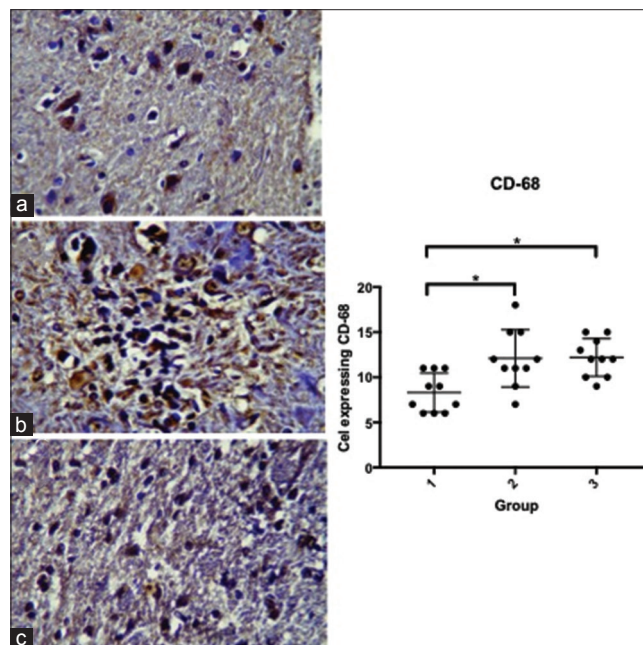


Figure 1: CD-68 expression in cortical brain slices from the sham control group (a), treatment Group 1 (b), and treatment Group 2 (c). Expression was significantly elevated in the treatment groups compared to the sham control group ($*p < 0.05$ by one-way ANOVA with post hoc Tukey tests), indicating sustained microglial activation following TBI. Group 1: Sham control group; Group 2: Trauma 1; Group 3: Trauma 2. Data are expressed as mean \pm SEM

Repetitive traumatic brain injuries caused prolonged tau phosphorylation

Immunoexpression of p-tau was also significantly enhanced in trauma Group 1 compared to the sham control group (Figure 2a and 2b). Further, expression was even higher after 1 week without TBI (Figure 2c).

Discussion

Hyperphosphorylation of tau protein is the hallmark of the neurodegenerative disorder induced

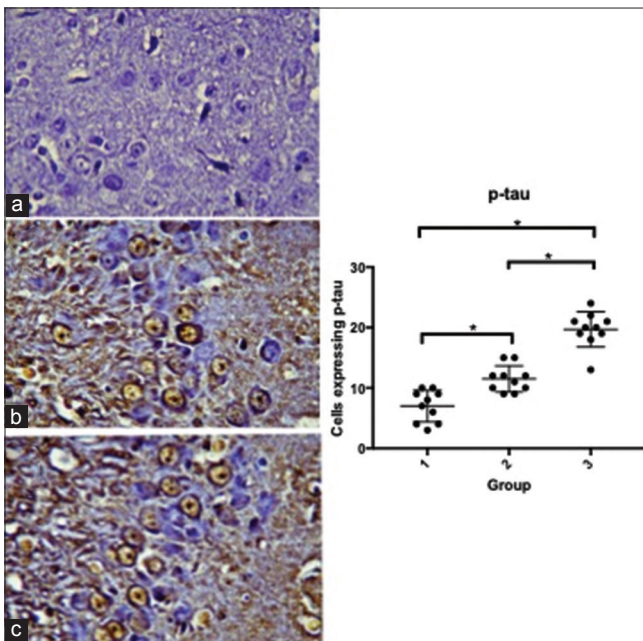


Figure 2: Expression of phosphorylated tau in cortical brain slices from the sham control group (a), treatment Group 1 (b) and treatment Group 2 (c). Expression was highest in treatment Group 2 (* $p < 0.05$ by one-way ANOVA with post hoc Tukey test). Group 1: Sham control group; Group 2: Trauma 1; Group 3: Trauma 2. Data are expressed as mean \pm SEM

by repetitive TBI [14]. Although it is still debated, there is growing evidence that p-tau accumulated following TBI will aggregate to form oligomers and ultimately neurotoxic neurofibrillary tangles [9]. In this study, same as the model we used, we found an increase of p-tau expression following repetitive TBIs [13].

The mechanism mediating TBI-induced phosphorylation and aggregation of tau protein is still uncertain, but microglial activation and ensuing neuroinflammation are strongly implicated [15], [16]. Indeed, activated microglia were observed before tau pathology in an animal model of tauopathy [17] and Maphis *et al.* (2015) report that reactive microglia are sufficient to drive tau pathology in an animal model of AD [18].

TBI will induce a complex inflammatory response involving proliferation and migration of resident microglia to the site of injury. Microglia then becomes activated and transforms to amoeboid-like cells that secrete pro-inflammatory or anti-inflammatory cytokines. Pro-inflammatory cytokines promote phagocytic activity and host defense, while anti-inflammatory cytokines promote repair and remodeling [19].

Chronic neuroinflammation has been observed following both moderate and severe head injury. Johnson *et al.* (2013) reported persistent inflammation and white matter degeneration years after single TBI [20]. Mild head injury itself may induce axonal disruption that, in turn, triggers a neuroinflammatory response to repair or limit the damage [21]. Following

a single mild head injury, this response will dissipate as the brain is repaired. However, repetitive TBI within a short interval induces a persistent pro-inflammatory state [22]. In this research, we demonstrated such a persistent inflammatory response after a week without TBI, as evidenced by a sustained increase in CD-68+ cells and a continued increase in p-tau expression.

Neuroinflammation also drives hyperphosphorylation of tau protein. In this study, there was a significant increase in AT-8 immunostaining during the rest period. This continued p-tau accumulation may result from persistent microglial activation. Alternatively, p-tau accumulation may become self-perpetuating through prion activity [23]. Tau may interact with the cell membrane to facilitate prion-like propagation, followed by uptake into recipient cells via macropinocytosis [24]. Although phosphorylation is reversible, tau seeding and propagation could promote further neurodegeneration [25].

The main limitation of this study is that AT-8 and CD-68 expression were not localized to specific cell types using double immunolabeling. Similarly, microglia polarization was not directly examined. Finally, the TBI-free period should be extended to better model repeated TBI in humans.

Conclusion

The repeated TBI-induced sustained microglial activation during the ensuing TBI-free period and a continued increase in p-tau expression. These sustained changes may account for the progressive nature of neurodegeneration during repeated TBI.

Acknowledgment

This study was funded by the 2020-2021 Fund Directorate of Research and Community Service-Ministry of Education Republic of Indonesia. We thank Wibi Riawan (Universitas Brawijaya, Malang, Indonesia) for helping in conducting the trauma. We thank Iskandar Japardi (Universitas Sumatera Utara, Medan, Indonesia) for comments on the manuscript.

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