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The Effects of MK-801 on Apoptosis in a Traumatic Brain Injury Model in Rat Pups

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Abstract: Traumatic brain injury (TBI) is an essential cause of morbidity and mortality during childhood. Trauma causes some changes that result in delayed and elongated damage known as secondary injury, which is characterized by neuronal apoptosis. Based on this information, the aim of this study was to investigate the effect of MK-801, a competitive NMDA receptor antagonist, on apoptosis against hippocampal damage in rat pups after TBI. Forty-two, 10-day-old Wistar Albino rats were randomly divided into three groups: Control, a Trauma, and Treatment groups, each having fourteen rats. TBI was created by blunt trauma model. MK-801, was injected intraperitoneally at the doses of 1 mg/kg of body weight immediately after induction of TBI. The hippocampus tissues were harvested 4 days after TBI. Then CA1 and dentate gyrus (DG) regions were evaluated in terms of immunoreactivity with BAX, cytochrome C, and caspase 3. Based on this evaluation, the control group showed weakly BAX and Cytochrome C immunoreactivities in hippocampus, but elevated reactions were observed in TBI group. Especially, it was determined that the cytochrome c immunoreactivity was granular form in the neurons of hippocampus DG region. In the Treatment group decreased BAX and Cytochrome C immunoreactivities. While a weak caspase-3 immunoreactivity was observed in control group, stronger immunoreactivity was determined both DG and CA1 region of hippocampus in TBI group. In the Treatment group, caspase-3 immunoreactivity decreased in hippocampus region when compared to TBI group. Our results showed that treatment with MK-801 may significantly decreased apoptosis through BAX, Cytochrome C and caspase-3 pathway.

Keywords: Traumatic brain injury, Apoptosis, MK-801

Introduction

Traumatic brain injury (TBI) is a major public health problem and an essential cause of morbidity and mortality during childhood. The brain damage from TBI can be divided into the two: Primary and secondary damage. Primary damage occurs immediately soon after trauma whereas secondary damage within several hours or days after trauma. Secondary damage is made up several of neuron damage and nervous dysfunction and biochemical alterations. Major pathophysiologic mechanisms such as excitotoxicity, calcium overload, free radical generation, apoptosis, and lipid peroxidation lead to secondary brain damage (Sönmez et al., 2015; Sönmez et al., 2007). Unfortunately, there is no available effective treatments to reduce secondary injuries. It has been shown that MK-801, a competitive NMDA receptor antagonist, has been reduced excitatory amino acid release following TBI (Han et al., 2009; Patner & Faden 1992). Although this effect of MK-801 have been shown, whether it has anti-apoptotic effect against to TBI is unknown. In this study, we investigated the anti-apoptotic effects of MK-801 on hippocampal damage on 10-day-old rat pups exposed to TBI injury.

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

Material and Method

Animals

All experiments were carried out in accordance with the National Institutes of Health Guide for the care and use of laboratory animals and were approved by Ethics Committee of the Research of Laboratory Animals, Dokuz Eylul University, Medical School, Izmir, Turkey. Forty-two Wistar Albino rats were randomised into three groups: Control, Trauma group, and treatment groups, each having fourteen rats.

MK-801 Application and Trauma Model

MK-80, was injected intraperitoneally at the doses of 1 mg/kg of body weight immediately after induction of traumatic injury. It has been used a modification of a well-described percussion trauma model in immature rats on P10, in an attempt to model infant and early childhood head trauma as described previously by Sonmez et al. (2007). All the pups were kept on a heating pad until returned to their mothers at 4 h after the trauma.

Tissue Harvesting

Four days after trauma, seven animals from each group were randomly separated for immunohistochemical assays evaluations, brain tissues were collected after cervical dislocation and fixed in 10% formalin solution for 24 h.

Immunohistochemical Assays

Collected tissues were embedded in paraffin blocks 5μm sections were taken using microtome. Sections were divided into two samples to be used for immunohistochemical assays. For BAX, Cytochrome C and caspase-3 immunoreactivity, sections were deparaffinized at 60 ◦C overnight and xylene for 30 min. Sections were first rehydrated in a series of baths with decreasing amounts of ethanol. After washing with distilled water and then phosphate buffered saline (PBS) for 10 min each, they were incubated with 2% tyripsin at 37 ◦C for 15 min. Sections were marked with a Dako pen and were added 3% H2O2 solution for 15 min to inhibit endogenous peroxidase activity. The primary antibodies were applied in a dilution of 1:100 at 4 ˚C overnight. After washing with PBS three times, and finally, the secondary antibodies biotinylated IgG and streptavidin-peroxidase conjugate (supplied ready to use) were incubated at 37 ˚C for 30 min. To quantify the number of cells that underwent apoptosis, 1000 apoptotic and normal cells were counted randomly in hippocampal damage of dentate gyrus and CA1 region and percentages of apoptotic cells were calculated (Gurgen et al., 2013).

Statistical Analysis

Data were analysed using a SPSS 15.0 for Windows program on a computer. Results were expressed as mean±S.E.M and analysed by using one-way analysis of variance followed by the Tukey HSD test. A p-value < 0.05 was considered statistically significant.

Results

The effect of MK-801 treatment on apoptosis was examined using active BAX, Cytochrome C and caspase-3 immunostaining. The Control group showed weakly BAX and Cytochrome C immunoreactivities in hippocampus but elevated reactions were observed in TBI group. Especially, it was determined that the cytochrome c immunoreactivity was granular form in the neurons of hippocampus DG region. In the Treatment group decreased BAX and Cytochrome C immunoreactivities (Figure 1,2). While a weak Caspase-3 immunoreactivity was observed in the Control group, stronger immunoreactivity was determined both DG and CA1 region of hippocampus in TBI group. In the Treatment group, caspase-3 immunoreactivity decreased in hippocampus region when compared to TBI group (Figure 3).

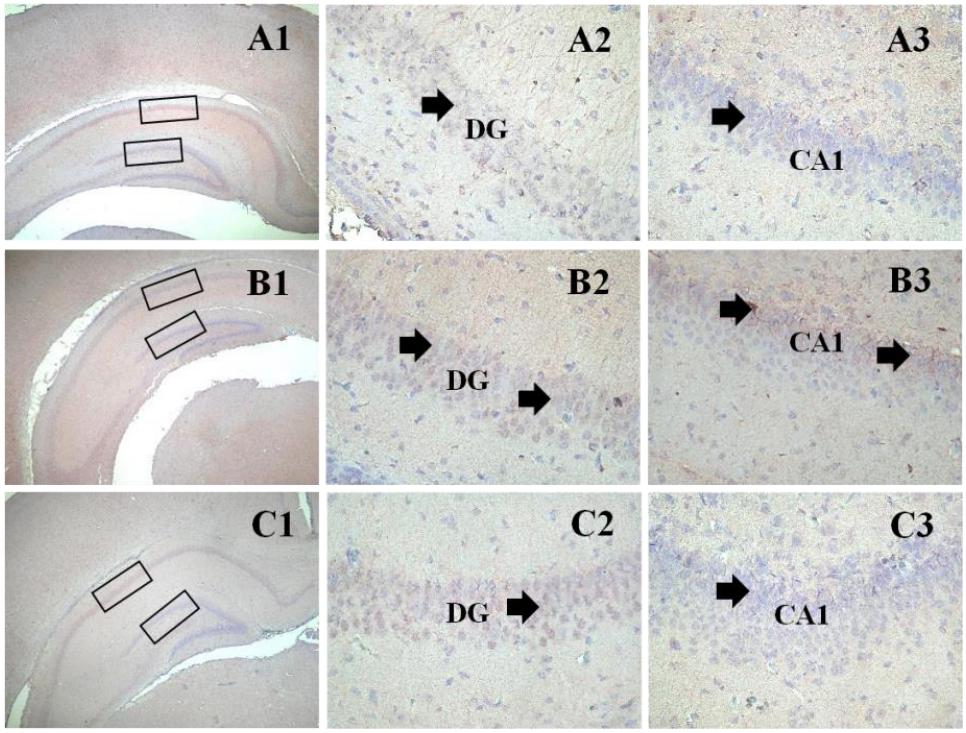

Figure 1. Effects of MK-801 on active BAX immunoreactivity in the dentate gyrus and Cornu Amnonis Area 1. Control (A), Trauma (B), Trauma-MK-801 (C). General Hippocampus (1),X40, DG: Dentate Gyrus (2), CA1: Cornu Ammonis Area1 (3)= X400. ➡: Positive cells. MK-801 treatment significantly reduced the number of apoptotic neurons.

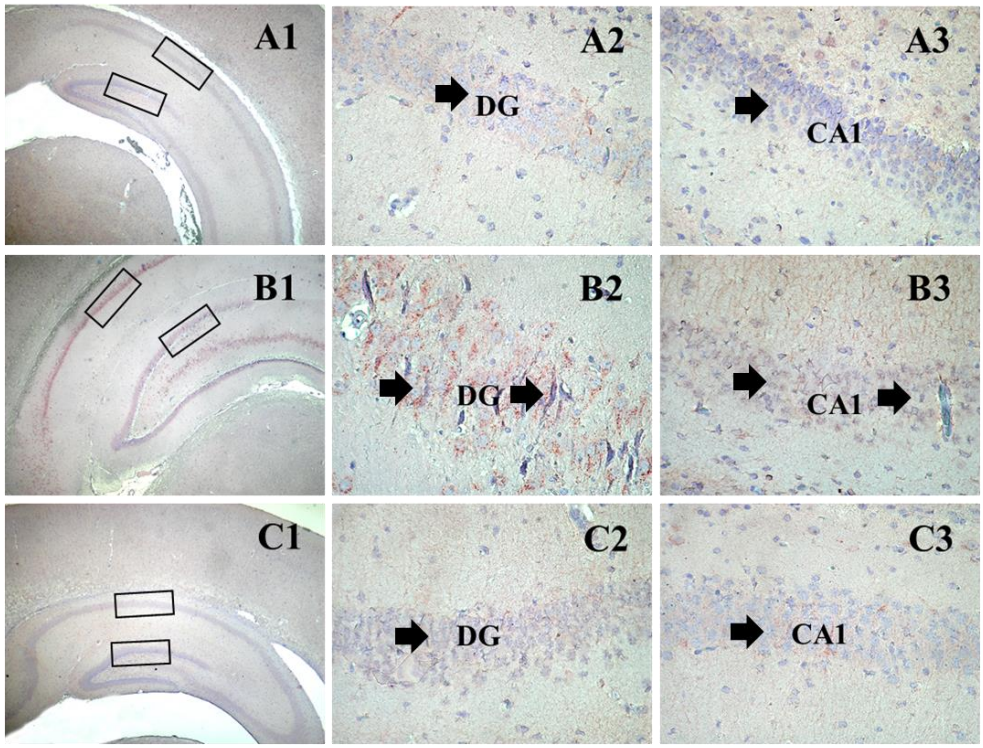

Figure 2. Effects of MK-801 on active Cytochrome C immunoreactivity in the dentate gyrus and Cornu Amnonis Area 1. Control (A), Trauma (B), Trauma-MK-801 (C). General Hippocampus (1)=X40, DG: Dentate Gyrus (2), CA1: Cornu Ammonis Area1 (3)= X400. ➡: Positive cells. MK-801 treatment significantly reduced the number of apoptotic neurons.

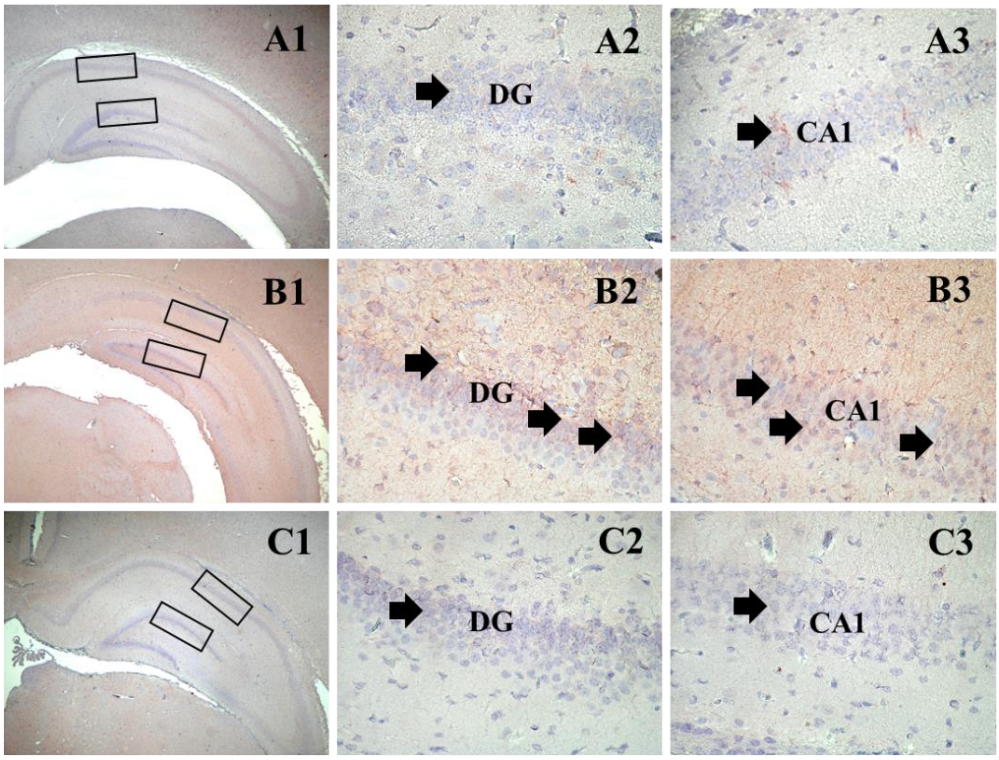

Figure 3. Effects of MK-801 on active caspase-3 immunoreactivity in the dentate gyrus and Cornu Amnonis Area 1. Control (A), Trauma (B), Trauma-MK-801 (C). General Hippocampus (1)=X40, DG: Dentate Gyrus (2), CA1: Cornu Ammonis Area1 (3)= X400. ➡: Positive cells. MK-801 treatment significantly reduced the number of apoptotic neurons.

Discussion

Our results showed that treatment with MK-801 has positive effects on apoptosis after the trauma in immature rats. We demonstrated that MK-801 has shown anti-apoptotic effects by reducing expressions of apoptotic proteins such as BAX, Cytochrome C and caspase-3 in traumatic brain injury in immature rats. Previous studies reported that administration of MK-801 to animal with head trauma injury resulted with better learning and memory scores in different animal studies (Han et al., 2009; Patner & Faden, 1992).

There was no data in the literature evaluating effects of MK-801 on apoptosis in a traumatic brain injury model in immature rats. To date, only a few studies have been completed assessing the MK-801 effects in traumatic brain injury in adult rats. Han et al. (2009) showed that MK-801 (0.5-2-10 mg/kg) could inhibit the neuronal caspase-3 expression in an adult rat model of traumatic brain injury. Also, Wang et al. (2014) MK-801 attenuated the increase BAX expression after in the rat fluid percussion traumatic brain injury model.

Our previous study showed that MK-801 administration has a neuroprotective role against trauma induced hippocampal neuron loss and associated cognitive impairment in immature rats (Sönmez et al., 2015). Our study is important for the evaluation of traumatic brain injury in childhood groups for the first time. Our study may also contribute to the prevention of complications that may occur in childhood head trauma with alternative treatment options.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

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