Ion transportation through Transitory water pores in cell membranes by using DMSO

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***Abstract*: It's well known that dimethyl sulphoxide (DMSO) increases membrane permeability, which makes it extensively used as a transport material to grease medicine delivery across natural membranes. Still, it is not yet understood that how DMSO increases membrane permeability. Lately, molecular dynamics simulations have demonstrated that DMSO can induce water pores in natural membranes, but no direct experimental substantiation is so far available to prove the simulation result. Using FluxOR Tl affluence assay and intracellular Ca2 imaging fashion, we studied the effect of DMSO on Tl and Ca2 saturation across cell membranes. Upon uses of DMSO on CHO-K1 cell line, Tl affluence was transiently goes up in a cure-dependent manner. The increased proportion in Tl permeability convinced by DMSO wasn't changed in the presence of blockers for K channel and Na-K ATPase, suggesting that Tl permeates through flash water pores convinced by the DMSO to enter into the cell. Adding to that, Ca2 permeability was increased upon operation of DMSO, indicating that those were non-selective pores which were convinced by DMSO from Fresh water pores. Likewise, analogous results could be attained from RAW264.7 macrophage cell line. Thus, this study handed experimental substantiation to support the logic that DMSO can induce flash water pores in cell membranes, which in turn facilitates the transport of active substances across membranes.**

***Keywords*: Cell Membrane, Dimethyl Sulfoxide (DMSO), Ion Permeation, Tl+ Permeation, Water Pore.**

Introduction-

DMSO (dimethyl sulfoxide) is frequently applied to facilitate the transport of active molecules across the biological membranes due to its ability to enhance membrane permeability [1, 2]. It is observed that transfection of exogenous DNA incubated with DMSO is more effective than that without any treatment [3]. DMSO is also used in percutaneous drug delivery as a chemical permeability enhancer [4]. With the help of DMSO, the internalization efficiency of arginine-rich cell penetrating peptides could be significantly enhanced [5, 6]. The widespread use of DMSO has led to numerous studies and hypotheses about its properties and interactions among the biological organisms [7]. X-ray diffraction measurement of biological membranes has suggested that the thickness of the bilayer membranes decreases with the increase in DMSO concentration [8].

The membrane thinning and increases the fluidity of the membrane's hydrophobic core induced by DMSO which is indicated by Molecular Dynamics simulations. DMSO can cause the membrane to become floppier, which would enhance the membrane permeability and enable the cell membrane to accommodate osmotic and mechanical stresses during cryopreservation [9, 10]. The formation of the transient water pores dramatically reduces the free energy barriers to permeation of ions through the membrane, so that ions are able to transport across the membrane, avoiding highly unfavorable contacts with the hydrophobic tails in the membrane interior [11, 12]. Though MD simulations provided detailed information at the atomic resolution on the mechanism of the action of DMSO [13], it is still lack of direct experimental evidence that supports the ability of DMSO to induce transient water pore in the biological membrane. In the recent period work, using intracellular ion imaging techniques, we studied the effect of DMSO on Tl+ and Ca2+ permeation across cell membranes [14, 15]. Our results provided compelling experimental evidence to support the prediction of the MD simulations that DMSO could induce transient water pores in cell membranes [16].

Materials and methods-

CHO-K1 cells were grown in Ham's F-12 nutrient mixture (Invitrogen, Co. Grand Island, NY, USA), while RAW 264.7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Both cell lines were supplemented with 10% fetal bovine serum and cultured in a humidified 37C incubator (5% CO2). The CHO-K1 cells were passaged twice weekly through exposure to 0.05% trypsin. For the cell imaging experiments, cells were transferred onto glass coverslips pretreated with poly-L-lysine in a culture medium at 37C incubator (5% CO2) to improve cell adhesion. After 2–4 days growth, the cells were transferred to a Hanks' balanced salts solution (HBSS, Sigma; in mM): 1.3 CaCl2, 0.8 MgSO4, 5.4 KCl, 0.4 KH2PO4, 136.9 NaCl, 0.3 Na2PO4, 10 D-glucose and 4.2 NaHCO3. The osmolality of all solutions was 290–310 mmol/kg asmeasured with a vapour pressure osmometer. For the real-time imaging of Tl+ fluorescence, the cells were loaded with a Tl+-sensitive fluorophore using the solution provided by the maker (FluxOR Thallium Detection Kit, Invitrogen). The experiments were carried out according to the provided protocols with slight modification. Briefly, the cells were loaded with a loading buffer containing 1% FluxOR reagent, in which the Tl+-sensitive dye was included, in the dark for 20–40 min. After loading, the cells were washed out with an assay buffer to remove the extracellular dye. Then, the solution was changed to a stimulus buffer containing 2 mM Tl+. The intracellular Tl+ fluorescence was measured with a Leica DMI4000B inverted microscope at 2 s intervals for 600–1800 s and analyzed with LAS AF 6000 software (Leica, Germany). DMSO was directly added into the bath solution. The Tl+ fluorescent dye has an excitation peak at 490 nm and an emission peak at 525 nm. The effect was evaluated by the calculated FluxOR fluorescence ratio normalized with the basal fluorescence level. All experiments were performed at room temperature (22–24C). For intracellular Ca2+ imaging, the cells were incubated in the culture medium containing 10 mM fluo-3/AM and 0.02% pluronic F-127 (Sigma Chemical Co.) for 30 min. Fluo-

3 intensity (480 ± 15 nm excitation, 530 ± 30 nm emission) was monitored every 2 s and was plotted as fluorescent intensity (Fmax) with regard to that of the basal calcium level (F0). The inhibitors for K+ channels and Na+-K+ ATPase were applied to the bath solution after the cells were loaded with the Tl+- sensitive fluorophore. Again, all experiments were performed at room temperature (20–25C).

Results-

DMSO induced transient Tl+ influx in CHO-K1 cells. In order to observe the fluorescent intensity of the individual cells, we did not use a microplate reader to detect Tl+ influx as the protocol suggested. Instead, we applied the intracellular ion imaging technique to detect Tl+ influx to judge whether DMSO could induce water pores in the cell membrane. Since Tl+ is not present in living cells in any considerable amount, there is a strong driving force for the extracellular Tl+ to enter into the cells so that even very small amounts of intracellular Tl+ can be detected. If DMSO can induce water pores in the cell membrane, it should be efficiently detected by fluorescent measurement of the Tl+ influx. The Tl+-sensitive dye was loaded into cells, which exhibited very low basal fluorescence in the absence of Tl+. Switching to the Tl+-containing buffer caused a gradual increase in fluorescence even in cells that were not exposed to DMSO, probably due to endocytosis or via some constitutive opening of endogenous ion channels. Approximately 10 s after application of 4% DMSO, a transient increase in intracellular Tl+ was observed. To obtain reproducible data, all the treatments were conducted after the basal fluorescence level getting stable in Tl+-containing buffer in the following experiments.

Conclusion-

DMSO is known to enhance the penetration of both hydrophilic and hydrophobic molecules (Williams and Barry 2004). The enhancement of hydrophilic compounds, such as ions, by DMSO is always difficult to explain. There are at least three mechanisms for ion transport across the biological membranes. First, ion transport is primarily mediated by specific proteins, such as ion channels, transporters and pumps. Secondly, ions penetrate the membrane according to the solubility-diffusion theory, which implies that ions partition into the membrane's hydrophobic core and diffuse across the membrane (Bordi et al. 2000). Thirdly, ion is transported via transient water pores in the biological membrane (Jansen and Blume 1995), which helps ions to evade the high-energy Born barrier associated with the solubility-diffusionmechanism.We noticed that the fluorescence levels decreased back to base level after the influx of Tl+ ions. A plausible explanation might be that the intracellular Tl+ concentration was transiently increased locally, near the sites of the pore, which was decreased as the Tl+ ions diffuse in the cytoplasm, resulting in the fluorescence levels decreased back to the base level. Nevertheless, the following pieces of evidence strongly suggest that the ions are transported across the cell membrane through DMSO-induced transient water pores in the plasma membrane.

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