Neuroprotective Effects of Vitamin E After Facial Nerve Injury

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Abstract
Objective: We investigated the possible neuroprotective effect of vitamin E on the facial motor nucleus (FMN) motoneurons after peripheral nerve avulsion.

Study Design: In adult rats, the right facial nerve was avulsed from the stylomastoid foramen. Following nerve avulsion, immunohistochemistry was used to investigate the effects of vitamin E on 4-hydroxynonenal (HNE) activity. FMN motoneurons and glial cells were counted bilaterally in sections stained with cresyl violet.

Results: Rats administered vitamin E exhibited clear suppression of injury-induced neuronal HNE expression in the ipsilateral FMN as compared to non-treated controls. Following nerve avulsion, the number of surviving motoneurons in the ipsilateral FMN was significantly greater among vitamin E-treated rats than non-treated controls.

Conclusion: The present study demonstrates the neuroprotective effect of vitamin E after peripheral facial nerve avulsion.

Significance: Antioxidants may have therapeutic potential in traumatic facial nerve dysfunction resulting from head injury, ear surgery, and parotid gland surgery.

Introduction
Primary injury of the facial nerve axon can trigger a cascade of secondary processes involving delayed and amplified injury to the facial motor nucleus (FMN) motoneurons, resulting in various grades of neuronal cell death. In severe cases of Bell’s palsy and traumatic nerve injury, irreversible damage to the FMN in the brainstem might occur due to retrograde degeneration. Oxidative stress and massive production of oxygen-derived free radicals, such as fatty acids in particular arachidonic acid from cell membranes, is believed to be one of the most important mechanisms of neuronal cell death following axotomy. One of the major biologically active products of arachidonic acid peroxidation is 4-hydroxynonenal (HNE). Inhibition of lipid peroxidation may provide protection from neuronal cell death produced by retrograde degeneration. Vitamin E (α-tocopherol) has excellent antioxidant capacity and toxicity due to accumulation is considered to be low. In the present study, we investigated the free radical scavenging activity and the possible neuroprotective effect of vitamin E on the FMN motoneurons after peripheral facial nerve avulsion.

Methods and Materials
Adult male SD rats were used in this study. The right facial nerve trunk was identified at the level of the stylomastoid foramen, then the nerve was avulsed from the temporal bone. Animals in the vitamin E-treated group were given free access to drinking water containing 20 µg/mL or 200 µg/mL vitamin E throughout the experimental period. The non-treated group were given free access to drinking water containing only 0.1% DMSO (n=4 for each group during the experimental period).

At 1, 2 and 4 weeks after facial nerve avulsion, animals were deeply anesthetized with sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially. Using a freezing microscope, samples were then cut into coronal sections that were 20 µm thick in the frontal plane. Cresyl violet staining was performed to evaluate the number of motoneurons and glial cells in the FMN. In order to evaluate the oxidation of neuronal membranes, we performed an HNE immunohistochemical study. The sections (3 µm thick) were incubated overnight at room temperature with 200 µg/mL vitamin E; in B and D, there was no treatment given.

Results
1. Amount of drinking water
Rats drank approximately 30 mL of water per day, resulting in the administration of 2.4 mg/kg/day vitamin E in 20 µg/mL group and 24 mg/kg/day vitamin E in 200 µg/mL group. The moderate vitamin E dose (20 µg/mL) was almost equivalent to the daily dose that is clinically used for humans.

2. Moderate vitamin E decrease neuronal degeneration and gliosis
In control animals that were given 0.1% DMSO (Fig. 1, right column), the outline of cell soma was quite unclear and there was a swelling of the cytoplasm and a strong neurogliosis around the cell somas observed at 2 weeks after the surgery. At 4 weeks after the surgery, many neurons disappeared (Fig. 2C). The survival ratios for the injured FMN were 81% at 1 week, 57% at 2 weeks and 34% at 4 weeks after the surgery, respectively (Fig. 3A filled circle).

Conclusions
Our result demonstrated that vitamin E suppressed the expression of HNE, retrograde degeneration and gliosis in the FMN following facial nerve avulsion. Results from the HNE immunohistochemical indicated that there was strong staining of the neurons in the FMN and that there was neuronal degeneration at 2 to 4 weeks after nerve avulsion in the control animals (DMSO group). These results suggest that lipid peroxidation leads to dysfunction of the cell membrane and subsequent neuronal cell death. It is interesting to note that the protective effect of the high dose (200 µg/mL) of vitamin E was worse than the moderate dose (20 µg/mL). There was also a significant decrease in the glial cell ratio in the group treated with 20 µg/mL vitamin E as compared to that which was seen in the 200 µg/mL vitamin E-treated group. One of the reasons for this paradoxical phenomenon might be that there is an injury effect related to the vitamin E radical itself. The radical form of vitamin E may be involved in oxidative stress. Our results suggested that there was a proper dose of vitamin E to show neuroprotective effect following facial nerve avulsion.

In the future, antioxidant substances might have considerable therapeutic potential in facial nerve injury that results from head trauma, ear surgery, and parotid gland surgery, or even in some cases with severe Bell’s palsy and Hunt syndrome.