ELECTROLYZED REDUCED WATER INDUCES DIFFERENTIATION IN K-562 HUMAN LEUKEMIA CELLS

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Abstract

Electrolyzed reduced water (ERW) is known to scavenge reactive oxygen species (ROS) which is related to malignant tumor phenotypes. We attempted to clarify the effect of ERW on tumor phenotypes of K562 human leukemia cells. Treatment of K562 cells with ERW resulted in growth arrest, morphological changes, and expression of CD41, a cell marker of megakaryocytic differentiation. However, the morphology of N-acetylcysteine (NAC)-treated cells was rather similar to that of non-treated control K562 cells. These results suggested that ERW, but not NAC can differentiate K562 cells into megakaryocytes. The induction of megakaryocytes from K562 cells by ERW was preceded by a rapid rise in the activity of MEK (MAP kinase /extra-cellular regulated kinases) that leads to sustained activation of ERK (extra-cellular regulated kinases; MAPK). However, In NAC-treated K562 cells, ERK activation was only transient. The different persistency of ERK activation induced by ERW and NAC might affect the cell fate.

1. Introduction

One of final goals for leukemia treatment is thought to be the eradication of every tumor cells. Anti-cancer agents have been produced by focusing upon the unusual multiplication nature of cancer cells, the execution ability of which was found to be clinically satisfactory. However, as the cytotoxic effect is stronger, serious side effects on normal cells have been shown to be negligible. Moreover, the potencies of anti-cancer agents vary in each stage of cancer cells, and tolerant cells against anti-cancer agents appears. To overcome these disadvantages of anti-cancer agents, exploitation of anti-cancer agent with a mild potency and no possible side effect has been desired. Recently, a dairy intake of ERW has been shown to improve pathophysiological conditions of various diseases including cancer. ERW produced near the cathode during electrolysis of water contains high dissolved molecular hydrogen, exhibits a high negative redox potential value and demonstrates a strong scavenging ability of reactive oxygen species (ROS) (Shirahata et al., 1997). Since ROS is known to be related to the occurrence and/or maintenance of malignant tumor phenotypes (Szatrowski and Nathan, 1991; Toyokuni et al., 1995), we expected that ERW would alter and/or diminish the tumor

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phenotypes of cancer cells via its scavenging ability of ROS. Actually, N-acetylcysteine (NAC), a known antioxidant, is reported to scavenge intracellular ROS, resulting in the cell cycle arrest and the inhibited proliferation of cancer cells (Sekharam et al., 1998) .Thus we expected that ERW can reduce the cellular redox level, leading to alter the tumor phenotypes such as high growth potential, because cancer cells maintain high cellular ROS level. Our previous study showed that the growth of human cancer cell lines incubated with ERW were significantly suppressed without affecting serious damage to normal cells. In the present study, we extended our work to another a human cancer cell line K562, which inhibit cell proliferation. In order to investigate the mechanism by which ERW inhibits the proliferation of K562 cells, we observed as initial step, the effect of ERW on inducing differentiation of K562 cells by morphological changes, expression of CD41. It was found that ERW induced the megakaryocytic differentiation of K562 under conditions in which cell proliferation was inhibited, but the precise modes of its differentiating and anti-proliferative actions require further elucidation before its clinical use is likely to be successful. We have focused on utilizing ERW induce differentiation in K562 cell line.

2. Materials and Methods

2.1 Preparation of ERW

Ultrapure water containing 0.002N NaOH was electrolyzed by an electrolyzing device equipped with platinum–coated titanium electrodes (TI-8000 and TI-200S: Nihon Trim, Tokyo, Japan). Electrolyzed water in the cathode was used as ERW. ERW was neutralized with HCl before use.

2.2 Cell culture

K562 human Leukaemia cells were obtained from the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer in Tohoku University. Cells were cultured in RPMI 1640 (Invitrogen, CA) supplemented with 10% inactivated fetal bovine serum (FBS) and ultrapure water or ERW at 37℃ under an atmosphere of 5%CO_2. Cells were seeded at the density of 1 x 10^5 cells/ml and counted every day.

2.3 Cell cycle analysis

Cell cycle distribution was analyzed by using flowcytometer (EPICS XL System Ⅱ-JK :Beckman Coulter). Cells (1 x 10^6 cells) were collected by centrifugation at 400 x g for 5 min, and washed twice in phosphate buffered saline (PBS). Cells were then resuspended in 1.0 ml of PBS containing 0.2% Triton-X (Sigma Aldrich corporation stlouismissouri) and 100 mg/ml RNase (Sigma Aldrich corporation stlouismissouri). After incubation at room temperature for 30 min, propidium iodide (Sigma Aldrich corporation stlouismissouri) was added at the final concentration of 20 mg/ml. After incubation at room temperature for 30 min, cell cycle distribution was analyzed by flowcytometer.

2.4 Examination of intracellular ROS level by flowcytomer

The amount of intracellular ROS, especially the intracellular H_2O_2 was determined by using a fluorescent dye, 2',7'-dichlorofluorescin-diacetate (DCFH-DA) (LeBel et al. 1992). K562 cells were pre-cultured for 10 min in Ca^{2+}, Mg^{2+}-free HBSS buffer with or