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Protective Effect of Curcumin on CA1 Region of Hippocampus in Rat Model of Ischemia/ Reperfusion Injury

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Abstract

Background: The brain is the most complex and vital organ of the human body. It requires 20-25 % of the total oxygen supply. Because of the limited oxygen and glucose reserves, brain tissue is sensitive to ischemic injury. Indeed, the tolerance of brain tissue for ischemic injury is fragile. Currently, few therapeutic strategies could provide complete neuroprotection. Despite decades of intense research, the beneficial treatment of stroke remains limited. Hence, we aimed to investigate the effect of curcumin on the CA1 region of the hippocampus in a rat model of ischemia/reperfusion (I/R) injury. **Materials and Methods:** In this experimental research, 24 male Wistar rats were randomly divided into three groups (n=8 per group) as control, I/R, and I/R plus curcumin. All rats underwent bilateral common carotid artery ligation followed by reperfusion. In the treatment group, curcumin (300 mg/kg) was injected 30 minutes before ischemia. Morphological changes of the hippocampus were assessed using Nissl staining, and apoptosis was determined via TUNEL immunohistochemical assays. **Results:** Nissl staining data showed that the administration of curcumin significantly ameliorated the CA1 pyramidal cell loss due to transient global I/R injury. TUNEL immunohistochemical assays demonstrated that the number of apoptotic cells was significantly lower in the curcumin group than in the I/R groups. **Conclusion:** Our study demonstrates that curcumin had beneficial activity against ischemia and played a neuroprotective role in the pathogenesis of I/R injury.

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Keywords: Brain Ischemia; Hippocampus; Curcumin; Neuroprotective; Reperfusion

Introduction

Cerebral ischemia has been responsible for one of the most common causes of morbidity and mortality world wide [1]. It is associated with various complications such as hemiplegia, coma, and even death [2]. Deprivation of blood and a decrease in oxygen supply is known to trigger some pathways, which increase cerebral injury,

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including inflammation, toxicity, oxidative stress, cell necrosis, apoptosis, and edema [2]. Among these pathways, inflammation is the most important reaction after ischemia [1]. The investigations show that reperfusion is responsible for the aggregation of cerebral injury by initiating the mentioned pathways [3]. Also, excess oxygen free radicals accumulation can cause cell function impairment, leading to death [3]. The increasing incidence of cerebral ischemia has motivated scientists to investigate agents with anti-oxidative and anti-inflammatory properties [1]. There are certain areas in the brain that are more susceptible to being affected during an ischemic attack, such as pyramidal cells of the hippocampus, and this is due to an excess of the protein that is responsible for apoptosis [3-6]. Curcumin is an active ingredient derived from *Curcuma Longa* (Turmeric). It has been indicated that curcumin has some neurotrophic effect against ischemia/reperfusion (I/R) injuries [7]. Some studies have focused on the extension of curcumin activities, such as anti-inflammatory, anti-apoptosis, and anti-oxidative stress pathways, which can be both protective and therapeutic effects of curcumin on cerebral I/R [1]. It also potentially influences neurological disorders associated with inflammation (e.g., Alzheimer Parkinson, and Huntington) [8]. Curcumin regulates the precursor mediators of inflammation, including interleukin-6 (IL6), IL-1 β, and tumor necrosis factor-α, by inhibiting the nuclear factor kappa B, which is the main regulator in inducing the expression of the inflammatory process. Also, recent studies confirmed that curcumin downstream the oxidative stress enzyme by ameliorating the Nrf2/ARE pathway and increasing the production of glutathione [9-11].

The aim of the present study was to determine the possible neurotrophic properties of curcumin in the CA1 area of the Wistar rat hippocampus assessed by histological outcome following I/R.

Materials and Methods

Animals

Study were performed on 24 male Wistar rats weighing between 250-300 g. Animals were kept in a controlled environment (25±2 °C and 50% humidity) with a 12/12 hours' light/dark cycle with food and water available *ad libitum*. All surgical procedures and care were approved by the ethics committee of the Islamic Azad University of Medical Sciences (approval code:6097).

Groups

Animals were randomly divided into three different groups (n=8 per each group) as described below:

-Control group: Rats only received sodium pentobarbital (40 mg/kg, intraperitoneal [I.P]) for anesthesia.

-Ischemic group: Rats were anesthesia and subjected to surgical procedure.

-Curcumin group: Rats were anesthesia and received curcumin (300 mg/kg, I.P) 30 minutes prior to the surgical procedure.

Surgical Procedure

A surgical procedure for bilateral common carotid artery occlusion was performed as previously described [6]. Briefly, Rats were anesthetized, a midline incision was made vertically in the neck region, and the common carotid arteries were exposed, care being taken to preserve the vagus nerves. The carotid arteries were ligated using non-traumatic micro-aneurysmal clips. Clamps were removed 20 min later to induce perfusion. During surgery, the temperature was maintained at 36-38 °C with a heating lamp. Animals were returned to their cage after surgery and kept for four days. Then, all brains were removed for histological assessments.

Nissl Staining

Nissl staining is commonly used for identifying the basic neuronal structure of necrotic neurons in the brain [6]. For Nissl staining, coronal sections at 10 μ thickness were mounted directly onto glass slides pre-coated by gelatin. The slides remained to dry and stained with 0.1% cresyl violet, dehydrated, and finally cover-slipped with Entellan. In stained glasses, Nissl bodies are seen as dark blue to purple subjects. Samples

were studied with light microscope (×400 magnification). Only pyramidal cells with clear nuclei and nucleolus were counted as alive neurons. Eight photomicrographs were prepared from each rat. Then, randomly three photomicrographs were collected, pyramidal cells were counted using the Image-Pro Plus (version e4.5.1; Media Cybernetics, Inc., Bethesda, Maryland, USA), and the mean number of positively stained cells was included.

Apoptosis Detection

The fluorescent terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL; Roche, Germany) was performed according to the manufacturer's protocol. In brief, slides were dried for 30 min followed by fixation in 10% formalin solution at room temperature. After washing in phosphate-buffered saline (PBS), sections were incubated in ice-cold ethanol-acetic acid solution (3:1), washed with PBS, and incubated with 3% Triton X-100 solution for 60 min at room temperature for permeabilization. Afterward, the sections were incubated in TdT buffer containing fluorescein-dUTP for 90 min at 37 °C. Negative control was performed using only the reaction buffer without TdT enzyme. Positive controls were carried out by digesting with 500 U/ml DNase grade I solution. To preserve cells for comparison, slices were covered with Vectashield® mounting medium containing 4',6'-diamino-2-phenylindole (DAPI). Five random slides were selected from each group, and five visual fields (at the hippocampus CA1 region) were randomly selected and immediately were observed after staining using an Axioskop 40 fluorescence microscope (Zeiss, Germany) at 460 nm and 520 nm for DAPI and TUNEL, respectively.

Statistical Analysis

Data are expressed as mean±standard deviation. One-way analysis of variance (ANOVA) followed by a post-hoc test was used to evaluate the differences among the groups using IBM SPSS Statistics for Windows, version 19 (IBM Corp., Armonk, N.Y., USA). A P-value<0.05 were considered significant.

Results

Survival of Neurons in the CA1 Region of the Hippocampus

We investigated the effect of curcumin on neuronal survival in the hippocampal CA1 region by Nissl staining. In Nissl staining, the pyramidal cells of the CA1 region of the control group had abundant cytoplasm and Nissl bodies, and the cell outlines were clear. In I/R group, dying neuronal cells in the CA1 showed shrunken cytoplasm and nucleic degeneration characterized by pyknotic and indistinct nuclei, and the number of surviving neurons was significantly decreased. However, the administration of curcumin (300 mg/kg) significantly increased the numbers of intact neuronal cells containing large, round, and transparent nuclei compared with I/R group (Figure-1 and -2).

Apoptosis in the CA1 Region of the Hippocampus

To investigate the possible mechanism of curcumin on the improvement of brain injury, we studied the effect of curcumin on apoptosis in the CA1 section of the hippocampus by TUNEL immunohistochemical assays. There were few TUNEL-positive cells in the control group, whereas many TUNEL-positive neurons were observed in the CA1 region of rats in the I/R group. In contrast, the number of apoptotic cells was significantly lower in the curcumin group than in the I/R groups (Figure-3).

Discussion

In this study, Nissl staining data indicated that induced transient global I/R resulted in damage to CA1 hippocampus pyramidal cells and showed shrunken cytoplasm and nucleic degeneration. Our findings indicated that the pretreatment of rats with curcumin significantly ameliorated the CA1 pyramidal cell apoptosis.

Brain ischemia is one of the common forms of ischemia, and stroke is the most important result of cerebral ischemia [12]. The important

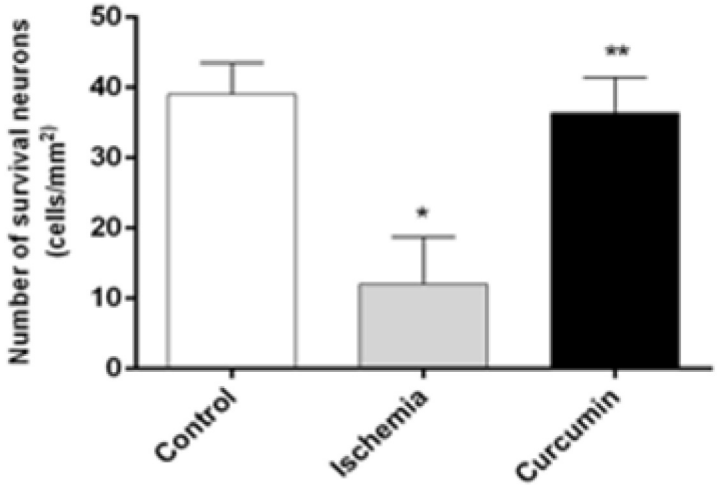

Figure 1. The relation of curcumin injection on ischemia/reperfusion (I/R) on various groups on the number of pyramidal cells. Data are presented as mean±SD. * P<0.05 vs. control group, ** P<0.05 vs. ischemia group

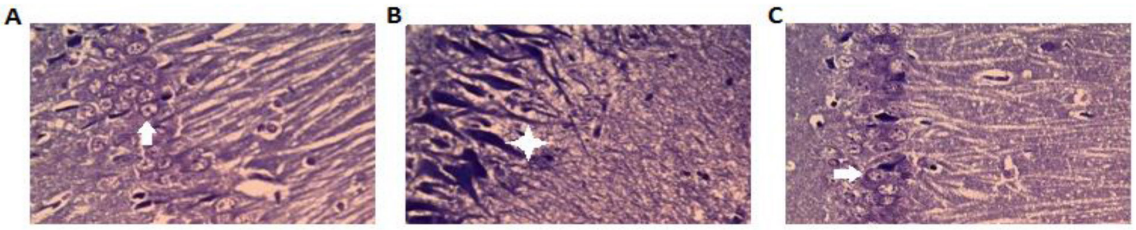

Figure 2. Photomicrograph of the CA1 region of the hippocampus following Nissl staining in different groups. In the control group (**A**), the number of viable neurons (arrows) are more than I/R (**B**) and curcumin (**C**) groups. Also, the number of degenerated neurons (asterisk) in the I/R group was significantly higher than in the curcumin group (×400 magnification).

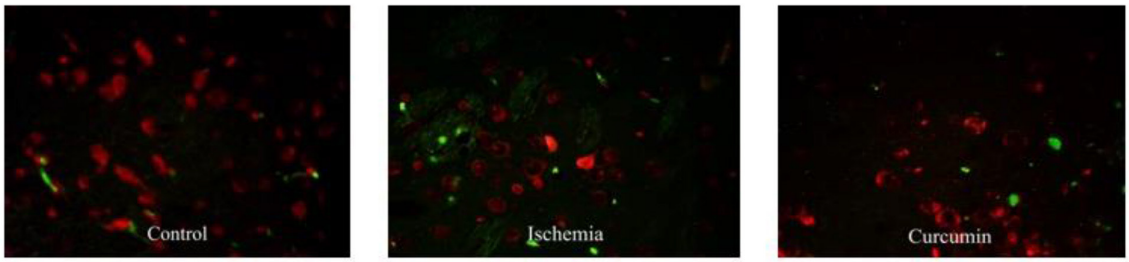

Figure 3. Representative photomicrographs of TUNEL staining in the hippocampal CA1 region of the Wistar rats in different groups. The green dye denotes apoptosis in neuronal cells (×400 magnification).

pathological mechanisms of ischemia-induced brain damage are oxidative stress, neuronal apoptosis, blood-brain barrier disruption, and inflammatory reactions [9]. Brain reperfusion leads to cell apoptosis due to exposure to oxygen-free radicals [13]. Certain brain areas are more vulnerable to oxidative stress, including the pyramidal neurons of the CA1 region of the hippocampus [14]. Our findings through Nissl staining and TUNEL immune-histochemical assays also confirmed diffuse neural death due to I/R. Considering the role of inflammation and oxidative stress in the ischemic-induced damage to the hippocampus,

previous studies have shown that drugs that have antioxidant and anti-inflammatory effects could prevent memory impairments due to cerebral ischemia in rats [15-18]. Numerous studies have demonstrated the antioxidant [19, 20] and anti-inflammatory [21] effects of curcumin, and its ability to decrease brain damage and apoptosis caused by oxidative stress and ischemia [7, 8, 10, 13, 22]. A study by Chhunchha *et al*. indicated that curcumin increases the expression of peroxiredoxin-6, leading to decrease oxidative stress response to I/R and apoptosis of hippocampal cells. Therefore, it can be used as a treatment for diseases associated with oxidative stress [23]. It can produce neuroprotective effects in diseases of the central nervous system, such as Alzheimer's disease, cerebral ischemia, and Parkinson's disease [24-26].

A study by Pan *et al*. showed that the administration of curcumin by gavage (oral) results in lower absorption than I.P. injection [27]. Another study by Zhao *et al*. evaluated the dose-dependent effect of I.P. injected curcumin, which showed that 300mg/kg could produce the best neuroprotective effect [28]. According to these findings, we evaluated the neuroprotective effect of I.P. injected curcumin on the dosage of 300 mg/kg.

A previous study by Fadhel *et al*. was done by performing forebrain ischemia [11]. This kind of ischemia causes a delayed type of hippocampal apoptosis [11]. In the current study, transient global ischemia was performed for sooner and better hippocampal apoptosis. In another study, induced focal cerebral ischemia was done for two hours, followed by 22 hours of reperfusion [22]. The duration of reperfusion in the mentioned study was insufficient to see hippocampal CA1 neuronal damage. We performed global cerebral ischemia instead of focal and a longer duration of reperfusion. Both mentioned studies evaluated some oxidative stress factors to investigate their elevation during I/R and the protective effect of curcumin on them, but neither had TUNEL immunohistochemical assays and histological Nissl staining, which can show neuronal damages and apoptosis.

Our findings were similar to the result of other studies and supported them [9, 28, 29].

TUNEL findings indicated more TUNEL-positive neurons in the CA1 region of I/R rats, and the numbers of apoptotic cells were significantly decreased by pretreatment of curcumin. It also supports the neuroprotective effect of curcumin, which was in line with the results of studies conducted in this field [28, 30, 31].

Conclusion

Curcumin pretreatment could significantly ameliorate the cerebral I/R-induced CA1 pyramidal cell apoptosis; therefore, it could be used as a neuroprotective agent in ischemia-associated neuronal disease.

Acknowledgment

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Conflict of Interest

The authors declare no conflicts of interest.

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