뇌 해마 절편 배양 모델에서 흥분 독성에 대한 비타민 E의 신경 보호 효과

Vitamin E protects neurons against kainic acid-induced neurotoxicity in organotypic hippocampal slice culture

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ABSTRACT

Kainic acid (KA), an agonist for kainate and AMPA receptors, is an excitatory neurotoxic substance. Vitamin E such as alpha-tocopherol and alpha-tocotrienol is a chain-breaking antioxidant, preventing the chain propagation step during lipid peroxidation. In the present study, we have investigated the neuroprotective effects of alpha-tocopherol and alpha-tocotrienol on KA-induced neuronal death using organotypic hippocampal slice culture (OHSC). After 15h KA treatment, delayed neuronal death was detected in CA3 region. Alpha-tocopherol and alpha-tocotrienol increased cell survival and reduced the number of TUNEL-positive cells in CA3 region. These data suggest that alpha-tocopherol and alpha-tocotrienol treatment have protective effects on KA-induced cell death

Keywords: Kainic acid, alpha-tochpherol, alpha-tocotrienol, hippocampus, rat

1. INTRODUCTION

Kainic acid (KA), an agonist for kainate and AMPA receptors, is an excitatory neurotoxic subastance. KA can induce characteristic limbic seizures and selective neuronal cell death in the hippocampal CA1 and CA3 subregion both after in vivo injection or in vitro exposure. The mechanisms involved in this pathogenesis appear to be linked to oxidative stress. The brain is highly susceptible to oxidative stress because of its high consumption of oxygen energy and glucose, large amount of peroxidizable polyunsaturated fatty acid and relatively low antioxidant capability.[1] Increased production of free radicals may play a key role in the neuronal injury caused by KA-induced lipid peroxidation. Therefore, scavanging or reducing the KA-induced free radical is important in preventing the harmful insults by KA treatment.[2]

Vitamin E is the term for a group of tocopherols and tocotrienols, each with alpha, beta, gamma and delta analogs, and is essential component of biological membranes where they have both antioxidant and non-antioxidant functions.[3] In the vitamin E group, alpha-tocopherol (ATPH) and alpha-tocotrienol (ATTN) are suggested to have the highest biological activity.[4,5] However, the functions

of tocotrienols have not been fully elucidated.

Organotypic hippocampal slice culture (OHSC) models represent a useful intermediate tool for studying chronic, progressive cell damage. As it maintains internal structure and function as in vivo yet, this allows an examination of the direct effect of an excitotoxic compound on the hippocampus that is not possible in vivo.

In the present experiments, whether KA-induced neuronal death in OHSC can be protected by ATPH and ATTN was investigated by measuring PI uptake.

2. MATERIALS AND METHODS

1. Organotypic slice cultures

7-day-old Sprague-Dawley rat pups were used and hippocampi were rapidly removed and transferred to cold dissection media : Gey's balanced salt solution (Sigma, Saint Louis, MO, USA) with 0.5% glucose and 3 mM KCl. Sections were cut to 350 μm with McIlwain tissue chopper (Vibratome, O'Fallon, MO, USA) and inspected and cut into slices under a dissection microscope. After inspection, six slices were transferred onto a Millicell-CM membrane insert (Millipore, Billreka, Massachusetts, USA) set in a 6-well plate on 1 ml of culture medium, composed of 50% Opti-MEM, 25% Hank's balanced salt solution (HBSS), 25% heat-inactivated horse serum, 6.5 mg/ml D-glucose (AMRESCO Inc, Solon, Ohio, USA), pH 7.2 (all from GIBCO BRL, Grand Island, NY, USA). Plates were kept in a 35°C humidified incubator with 5% CO_2. Medium was then changed three times a week. Slices were grown for 20-24 days in vitro (DIV) prior to drug treatments.

2. Drug treatment and assessment of neuronal injury

KA (5 μM) was applied for 15 h after mature cultures. Stock solutions (10^3 x working concentration, 100 mM) of α-tocopherl (ATPH) and α-tocotrienol (ATTN) were prepared in ethanol. ATPH (100 μM) and ATTN (100 μM) were added to the culture dishes during KA treatment or after KA treatment for 24 h and 48 h. For detection of neuronal cell death, cellular uptake of the fluorescent dye propidium iodide (PI, Sigma, Saint Louis, MO, USA) was recorded. PI was added to the culture medium to achieve a final concentration of 5 μg/ml. PI uptake was recorded by fluorescence microscopy (IX-71, Olympus, Tokyo, Japan) using a standard rhodamine filter and digital camera. After exposing the cultures to the drugs, digital fluorescent micrographs of the cultures were taken at different time points during the experiments. The fluorescence images were analysed with the MetaMorph Imaging System (Universal Imaging, Downingtown, PA, USA). Pixel intensity was measured in selected areas of CA1, CA3, and dentate gyrus (DG), using a standardizing software features (Fig. 2). Slices were killed by exposure to 100 μM NMDA for 24 h in normal medium with PI. Then PI fluorescence image (F_{fk}) was obtained and was considered to represent 100% cell death. PI uptake was expressed as a percentage in the following equation : % of PI uptake $= 100(F_t - F_{pre}) / (F_{fk} - F_{pre})$ where F_{pre} is the fluorescence taken before the drug treatment and F_t is at 24 h or 48 h recovery after KA exposure.

3. RESULTS AND DISCUSSIONS

To assess the effects of ATPH and ATTN on KA-induced cell death, PI fluorescence were observed. When exposed to 5 μM KA for 15 h, the PI uptake in CA3 region was significantly higher than the uptake in CA1 region (Fig. 1A). ATPH (100 μM) and ATTN (100 μM) significantly reduced the level of PI fluorescence in CA3 region compared with KA, although it remained much higher than in control

(Fig. 1). In co-treatment of ATPH and ATTN with KA, ATTN tends to be more effective than ATPH in protecting pyramidal cell death of CA3 region (Fig. 1B). And the same tendency was also shown in post-treatment of ATPH and ATTN after KA for 15 h. However, there's no significant difference between co-treatment and post-treatment in each drugs.

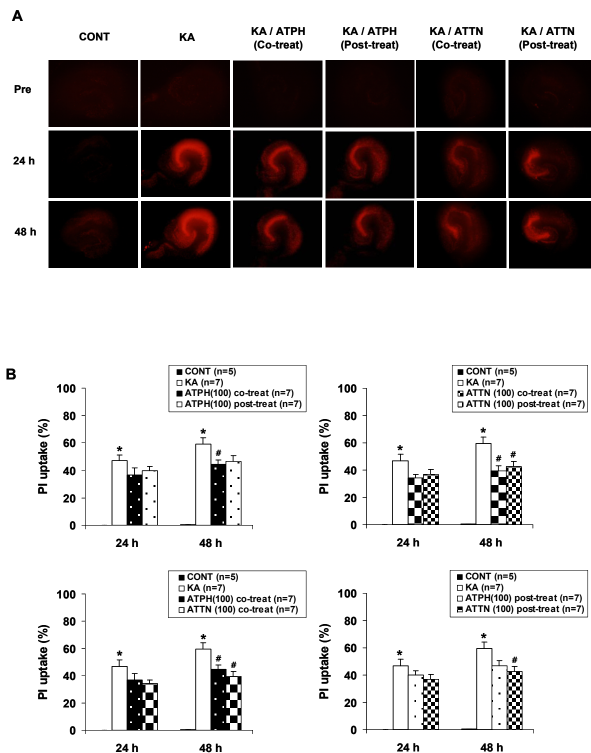

Figure 1. Effects of ATPH and ATTN on KA-induced PI uptake in OHSC. A. Representative PI uptake images from CONT (untreated), KA (5μM KA only treated), KA/ATPH Co-treat (100 μM ATPH with KA), KA/ATPH Post-treat (100 μM ATPH after KA), KA/ATTN Co-treat (100 μM ATTN with KA), and KA/ATTN Post-treat (100 μM ATTN after KA) slices. B. Quantification of PI images. Data are presented as means ± S.E.M. of 5 to 7 experiments. * indicates statistically significant difference from control ($^{*}p < 0.001$); # indicates statistically significant difference from KA-treated cultures ($^{\#}p < 0.05$)

These data suggest that ATPH and ATTN treatment have protective effects on KA-induced cell death in OHSC.

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