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The Induction and Control of Oxidation Damage caused by arsenic and UV Radiation exposure to *Euglena gracilis* SMZ

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Arsenic (As) is a ubiquitous element in the environment. Chronic As poisoning from natural and anthropogenic sources has become one of the most significant environmental health problems affecting over 100 million people worldwide[1)]. As leads human carcinogen. It causes increased cancer risk of skin, lung, bladder and other internal organ systems[2)]. There had been many failed attempts, as review by Wang *et al.*[3)], to demonstrate As carcinogenicity in *in- vivo* animal systems until recent years. Chronic exposure of sodium arsenate caused multiple tumours in C57Bl/6J mice[4)]. Waalkes *et al.*[5)] were able to demonstrate transplacental carcinogenic effects of arsenite dosed to the pregnant C3H mice resulting in a higher incidence of cancers in the offspring at their late stage of life.

Solar ultraviolet radiation (UVR) is responsible for the induction of more than 90% of non-melanoma skin cancers[11)]. This is of particular concern in view of the consequences of stratospheric ozone depletion. It has been estimated that every 1% depletion of ozone concentration results in a 2% increase in the amount of UVR the earth[6)].

It has been demonstrated that As enhanced the number of skin cancers in mice induced by UV exposure[7)]. Since people who live in As-endemic areas are also exposing to UV radiation. The significance of the combined effect of arsenic and UV radiation need further investigation.

Euglena gracilis SMZ strain,has been shown as a useful model for the study of oxidative damage induced by a number of metals including mercury, lead and cadmium[8, 9)]. In this study, we investigate the oxidative effect of arsenic and/or UVB exposure in *E. gracilus* SMZ strain.

Material and methods

Cultivation of *E. gracilis* SMZ cell with or without exposure to sodium arsenite ($NaAsO_2$) and/or ultraviolet B (UVB) Achlorophyllous *Euglena gracilis* SMZ strain was cultivated in a Koren-Hutner medium (K.H.) pH 3.5 at 28℃ under illumination (2800 lx) using fluorescent light for plant growth on a light/dark cycle 12/12h for 5 days until reaching an early stationary phase of growth. The cells were harvested aseptically, treated with $NaAsO_2$ (nacalai tesque LTD., Kyoto, Japan) at a final concentration of 500 μM in K.H. (pH 5.5). The cells were incubated at 28℃ under illumination (2800 lx) for 0, 3 and 6h.

For UVB exposure, cells suspended in the Petri-dishes were exposed to UVB radiation from a UVB lamp (model UVM-57, 302 nm, 6W, equipped with 2 UVG filter to remove UVC; UVP Inc., Upland, CA, USA) at 4W/m^2 equivalent to an UVB dose of 0.7J/cm^2 in 30 min. The irradiated UVB energy was monitored simultaneously using a radiometer (VLX-3W Vilber lourmat, Torcy, France). The UVB irradiation experiments were carried out between 2 p.m. and 4 p.m.

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Examination of cell viability

To estimate cell viability, 200 μl of *E. gracilis* SMZ cell suspensions were taken from each Petri-dish, centrifuged at 1000 × g (21℃ for 3 min), washed twice with 200 μl of PBS (pH6.4), and then stained with 100 μl of 20 μg/ml Fluorescein diacetate (FDA, Molecular Probes Inc., Eugene, OR, USA) and 30 μl of 20 μg/ml Propidium iodide (PI) at room temperature for 30 min in darkness. After staining, cells were washed again with 200 μl of PBS solution. The viable and non-viable cells were detected by as fluorescence microscope (model BX 51; Olympus, Tokyo, Japan).

Cell viability was expressed as the percentage of viable cells over control viable cells.

Effect of preliminary beta- carotene supplementation on the protection of cell viability induced by As and/or UV-B exposure

To assess whether the As- and UVB-induced cell death could be prevented by beta-carotene, 10ppm beta-carotene modified as water soluble form (Sunactive V-BC 10, Taiyo Kagaku Co. Ltd.,) was added to *E. gracilis* SMZ cells immediately before exposure to As and UVB.

Briefly, *E. gracilis* SMZ cells, grown in the K.H. medium (pH5.5), were harvested, re-suspended in the K.H. medium supplemented with modified beta-carotene at 10ppm as the final concentration. In the similar manner as described in the previous section, Aswas exposed to the cells for 3 and 6h in the cell suspension and washed twice with K.H. medium (pH5.5). Cells were treated with beta-carotene again then exposed to UVB for 30 min as described above.

Results and discussion

The purpose of the present study was to investigate how the sole or combined exposure of As and/or UVB would affect the viability of achlorophyllous *E. gracilis* SMZ strain that can be regarded as protozoan model, and the effect of beta-carotene supplementation on the cell death induced by As and/or UVB.

The authors previously reported that cell growth of *E. gracilis* SMZ was inhibited by 50% with long-term exposure of 500 μM $NaAsO_2$[10]. In another study, the authors examined the effect of single or combined exposure of herbicide, methyl-viologen and/or UVA or UVB on the cellular damage of *E. gracilis* Z and SMZ[11].

However, cell viability under the combined exposure of As and UVB has never been examined neither by short-term nor long-term exposure.

When *E. gracilis* SMZ cells were cultivated with 500 μM $NaAsO_2$ for 0, 1, 3, 6 and 12h, the uptake of arsenic was proportionally increased with increased incubation time (data not shown).

The percentages of viable cells after As and/or UVB-exposure are shown in the Figure 1.

By the As-exposure alone, viable cell number was 90% of the total cells, and there was no significant difference from that of the control cells. However, the number of dead cells significantly increased by combined As and UVB exposure. The most remarkable cell death was observed with 500 μM $NaAsO_2$ exposure for 3h and short term UVB irradiation showing 50% cell dead in the cell population.

Interestingly, more cell death was observed at As3h+UVB than As6h+UVB (Fig. 1). Considering the reason for higher mortality at As3h+UVB than As6h+UVB, it may be due to the toxicity of metabolites of $NaAsO_2$, exhibiting synergistic effect with UVB in the SMZ cells. Namely, metabolites in the SMZ strain after 3h of exposure might be more reactive, or toxic than that formed after 6h of incubation. To affirm this postulation, analysis of As metabolites should be essential, and is now under way.

Rossman *et al.* reported that no tumors appeared in mice given aresnite alone. The tumors were mostly squa-

mous cell carcinomas, and those occurring in mice given UV radiation plus arsenite appeared earlier and were much larger and more invasive than in mice given UV radiation alone[7].

Seeking the way to reduce the combined arsenic and UVB-induced cellular damage is also important. To control the cell death that were induced by As and UVB exposure, we used modified beta-carotene as an antioxidant. Figure 2 shows the effect of beta-carotene on the cell viability of As andUVB exposed *E. gracilis* SMZ. The dead cell number was decreased by 30% in cells exposed to As for 3h and subsequent UVB irradiation. Similar effect was

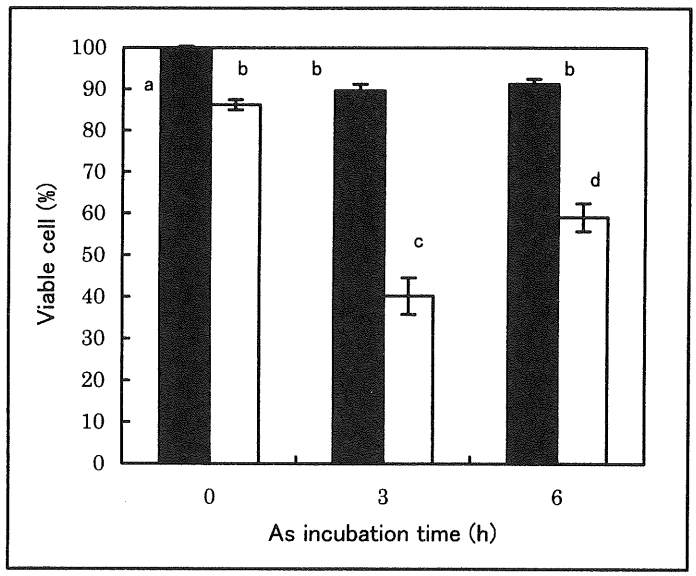

Fig. 1 Effect of As and/or UVB exposure on the cell viability of *E. gracilis* SMZ. (■- As exposure without UVB irradiation; □- UVB irradiation for 30min after As exposure for 3h and 6h). Each bar represents the mean ± S.E. (n=30). Letters a, b, c and d indicate significant differences ($p<0.01$).

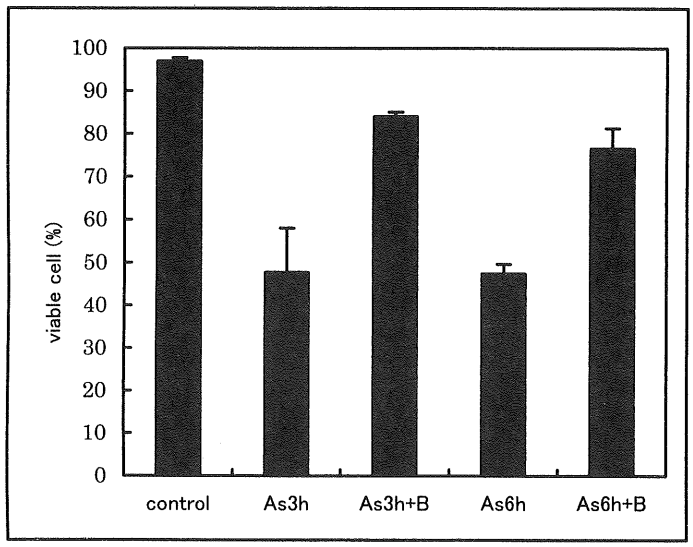

Fig. 2 Effect of β-carotene (an antioxidant) on cell viability of As and UVB exposed *E. gracilis* SMZ. Where As3h or 6h = UVB irradiation after As exposure without the treatment of β-carotene; As+B = As and UVB exposure in the presence of β-carotene.

observed in the cells receiving 6h of As treatment and subsequent UVB irradiation. These data suggest that As and UVB-induced cell death was not completely but partially suppressed by preliminary supplementation treatment with beta-carotene. Beta-carotene has been reported as an anti-oxidant micronutrient and singlet oxygen quencher in human skin by protecting against UVA-induced cell damage[12, 13] They presented data to support beta-carotene as a singlet oxygen quencher.

Petersen et al. studied UVA-induced DNA damage and its prevention by antioxidant[14]. However, their data were not those as supporting protective function of beta-carotene as singlet oxygen quencher. Petersen et al. strongly suggested the conversion of H_2O_2 to hydroxil radical would be the most important step in UVA-induced generation of strand breaks. In UVB irradiation, hydroxyl radical is believed to be the reactive oxygen species (ROS) participating in cellular damage. Our data has not dealt with DNA strand breakage by UVB irradiation, however, the results definitely show that preliminarily treatment of beta-carotene significantly reduced the cell death caused by the combined exposure of $NaAsO_2$ for 3h and 6h followed by UVB irradiation for 30 min. We have not revealed the suppression mechanism of the modified beta-carotene against cell death by arsenite and UVB. Because UVB irradiation does not generate singlet oxygen but hydroxyl radical, our data suggest the radical scavenging effect of the modified beta-carotene. Study on the mechanism of modified beta-carotene is now under way.

Euglena gracilis SMZ has highly developed subcellular organelles equivalent to those of higher mammals. From the ecological perspective in keeping primary aqua-sphere productivity supporting micro-biota such as prankton, data obtained in this study can be a useful model for the investigation of environmental risk assessment of single and combined arsenite, its metabolites and UVB ray. It is also a tool for the discovery of potential remedies against As and UVB damage.

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