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Dimethyl sulfoxide attenuates nitric oxide generation via modulation of cationic amino acid transporter-1 in human umbilical vein endothelial cells

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ABSTRACT

Dimethyl sulfoxide (DMSO) is a solvent that is commonly used in medicine. Conflicting data exist as to its effects on endothelial function. Endothelial cell dysfunction (ECD) is characterized by decreased endothelial nitric oxide synthase (eNOS) activity. Cationic amino acid transporter-1 (CAT-1), the specific arginine transporter for eNOS, has been shown to modulate eNOS activity. We hypothesize that DMSO inhibits eNOS activity through modulation of its selective arginine supplier CAT-1. We studied the effect of DMSO on arginine transport, NO_2/NO_3 generation as an index of NO production, as well as CAT-1 and Protein Kinase C alpha (PKC-α) (CAT-1 inhibitor) protein expression in human umbilical vein endothelial cell cultures (HUVECs). DMSO 2.5% and 3.5% (v/v) significantly attenuated arginine transport, a phenomenon which was prevented by co-incubation with L-arginine (1 mM). The aforementioned findings were accompanied by a decrease in NO_2/NO_3 generation. DMSO significantly increased the abundance of phosphorylated CAT-1 (the inactive form) and phosphorylated PKC-α protein, an effect that was attenuated by L-arginine. GO 6976 (PKC-α antagonist) prevented the decrease in arginine transport caused by DMSO. DMSO also induced profound transient morphological changes in HUVECs' structure but these were not related to its effect on arginine transport. In conclusion, DMSO inhibits NO generation by endothelial cells through modulation of CAT-1 activity.

1. Introduction

Dimethyl sulfoxide (DMSO) is a widely used substance, both in vitro and in vivo. It is being utilized for preservation of hematopoietic cells, in patients undergoing bone marrow or stem cell transplantation [15,21] and has been proposed as an essential ingredient in cardiovascular grafts [1,5]. DMSO is also used as a solvent for chemotherapeutic drugs and due to its anti-inflammatory properties has been utilized for treating rheumatic, pulmonary, neurological and urinary disorders [15].

Conflicting data exist as to the effects of DMSO on endothelial function (Table 1). Eter et al. have shown that DMSO interferes with endothelial cell proliferation [6] and Koizumi et al. have found that it exhibits anti angiogenic properties [11]. In contrast, beneficial effects of DMSO on endothelial cell function have also been described. DMSO exhibited anti thrombotic properties in human aortic endothelial cells following incubation with tumor necrosis factor alpha (TNF-α) [5]. Moreover, exposing Human Umbilical Vein Endothelial Cells (HUVECs) to DMSO increased heme oxygenase-1 expression, which leads to generation of potent antioxidant metabolites [12].

Endothelial cell dysfunction is characterized by impaired capacity of the constitutive, Ca^{2+}/calmodulin-sensitive endothelial nitric oxide synthase (eNOS) to generate adequate quantities of nitric oxide (NO) [7]. Delivery of transported arginine to membrane-bound eNOS, selectively by cationic amino acid transporter-1 (CAT-1) has been shown to be a predominant factor governing eNOS activity [3,8,9,13,14,18,19].

While using DMSO as a solvent in our lab we were intrigued by its effect on arginine uptake in endothelial cells. The experiments reported herein were designed to study the effects of DMSO exposure on arginine uptake and NO generation by HUVECs and elucidate a molecular mechanism to explain our findings.

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Table 1
Data from previous studies on the mechanism and effect of DMSO on endothelial function.

Source	Type of effect	Type of endothelial cell	DMSO conc.	Cell viability	Effect and mechanism
Eter et al. [6]	DMSO is detrimental to endothelial function	Bovine choriocapillary endothelial cells	0.1%	Not examined	DMSO Interfered with endothelial cell proliferation, mechanism not identified.
Koizumi et al. [11]		Human aorta endothelial cells	1–3%	DMSO was not toxic as evidenced by MTT assay (data not shown)	DMSO (1–3%, 4 h) interfered with endothelial cell tube formation (a marker of angiogenesis and inflammatory reaction) via inhibition of matrix metalloproteinase-2 production.
Camici et al. [5]	DMSO enhances endothelial function	Human aortic endothelial cells	0.1 −1%	DMSO (1%, 6 h) did not result in morphological changes suggestive of cytotoxicity, no evidence of cell death by LDH and trypan blue exclusion tests	DMSO (0.1–1%, 1 h) inhibited Tumor Necrosis Factor alpha (TNF-α) and thrombin mediated Tissue Factor (TF) induction via a reduction in the phosphorylation of MAPK proteins cJun-N-terminal kinases (JNK) and p38 and reduced TF mRNA expression.
		Carotid artery thrombosis model in mice	80 mg/kg		In vivo treatment with DMSO reduced Tissue Factor activity and prevented occlusion in a mouse model of carotid arterial injury.
Liang et al. [12]		Human umbilical vein endothelial cells (HUVECs)	0.1 −0.8%	Not examined	DMSO (0.1–0.8%, 1–12 h) induced dose and time dependent heme oxygenase 1 (HO-1) mRNA and protein expression. DMSO induced HO-1 activity through c-Jun-N-terminal kinases (JNK) phosphorylation. Heme derived metabolits generated by HO-1 catalysis have potent antioxidant and cytoprotective activities.

2. Material and methods

2.1. Materials

Fresh unpassaged HUVECs were obtained from Promo Cell GmbH, Heidelberg, Germany. Upon arrival, the cells were cultured according to the manufacturer's instructions in Endothelial cell M2 growth medium containing 2% fetal calf serum (FCS), penicillin/streptomycin (100 units/ml), and amphotericin B (0.05 μg/ml) (Life Technologies, Inc.) at 37 °C, in a humidified atmosphere containing 5% CO_2. The medium was changed 3 times weekly. Cells were used for experiments when they were 70–80% confluent, between passages 3 and 5. 24 h before each experiment, the incubation medium was changed to 5% bovine serum albumin (BSA). DMSO and all standard reagents were obtained from Sigma-Aldrich (St Louis, MO, USA) unless indicated otherwise. L-[H^3] arginine was supplied by PerkinElmer Life and Analytical Sciences Inc. (Boston, MA, USA).

2.2. L-Arginine transport

Uptake of radiolabeled L-arginine in HUVECs was measured according to previously described methods [16]. The cells were seeded onto 6-well plates (Corning) at a density of 10^6 cells/well. When confluent, cells were washed with 1 ml HEPES buffer, pH 7.4 at 37 °C. L-[H^3 arginine] and L-arginine, in a final concentration of 100 μM, were added to a total volume of 1 ml for 1 min. The duration of 1 min was chosen since it was within the linear portion of the uptake curve (data not shown). Transport was terminated by rapidly washing the cells with ice-cold PBS buffer (4 times, 1 ml/well). The cells were then dried and solubilized in 1 ml of 0.5% SDS in 0.5 N NaOH. 700 μl of the lysate were used to monitor radioactivity by liquid scintillation spectrometry (Betamatic; Kontron). The remaining 300 μl were used for protein content determination by the Lowry method (Lowry Assay Kit; Sigma Co.). Results are expressed as means ± SE of at least five different experiments.

2.3. Nitrite/nitrate determination

The level of nitrite/nitrate concentrations in the conditioned medium was measured using a nitric oxide detection kit (ENZO Life Sciences) according to the manufacturer's instructions. In brief, following filtration in a 10 Kd spin column (Biovision INC. CA, USA) 50 μl of the culture medium was diluted with 50 μl of reaction buffer containing 50 μM L-N6-(1-iminoethyl) lysine hydrochloride (L-NIL, a selective inducible NOS inhibitor), and mixed with 25 μl NADH and 25 μl nitrate reductase. Following incubation to convert nitrate to nitrite the medium was exposed to Griess reagents (Sulfanilamide in 2M hydrochloric acid and N-1-naphthylethylenediamine in 2M hydrochloric acid) which reacted with nitrite to yield a diazochromophore. Total nitrite was measured at 540 nm. A standard curve was generated and each sample was analyzed in triplicates. Each measurement was corrected for protein content (using the Lowry assay). Results of at least six experiments were used for statistical analysis.

2.4. Protein quantification by western blotting

Human CAT-1 (hCAT-1), PKC-α, and phosphorylated PKC-α (p-PKC-α) protein content was determined by immunoblotting. Cells were separately placed in ice-cold PBS lysis buffer (pH 7.4), containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 4.5 μM leupeptin, 5 μM aprotinin) (ICN Biomedicals, Inc.), 0.01% Triton X-100 and 0.1% SDS, then mechanically homogenized and left on ice for 45 min. Homogenates were subsequently centrifuged (13,000 rpm, 10 min, 4 °C). Cell lysates were stored in aliquots at −80 °C. A membrane fraction was obtained by adding to the pellet an equal volume of lysis buffer supplemented by Tween-20 (0.25%) to solubilize. The protein content of each sample was determined by the method of Lowry. Equal amounts of protein (30 μg) were prepared in a sample buffer (2% SDS, 0.01% bromophenol blue, 25% glycerol, 0.0625 M Tris-HCl, pH 6.8, 5% mercaptoethanol) and analyzed on a 7.5% SDS-PAGE gel. The gel was transferred onto Hybond ECL nitrocellulose membranes (Amersham Corp.) and blocked in PBS-T containing 5% non-fat dried milk at room temperature. Membranes were then incubated with rabbit anti-human CAT-1, p-PKC-α and mouse monoclonal anti-human PKC-α (all from Santa Cruz Biotechnology, Inc., Calif., USA) for 1 h at room temperature, washed, and incubated with secondary horseradish peroxidase-conjugated goat anti-rabbit and goat anti-mouse antibodies (1:10,000) in PBS-T for 1 h. Membranes were subsequently washed 3 times, 5 min each, in