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Modification of vulnerability to dodecylbenzenesulfonate, an anionic surfactant, by calcium in rat thymocytes

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Abstract

We have previously reported that cremophor EL, a nonionic surfactant, at clinical concentrations significantly decreases the cell viability of rat thymocytes with phosphatidylserine-exposed (PS-exposed) membranes under in vitro condition. It is reminiscent of a possibility that sodium dodecylbenzenesulfonate (DCBS), an anionic surfactant world-widely used for detergents, also affects the cells in the similar manner. To test the possibility, the effect of DCBS on rat thymocytes has been examined using a flow cytometer with fluorescent probes. Exposure of PS on outer surface of cell membranes was induced by A23187, a calcium ionophore to increase intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). DCBS at 1 μg/mL (2.87 μM) significantly decreased the viability of cells with PS-exposed membranes, but not with intact membranes. DCBS also significantly decreased the viability of cells exposed to H_2O_2, an oxidative stress increasing the $[Ca^{2+}]_i$. On the other hand, the decrease in extracellular Ca^{2+} concentration ($[Ca^{2+}]_e$) increased the cell vulnerability to DCBS and vice versa. Intact membrane lipid bilayer and extracellular Ca^{2+} are required to maintain membrane integrity. Therefore, the change of membrane property by manipulation of $[Ca^{2+}]_i$ and $[Ca^{2+}]_e$ is one of causes for the augmentation of DCBS cytotoxicity.

Keywords: Ca^{2+}; Cytotoxicity; Dodecylbenzenesulfonate; Thymocytes

1. Introduction

Linear alkylbenzene sulfonates (LABS) are the most important group of synthetic anionic surfactants. LABS consist of an alkyl chain attached to a benzene ring in *para* position to sulfonate group. The worldwide production capacity of LABS in 2002 was estimated at 3 million metric tonnes (Modler et al., 2003). Therefore, there is a concern over adverse effects of LABS on human and wild animals due to large quantity in use.

Dodecylbenzenesulfonate (DCBS) is one of LABS, mainly used in household detergents because of its good performance and relatively low cost. The toxicological study for DCBS indicates no severe toxic effects after repeated exposure in experimental animals (IPCS, 1996). Thus, it is likely that the toxicity of DCBS is relatively low in human and wild animals.

We have previously reported that cremophor EL and polysorbate 80, nonionic surfactants used for intravenous injection of water-insoluble drugs, promote the process of cell death induced by A23187, a calcium ionophore, in rat thymocytes (Tatsuishi et al., 2005; Yamaguchi et al., 2005). In brief, A23187 increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), leading to exposure of phosphatidylserine (PS) on outer surface of membranes probably by activation of scramblase, which exhibits Ca^{2+}-activated phospholipid scrambling activity (Comfurius et al., 1996; Zhou et al., 1997). The cells with PS-exposed membranes are more vulnerable to nonionic surfactants. Therefore, DCBS may decrease the viability of cells treated with A23187 because of possible change in membrane property. Furthermore, the membrane property is modified by change in extracellular Ca^{2+} concentration ($[Ca^{2+}]_e$) because of Ca^{2+} action to screen charges on membrane phospholipids (Papahadjopoulos et al., 1977). Therefore, the cytotoxic action of DCBS may be influenced by external Ca^{2+}. In present study, to test the possibility, we have examined the action of DCBS on rat thymocytes under

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various conditions. Such a study is important because of following reasons. First, the exposure of PS on outer surface of membranes occurs during an early phase of apoptosis (Koopman et al., 1994; Vermes et al., 1995). Apoptosis is a process during normal development in vertebrates and invertebrates (Haanen and Vermes, 1996; Brill et al., 1999). Thus, DCBS exhausted to the environment may affect the cells with PS-exposed membranes, resulting in abnormal development. Secondly, the requirement of external Ca^{2+} is explained by its actions on membrane permeability (Tupper and Maloff, 1973; Kolb and Adam, 1976; Orlov et al., 2005). The membranes under low $[Ca^{2+}]_e$ condition may be vulnerable to the surfactants. Furthermore, Ca^{2+} has a key role in cytotoxicity induced by chemical compounds (Viarengo and Nicotera, 1991; Kass and Orrenius, 1999).

2. Materials and methods

2.1. Reagents

Sodium dodecylbenzenesulfonate (DCBS, CAS No. 025155-30-0, $C_{12}H_{25}C_6H_4SO_3Na$, molecular weight 348.48) was purchased from Tokyo Kasei Co. (Tokyo, Japan). Other chemicals except for fluorescent probes, described above, were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Cell preparation

The procedure to prepare the cell suspension was similar to that previously reported (Chikahisa and Oyama, 1992; Chikahisa et al., 1996). In brief, thymus glands dissected from 3- to 4-week-old Wistar rats were sliced at a thickness of 400–500 μm. The slices were triturated in chilled Tyrode's solution (in mM: NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, glucose 5, HEPES 5, with an appropriate amount of NaOH to adjust pH to 7.3–7.4) to dissociate thymocytes. Thereafter, the Tyrode's solution was passed through a mesh (a diameter of 10 μm) to prepare the cell suspension (10^5 to 10^6 cells/mL). The cells were incubated at 35–36 °C for 1 h before use.

2.3. Fluorescence measurements of cellular parameters

The methods for measurements of cellular parameters using a flow cytometer equipped with an argon laser (CytoACE-150, JASCO, Tokyo, Japan) and fluorescent probes were similar to those described previously (Chikahisa and Oyama, 1992; Chikahisa et al., 1996; Nakata et al., 1999; Oyama et al., 1999). Fluorescence was analyzed by JASCO Ver.3XX software (JASCO, Tokyo, Japan).

To assess the population of dead cells, propidium iodide (Molecular Probe Inc., Eugene, OR, USA) was added to cell suspension to achieve a final concentration of 10 μM. Since propidium stains dead cells, the measurement of propidium fluorescence from the cells provides a clue to estimate the viability. Propidium fluorescence was measured at 1–2 min after the application by a flow cytometer. Excitation wavelength for propidium was 488 nm and emission was detected at 600 ± 20 nm.

The exposure of PS on outer membranes of rat thymocytes was detected by using annexin V-FITC (Roche Diagnosis, Basel, Switzerland) (Nakata et al., 1999; Oyama et al., 1999). The excitation was 488 nm. The emission was detected at 530 ± 20 nm for FITC (annexin V binding to membranes).

5-Chloromethylfluorescein diacetate (CMF-DA, Molecular Probes Inc.) was used to estimate cellular content of glutathione in thymocytes (Chikahisa et al., 1996). CMF-DA was added to cell suspension to achieve a final concentration of 1 μM. The cells were incubated with CMF-DA for 30 min before the measurement. The excitation was 488 nm. CMF fluorescence at 530 ± 20 nm was recorded from intact living cells.

2.4. Statistics

Statistical analysis was performed by a paired Student's *t*-test and/or an overall test of significance using an *F*-ratio derived from analysis of variance (ANOVA). Significance between test groups was confirmed by Dunnet's test. A *P*-value of <0.05 was considered significant.

3. Results

3.1. Effect of DCBS on rat thymocytes in absence or presence of A23187

The cells were incubated with DCBS at concentrations ranging from 0.3 to 3 μg/mL (from 0.86 to 8.6 μM) for 1 h. As shown in Fig. 1A, the population of cells stained with propidium iodide (the population of dead cells) was significantly increased in the presence of DCBS at 3 μg/mL or more.

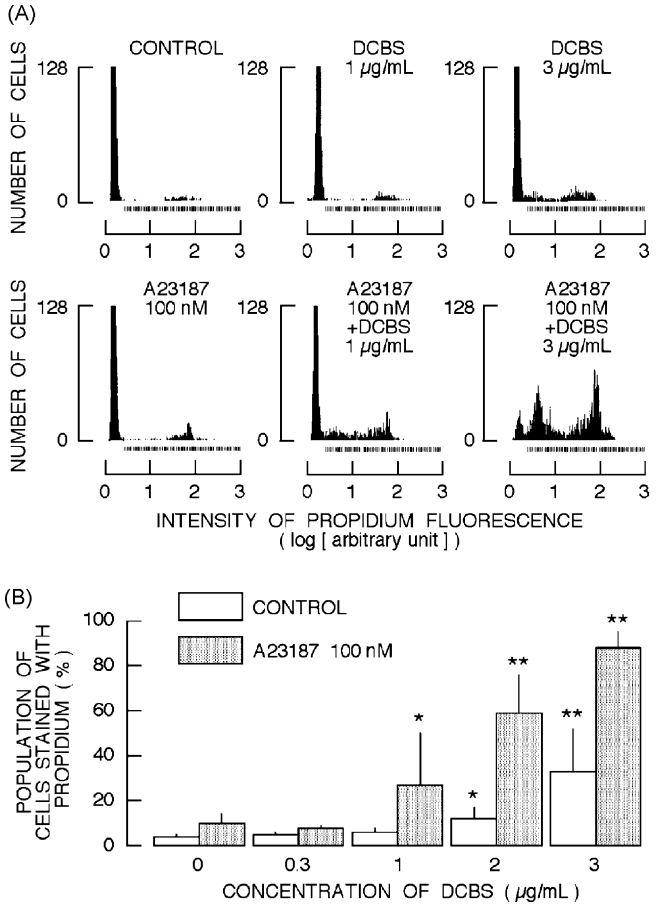

Fig. 1. Effect of dodecylbenzenesulfonate (DCBS) on rat thymocytes simultaneously incubated with or without A23187. (A) Effect of DCBS on the histogram of propidium fluorescence monitored from 2500 cells. (Upper panels) Effect of DCBS on the cells under control condition. (Lower panels) Effect of DCBS on the cells simultaneously incubated with A23187. The dotted line under the histogram indicates the cells exhibiting propidium fluorescence. (B) Concentration-dependent change in percentage population of cells stained with propidium by DCBS in absence (CONTROL) or presence of A23187 (A23187 100 nM). Column and bar, respectively, indicate mean and standard deviation of four to eight experiments. Asterisks (* and **) indicate significant difference ($P < 0.05$ and $P < 0.01$, respectively) between the control (0 μg/mL DCBS) and test group (0.3–3 μg/mL DCBS) in absence or presence of A23187.