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Sevoflurane-induced oxidative stress and cellular injury in human peripheral polymorphonuclear neutrophils

Chung Hang Wong [a], Tsan-Zon Liu [b], Soi-Moi Chye [c], Fung-Jou Lu [d], Ya-Chen Liu [e], Zhao-Cen Lin [f], Ching-Hsein Chen [e,*]

[a] *Department of Anesthesiology, Chang Gung Memorial Hospital at Chia Yi, and Chia Yi School, Chang Gung Institute of Technology, Puzih City, Chia Yi County 613, Taiwan, ROC*
[b] *Center for Gerontological Research and Graduate Institute of Medical Biotechnology, Chang Gung University, Kwei-Shan, Taoyuan 333, Taiwan, ROC*
[c] *Faculty of Engineering and Science, University Tunku Abdul Rahman, Malaysia*
[d] *Department of Applied Chemistry, Chung Shan Medical University, Taichung 402, Taiwan, ROC*
[e] *Department of Medical Technology, Fooyin University, 151 Ching-Hsueh Rd., Ta-Liao, Kaohsiung Hsien 831, Taiwan, ROC*
[f] *Department of Nutrition and Health Science, Fooyin University, Taiwan, ROC*

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Abstract

Sevoflurane is an inhalation anesthetic used for general anesthesia. Several studies have demonstrated that reactive oxygen species (ROS) exist in cardioprotection when preconditioned with sevoflurane. Moreover, sevoflurane can also directly trigger the formation of peroxynitrite. Up to now, information pertinent to the effect of sevoflurane on cellular injuries in human polymorphonuclear neutrophils (PMN) is scant. In this study, we demonstrated that sevoflurane significantly increases intracellular H_2O_2 and/or peroxide, superoxide, and nitric oxide (NO) in PMN within 1 h treatment. Intensification of intracellular glutathione (GSH) depletion in PMN has been demonstrated with the presence of sevoflurane. Inhibition of sevoflurane-mediated intracellular H_2O_2 and/or peroxide in PMN by catalase, mannitol, dexamethasone, *N*-acetylcysteine (NAC) and trolox, but not superoxide dismutase (SOD) pretreatment, was observed. Among them, catalase has the best effect scavenging intracellular H_2O_2 and/or peroxide, suggesting that H_2O_2 is the major ROS during sevoflurane treatment. Two apoptotic critical factors—lowering of the mitochondrial transmembrane potential ($\Delta\Psi_m$) and activation of caspase 3/7—were significantly increased after 1 h of sevoflurane treatment. Apoptosis of PMN were determined by comet assay and flow cytometric analysis of annexin V-FITV protein binding to the cell surface. Exposure of PMN to sevoflurane markedly increased apoptosis in a dose-dependent manner. In summary, these results are important for demonstrating the oxidative stress and cellular injury on sevoflurane-treated human PMN.

Keywords: Sevoflurane; Oxidative stress; Glutathione; Mitochondrial transmembrane potential; Apoptosis

1. Introduction

Sevoflurane is an inhalation anesthetic used for general anesthesia and is part of the "flurane" family (Burrows et al., 2004). It is applied by vaporization and typically given coexistent with other anesthetics to carry out the preferred level of anesthesia (Malviya et al., 2004). Recently, several studies have shown that ROS exist in sevoflurane-induced cardioprotection (de Ruijter et al., 2003; Bouwman et al., 2004). Moreover, sevoflurane can directly trigger the formation of peroxynitrite, inducing nitrolysation of

Abbreviations: DCF, dichlorofluorescein; GSH, glutathione; ROS, reactive oxygen species; DCFH-DA, 2′,7′-dichlorodihydrofluorescein-diacetate; HE, hydroethidine; DAF-2, 4,5-diaminofluorescein; CMF-DA, chloromethylflourescein diacetate; PI, propidium iodide; NO, nitric oxide; PMN, polymorphonuclear neutrophils; SNAP, *S*-nitroso-*N*-acetylpenicillamine; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

* Corresponding author. Tel.: +886 7 7811151x629; fax: +886 7 7827162.
E-mail address: sc111@mail.fy.edu.tw (C.-H. Chen).

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sarcolemmal proteins (Bouwman et al., 2004). Other reports also demonstrated that sevoflurane exposure induces an increase of ROS, resulting in attenuation of mitochondrial electron transport as a fire of anesthetic preconditioning in fresh guinea pig heart experimental model (Riess et al., 2004, 2005).

ROS has been implicated to be a causative factor in many human degenerative diseases of aging (Balaban et al., 2005; Stadtman et al., 2005). Many clinical conditions, such as atherosclerosis (Kovacic and Thurn, 2005; van Oostrom et al., 2004), carcinogenesis (Valko et al., 2004), ischemia/reperfusion damage (Szocs, 2004), cataractogenesis (Spector, 1995), neurodegenerative disorders (Rego and Oliveira, 2003), and rheumatoid arthritis (Hadjigogos, 2003) are related with ROS production. Furthermore, ROS also exist in inflammatory periods (Lin et al., 2005). The oxidative stress induced by increasing ROS is believed to adversely affect the state of normal human health.

Sevoflurane is a widely used inhalation anesthetic. However, it will distribute to blood stream and affect leukocytes. Up to now, information pertinent to the effect of sevoflurane on oxidative stress in human peripheral leukocytes is scant. In peripheral leukocytes, the polymorphonuclear neutrophils (PMN) are the major group that defeat pathogenic microorganisms by phagocytosis and generate extremely high amounts of ROS (Amer and Fibach, 2005). Many proinflammatory substances and chemical stimulants can provoke the production of ROS in PMN (Braga et al., 2003; Tripathy et al., 2003; Kopprasch et al., 2004). For that reason, to evaluate oxidative stress and cellular injury in sevoflurane treated PMN, we designed to investigate those factors of oxidative stress including intracellular hydrogen peroxide, superoxide and nitric oxide (NO) production; intracellular glutathione (GSH) content; lowering of the mitochondrial transmembrane potential ($\Delta\Psi_m$); activation of caspase 3/7; and apoptosis.

2. Materials and methods

2.1. Reagents

The hydroethidine (HE), chloromethylflourescein diacetate (CMF-DA) utilized herein was acquired from Molecular Probes, Inc. (Eugene, OR, USA). The Apo-one™ homogeneous caspase-3/7 assay kit was purchased from Promega Company (Madison, WI, USA). Annexin-V-FLUOS staining kit was obtained from Roche Applied Science Company (Penzberg, Germany). Propidium iodide (PI), 2′,7′-dichlorodihydrofluorescein-diacetate (DCFH-DA), 4,5-diaminofluorescein (DAF-2), *S*-nitroso-*N*-acetylpenicillamine (SNAP), catalase, mannitol, superoxide dismutase (SOD), dexamethasone, *N*-acetylcysteine (NAC), trolox, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), Dulbecco's modified Eagle's medium (DMEM), MTT, and other chemicals were bought from Sigma Chemical Co. (St. Louis, MO, USA). Sevoflurane was bought from Abbott (Taiwan) Co. (Abbott Laboratories, North Chicago, IL).

2.2. PMN preparations and treatments

Peripheral whole blood was obtained from volunteers (25 ± 5 years), none of whom suffering from any known acute or chronic disease, showing any symptoms of infection or inflammation, nor taking any drugs recognized to influence the immune system. They had body mass indices within the normal range (20–25 kg/m^2), and did not use special dietary regimen nor take antioxidant supplement. Peripheral whole blood samples were collected into vacutainer tubes containing EDTA. Red blood cells were hemolyzed using ammonium chloride lysing solution, (150 mM NH_4Cl, 10 nM $KHCO_3$, 1 nM Na_4EDTA, pH 7.4). Leukocytes were then washed twice with incubation buffer (140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM D-glucose, 10 mM Hepes, pH 7.4), and then incubated in the same buffer with various concentrations of sevoflurane and other drugs. The population of PMN was measured using flow cytometry gating (see Fig. 1). For the comet assay, 5 ml whole blood was stratified on a discontinuous Ficoll–Hypaque density gradient (HistopaqueR 1077 and 1119, Sigma–Aldrich) and centrifuged (700 g, 30 min, 20 °C) to obtain PMN. PMN were then collected on the corresponding layer (1.077 < density < 1.119).

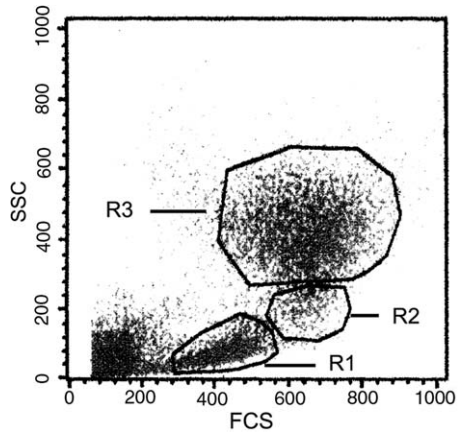

Fig. 1. Gating PMN by CellQuest software in flow cytometric analysis. The representative plots depict forward scatter (FSC) on the *X*-axis and side scatter (SSC) on the *Y*-axis. The leukocytes cells were sorted as three groups, including lymphocytes (R1), monocytes (R2), and PMN (R3) by the CellQuest software. The R3 region was gated and evaluated in all of present studies.

2.3. Flow cytometry analysis of H_2O_2 and/or peroxide content in PMN

The H_2O_2 and/or peroxide assay was performed following the method of Hsieh et al. (2004). PMN (10^6 cells/ml) were preincubated with 20 μM DCFH-DA in 5% CO_2 incubator for 15 min. DCFH-DA is freely permeable to cellular membrane. In cytoplasm, esterase catalyzes DCFH-DA to DCFH, which is then oxidized by H_2O_2 and/or peroxide into dichlorofluorescein (DCF) and emits a bright green fluorescence. After the preincubation, PMN were stimulated with 1% and 3% sevoflurane for 1 h at 37 °C in CO_2 incubator. In order to check the experimental system, we used 2 mM H_2O_2 to treat PMN for 10 min as the positive control group. H_2O_2 is freely permeable to the cellular membrane and into cytoplasm. In cytoplasm, H_2O_2 directly converts DCFH-DA to DCF. For antioxidants and NO inhibitor studies, PMN was first pretreated with either one of catalase (200 U/ml), mannitol (50 mM), SOD (10 U/ml), dexamethasone (10 μM), NAC (10 mM), or trolox (50 μM) for 15 min, followed by incubation with 20 μM DCFH-DA for another 15 min, and finally treated with 3% sevoflurane for 1 h. After drugs treatment, PMN were washed with incubation buffer, collected by centrifugation, suspended in the same buffer and analyzed by flow cytometry.

2.4. Flow cytometry analysis of superoxide content in PMN

PMN (10^6 cells/ml) were preincubated with 10 μM of HE in the 5% CO_2 incubator for 15 min. HE is freely permeable to cellular membrane,