ELECTROLYZED REDUCED WATER WHICH CAN SCAVENGE ACTIVE OXYGEN SPECIES SUPRESSES CELL GROWTH AND REGULATES GENE EXPRESSION OF ANIMAL CELLS.

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Abstract:

Active oxygen species are considered to cause extensive oxidative damage to biological macromolecules, which bring about a variety of diseases as well as aging. Reduced water produced near cathode during electrolysis of water exhibits high pH, low dissolved oxygen, extremely high dissolved molecular hydrogen, and extremely negative redox potential values. Recently we found that strongly electrolyzed reduced water scavenges active oxygen species and protects DNA from oxidative damage (Shirahata, S. *et al*., *Biochem. Biophys. Res. Commun.*, **234,** 269-274 (1997)). Electrolyzed reduced water suppressed the growth of human normal fibroblast TIG-1, human lung adenocarcinoma A549, and human uterine cervix cancer HeLa, indicating that reduced water affects the signaling pathway of cell cycle. The expression of the interleukin-6 gene was enhanced by reduced water as well as ascorbic acid, (+)-catechin and tannic acid when added to the culture of human osteosarcoma MG-63 cells, suggesting that reduced water acts as a reductant to cells.

Introduction

Active oxygen species such as singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) are considered to cause extensive oxidative damage to biological macromolecules (DNA, membranes, enzymes and so on), which bring about a variety of diseases as well as aging (1, 2). Antioxidative enzymes such as superoxide dismutase, catalase, and glutathione peroxidase can scavenge active oxygen species. Daily intake of antioxidants such as vitamin C, vitamin E, β-carotene, (+)-catechin is also important to protect cells from oxidative damage. In spite of these protective mechanism, a chronic state of oxidative stress exists in cells because of an imbalance between prooxidants and antioxidants. Recent heavy environmental pollution seems to strengthen oxidative stress to our bodies.

Water is a most abundant compound on the earth and indispensable for existence of life. More than a half century ago, domestic devices to reform water have been developed in Japan. The principal is to separate reduced water near cathode from oxidized water near anode by semipeamiable membrane during electrolysis of water. Electrolyzed reduced water exhibits alkaline pH and negative redox potential. The devices to reform water are admitted as therapeutic devices by the Japanese ministry of public welfare because reduced water is effective to suppress abnormal fermentation in intestine and hyperacidity, although the action mechanism is unclear. Based upon the interesting clinical improvement of a variety of diseases by intake of reduced water, Hayashi proposed the hypothesis “water regulating theory’ since 1985 (3). Recently we found that electrolyzed reduced water contains high concentration of dissolved hydrogen (DH), scavenges active

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oxygen species and protects DNA from oxidative damage (4). We proposed a new hypothesis that active hydrogen in reduce water may scavenge active oxygen. Here we report that electrolyzed reduced water suppresses the cell growth and regulates the gene expression in animal cells.

Materials and methods

Electrolysis of water. Ultrapure water produced by an ultrapure system (ULTRAPUR LV-10T, TORAY, Tokyo) was added 0.1 g/l NaCl to elevate electrical conductance (EC) to about 20 ms/m. The water was then electrolyzed with various voltages (0 - 40 V) by an electrolyzing device (Type TI-7000S and TI-7000SL, Nihon Trim Co., Osaka) equipped with platina-coated titanium electrode to produce reduced water which exhibited various RP. Ultrapure water containing NaCl was sent to the electrolyzing device using a water current pump at a rate of 0.5 l/min to 1.0 l/min. Electrolyzing voltage was changed to several to several tens V and currents several to 10 A. RP, EC, dissolved oxygen *(DO)* and DH were measured using a RP meter (type, HM-14P), a EC meter (CM-14P), a DO meter (DO-14P) and a DH meter (DHDI-1) of Toa Electronics Ltd. (Tokyo) at 25°C. pH was measured using a pH meter (Beckman, Type pHI32) at 25°C.

Assay of scavenging effect of reduced water against superoxide onion radical. The scavenging effect of reduced water against $O_2^{\cdot-}$ was examined by hypoxanthine-xanthine oxidase system using luciferine analog and a chemiluminometer as described previously (4). All reactions were performed in neutralized condition using 40 mM sodium phosphate buffer (pH 7.0).

Measurement of growth curve of human normal and cancer cell lines. Normal human fibroblast cell line TIG-1, human lung adenocarcinoma cell line A549 and human uterine cancer cell line HeLa were inoculated into 24-well microplates and cultivated in 10% fetal calf serum-MEM medium containing electrolyzed reduced water at 37°C under an atmosphere of 5% CO_2. Since strongly electrolyzed reduced water often contains HOCl which was produced near anode, the effects of reduced water on the cell growth were compared with those of HOCl of the same concentration. Tap water must contains more than 0.1 ppm of residual chlorine. The concentration of HOCl was determined by o-tolidine method.

Analysis of the interleukin-6 gene expression in MG-63 cells. Human osteosarcoma MG-63 cells were cutured in 5% FBS-MEM medium at 37°C under an atmosphere of 5% CO_2. The cells were treated in PBS (+) containing 2.7% reduced water, 0.1 μM ascorbic acid, 0.33 μg/ml (+)-catechin or 40 μM tannnic acid in the presence of $MgCl_2$ and $CaCl_2$ for 2 hour. RT-PCR was performed after extraction of mRNA.

Results and discussion

Characteristic of electrolyzed reduced water

Strongly electrolyzed reduced water exhibited high pH, low DO, high DH and extremely negativeRP values. Marked changes in these values occur in water after electrolysis. For example, the value of RP +350 mV in water before electrolysis changes to -659 mV after electrolysis; 4.08 of pH changes to 10.47; 4.46 mg/1 of DO changes to 3.38 mg/l and 0.0046 mg/l of DH changes to 0.467 mg/l, respectively. It should be noticed that DH value is higher in reduced water than in the original water by two orders of magnitude. Strongly reduced water completely scavenged $O_2^{\cdot-}$ produced by hypoxanthine-xanthine oxidase system (4). Reduced water was shown to scavenge H_2O_2 (4). It prevented single-strand breakage of plasmid DNA caused by the Cu(II)-catalyzed oxidation of ascorbic acid, suggesting that reduced water can also scavenge OH and $^1O_2$(4). The scavenging activity of reduced water against $O_2^{\cdot-}$ was stable in closed bottle at 4 °C for about a month