Induction of Caspase-3-dependent Apoptosis by Electrolyzed Reduced Water/platinum Nanoparticles in Cancer Cells

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Abstract: Electrolyzed reduced water (ERW) supplemented with platinum nanoparticles (Pt Nps)(ERW/Pt Nps) exhibited strong antioxidative and active hydrogen donating activities. ERW/Pt Nps-containing medium caused lowering of cell viability. Analysis of cell cycle and activity of caspase-3 suggested that ERW/Pt Nps induced apoptosis in cancer cells. This apoptosis inducibility ERW/Pt Nps was lost by using degassed ERW and recovered by bubbling of hydrogen gas. These results suggested that active hydrogen produced from molecular hydrogen in ERW by catalysis of Pt Nps induced apoptosis of cancer cells.

Key words: Antioxidant, Apoptosis, Platinum, Nanoparticles, Reactive oxygen species

1. INTRODUCTION

We previously reported electrolyzed reduced water (ERW), produced near the cathode by electrolysis, has a reductive activity (Shirahata *et al*., 1997). We also revealed that ERW contains platinum nanoparticles (Pt Npps) derived from Pt-coated titanium electrodes in addition to high concentration of dissolved molecular hydrogen. Pt Nps exhibited strong reactive oxygen species (ROS) scavenger activity and catalysis activity converting molecular hydrogen to active hydrogen. Therefoe, ERW supplemented with Pt Nps (ERW/Pt Nps) is expected to has a strong

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antioxidative activity. A hypothesis has been proposed that high level of intracellular ROS promotes malignant properties of cancer cells such as activation of growth signals, apoptosis tolerance, metastasis, and angiogenesis. Here, we investigated apoptosis inducibility of ERW/Pt Nps on cancer cell lines.

2. EXPRIMENTAL PROCEDURE

2.1 Cell culture and measurement of cell proliferation

Normal cell lines; human diploid embryonic lung fibroblasts (TIG-1), human diploid fibroblasts (WI-38) and human diploid embryonic lung cell line (MRC-5), and cancer cell lines; cervical carcinoma cells (HeLa) and human hepatocellular carcinoma cell line (HepG2) were obtained from the Japanese Collection of Research Bioresources (JCRB, Osaka, Japan). Adherent cell cells were cultured in Eagle's MEM medium supplemented with 10% fetal bovine serum (FBS) at 37°C in a 95% air/5% CO_2 atmosphere. Briefly, cells (floating cells, 1.25 x 10^4 cells/cm^2 and adherent cells, 2.5 x 10^4 cells/cm^2) were seeded in 24-well plates. The cultures were incubated with ERW supplemented with different concentrations of Pt Nps for 24 hours. Cytotoxicity was determined using the 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetra-zolium,monosodium salt (WST-1) assay.

2.2 Measurement of intracellular ROS by flowcytometer

The amount of intracellular ROS, especially the intracellular H_2O_2, was determined by using a fluorescent dye, 2',7'-dichlorofluorescin-diacetate (DCFH-DA). Cells were pre-cultured for 10 min in Ca^{2+}, Mg^{2+}-free HBSS buffer with ERW/Pt Nps. After the removal of the supernatant, 5 μM DCFH-DA was added and incubated for 10 min. After resuspended in PBS, intracellular redox state of cells was analyzed immediately using a flowcytometer.

3. RESULT AND DISCUSSION

ERW/Pt Nps exhibited strong antioxidative and active hydrogen donating activities. Pt Nps did not cause lowering of cell viability (Fig. 1). However, ERW/Pt Nps exhibited strong cytotoxicity on cancer cells but not on normal

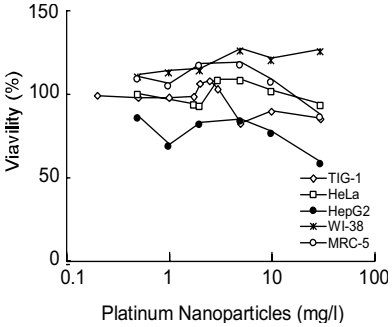

Figure 1. Cytotoxicity test of ERW/Pt Nps.

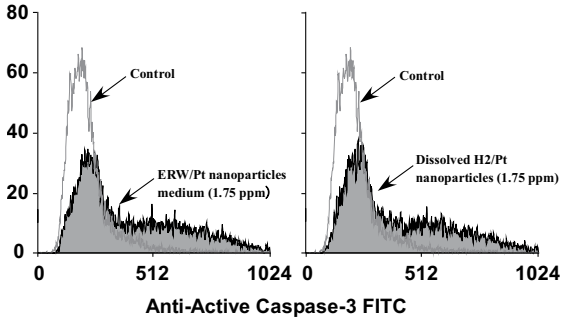

Figure 2. Determination of activated caspase-3 in HeLa cells.

cells (data not shown). Analysis of cell cycle and caspase-3 activity suggested that ERW/Pt Nps induced apoptosis in cancer cells. This apoptosis inducibility of Pt Nps/ERW was lost by using degassed ERW and recovered by bubbling of hydrogen gas (Fig. 2). The cytotoxicity of ERW/Pt Nps on cancer cells was rapidly lost in high concentrations of Pt Nps, suggesting that the phenomenon is due to agglutination of Pt Nps. These results suggested that active hydrogen produced from molecular hydrogen in ERW by catalysis of Pt Nps induced apoptosis of cancer cells. ERW/Pt Nps scavenged intracellular ROS in cancer cells. Investigation on the relationship between ROS-scavenging activity and apoptosis is undergoing.