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– Note –

Effects of N,N-dimethyldodecylamine-N-oxide on some cellular parameters of rat thymocytes

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Abstract

N,N-Dimethyldodecylamine-N-oxide (DDAO) is an amphoteric surfactant used in many detergents for kitchens. In this study, the effects of DDAO (10–100 μM) on cell lethality, intracellular Ca^{2+} level, intracellular Zn^{2+} level, and cellular content of nonprotein thiol were examined in mammalian cells (rat thymocytes) to further characterize its cytotoxicity. DDAO at the concentration of 100 μM (22.9 mg/L) slightly, but significantly, increased the parameters described above, and it showed no significant effect at the concentrations of 30 μM (6.87 mg/L) or less. Therefore, it is unlikely that DDAO at environmentally-relevant concentrations (< 10–70 ng/L) exerts toxic actions on wild mammals and humans.

Keywords: N,N-dimethyldodecylamine-N-oxide (DDAO); cytotoxicity; lymphocyte

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N,N-Dimethyldodecylamine-N-oxide (DDAO) is an amphoteric surfactant that is well used as one of main ingredients in many detergents for kitchens. Japan Soap and Detergent Association (JSDA, Tokyo, Japan), the industrial association of 23 manufacturers of soaps, detergents and oils and fats, further conducted the risk assessment of DDAO and announced that the impact of DDAO on human health and environment was small under common conditions (2010). The report shows that the toxicity of observed at the concentrations of 0.04–5.3 mg/L in several algal species, and 0.36 mg/L in water flea (crustacean). In vertebrate, the 50% lethal concentration of DDAO after 96 h incubation is 29.9 mg/L (130.3 μM) in medaka (*Oryzias latipes*) (JSDA, 2010) and 2.67–3.46 mg/L (11.6–15.1 μM) in fathead minnow (*Pimephales promelas*) (Procter & Gamble Company, 1976). The 50 % lethal doses of DDAO via intraperitoneal injection are 375 mg/kg for mice and 271 mg/kg for rats, respectively (JSDA, 2010). Thus, it is reminiscent of the possibility that DDAO exerts low toxicity on mammals from the report of JSDA (2010). In this study, the effects of DDAO on some cellular parameters were examined in mammalian cells to further characterize its cytotoxicity. It is noted that we have no conflicts of interests to declare. This study was performed with annual educational and research expenditure of the University of Tokushima.

This study was approved by The University of Tokushima (Registered No. 05279). All methods employed in this study were described in our previous papers (Chikahisa and Oyama, 1992; Matsui et al., 2008). In brief, thymus glands dissected from ether-anesthetized Wistar rats were sliced with a blade under ice-cold conditions. The slices were triturated by gently shaking in chilled Tyrode's solution to dissociate the thymocytes. Thereafter, the beaker containing the cell suspension was incubated in a water bath at 36–37°C for 1 h before the experiment. Cells were incubated with DDAO for 1 h throughout the experiments, then cellular parameters were measured. Fluo-3-AM (Molecular Probes Inc., USA) was used to monitor changes in the intracellular Ca^{2+} level (Kao et al., 1989). FluoZin-3-AM (Molecular Probes Inc., USA) was employed to estimate the change in intracellular Zn^{2+} level (Gee et al., 2002). 5-Chloromethylfluorescein

(5-CMF) diacetate (Molecular Probes Inc., USA) was applied to examine the change in cellular content of nonprotein thiols (mainly, glutathione) (Chikahisa et al., 1996). Propidium iodide was used to stain dead cells or the cells with compromised membranes (Yeh et al., 1981). The cells were incubated with 500 nM Fluo-3-AM or 500 nM FluoZin-3-AM in the simultaneous presence of 5 μM propidium iodide (Molecular Probes Inc., USA) for 60 min prior to any fluorescence measurements. 5-CMF diacetate (500 nM) was applied to the cells just 30 min before the measurement. Fluo-3, FluoZin-3, and 5-CMF fluorescence were measured in the living cells that were not stained with 5 μM propidium iodide. The excitation wavelength used for all fluorechromes was 488 nm, and the emission was detected at 530 ± 15 nm for Fluo-3, FluoZin-3, and 5-CMF fluorescence and at 600 ± 20 nm for propidium fluorescence. The fluorescence was analyzed by JASCO software (Version 3.06; JASCO, Japan). Statistical analysis was performed with Tukey's multivariate analysis. A *P* value of <0.05 was considered significant. Values (columns and bars in figures) are expressed as the mean and standard deviation, respectively.

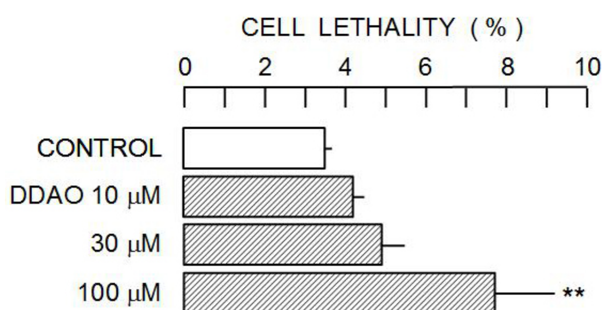

Figure 1. Change in cell lethality (the percentage population of cells stained with propidium iodide) induced by DDAO. Column and bar indicate mean and standard deviation of four samples. Symbol (**) shows significant difference (P < 0.01) between CONTROL group and DDAO group.

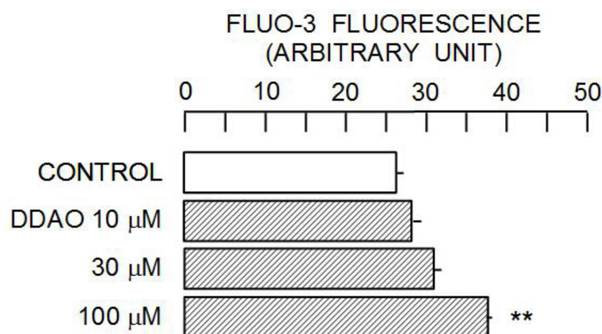

Figure 2. Change in intracellular Ca^{2+} level (the intensity of fluo-3 fluorescence) by DDAO. Column and bar indicate mean and standard deviation of four samples. Symbol (**) shows significant difference (P < 0.01) between CONTROL group and DDAO group.

The incubation of rat thymocytes with DDAO (10–100 μM) for 1 hr increased the population of cells exerting propidium fluorescence in a concentration-dependent manner (Fig. 1). The significant increase in cell lethality was observed at the concentration of 100 μM. The concentration of 100 μM DDAO is equivalent to 22.94 mg/L DDAO. It is unlikely that the blood DDAO concentrations in wild mammals and humans reach lethal levels in Japan because the monitoring concentrations of N,N-dimethylalkylamine-N-oxides in some rivers are < 0.01–0.07 μg/L (JSDA, 2010) and because the estimated human exposure is 15.41 μg/kg/day (JSDA, 2010).

The changes in some cellular parameters such as intracellular Ca^{2+} and Zn^{2+} levels can be observed when the cells are incubated with sublethal concentrations of cytotoxic substances. Therefore, the effects of DDAO on Fluo-3 and FluoZin-3 fluorescence were tested. The effects were examined at 60 min after the start of DDAO application. As shown in Fig. 2, DDAO (10–100 μM) augmented the intensity of Fluo-3 fluorescence in a concentration-dependent manner. Significant augmentation of Fluo-3 fluorescence was observed when the concentration of DDAO was 100 μM. Thus, it is suggested that the intracellular Ca^{2+} levels of wild mammals and humans are not affected by DDAO at environmentally-relevant concentrations. Similar suggestion is also applied to the case of intracellular Zn^{2+} level since DDAO at 100 μM significantly increased the intensity of FluoZin-3 fluorescence, suggesting an increase in intracellular Zn^{2+} level, while it was not the case for 10–30 μM DDAO (Fig. 3).

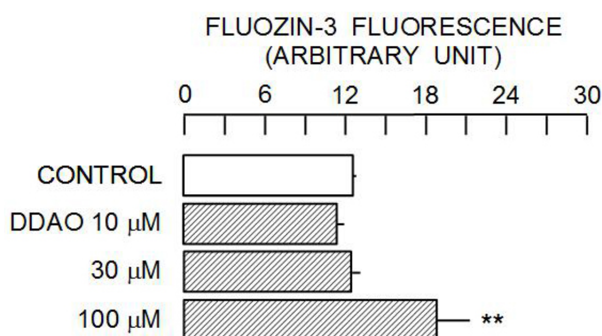

Figure 3. Change in intracellular Zn^{2+} level (the intensity of FluoZin-3 fluorescence) by DDAO. Column and bar indicate mean and standard deviation of four samples. Symbol (**) shows significant difference ($P < 0.01$) between CONTROL group and DDAO group.

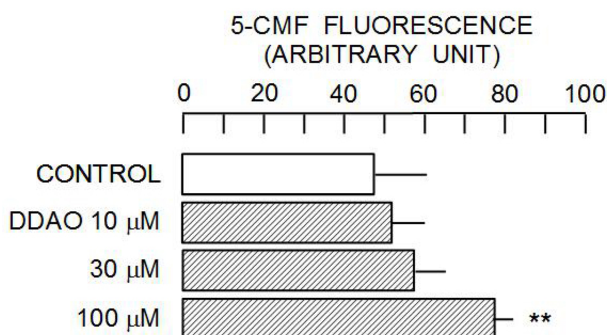

Figure 4. Change in cellular content of nonprotein thiols (the intensity of 5-CMF fluorescence) by DDAO. Column and bar indicate mean and standard deviation of four samples. Symbol (**) shows significant difference ($P < 0.01$) between CONTROL group and DDAO group.

It is proposed that the increase in intracellular Zn^{2+} level induces cellular content of nonprotein thiols or that the decrease in cellular content of nonprotein thiols increases intracellular Zn^{2+} level (Kinazaki et al., 2011). To see if DDAO induces the change in cellular content of nonprotein thiols, the effect of DDAO on the intensity of 5-CMF fluorescence was examined. As shown in Fig. 4, DDAO increased the intensity of 5-CMF fluorescence in a concentration-dependent manner. Significant augmentation of 5-CMF fluorescence was observed in the case of 100 μM DDAO. Thus, it is suggested that DDAO increases the cellular content of nonprotein thiols. However, like other parameters,

it must not occur in the cells of human and wild mammals as long as DDAO is used normally and its environmental concentration is maintained.

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