

Preliminary Study on the Use of Electrolyzed Reduced Water as Radioprotector

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1. Introduction

Hydrogen molecules are inert gases that selectively reduce the relatively oxidative $\cdot\text{OH}$ and ONOO^- [1]. Electrolyzed reduced water (ERW), produced near the cathode during electrolysis of water, contains hydrogen molecules and may contain small amount of platinum nanoparticles being off the platinum-coated titanium electrodes. Although no exact mechanism is known, it has been demonstrated in an earlier study that hydrogen-enriched water and ERW have an ROS-scavenging and thus radio-protective effect [2].

ERW has been known for its protection against various chemicals such as H_2O_2 and also against UV through precedent *in vitro* and *in vivo* experiment studies, but there is no study yet to have observed the protection by ERW against ionizing radiation [3]. It can be expected that ERW would have a protection effect against ionizing radiation like against UV.

In this study, we investigated the feasibility of ERW being utilized as a radioprotector, in terms of ROS scavenging and cell saving.

2. Methods

2.1 Cell culture and irradiation

Rat diencephalon cells (RDCs) [CRL-2005, ATCC, Manassas, VA, USA] were cultured with Dulbecco's modified Eagle medium (DMEM) (GIBCO, Grand Island, NY, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (GIBCO) and incubated at 37 °C in a humidified incubator with 10% CO_2 .

Cells were exposed to X-rays generated from the beam tube (model 450-D08, YXLON, Germany) operating at up to 150 kVp and at 10 mA in the facility installed in the Radiation Bioengineering Laboratory at Seoul National University [4].

2.2 Preparation of ERW

Distilled water (MilliQ, Merck Millipore, Darmstadt, Germany) was electrolyzed with NaCl added at 0.5 M and applying 30 V power supply on the platinum-coated titanium electrodes. ERW was collected from the cathode chamber. The pH of ERW was approximately 11~12 initially and turned to 7.0~7.4 after being put in the incubator for an hour. ERW was added to the cell culture medium in the ratio of 1 to 1.

2.3 DCFDA assay

Chemical- or radiation-induced ROS are detected in the process of the oxidation of 2', 7'-dichlorofluorescein diacetate (DCFDA) to fluorescent 2', 7'-dichlorofluorescein (DCF). The non-fluorescent DCFDA compound is oxidized by ROS into the highly fluorescent DCF, which can be detected by fluorescence spectroscopy. A cellular ROS detection assay kit (ab113851, Abcam, Cambridge, UK) was used for the oxidation of DCFDA to DCF and the fluorescence was measured at 495 nm and 529 nm of maximum excitation and maximum emission wavelengths, respectively. The fluorescence spectroscopy was carried out by using the FACS Aria-II (BD Bioscience, San Jose, CA, USA).

2.4 Clonogenic assay

Cells were treated with ERW for 0.5 h prior to irradiation. Clonogenic surviving fractions of the control cells treated with neither radiation nor ERW were normalized to 1. Clonogenic surviving fractions from radiation exposures were compared among the cells in ERW-free media and those in ERW-added media.

2.5 ICP Mass Spectrometry

The concentrations of elements contained in distilled water (MilliQ) and ERW were measured using an ICP-Mass Spectrometer (NexION 350D, PerkinElmer SCIEX). Distilled water and ERW are known to differ in the concentrations of iron (Fe), platinum (Pt), titanium (Ti), silver (Ag) and hafnium (Hf) [2].

3. Results

3.1 Cytotoxicity of ERW

As shown in Fig. 1, ERW alone did not cause any significant cytotoxic effect on RDCs.

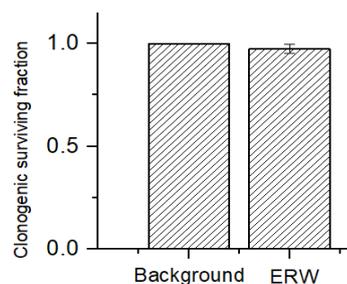


Fig. 1. Clonogenic surviving fractions of RDCs with or without ERW added to the culture medium.

3.2 Effect of ERW on radioresponse of RDCs

Fig. 2 presents that radiation exposure resulted in intracellular ROS production and ERW added to the culture media lowered the ROS concentration in the cells. The ratios of DCF signal reduction by ERW were comparable regardless of radiation dose (Fig. 2(a)). ERW reduced the DCF signal by a greater extent in the cells irradiated at a lower dose rate (Fig. 2(b)). The reduction ratio of DCF signal from the cells irradiated for the same dose (1 Gy) was greater with the X-rays of lower end energy (Fig. 2(c)).

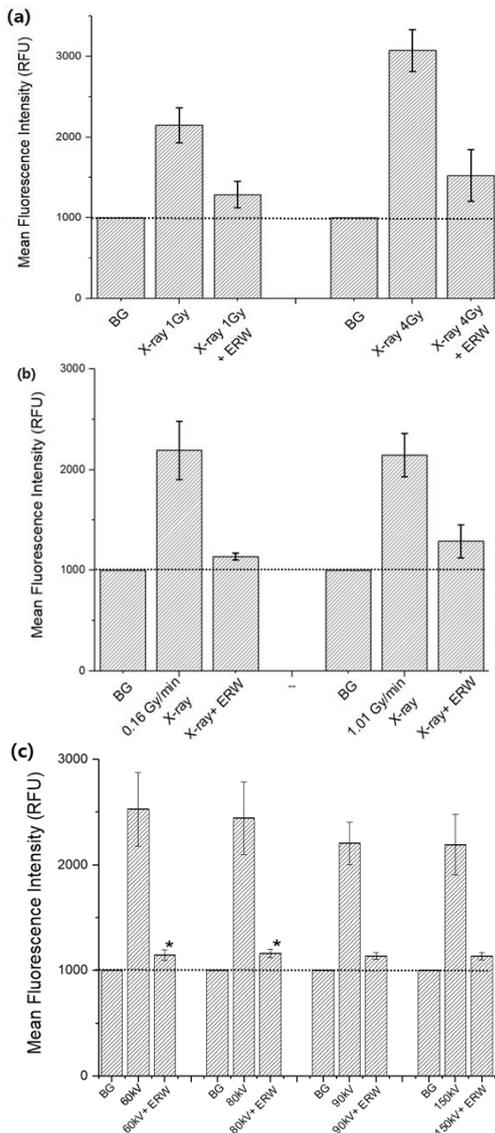


Fig. 2. The relative ROS signals due to X-ray exposures with or without ERW treatment of the culture medium: (a) at different doses (1 or 4 Gy) from 150 kVp X-rays by the same dose rate of 1.01 Gy/min, (b) by different dose rates (0.16 or 1.01 Gy/min) for the same total dose of 1 Gy from 150 kVp X-rays, and (c) at the same dose rate of 0.16 Gy/min for the same total dose of 1 Gy but from X-rays of different end energies (60, 80, 90 or 150 keV). The average DCF fluorescence intensity of the control cells were normalized to 1000. The error bars represent one standard errors.

Fig. 3 depicts the clonogenic surviving curves of RDCs decreasing with an increasing dose delivered by X-rays at different end energies (150 keV vs. 60 keV). Cells were slightly more sensitive to X-rays of higher end energy (150 keV) and higher dose rate (1.01 Gy/min). The cell saving by ERW treatment seemed more efficient for the cells exposed to X-rays of lower end energy (60 keV).

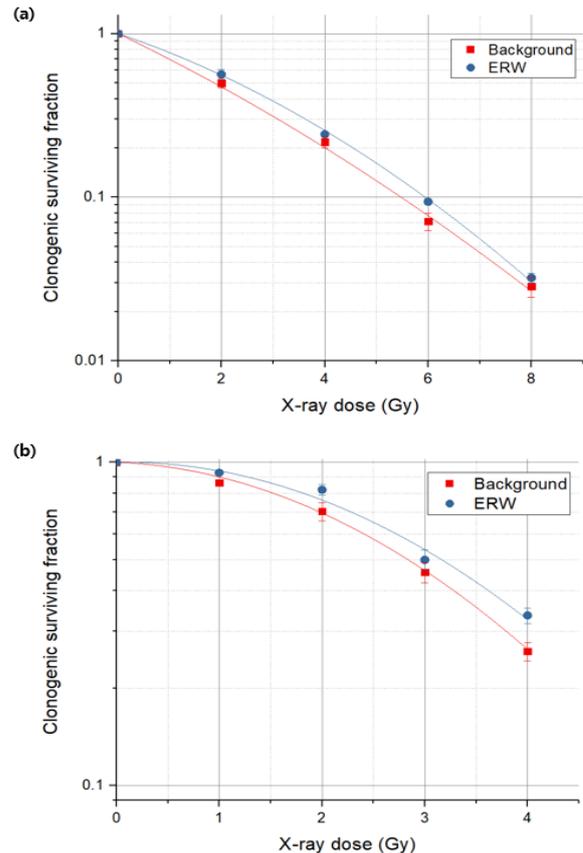


Fig. 3. Clonogenic surviving fractions of RDCs from exposures (a) at 1.01 Gy/min to X-rays of end energy at 150 keV and (b) at 0.16 Gy/min to X-rays of end energy at 60 keV.

3.3 Influence of nanoparticles in ERW

In a previous study, platinum nanoparticles were suspected to have a protective effect by ROS elimination for the cells treated with chemicals [5]. Considering that the high-Z platinum is highly effective in photoelectron production, dose enhancement by platinum possibly contained in ERW would make a conflict with the intention of employing ERW to protect the cells from radiation damage. According to the results of ICP Mass spectrometry (Table 1), ERW used in this study contained iron, platinum, titanium, silver and hafnium at concentrations less than 100 ppb. Other elements than platinum are also suspected to enhance cellular dose. In fact, the K-edge energies of those metal nanoparticles are 78.395, 85.530 and 7.122 keV for platinum, titanium and iron, respectively.

In Fig. 2(c), the ROS signal from the cells was reduced by greater extents when the end energies (60 and 80 keV) of X-ray beam were lower than K-edges of the ingredient platinum (~78 keV) and titanium (~85 keV) nanoparticles, respectively, in ERW.

Table 1. Concentrations in ppb of metal elements in ERW.

element	distilled water	filtered ERW
Fe	0.85	70.72
Pt	< 0.1	7.87
Ti	< 0.1	27.54
Ag	< 0.1	< 0.1
Hf	< 0.1	< 0.1

4. Conclusion

ERW showed a protective effect on RDCs against X-ray exposure, by a greater extent for X-ray beam of a lower end energy and at a lower dose rate. The dose level did not matter regarding the extent in protective effect of ERW. The ingredient high-Z nanoparticles in ERW may offset the protective effect of ERW by enhancing cellular doses through photoelectric interactions, on the condition that the end energy of X-ray beam is higher than the K-edges of those nanoparticles.

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