Suppression of glutamate-induced neural cell death by electrolyzed-reduced water

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Abstract

Electrolyzed-reduced water (ERW) produced by electrolyzing water in cathode side has the ability to scavenge reactive oxygen species (ROS). Here, we investigated the effect of ERW on oxidative stress-induced neural cell death by glutamate. When cell viability assay was performed using primary rat cerebral cortical culture as neural model, ERW suppressed neural cell death by glutamate. Furthermore, intracellular ROS levels were reduced by ERW, suggesting that suppressive effect of ERW on the glutamate-induced neural cell death was due to the suppression of glutamate-induced ROS augmentation by ERW.

1. Introduction

Neural cell death associates with aging and neurodegenerative diseases including Alzheimer's disease (Miranda et al., 2000), Parkinson's disease (Koutsilieri et al., 2002), Huntington's disease (Browne et al., 1999) and pathological conditions such as ischemia, stroke and excitotoxicity (Love, 1999; Coyle et al., 1993). In these diseases, it is considered that oxidative stress is one of the causes of the neural cell death. Oxidative stress is caused by reactive oxygen species (ROS) such as hydroxyl radical,

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hydrogen peroxide and superoxide anion radical generated in the process of respiration, associated with mitochondrial electron transport. Glutamate is a major excitatory neurotransmitter in the central nervous system (CNS) and has an important role especially in memory. On the contrary, glutamate has neurotoxicity and is associated with oxidative stress. When ischemia-reperfusion injury is caused, neural cell release excess glutamate, triggering calcium ion influx, followed by increase in nitric oxide (NO) and ROS-induced neural cell death. Here, we investigated whether ERW exhibiting antioxidative activity could suppress neural cells death by oxidative stress, which might cause neurodegenerative diseases.

2. Materials and Methods

2.1. Cell culture

Rat pheochromocytoma PC12 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% horse serum, 5% fetal bovine serum, 100 unit/ml penicillin and 100 μg/ml streptomycin. Primary cell cultures were obtained from the cerebral cortex of fatal rats. The embryos (17 to 18 days gestation) were removed under sterile conditions and placed into ice-cold 1 : 1 mixture of PBS and DMEM. Cerebral cortex was dissected, and incubated for 15 min in 0.05% trypsin and phosphate-buffered saline (PBS). Single cells dissociated from the cerebral cortex were plated on poly-D-lysine coated plastic 24 well plates (0.5-1 x 10^5 cells/ml). Cultures were incubated in DMEM supplemented with 5% horse serum, 5% fetal bovine serum, 100 unit/ml penicillin and 100 μg/ml streptomycin. After 2 days plating, non-neural cells were removed by addition of 10 μM cytosine arabinoside (Ara-C). Experiments were performed on mature cultures incubated for 7-10 days *in vitro*.

2.2. Electrolysis of water

ERW was prepared by electrolysis of ultrapure water containing 0.002 N NaOH using an electrolysis device TI-200S (Nihon Trim Co., Ltd., Osaka) equipped with platinum-