Turmeric Extract Supplementation Reduces Tau Protein Level in Repetitive Traumatic Brain Injury Model

Andre Marolop Pangihutan Siahaan[1*], Iskandar Japardi[1], Aldy Safruddin Rambe[2], Rr Suzy Indharty[1], Muhammad Ichwan[3]

[1]*Department of Neurosurgery, Medical Faculty Universitas Sumatera Utara, Medan, Indonesia;* [2]*Department of Neurology, Medical Faculty Universitas Sumatera Utara, Medan, Indonesia;* [3]*Department of Pharmacology and Therapeutic, Medical Faculty Universitas Sumatera Utara, Medan, Indonesia*

Abstract

BACKGROUND: Repetitive traumatic brain injury (RTBI) has gained much attention in this decade, especially in contact sports athletes and military personals. This injury is correlated with early neurodegenerative changes that are marked with the increased of tau protein. Turmeric extract (TE) is a well-known anti-inflammation and antioxidant that decreases tau protein expression in neurodegenerative disease.

AIM: This study aimed to prove the effect of TE on tau protein level after RTBI.

METHODS: Forty Sprague Dawley mice were divided into four groups, i.e. negative sham control group, the control group, and two treatment groups. A weight drop model was used by applying a 40-gram mass that was dropped from a 1-meter height onto the vertex of the head, with a total frequency of 12 times, divided into 4 days (day 0, 1, 3, and 7; 3 traumas on each day). TE was given to all treatment groups with 500 mg/kg BW doses once daily. The first treatment group had TE for seven days along the trauma. The second treatment group had pretreatment TE extract, given from seven days before first trauma and continued along the trauma protocol days. Tau protein level was measured on brain and serum using ELISA method.

RESULTS: There was a significant reduction of tau protein level in both treatment groups compared to trauma group, either in serum or brain, but we also found significant differences regarding brain tau level between the treatment and pretreatment group.

CONCLUSION: This study might provide evidence of with the role of pretreatment TE in RTBI.

Introduction

Repetitive traumatic brain injury (RTBI), particularly concussion, is frequently happened in some populations, like contact sports athletes and military personals. In a survey involving 222 professional football players, the incident of head impact is very diverse, from four times to 125 times in two weeks. Another report said that around 30% of football players do heading for more than 1000 times annually [1]. In the military, it is estimated that up to 35% of American service personnel in Iraq and Afghanistan have sustained a concussion [2]. Almost all sports-related traumatic brain injury is minor, with spontaneous recovery within days to weeks, even a small number of individuals develop long-lasting and progressive symptoms.

Following traumatic brain injury, even in mildest form, ionic flux, hyper glycolysis, and metabolic uncoupling will result in an energy crisis that can be lasted until 7 to 10 days [3]. The time needed for recover may render the brain vulnerable to the second event. RTBI has been shown they exacerbate the impairment of the brain's cellular oxidative metabolism similar to severe traumatic brain injury [4]. RTBI itself will result in progressive white matter injury

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and behavioural dysfunction [5]. Around 17% of individuals with RTBI will develop a constellation of several dysfunctions in behaviour, mood, cognitive, and motoric [6]. Over the last several decades, it is known that these symptoms are correlated with progressive neurodegeneration process that happens in this population. This early neurodegenerative process is named chronic traumatic encephalopathy (CTE). The epidemiology of CTE is unknown since microscopic examination after an autopsy is the only widely accepted way to diagnose this condition [7].

As in another neurodegenerative process, there are several proteinopathies that can be found in CTE. Protein tau is the main proteinopathy. Hyperphosphorylation will dissociate tau from tubulin and expose new phosphorylation site. In this state, protein tau will be insoluble, accumulated into oligomers and neurofibrillary tangles [8]. This condition resembles Alzheimer's disease (AD), with different in tau distribution. In CTE, tau is most commonly found in the superficial layer, compared to layers III and V in AD [9] [10]. Factors that initiate the development of tau pathology are still unknown but associated with chronic neuroinflammation. Neuroinflammation is a common finding after TBI, especially in moderate and severe TBI. In mild TBI, neuroinflammation is usually short-lived, but RTBI may lead to chronic neuroinflammation due to self-perpetuating inflammatory cycle, including the sustained release of inflammatory mediators. It is shown that microglial neuroinflammation contributes to tau accumulation in CTE [11].

To this date, there is no approved therapy for CTE. Rest and symptomatic therapy are the main treatment approaches. The answer may lie in understanding the principle of tau proteins, where tau proteins aggregate through nucleation, templating, growth, multiplication, and spread. Each of these mechanisms represents a potential target for therapeutic intervention. Therapies that target tau phosphorylation, tau aggregation, and microtubule stabilisation are already in clinical trials [12].

For a long time, practitioners of Ayurvedic, traditional Chinese medicine, and Southeast Asian medicine have used turmeric root extract (TE) for the culinary, religious, and medical condition. Curcumin, the major active compound of TE, is a strong anti-inflammatory and antioxidant properties [13]. The use of curcumin in the AD has been studied extensively. Curcumin reduces accumulation of soluble tau aggregates via inhibition the kinase [14]. Even so, all human clinical trials fail to prove the role of curcumin in the AD, regarding cognitive function [15]. The main problem is curcumin's low bioavailability [16].

The present study was undertaken to evaluate the effects of turmeric extract in RTBI concerning protein tau level, both in brain and plasma.

Materials and Methods

The subjects of this experiment were 6-8 weeks old Sprague Dawley rats weighing 300-350 g (n = 40). This experimental protocol was approved by an Institutional Ethics Committee in Universitas Sumatera Utara, Medan, Indonesia. The animals were housed with 12 h light/12 h cycle and given access to food and water ad libitum.

The animals were exposed to the weight-drop model of TBI that had been described before [17]. A 40 g mass was dropped from 1 m high unto the vertex. A 2.5 cm metal plate was placed on the vertex to prevent skull fracture. The total cumulative traumas were 12 times, divided 3 times each day on day 0, 1, 3, and 7. We did not use anaesthesia in this protocol.

The rats were randomly allocated into four groups (n = 10) as follow: a control (sham-operated) group, a trauma group, and two group treatments, i.e., treatment 1 (after trauma), and treatment 2 (pretreatment) groups. The control group underwent neither trauma nor TE. The trauma group underwent trauma only, but no TE. The first treatment group received TE for seven days along the trauma and the second treatment group received TE started from seven days before the trauma and along the trauma.

Turmeric extract (Sido Muncul, Indonesia, 18% curcumin) was suspended in distilled water and administered to animals via oral gavage in 2 cc solution. Turmeric extract was given per oral with dose 500 mg/kgBW once daily. The animals were weighed after the acclimatisation. Turmeric extract was administered in the morning, 1 hour before the first trauma.

As a marker of neurodegenerative process, we investigated AT-8 expression. The expression of all markers was investigated on paraffin-embedded sections using the avidin-biotin-peroxidase complex method. Four-millimetre-thick paraffin sections were dewaxed, rehydrated, and microwave for 10 minutes. The endogenous peroxidase activity of the investigated specimens was blocked with 3 per cent H_2O_2 for 10 minutes, followed by 25 minutes washing with phosphate-buffered saline (PBS). The tissue sections were incubated with normal rabbit serum for 10 minutes, and then the slides were incubated at room temperature with monoclonal mouse AT-8 (Santa Cruz). Sections were washed with PBS and incubated with a secondary antibody for 30 minutes. Sections were washed twice with PBS, developed with 0.05% 3, 3 diamino-benzinetetrahydrochloride for five minutes, and slightly counterstained.

All samples were evaluated by the first author (not blinded to specimen). A positive signal for AT-8 in brain tissue was quantitatively estimated by the distribution of positively stained cells in the cortical brain. Cell counts were carried out using a light

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binocular microscope with 1000 times magnification in 20 high power fields.

Protein tau level was measured in plasma and brain. Animals were sacrificed one day after the last protocol day. After anaesthesia, rats were decapitated, and brains were dissected. Brain tissues were homogenised in ice-cold homogenization buffer (50 mM Trizma base/HCl buffer, pH 7.4 containing protease inhibitors and 2 ÌM of the phosphatase inhibitor okadaic acid), these brain homogenates were placed in -20°C.

Blood was taken from the left ventricle. After collection of the whole blood, blood was left undisturbed at room temperature for 15-30 minutes. The clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes. The plasma then was maintained at -20°C. Blood and plasma levels of total tau were determined using a commercially available sandwich ELISA system (Cusabio).

Values were expressed as means ± SD. The results were computed statistically using one-way analysis of variance for each group. The Tuckey post hoc testing was performed for intergroup comparisons. A difference was considered significant at the p < 0.05 level.

Results

There was no mortality rate in this protocol. We also did not find a decrease in body weight (Table 1). There was no significant difference regarding body weight on all three groups (Table 1), either in negative sham control group (377.22 ± 29.72 gr vs. 378.44 ± 29.66 gr), trauma group (351.78 ± 29.89 gr vs. 349.33 ± 38.90 gr), or in treatment group (367.89 ± 36.70 gr vs. 357.89 ± 39.89 gr).

Table 1: Weight changes in subjects (Mean ± SD)

Group	Day 0 (gr)	Day 7 (gr)	p
Negative sham	377.22 ± 29.72	378.44 ± 29.66	0.910
Trauma	351.78 ± 29.89	349.33 ± 38.90	0.482
Treatment	367.89 ± 36.70	357.89 ± 39.89	0.950

Paired t-test. Significant if p < 0.05

There was significantly difference between tau level between the negative sham group and trauma group, either in the brain (Figure 1A) or plasma (Figure 1B). In the negative sham control group, brain protein tau level was 4.21 ± 0.22 pg/ml while plasma tau protein level was 4.36 ± 0.13 pg/ml). There was significant increase of tau protein level following RTBI, either in brain (17.76 ± 2.06 ng/ml, p < 0.05) or plasma (6.16 ± 1.11 ng/ml, p < 0.05).
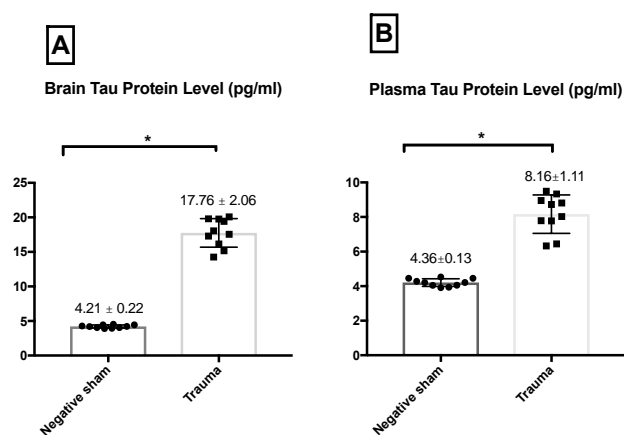

Figure 1: Change in protein tau level following RTBI in the brain (A) and plasma (B). There was significant protein tau level difference between trauma and negative sham group both in the brain and in plasma. Data was shown as Mean ± SD. T-test; significant if p < 0.05

There was a significant decrease of tau protein level in both treatment groups compared to the trauma group, either in the brain (Figure 2A) or in plasma (Figure 2B). In treatment 1 group, the brain tau protein level was 13.31 ± 1.45 pg/ml and the plasma tau protein level was 5.25 ± 0.77 pg/ml.

These were significantly different compared to in trauma group (p < 0.05). In treatment 2 group, either brain tau protein level (3.60 ± 0.23 pg/ml) or plasma tau protein level (4.99 ± 0.53 pg/ml) was also significantly different compared to trauma group (p < 0.05). There was significant brain tau protein level between treatment 1 group and treatment 2 group, which was not seen at the plasma.
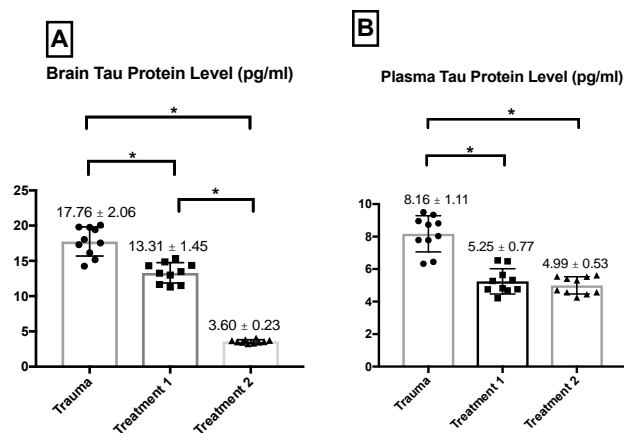
Figure 2: Change in protein tau level after TE supplementation on the brain (A) and plasma (B). There was significant protein tau level difference among either treatment 1 or treatment 2 group with trauma group in brain and plasma. There was also a significant difference regarding protein tau level between treatment 1 and treatment 2 group in bran. The significant difference was not found in plasma. Data was shown as Mean ± SD. T-test; significant if p < 0.05

Discussion

We reported no mortality rate in this protocol. Xu *et al.,* (2006) report the same finding. The mortality rate is increased along the increased weight and height. With the 50 g-1 m impact, mortality is 18.2%, and with the 60 g-1 m impact, mortality is increased up to 40% [17].

We also did not find a decrease in body weight (Table 1). In this RTBI model, the animals underwent injury schedule at days 0, 1, 3, and 7, with three hits daily. It made a total of 12 TBI events. The use of this model was meant to replicate the pattern of multiple concussion in contact sports athlete. This model did not show evidence of weight loss, compared to the more "severe" model. Samini *et al.,* (2013) find significant weight decrease following a weight-drop model of TBI. The body weight decrease was probably due to injury affecting feeding behaviour and injury to the anterior hypothalamus, even though multi-factorial mechanisms are involved in TBI [18].

Tau is a soluble intracellular protein with little secondary structure. One of protein tau's functions is to modulate the stability of microtubules. Tau is localised in neuronal cells, but it is not released. Therefore, CSF and blood tau levels in healthy subjects are expected to be low or non-existent.

In pathologic conditions, associated with tauopathy, the soluble protein tau becomes hyperphosphorylated at specific site forming an insoluble form. The brains of some individuals who experience RTBI and develop CTE display tauopathy, predominantly perivascular and in the cortex [19]. Several pathogenic mechanisms have been suggested to contribute to this tau-specific pathology profile, include an imbalance in the activity levels of the tau-specific kinase, dysfunction in protein clearance mechanism, prolonged neuroinflammation, and increased genetic susceptibility (ApoE4 allele) [20]. Increased of protein tau's level in the brain is reported in neurodegenerative diseases, such as AD (21). High protein tau level is also found in the brain's interstitial fluid [22], CSF [23], and blood [24].

Neselius *et al.,* (2013) reported that tau plasma levels were significantly increased in Olympic boxers, even without a symptom of concussion [25]. In mild TBI, tau is a poor predictor of CT lesions and post-concussion syndrome [26]. There are inconsistencies regarding the evaluation of protein tau across the literature, i.e. in the form of cleaved-tau, total-tau, and phosphorylated tau. This inconsistency may also be a result of many factors, including the sensitivity and specificity of the tau assays used and the timing of the sample [27].

Penetration of protein tau into the blood suggested that this protein might enter the circulation through the blood-brain barrier (BBB) disruption. Although the integrity of BBB was not assessed, the disruption of BBB in the previous report with the same trauma protocol was minimal [17]. Kane *et al.,* (2012) also reported minimal BBB disruption when using 95-g mass dropped from a 1.5-m height [28]. Other hypotheses that could be proposed are release via glymphatic system or a more complex intracellular transport via macrophages of phagocytic microglia (24). However, these hypotheses require further studies.

However, the mechanism behind tau pathology in repetitive concussive head injury is unknown. Although protein tau is widely used as a biomarker for the diagnosis of CTE, it is not yet clear that tau is the principal mediator of pathogenesis. Protein tau deposition could be just a result of cumulative biochemical changes that happen after RTBI [29].

In this study, treatment groups were divided into two groups. The first treatment group received TE along the trauma for seven days. The second group was pretreatment group that received TE seven days before the trauma was started and continued along the trauma. Protein tau level was significantly lower in either first or second treatment group compared to the trauma group. Nevertheless, protein tau level on the brain was significantly lower on the second treatment group compared to the first. The same difference was not found in plasma (Figure 2A and 2B).

The potential role of TE in degenerative disease is supported by epidemiological evidence. It is reported that people age 70-79 years old in India has a 4.4-fold less prevalence of AD compared to the same population age in the US. The regular intake of turmeric in common Indian diet has been thought as the primary reason for this finding [30]. In Singapore, it was also found that people who regularly ate curry have better cognitive performance than people who rarely or never ate curry [31].

Curcumin was shown to affect protein kinase modulation, such as Akt [32], JNK [33], and GSK-3β [34]. Since protein kinase has a crucial role in protein tau phosphorylation, it may explain why tau protein level was significantly decreased in both treatment group. In neurodegenerative disease, there is also dysfunction in molecular chaperone. Molecular chaperones, such as heat shock proteins (HSPs), play a significant role in the removal of misfolded or mutant proteins. Curcumin affects augmenting expression or function of HSPs in the cell [35]. The anti-inflammatory properties of curcumin also play a significant role in this effect. Curcumin also induces M2 macrophage polarisation in experimental autoimmune myocarditis [36]. M2 macrophages are associated with the production of anti-inflammatory cytokines, increased phagocytic activity, as well as regulation of tissue repair and remodelling [37].

In this research, we found significant protein tau level difference between the first and second treatment group. The second treatment group was a

pre-treatment group that received TE for one week before TBI. Curcumin retreatment will induce Nrf2 that is a regulator of cellular resistance to oxidants [38]. In a model of traumatic brain injury, curcumin pre-treatment also decreases malondialdehyde expression, decreases the lesion size, and improves neurological function [18]. This finding might also support the role of curcumin as a neuroprotective agent, not as a treatment agent. It also could explain the reason behind the failure of curcumin in a human study regarding Alzheimer's disease [15].

There was no significant difference regarding total plasma tau protein level between the two treatment groups. The expression of tau is predominantly in brain, but tau can also be found at both mRNA and protein level in salivary glands and kidney. This is a potential confounder that may help explain the difference of total tau protein level between brain and plasma. In plasma, the half-life of tau appears to be shorter (hours) than in cerebrospinal fluid (weeks) [39].

In conclusion, turmeric extract supplementation reduces tau protein level in brain and plasma following repetitive traumatic brain injury. The most significant effect was found in the pre-treatment group. Further studies to demonstrate the effect of turmeric extract on the clinical outcome will be needed.

Acknowledgements

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