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Efficacy of Neuroprotection from Curcumin through Heat Shock Protein 70 Induction in Traumatic Brain Injury – Rat Model

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Abstract

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BACKGROUND: Traumatic brain injury (TBI) is the most common problem that caused morbidity and mortality in the world. Secondary brain injury is a complex cascade that causes brain cell apoptosis. Curcumin is a natural product that has neuroprotective properties.

AIM: This study aimed to investigate the effect of curcumin toward heat shock protein 70 (HSP 70) expression against the expression apoptosis marker (apoptosis-inducing factor [AIF], caspase-3, and TUNEL assay) in brain tissue after TBI.

METHODS: Thirty-three Sprague Dawley rats were randomized into three treatment groups, that is, sham-operated controls, closed head trauma (CHT), and CHT with curcumin extract (treatment group). In the treatment group, curcumin was given 500 mg/kg per oral for 7 days, then brain tissues were investigated (marker AIF, caspase-3, TUNEL assay, and HSP 70) through immunohistochemistry. Statistical test using one-way ANOVA test and Tukey honestly significant difference as *post hoc* test.

RESULTS: The mean of positive AIF stained cells in Group A was 5.36 ± 2.11 , Group B was 12.82 ± 1.40 , and Group C was 3.82 ± 1.40 , with a significant difference of AIF expression between Groups C and B ($p < 0.05$). Mean of positive caspase-3 stained cells in Group A was 5.45 ± 2.30 , Group B was 13.82 ± 2.44 , and Group C was 3.82 ± 1.54 , with a significant difference of caspase-3 expression between Groups C and B ($p < 0.05$). Mean of positive TUNEL assay stained cells in Group A was 4.82 ± 2.04 , Group B was 11.55 ± 1.51 , and Group C was 3.55 ± 1.70 , with a significant difference between Groups C and B ($p < 0.05$). Mean of positive HSP 70 stained cells in Group A was 6.82 ± 2.14 , Group B was 3.91 ± 2.26 , and Group C was 10.27 ± 2.45 with a significant difference of HSP 70 expression distribution within groups ($p < 0.05$).

CONCLUSION: Curcumin may protect brain cells from apoptosis after close head trauma by upregulated HSP 70 expression.

Introduction

Traumatic brain injury (TBI) is still one of the leading causes of morbidity and mortality in the world. TBI also causes socioeconomic problems to the family and country. The prevalence of TBI was different between countries and most commonly cause by traffic injury, falls, struck by an object, and assaults [1], [2]. Traffic injury is the most common cause of head injury in low- and middle-income countries. It was estimated that 69 million lives had TBI each year with the most burden in Southeast Asia and Western Pacific countries [3].

TBI has primary and secondary injury, which primary injury is a direct lesion after injury such as brain hemorrhage and axonal shearing, while secondary injury is an activation of apoptosis caused by complex inflammation process after primary brain injury. Complex inflammation process can activate caspase dependent and caspase independent. Current treatment of TBI focused on avoiding or mitigating secondary injury process rather than repair the damage caused by primary injury [2], [4].

Curcumin is one of the active components from the extract of turmeric that has effects as antioxidant, anti-inflammation, antimicrobial, anticarcinogenic, and neuroprotective effect [5], [6], [7]. Curcumin can reduce free radical's effect in brain. From research, curcumin can protect neuron by reduced neurodegenerative processes due to oxidative stress. Curcumin has been proved to decrease neuronal apoptosis process in patient with human immunodeficiency virus type 1 (HIV-1) by increase heat shock protein 70 (HSP 70) expression [8]. Therefore, this study was purpose to evaluate curcumin effect in neuronal apoptosis due to TBI.

Materials and Methods

This study used animal model with post-test only control group design. This research has been approved by the Health Research Ethical Committee,

Medical Faculty of Universitas Sumatera Utara/H. Adam Malik General Hospital. Curcumin extract was collected from extraction process from turmeric. Animal model used in this research was 33 healthy Sprague-Dawley rats aged 2.5–3 months old with weight between 280 and 320 g. Animal was collected from Faculty of Veterinary Medicine, Institut Pertanian Bogor. All samples have been adapted to laboratory environment for 7 days before the study conducted (acclimatization).

Samples will be divided into three groups with 11 rats in each group: Group A, sham-operated controls group (negative control); Group B, closed head trauma (CHT) group (positive control); and Group C, treatment group (CHT+Curcumin extract). CHT model was done, using modification of Feeny TBI weight-drop model. Forty-milligram metal mass was dropped from 1.5 m height to exposed rats' mid frontal head. For Group C, all samples will be given curcumin extract 500 mg/kg BW orally once daily from the 1st day after CHT for 7 days. Then, all samples (Groups A, B, and C) were sacrificed on the 8th day, using cervical dislocation methods with general anesthesia. After that, all brains were harvest for immunohistochemistry process.

Immunohistochemistry staining

The expression of all markers (apoptosis-inducing factor [AIF], caspase-3, TUNEL assay, and HSP 70) was investigated on paraffin-embedded sections using the avidin-biotin peroxidase complex method. A 5 mm thickness paraffin-embedded sections were dewaxed, rehydrated, and microwave for 10 min. About 3% H₂O₂ was used to block the activity of endogenous peroxidase then rinsed with phosphate-buffered saline (PBS). The tissue section was incubated with normal rabbit serum and incubated with a monoclonal antibody (Santa Cruz) at room temperature. These samples were rinsed with PBS and incubated with secondary antibody for 30 min. Tissue sections were rinsed twice with PBS and developed with 0.05% 3, 3-diamino-benzinetetrahydrochloride and slightly counterstained. All samples were evaluated by one pathologist (blinded) and the first author (not blinded). Positive signal of IHC was found in the cytoplasm and counted with a binocular microscope with ×1000 magnify in 10 high-power fields.

Statistical analysis

The total stained cells were reported in mean and standard deviation. When comparisons were made between groups, significance in between-group variability was analyzed using the one-way ANOVA test with Tukey as *post hoc* test. Differences were considered statistically significant at $p < 0.05$.

Results

Thirty rats were included in this study, divided into three groups, that is, sham-operated controls group, CHT, and CHT+Curcumin extract. Curcumin extract was given once daily. After trauma procedure, the brain was harvest after craniocervical dislocation.

Expression of AIF, caspase-3, TUNEL assay, and HSP 70 after TBI

Immunohistochemistry was used to detect the expression of AIF, caspase-3, and HSP 70 on brain tissue after the 7th day CHT. As expected AIF, caspase-3, TUNEL assay, and HSP 70 were localized to the cytoplasm. All of the immunopositive cells were present in the dentate gyrus (Figure 1).

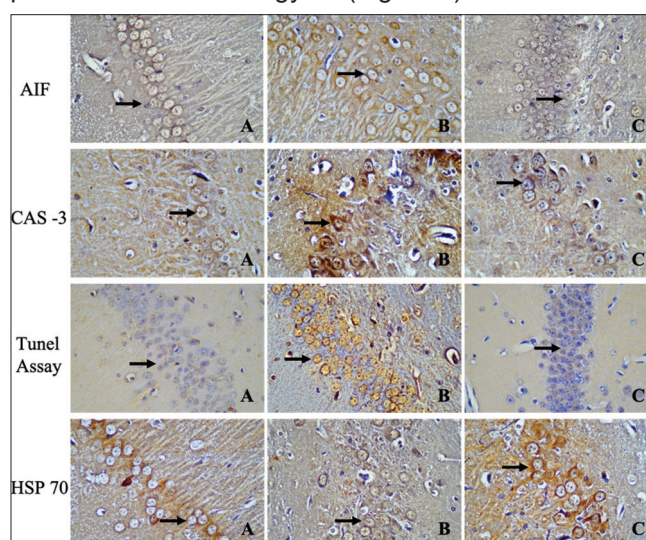


Figure 1: Expression of AIF, caspase-3, TUNEL assay, and HSP 70 in brain tissue after CHT; (a) sham-operated control group; (b) CHT group; (c) CHT+ Curcumin extract group

The mean of immunopositive cells (Table 1) to AIF in sham-operated controls was 5.36 ± 2.11 and in CHT was 12.82 ± 1.40 ; it was significantly upregulated in the CHT group ($p = 0.001$). In addition, the mean of immunopositive cells to caspase-3 in sham-operated controls was 5.45 ± 2.30 and in CHT was 13.82 ± 2.44 . It was significantly upregulated in the CHT group ($p = 0.001$). The mean of immunopositive cells to TUNEL assay in sham-operated controls was 4.82 ± 2.04 and in CHT was 11.55 ± 1.51 . It was significantly upregulated in the CHT group ($p = 0.001$). The mean of immunopositive cells to HSP 70 in sham-operated controls was 6.82 ± 2.14 and in CHT was 3.91 ± 2.26 . It was significantly downregulated in the CHT group ($p = 0.015$). We found that AIF, caspase-3, and TUNEL assay were significantly upregulated in the CHI group compared to the sham-operated group ($p < 0.05$); and HSP 70 was significantly downregulated in the CHI group compared to the sham-operated group ($p < 0.05$).

Table 1: Expression of AIF, caspase-3, TUNEL assay, and HSP 70 in sham-operated control group, CHT, and CHT+Curcumin extract group

Group	AIF	Caspase-3	TUNEL assay	HSP 70
Sham-operated control	5.36 ± 2.11 [#]	5.45 ± 2.30 [#]	4.82 ± 2.04 [#]	6.82 ± 2.14 [#]
CHT	12.82 ± 1.40 [*]	13.82 ± 2.44 [*]	11.55 ± 1.51 [*]	3.91 ± 2.26 [*]
CHT+Curcumin extract	3.82 ± 1.40 [#]	3.82 ± 1.54 [#]	3.55 ± 1.7 [#]	10.27 ± 2.45 [*]

One-way ANOVA; *p<0.05; [#]p>0.05; CHT: Closed head trauma, HSP: Heat shock protein 70, AIF: Apoptosis-inducing factor.

Curcumin modulated the expression of caspase-3, TUNEL assay, and HSP 70 after TBI

After 7 days treatment of curcumin extract, it showed that curcumin downregulated the expression of AIF, caspase-3, and TUNEL assay, upregulated the expression of HSP 70, respectively (Table 1). The mean of immunopositive cells to AIF was 3.82 ± 1.40; caspase-3 was 3.82 ± 1.54, and TUNEL assay was 3.55 ± 1.7. It was significantly compared to the CHT group (p = 0.001) and insignificant compared to the sham-operated group (AIF, p = 0.186, caspase-3, p = 0.093, and TUNEL assay, p = 0.224). Mean of immunopositive cells to HSP 70 was 10.27 ± 2.45. It was significantly upregulated compared to the sham-operated group (p = 0.004) and CHT group (p = 0.001) (Figure 1).

Discussion

Curcumin is a hydrophobic polyphenol and a derivate from turmeric's root which has several effects, that is, antioxidant, anti-inflammation, antimicrobial, and anticarcinogenic properties. Several studies demonstrated that curcumin has neuroprotective effect [5], [6], [7]. A study by Dong *et al.* with mice model that given TBI using Feeney weight-drop contusion model showed reduction and degeneration of neuron through Nrf2 signaling induction [5]. Huang *et al.* study also supported the previous study. Experimental rats were subjected with a diffuse axonal injury model by head rotational acceleration that treated with curcumin (20 mg/kg BW intraperitoneal) and also showed protection to abnormal protein and neuronal apoptosis through the activation of Nrf2 signaling [6]. Other study from Zhu *et al.* showed that curcumin also can prevent neuronal apoptosis by reducing activation of microglia by mechanism including TLR4/MyD88/NF-κB pathway [7].

In this study, the treatment group (CHT+Curcumin extract) showed low caspase-3 expressions as the sham-operated control group but has significant downregulation than the CHT group. AIF expression in the treatment group (CHT+Curcumin extract) also showed low expression as the sham-operated control group but has significant downregulation compare to the CHT group. This result showed that curcumin has anti-apoptosis effect to neuronal after TBI through inhibition in

caspase-dependent and -independent pathways. Inhibition to caspase-dependent and -independent pathways will mitigate apoptotic downstream event and finally prevent neuronal apoptosis. TUNEL assay level in this study was significantly downregulated in the treatment group (CHT+Curcumin extract) compare to the CHT groups and also has TUNEL assay level as low as the sham-operated control group. TUNEL assay result proved that curcumin has potential effect in mitigate apoptosis after TBI.

Several studies have proved that HSP 70 has neuroprotective effect in brain injury and cerebral ischemic. Caspase-dependent and -independent pathways activation can be inhibited by HSP 70 through prevention of cytochrome c, Apaf-1, and caspase-3 release [9], [10]. Animal experiment by Xia *et al.* using rats that infected with HIV-1 then administrated curcumin showed elevated HSP 70. Elevated HSP 70 expression in gp120 V3 loop peptide induced in primary rat cortical will prevent neuronal cell dysfunction and apoptosis caused by invasion of HIV-1 in central nervous system [8]. In our experiment, HSP 70 also upregulated significantly in the treatment group (CHT+Curcumin extract) than the CHT group and also the sham-operated control group. Our study synchronous with the previous study that curcumin can increase HSP 70 expression in the brain injury area. According to this data, we proposed that curcumin has neuroprotective effect by HSP 70 induction, besides from Nrf2 signaling and TLR4/MyD88/NF-κB pathway.

Conclusion

Caspase-3, AIF, and TUNEL assay expression downregulated significantly after the administration of curcumin compare to the CHT group (p < 0.05). Curcumin also elevated HSP 70 expression even higher than control group. Propolis has been proved and has potential neuroprotective effect after brain injury by increasing HSP 70 expression in addition to Nrf2 signaling and TLR4/MyD88/NF-κB pathway induction. However, further clinical trials were required for clinical use of curcumin.

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