비타민 C의 신경 보호 효과

Neuroprotective effects of vitamin C

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ABSTRACT

Vitamin C ascorbic acid (AA) and dehydroascorbic acid (DHA) as an antioxidant have been shown to have protective effects in experimental neurological disorder models such as stroke, ischemia, and epileptic seizures. The present study was conducted to examine the protective effect of AA and DHA on Kainic acid (KA) neurotoxicity using organotypic hippocampal slice cultures (OHSC). After 12h KA treatment, significant delayed neuronal death was detected in CA3 region, but not in CA1. Intermediate dose of AA and DHA pretreatment significantly prevented cell death and inhibit ROS level, mitochondrial dysfunction and capase-3 activation in CA3 region. In the case of low or high dose, however, AA or DHA pretreatment were not effective. These data suggest that both AA and DHA pretreatment have neuroprotective effects on KA-induced neuronal injury depending on the concentration, by means of inhibition of ROS generation, mitochondrial dysfunction, and caspase-dependent apoptotic pathway.

Keyword: vitamin C, ascortic acid, dehydroascorbic acid, kainic acid, organotypic hippocampal slice culture

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1. Introduction

Vitamin C ascorbic acid (AA) has been known to be a potent antioxidant and accumulates in the brain at a much higher concentration than it dose in any other organs [1]. However, within the brain, AA levels are not homogeneous, with the highest levels being found in the amygdala, hippocampus and hypothalamus [2]. Neuroprotection by AA has been demonstrated in several recent studies, both in vitro and in vivo. AA protects the brain against injury resulting from ischemia and excitatory amino acid toxicity [2-4]. The role of AA in protecting against oxidative stress is controversial because AA also has pro-oxidant property in the presence of free transition metals in vitro [5]. Several studies have shown that AA induced lipid peroxide production and cell death in cortical slices [6] or PC12 cells [7]. Therefore, the antioxidant or prooxidant properties of AA are not determined yet.

DHA is an oxidized form of AA. It has been shown that DHA treatment circumvents the pro-oxidant effects of AA [8]. DHA are taken up by glucose transporter [9] and is regenerated into AA at the expense of the glutathione [10]. Pathological conditions that inhibit DHA recycling may decrease AA concentrations and thereby impair AA-dependent enzymatic and antioxidant activities [11]. DHA administration has resulted in normalization of oxidative stress markers and inflammation in hyperglycemic stroke models [12]. In vitro preparation, DHA inhibited mitochondrial damage and cell death against oxidative injury [8]. In primary astrocytes, we observed that DHA prevents H_2O_2-induced cell death by increasing the glutathione (GSH) levels [13].

Organotypic hippocampal slice cultures (OHSCs) have advantages for examining hippocampal function by maturation of synapses, receptors, and intrinsic fiber pathways for a number of weeks in a well-controlled in vitro environment. These cultures can apply experimental manipulation that is not possible in vivo, because OHSCs allow to apply a precise concentration drugs or factors at precise time and visualize cell morphology and function, using fluorescent markers/probes within the same cultures for long-term periods. Using OHSCs, the present study was conducted to assess the effects of AA and DHA on KA-induced neuronal death via production of ROS and to assess the possible involvement of a loss of MMPs and apoptosis in these processes.

2. Materials and Methods

Preparation and maintenance of OHSC. All animal experiments were approved by the Institutional Animal Care and Use Committee of Yonsei University College of Medicine. OHSC were prepared by the method of Stoppini et al. [14].

Drug treatment. KA (5 μM) was applied for 12 h after mature cultures were incubated in serum-free culture medium overnight. After KA treatment, cultures were allowed to recover for 48 h in fresh serum-free medium. AA (Sigma, Saint Louis, MO, USA) and DHA (Sigma, Saint Louis, MO, USA) were dissolved in 0.1 M phosphate buffered saline (PBS). Cultures were pretreated with AA or DHA at different concentrations for 1 h before KA treatment.

Assessment of neuronal injury. Neuronal injury was assessed by using the fluorescence cell death marker propidium iodide (PI, Sigma, Saint Louis, MO, USA) that is a very stable dye.

3. Results and Discussions

KA neurotoxicity in OHSC. An initial experiment

was conducted to determine relationship of KA and neuronal death in OHSC (Fig. 1). To exhibit the temporal development of cell death following 12 h exposure to KA (5 μM), representative PI fluorescence images of dead cells captured at 0 and 48 h recovery time are shown in Fig. 1A. In untreated slices, no noticeable PI fluorescence was observed (Fig. 1A). The treatment of slices with KA resulted in neuronal death which showed a selective uptake of PI fluorescence in CA3 region, while faint PI staining was observed in CA1 region after 0 and 48 h of recovery time (Fig. 1A). The PI-staining area significantly continued to increase until 48 h of recovery time in CA3 region (Fig. 1B). However, in the case of CA1 region did not show significant cell death at every recovery time (Fig. 1B).

Effects of AA and DHA on cell death induced by KA. The effects of AA and DHA on KA-induced cell death were observed by fluorescence microscopy (Fig. 2). Representative PI-stained images of increasing dose of AA and DHA (0, 10, 100, 500 and 1000 μM) are shown in Fig. 2A and B. In control cultures, the PI uptake was very low, while in KA only cultures, area of PI-incorporated cell death was broad and deep in CA3 region as compared with the rest of groups (Fig. 2A and B). Pretreatment with AA reduced the area of PI uptake in pyramidal cells at 500 μM in CA3 region (Fig. 2A). The neuroprotective effect of DHA pretreatment appeared at 100 and 500 μM in CA3 region (Fig. 2B). In addition, pretreatment with 500 μM AA significantly prevented cell death after 24 and 48 h recovery in CA3 region, but the rest of groups did not prevent it in same region (Fig. 2C). Furthermore, pretreatment with 100 μM DHA significantly prevented cell death at 12, 24 and 48 h of recovery in CA3 region (Fig. 2D). Moreover, pretreatment with 500 μM DHA significantly reduced cell death at 24 and 48 h of recovery in CA3 region (Fig. 2D). However, low- and high-dose of AA and DHA pretreatment did not prevent cell death at every recovery time.

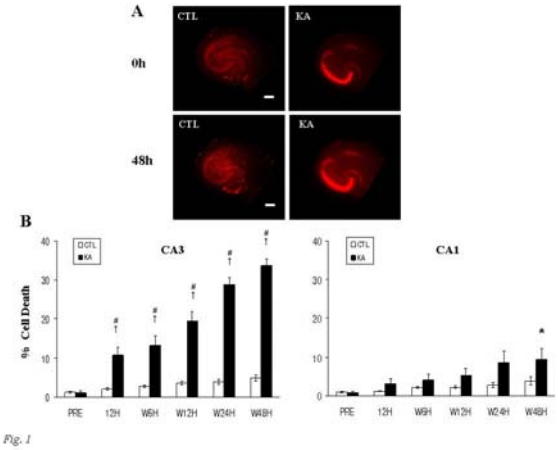

Fig. 1. KA-induced neuronal death in OHSCs. A: PI fluorescence images. B: Quantification of PI-incorporated area following withdrawal of exposure to KA in the CA3 and CA1 regions at each recovery time.

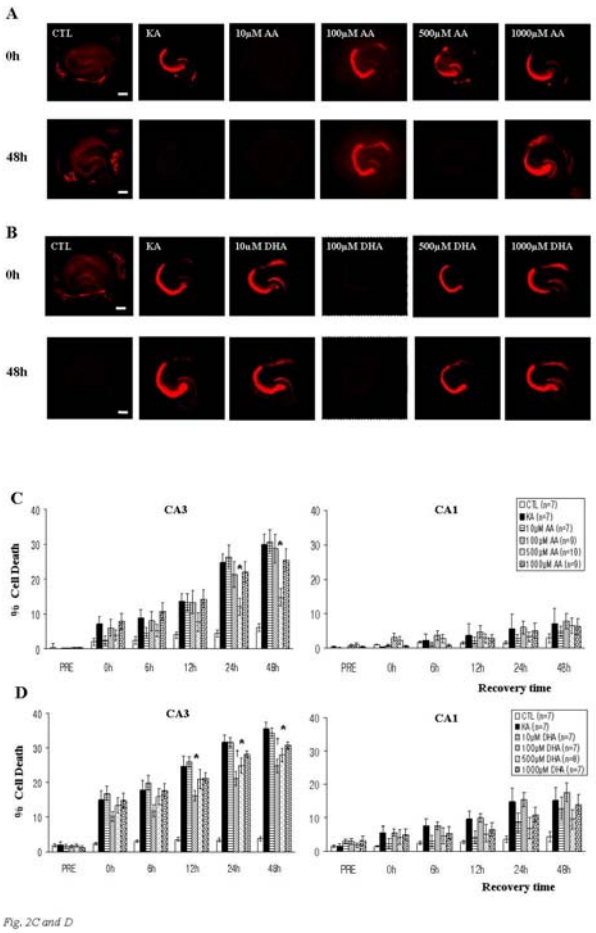

Fig. 2. The effects of AA and DHA on KA-induced PI

uptake in OHSCs. A and B: Representative fluorescence images. Slices were captured following pretreatment of different concentration of AA (A) or DHA (B) and withdrawal of KA at 0 and 48 h recovery time. C and D: Quantification of the effects of AA and DHA on KA-induced PI uptake in OHSCs. C: Quantification of PI-incorporated cell death following pretreatment with AA and withdrawal of KA in the CA3 and CA1 regions. D: Quantification of PI-incorporated cell death following pretreatment with DHA and withdrawal of KA in CA3 and CA1 regions.

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