行政院國家科學委員會專題研究計畫 成果報告

吸入性麻醉劑七氟烷對支氣管上皮及血管內皮細胞之影響--探討自由基生成機制、發炎及細胞貼附反應(第2年)
研究成果報告(完整版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 96-2320-B-040-008-MY2
執 行 期 間 ：97年08月01日至98年07月31日
執 行 單 位 ：中山醫學大學應用化學系（所）

計畫主持人：張雯惠
共同主持人：陳俊憲、呂鋒洲
計畫參與人員：大專生-兼任助理人員：馮玟菲
大專生-兼任助理人員：張文菁
大專生-兼任助理人員：徐琬婷
大專生-兼任助理人員：葉玟蘭

報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫涉及專利或其他智慧財產權，2年後可公開查詢

中 華 民 國 98年10月28日

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告 □ 期中進度報告

吸入性麻醉劑七氟烷對支氣管上皮及血管內皮細胞之影響--探討自由基生成機制、發炎及細胞貼附反應

計畫類別：■ 個別型計畫　　□ 整合型計畫
計畫編號：NSC 96－2320－B－040 －008－MY2
執行期間：　96　年　8　月　1　日至　98　年　7　月　31　日

計畫主持人：張雯惠
共同主持人：呂鋒洲、陳俊憲
計畫參與人員：　張文菁、馮玟菲、徐琬婷、葉玟蘭

成果報告類型(依經費核定清單規定繳交)：□精簡報告　■完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
■出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年■二年後可公開查詢

執行單位：中山醫學大學應用化學系

中　華　民　國　98　年　10　月　28　日

中文摘要：

七氟烷是新一代吸入性麻醉劑，用於成年及小兒病患之住院或門診手術之全身性麻醉的誘發及維持，使用量為 0.5～5.0%；過去吾人研究發現：七氟烷會造成血液單核白血球細胞(PMN)產生大量自由基及細胞傷害，然而關於七氟烷對於內皮細胞的影響並不清楚，故本研究以人類臍帶內皮及小鼠內皮細胞進行實驗，以釐清細胞內超氧自由基、過氧化氫、一氧化氮、其他過氧化物及穀胱甘肽的含量是否如 PMN 一樣會受七氟烷影響。研究結果顯示七氟烷使用劑量達 3%時，細胞經處理 15 分鐘以上就產生明顯死亡的情況，七氟烷對血管內皮細胞的傷害更甚於 PMN，胞內過氧化氫、超氧自由基、一氧化氮及其他過氧化物含量明顯增加，但胞內重要抗氧化物-穀胱甘肽則顯著被消耗；吾人首次發現七氟烷可以直接誘發多種細胞產生自由基並且消耗細胞抗氧化能力。此外，本研究亦七氟烷會促進內皮細胞產生一氧化氮(NO)、並影響核轉錄因子訊息傳遞途徑之相關蛋白質(如 NF-kB、IkB、IKK 等)的表現量。

關鍵字：七氟烷、人類臍帶內皮細胞、小鼠內皮細胞、超氧自由基、過氧化氫、一氧化氮

英文摘要：

Sevoflurane is an inhalation anesthetic using 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 vascular endothelium is scant. In this study, we demonstrated that sevoflurane significantly increased intracellular H2O2 and/orperoxide, superoxide, and nitric oxide (NO) in various types of cells. Intensification of intracellular glutathione(GSH) depletion was also observed. Our current data showed that the expression of NF-κB, I-κB, IKKγ, IRAK, and p68 was affected by sevoflurane.
Keywords: sevoflurane, HUVEC, SVEC4-10, hydrogen peroxide, superoxide, nitric oxide.

目錄

中文摘要……………………………………………………………………………………I
英文摘要……………………………………………………………………………………II
報告內容
前言、研究目的、文獻探討………………………………………………………………1
研究方法……………………………………………………………………………………2
結果…………………………………………………………………………………………3
討論…………………………………………………………………………………………4
參考文獻……………………………………………………………………………………6
附圖…………………………………………………………………………………………9
計畫成果自評………………………………………………………………………………11

報告內容：

Introduction:

Sevoflurane is an inhalation anesthetic using for general anesthesia. The advantages of sevoflurane include a more rapid onset and recovery due to its lower solubility in blood and a relatively less irritation to the airway thus making it well suited as an induction drug for children. The minimum alveolar concentration (MAC) of sevoflurane is reported to be between 1.71% and 2.05%. The MAC for sevoflurane is somewhat higher in children. Typical values are 2.6% in children and 3.3% in neonates. Previous study showed that sevoflurane inhibits NADH/ubiquinone oxidoreductase activity, suggesting that complex I is a likely target. Other studies demonstrated that reactive oxygen species (ROS) exist in cardioprotection when preconditioned with sevoflurane. It also directly triggers the formation of peroxynitrite, inducing nitrolysation of sarcolemmal proteins. In contrast to small quantities of ROS required to initiate sevoflurane-induced preconditioning, large amounts of ROS play a major role in the pathophysiology of reperfusion injury. Sevoflurane also produces cardioprotection by attenuating the adverse effects of ROS burst upon reperfusion. Because of its cardioprotective effects, researches of sevoflurane focus mainly on heart or myocardium. Indeed, sevoflurane distributes to circulation and affects leukocytes within. In peripheral, polymorphonuclear neutrophils (PMN) are a major component of non-antigen-specific, cell-mediated immune system. They generate extremely high amounts of ROS to defeat pathogenic microorganisms. Current studies on neutrophils treated with sevoflurane have demonstrated that neutrophils lost their ability to cause cardiac dysfunction and inflammation as well as reduce superoxide production and adherence to the endothelium. Surprisingly, all these experiments carried out a neutrophil-stimulated or primed condition by using fMLP, PAF, or PMA. Without these activations, they couldn't observe any benefits of sevoflurane on neutrophils or explain the virtue of anesthetic preconditioning (APC). Some reports even used hearts, neutrophils, or endothelia from different species to execute experiments. They couldn't rule out interaction between species.

To clarify the exact physical properties of PMN following sevoflurane treatment, we used whole blood from healthy volunteers to execute our experiments in our previous NSC program (NSC 95-2320-B-040-014). The results have showed that sevoflurane significantly increased intracellular H_2O_2 and/or peroxide, superoxide, and nitric oxide in PMN within 1 h treatment. Intensification of intracellular glutathione (GSH) depletion in PMN was also demonstrated. These results were of our expectations and interest us to evaluate the following issues. (1) Effects of sevoflurane have been carried out in epithelium, endothelium, myocardium, platelets, and vascular smooth muscle cells, and many pharmacological properties were recorded therein. However, information in regard to the effects of sevoflurane on oxidative stress of these cells is scant. Thus, we would like to evaluate the oxidative status of other cells, especially those contact sevoflurane directly such as bronchial epithelial and endothelial cells, to clarify the ROS-promoting properties are general or cell-type specific. Here, we design 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, and oxidative related apoptosis and necrosis.

Materials and Methods:

1.1 Cell line and reagents

Mouse lymph node endothelial cell (SVEC4-10) was purchased from The Food Industry Research and Development Institute (FIRDI) (Hsinchu, Taiwan). The hydroethidine (HE), chloromethylflourescein diacetate (CMF-DA) utilized herein were acquired from Molecular Probes, Inc. (Eugene, OR, USA). Dulbecco's modified Eagle's medium (DMEM), Propidium iodide (PI), 4,5-diaminofluorescein (DAF-2), 2',7'-dichlorodihydrofluorescein-diacetate (DCFH-DA), and other chemicals were bought from Sigma Chemical Co. (St. Louis, MO, USA).

1.2 Culture of SVEC4-10

The basal medium for SVEC4-10 is 90% Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose + 10% heat-inactivated fetal bovine serum. The cell cultures were incubated at 37°C and 95% humidity in room air with added 5% carbon dioxide. The culture of BEAS-2B was not carried out due to the limitation of legal rules. The import of bovine pituitary extract was not available right now. Thus, we will seek another cell lines to perform the experiment in the future.

1.3 Flow cytometry analysis of superoxide content in SVEC4-10

SVEC4-10 cells (10^6 cells/ml) were preincubated with 10 μM of HE in the 5% CO_2 incubator for 15 minutes. HE is freely permeable to cellular membrane, and after being oxidized by superoxide, it turns into ethidium and emits a bright red fluorescence. SVEC4-10 cells were then be stimulated with 1% and 3% of sevoflurane for 1 h at 37_0C in the CO_2 incubator. The intracellular NAD(P)H oxidase can react with menadione to generate superoxide. After drugs treatment, SVEC4-10 cells were washed with incubation buffer, collected by centrifugation, suspended in the same buffer and analyzed by flow cytometry.

1.4 Flow cytometry analysis of H_2O_2 and/or peroxide content in SVEC4-10

SVEC4-10 cells (10^6 cells/ml) were preincubated with 20 μM of DCFH-DA in the 5% CO_2 incubator for 15 minutes. DCFH-DA is freely permeable to cellular membrane. In cytoplasm, esterases catalyze DCFH-DA to DCFH, which is then oxidized by H_2O_2 and/or peroxide into dichlorofluorescein (DCF) and emits a bright green fluorescence. SVEC4-10 cells were stimulated with 1% and 3% of sevoflurane for 1 h at 37℃ in CO_2 incubator. H_2O_2 is freely permeable to the cellular membrane and into cytoplasm. In cytoplasm, H_2O_2 directly converts DCFH-DA to DCF. After drugs treatment, SVEC4-10cells were washed with incubation buffer, collected by centrifugation, suspended in the same buffer and analyzed by flow cytometry.

1.5 Flow cytometry analysis of NO content in SVEC4-10 and HUVECs

SVEC4-10 cells (10^6 cells/ml) were preincubated with 1 μM of DAF-2 in the 5% CO_2 incubator for 15 minutes. SVEC4-10 and HUVECs will then be stimulated with 1% and 3% of sevoflurane for 1 h at 37℃ in the CO_2 incubator. After drugs treatment, SVEC4-10 cells were washed with incubation buffer, collected by centrifugation, suspended in the same buffer and analyzed by flow cytometry.

1.6 Western blot analysis

Western blotting was performed as previously described (Melchjorsen et. al., 2002). Cell lysates were fractionated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Blocked membranes were then incubated with the indicated Abs, and the immunoreactive bands were visualized using a chemiluminescent substrate. Briefly, cells were cultured in 60-mm

tissue-culture dishes. The culture medium was replaced with new medium when the cells were 60% confluent and then exposed to various concentrations of sevoflurane for indicating times. After drug treatment, cells were washed twice with PBS, scraped with a rubber policeman, and extracted using CelLytic^{TM}-M lysis reagent. The quantity of total protein present was assessed by Bio-Rad protein assay reagent, with 50 μg of protein being loaded onto each lane of a 12% SDS-polyacrylamide gel. The separated proteins were blotted onto a hybond-polyvinylidene difluoride (PVDF) membrane. Non-specific binding was blocked by overnight incubation in 1% BSA, 0.1% Tween 20, in PBS at 4°C. The membranes were then stained with mouse anti-iNOS antibody (diluted 1: 500 in PBS containing 1% BSA and 0.1% Tween 20) for 1 h at room temperature using gentle agitation. After being washed three times with PBS containing 0.1% Tween 20, the membrane was incubated with 1:5,000 diluted horseradish peroxidase-coupled anti-mouse IgG for 1 h at room temperature. After having been washed three times with PBS containing 0.1% Tween 20, the specific proteins were then identified using an enhanced chemiluminescent-based detection system. Membranes were subsequently exposed to X-ray films.

1.7 Antibodies and concentrations used in Western blot analysis

The following Abs and concentrations were used: mouse monoclonal anti-IκB (H-4), 1/200; mouse monoclonal anti-p-IκB (B-9) 1/100; rabbit polyclonal anti-IKK (H470), 1/200 (all Santa Cruz Biotechnology); mouse monoclonal anti-β-actin (AC-15), 1/5000 (Sigma-Aldrich); HRP-conjugated goat polyclonal anti-mouse IgG, 1/2000; HRP-conjugated goat polyclonal anti-rabbit IgG, 1/2000 (both BD Transduction Laboratories, Lexington, KY).

Results

First, the effect of sevoflurane on the SVEC4-10 cells was examined by MTT assay. As shown in Figure 1, cell viability for SVEC4-10 cells treated with sevoflurane at doses of 0.125, 0.25, 0.5, and 1% was 91.10, 80.52, 63.84, and 52.27%, respectively, indicating that sevoflurane significantly reduction of cell viability through a dose-dependant manner in SVEC4-10 cells.

To evaluate the oxidative stress of sevoflurane in SVEC4-10 cells, we first tested the production of intracellular H_2O_2 and/or peroxide at the concentration of 0.125, 0.25, 0.5, and 1% of sevoflurane for 30 min by flow cytometry and DCFH-DA staining. The DCF fluorescence intensity expresses the related intracellular H_2O_2 and/or peroxide level. As shown in Figure 2, the DCF fluorescence increased significantly in SVEC4-10 cells after 0.125% of sevoflurane treatment as compared with untreated control cells. By contrast, the DCF fluorescence decreased significantly in SVEC4-10 cells after 0.25, 0.5, and 1% of sevoflurane treatment.

Nitric oxide (NO) has many important biologic functions, but it produces many damaging effects on several biological molecular, such as lipids, proteins and DNA through switch into reactive nitrogen oxide species including nitrogen dioxide, dinitrogen trioxide, and peroxynitrite. To investigate whether NO appears during sevoflurane treatment, we examined the production of NO by DAF-2 staining, a NO specific probe and flow cytometry. As shown in Figure 3, the DAF fluorescence slightly decreased in SVEC4-10 cells after 0.125% of sevoflurane treatment as compared with untreated control cells. By contrast, the DAF fluorescence increased significantly after 0.25, 0.5, and 1% of sevoflurane treatment.

Many studies have reported that GSH depletion is a key factor on chromosomal DNA fragmentation and apoptosis or necrosis in oxidative stress-induced cell death. The effect of

sevoflurane on intracellular GSH depletion is thus worth investigating. The intracellular GSH level was analyzed by CMF-DA staining, a GSH specific probe and flow cytometry. The CMF fluorescence is directly related to intracellular GSH level. As shown in Figure 4, the CMF fluorescence decreased in SVEC4-10 cells after 0.125, 0.25, 0.5, and 1% of sevoflurane treatment as compared with untreated control cells.

Experimental data from other studies have demonstrated that ROS can disrupt $\Delta\Psi m$. Because sevoflurane changed ROS levels in SVEC4-10 cells, we further studied the $\Delta\Psi m$ by using rhodamine 123 staining. It is interesting to note that 0.25 to 1% of sevoflurane resulted significantly increasing of $\Delta\Psi m$ after 30 min treatment as compared with their untreated cells. This is a novel phenomenon and the first discovery in the field of anesthetic practice Figure 5.

To evaluate the molecular mechanism and signal transduction pathway corresponding to the generation of ROS, immunoblotting analysis was performed and the results were shown in Figure 6. Nuclear factor-κB (NF-κB) pathway, one of the key regulatory molecules in oxidative stress-induced cell activation, is of our interest in this study. Our data shows that the expression of NF-κB, I-κB, IKKγ were regulated after sevoflurane treatment in SVEC4-10**.**

In conclusion, we demonstrated that sevoflurane significantly caused cell death in mouse lymph node endothelial cell (SVEC4-10) within 30 minutes treatment. The process was accompanied with an increase of intracellular superoxide (data not shown), nitric oxide (NO) and mitochondrial membrane potential ($\Delta\Psi m$) and a decrease of H_2O_2 and/or peroxide and intracellular glutathione (GSH) levels. These results were consistent with our findings in polymorphonuclear neutrophils (PMN), suggesting that the ROS-promoting properties of sevoflurane are general phenomena in many kinds of cells. In addition, activation of NF-kB signaling pathway may be responsible for the generation of ROS.

Discussion

In this study we have demonstrated that sevoflurane, at a clinically attainable concentration, induces cellular damage in SVEC4-10 within 15 and 30 minutes treatment by disturbing the generation of superoxide, ROS and NO. ROS have become known as major signaling molecules in the modulation of various cellular processes. Immoderate generation of ROS may result in attack of many diseases and harm to most intracellular and extracellular biomolecules in a living organism. Moreover, ROS can directly prompt and/or adjust apoptosis and necrosis (Canakci et al., 2005). Mitochondria are major producers of ROS, which mainly consist of superoxide anion, hydrogen peroxide, and hydroxyl radical (Larochette et al., 1999; Brand and Murphy, 1987). The main source of ROS in most cell types is possibly the electron leakage from mitochondrial electron transport chain that diminishes molecular oxygen to superoxide anion. Superoxide dismutase (SOD) will convert superoxide anion to hydrogen peroxide and then produce hydroxyl radical rapidly via either Fenton reaction (Hsieh et al., 2004) or Harber-Weiss reaction (Hsieh et al., 2004). Our results found that intracellular superoxide level was increased at 15 minutes (Fig. 3) in sevoflurane-treated SVEC4-10. In the future, we will demonstrate that whether pretreatment with SOD affects sevoflurane-treated HUVEC and SVEC4-10. These results will help to explain that the superoxide induced by sevofluane may be rapidly converted by intracellular SOD to hydrogen peroxide, and then reacted with non-fluorescence of DCFH to a fluorescence substance, DCF. NO is known to modulate various function of HUVEC and SVEC4-10 such as chemotaxis,

adherence, aggregation, and generation of ROS. We will next examine that the effect of dexamethasone, a NO inhibitor, in sevoflurane-induced intracellular DCF fluorescence. We propose that part of intracellular ROS induced by sevoflurane is coming from NO generation.

The $\Delta\Psi_m$ reflects the energy accumulated in the electrochemical gradient across the inner mitochondrial membrane, which in turn is used by F0F1-ATPase to change adenosine 5'-diphosphate to ATP throughout oxidative phosphorylation. Mitochondrial hyperpolarization and transient ATP depletion represent early and reversible steps in T-cell activation and apoptosis (Perl et al., 2004). The $\Delta\Psi_m$ increase often implies mitochondrial hyperpolarization. Several studies demonstrated that mitochondrial hyperpolarization induced by some substances, such as lysophophatidylcholine, linoleic acid or farnesol usually accompanies with ROS overproduction (Machida and Tanaka, 1999; Lee et al., 2005). These phenomena excited us to discover the relationship between ROS production and transient mitochondrial hyperpolarization in sevoflurane-treated SVEC4-10. Using CCCP pretreatment to inhibit the mitochondrial hyperpolarization, we will evaluate the intracellular DCF fluorescence by flow cytometry in sevoflurane-treated SVEC4-10.

Cells contain several antioxidant systems to protect the injury induced by increased intracellular ROS. Among them, GSH is an antioxidant that it defends cells from ROS-induced apoptosis (Yu, 1994). Many studies point out the GSH depletion is an importance factor that increases percentage of cells undergoing apoptosis; on other contrary, NAC, a GSH precursor, decreases apoptosis induced by GSH depletion (Beaver and Waring, 1995; Kito et al., 2002). Other reports demonstrated that caspase 3/7 activation is an important step in glutathione depletion-induced apoptosis in resting and inflammatory neutrophils (O'Neill et al., 2000). In the future, we will observe that the effect of sevoflurane on intracellular GSH content in SVEC4-10.

NF-κB comprises a family of inducible transcription factors that serve as important regulators of the host immune and inflammatory response (Baldwin, 1996; Pahl, 1999; Gerondakis et. al., 1998; Ghosh et. al., 1998). It also involves in protecting cells from undergoing apoptosis in response to DNA damage or cytokine treatment. Stimulation of the NF-κB pathway is mediated by diverse signal transduction cascades. These signals activate the IκB kinases, IKKα and IKKβ, which phosphorylate inhibitory proteins known as IκB to result in their ubiquitination and degradation by the proteasome. The degradation of IκB results in the translocation of NF-κB from the cytoplasm to the nucleus where it activates the expression of specific cellular genes. Cytokines and chemokines, receptors involved in immune recognition such as members of the MHC, proteins involved in antigen presentation, and receptors required for neutrophil adhesion and migration are of NF-κB regulation (Pahl, 1999). NF-κB also stimulates the expression of enzymes whose products contribute to the pathogenesis of the inflammatory process, including the inducible form of nitric oxide synthase (iNOS), which generates nitric oxide (NO), and the inducible cyclooxygenase (COX-2), which generates prostanoids. "Does ROS generation and downstream signal transduction pathway result in the formation of proinflammatory mediators or expression of adhesion molecules?" are of our interests to investigate in the future.

References

Behne M, Wilke HJ, Harder S. Clinical pharmacokinetics of sevoflurane. Clinical Pharmacokinetics 1999; 36: 13-26.

Bouwman RA, Musters RJ, van Beek-Harmsen BJ, de Lange JJ, Boer C. Reactive oxygen species precede protein kinase C-delta activation independent of adenosine triphosphate-sensitive mitochondrial channel opening opening in sevoflurane-induced cardioprotection. Anesthesiology 2004; 100: 506-14.

Burrows DL, Nicolaides A, Stephens GC, Ferslew KE. The distribution of sevoflurane in a sevoflurane induced death. J Forensic Sci 2004; 49: 1-4.

Coetzee JF, le Roux PJ, Genade S, Lochner A: Reduction of postischemic contractile dysfunction of the isolated rat heart by sevoflurane: Comparison with halothane. Anesth Analg 2000; 90: 1089-97.

de Hert SG, ten Broecke PW, Mertens E, van Sommeren EW, de Blier IG, Stockman BA, Rodrigus IE: Sevoflurane but not propofol preserves myocardial function in coronary surgery patients. Anesthesiology 2002; 97: 42-9.

Finkel T: Oxygen radicals and signaling. Curr. Opin. Cell Biol. 10 (1998), pp. 248 – 253.

Hanley P, Ray J, Brandt U, Daut J. Halothane, isoflurane and sevoflurane inhibit NADH:ubiquinone oxidoreductase (complex I) of cardiac mitochondria. J Physiol 2002; 544: 687-93.

Heind B, Reichle FM, Zahler S, Conzen PF, Becker BF. Sevoflurane and isoflurane protect the reperfused guinea pig heart by reducing postischemic adhesion of polymorphonuclear neutrophils. Anesthesiology 1999; 91: 521-30.

Heind B. Reichle F. Beckert BF. Sevoflurane but not isoflurane can reduce prostacyclin production of endothelial cells. European Journal of Anaesthesiology. 20(2):116-9, 2003

Hu G, Salem MR, Crystal GJ. Role of adenosine receptors in volatile anesthetic preconditioning against neutrophil-induced contractile dysfunction in isolated rat hearts. Anesthesiology 2005; 103: 287-295.

Kakutani T. Ogawa K. Iwahashi S. Mizumoto K. Hatano Y. Sevoflurane enhances nitroglycerin tolerance in rat aorta: implications for the desensitization of soluble guanylate cyclase possibly through the additive generation of superoxide anions and/or hydroxyl radicals within vascular smooth muscle. Anesthesia &Analgesia. 101(4):1015-22, 2005

Kanna T, Akata T, Izumi K, Nakashima M, Yonemitsu Y, Hashizume M, Takahashi S. Sevoflurane and bradykinin-induced calcium mobilization in pulmonary arterial valvular endothelial cells in situ: sevoflurane stimulates plasmalemmal calcium influx into endothelial cells. J. Cardiovasc. Pharmacol. 2002; 40: 714-24.

Kevin LG, Novalija E, Riess ML, et al. Sevoflurane exposure generates superoxide but leads to decreased superoxide during ischemia and reperfusion in isolated hearts. Anesth Analg 2003; 96: 949-55.

Kowalski C, Zahler S, Becker BF, Flaucher A, Conzen PF, Gerlach E, Peter K: Halothane, isoflurane and sevoflurane reduce postischemic adhesion of neutrophils in the coronary system. Anesthesiology 1997; 86:188-95

Lefer DJ, Nakanishi K, Johnston WE, Vinten-Johansen J: Antineutrophil and myocardial protecting actions of a novel nitric oxide donor after acute myocardial ischemia and reperfusion

in dogs. Circulation 1993; 88:2337-50

Lerman J. Sevoflurane in pediatric anesthesia. Anesth Analg 1995; 81:S4-10.

Lucchesi BR, Mullane KM: Leukocytes and ischemia-induced myocardial injury. Annu Rev Pharmacol Toxicol 1986; 26:201-24

McCord JM: Oxygen derived free radicals in postischemic tissue injury. N Engl J Med 1985; 312:159-63

Mobert J, Zahler S, Becker BF, Conzen PF. Inhibition of neutrophil activation by volatile anesthetics decreases adhesion to cultured human endothelial cells. Anesthesiology 1999; 90: 1372-81.

Morisaki H, Suematsu M, Wakabayashi Y, Moro-oka S, Fukushima K, Ishimura Y, Takeda J: Leukocyte-endothelium interaction in the rat mesenteric microcirculation during halothane or sevoflurane anesthesia. Anesthesiology 1997; 87:591-8

Mullenheim J, Ebel D, Frabetadorf J, et al. Isoflurane preconditions myocardium against infarction via release of free radicals. Anesthesiology 2002; 96: 934-40.

Niemczyk E, Majczak A, Hallmann A, Kedzior J, Wozniak M, Wakabayashi T. A possible involvement of plasma membrane NAD(P)H oxidase in the switch mechanism of the cell death mode from apoptosis to necrosis in menadione-induced cell injury. Acta. Biochim. Pol. 2004; 51: 1015-22.

Novalija E, Varadarajan SG, Camara AKS, et al. Anesthetic preconditioning: triggering role of reactive oxygen and nitrogen species in isolated hearts. Am J Physiol Heart Circ Physiol 2002; 283: H44-52.

Ozes, O. N., L. D. Mayo, J. A. Gustin, S. R. Pfeffer, L. M. Pfeffer, D. B. Donner. 1999. NF-B activation by tumour necrosis factor requires the Akt serine-threonine kinase. Nature 401:82.

Park KW. Dai HB. Lowenstein E. Sellke FW. Epithelial dependence of the bronchodilatory effect of sevoflurane and desflurane in rat distal bronchi. Anesthesia &Analgesia. 86(3):646-51, 1998

Pinsky MR, Vincent JL, Deviere J, et al. Serum cytokine levels in human septic shock; relation to multiple-system organ failure and mortality. Chest 1993; 103:565-575.

Preckel B, Schlack W, Comfere T, Obal D, Barthel H, Thamer V. Effects of enflurane, isoflurane, sevoflurane and desflurane on reperfusion injury after regional myocardial ischaemia in the rabbit heart in vivo. Br J Anaesth 1998; 81: 905-12.

Riess ML, Camara AK, Chen Q. Altered NADH and improved function by anesthetic and ischemic preconditioning in guinea pig intact hearts. Am J Physiol Heart Circ Physiol 2002; 283: H53-60.

Riess ML, Camara KS, Novalija E, et al. Anesthetic preconditioning attenuates mitochondrial Ca^{2+} overload during ischemia in guinea pig intact hearts: reversal by 5-hydroxydecanoic acid. Anesth Analg 2002; 95: 1540-6.

Riess ML, Eells JT, Kevin LG, Camara AK, Henry MM, Stowe DF. Attenuation of mitochondrial respiration by sevoflurane in isolated cardiac mitochondria is mediated in part by reactive oxygen species. Anesthesiology 2004; 100: 498-505.

Riess ML, Kevin LG, McCormick J, Jiang MT, Rhodes SS, Stowe DF. Anesthetic preconditioning: the role of free radicals in sevoflurane-induced attenuation of mitochondrial electron transport in Guinea pig isolated hearts. Anesth. Analg. 2005;100: 46-53.

Riess ML, Novalija E, Camara AK, et al. Preconditioning with sevoflurane reduces changes in

nicotinamide adenine dinucleotide during ischemia-reperfusion in isolated hearts: reversal by 5-hydroxydecanoic acid. Anesthesiology 2003;98:387-95.

Schlack W, Preckel B, Stunneck D, Thamer V. Effects of halothane, enflurane, isoflurane, sevoflurane and desflurane on myocardial reperfusion injury in the isolated rat heart. Br J Anaesth 1998; 81: 913-9.

Sheeran P, Hall GM. Cytokines in anaesthesia. Br J Anaesth 1997; 78:201-219.

Tanaka K, Weihrauch D, Kehl F, et al. Mechanism of preconditioning by isoflurane in rabbits: a direct role for reactive oxygen species. Anesthesiology 2002; 97: 1485-90.

Thorlacius K. Bodelsson M. Sevoflurane promotes endothelium-dependent smooth muscle relaxation in isolated human omental arteries and veins. Anesthesia & Analgesia. 99(2):423-8, 2004

Varadarajan SG, An J, Novalija E, Stowe DF: Sevoflurane before or after ischemia improves contractile and metabolic function while reducing myoplasmic Ca^{2+} loading in intact hearts. Anesthesiology 2002; 96: 125-33.

Yu J. Ogawa K. Tokinaga Y. Hatano Y. Sevoflurane inhibits guanosine 5'-[gamma-thio] triphosphatestimulated, Rho/Rho-kinase-mediated contraction of isolated rat aortic smooth muscle. Anesthesiology. 99(3):646-51, 2003

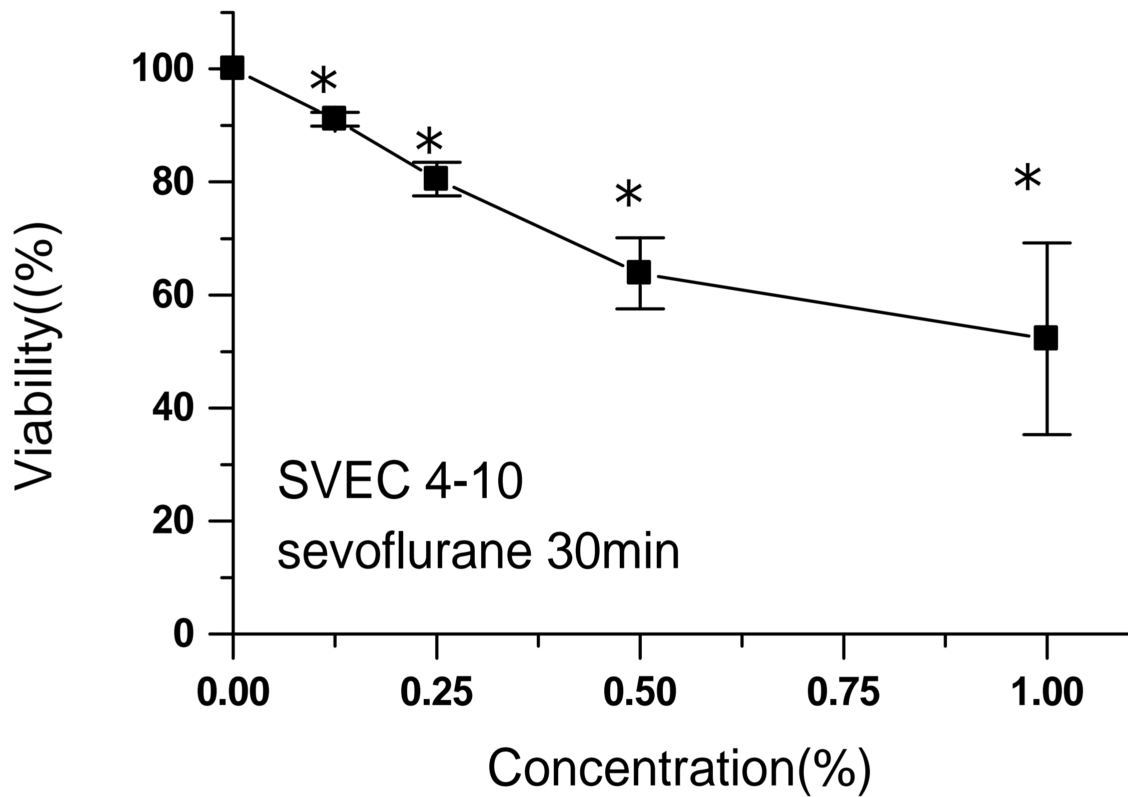

Fig 1. Effects of sevoflurane on SVEC4-10 cells. SVEC4-10 cells were treated with varying levels of sevoflurane (0, 0.125, 0.25, 0.5, and 1%). After treatment for 30 min, the cell viability was determined by MTT assay. The control condition was defined as media alone. Each experiment was done in triplicate. Bars indicate mean ± standard errors. All comparisons were made relative to controls and significant difference was indicated as * $p < 0.05$ using Student's t test

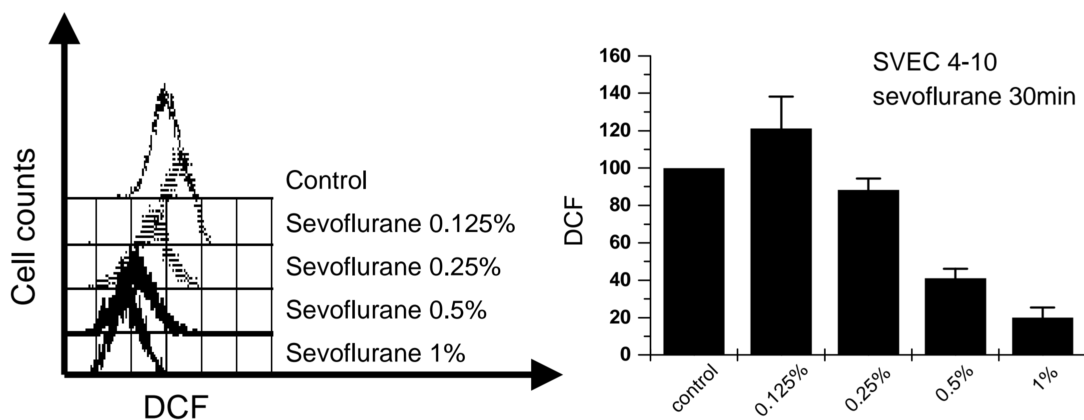

Fig 2. Effects of sevoflurane on intracellular H_2O_2 and/or peroxide level in SVEC4-10.

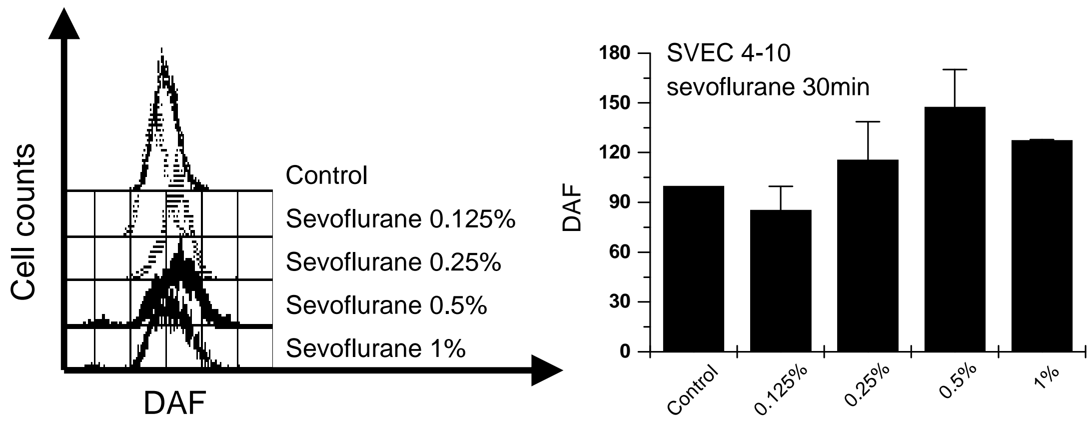

Fig 3. Effects of sevoflurane on NO level in SVEC4-10 cells.

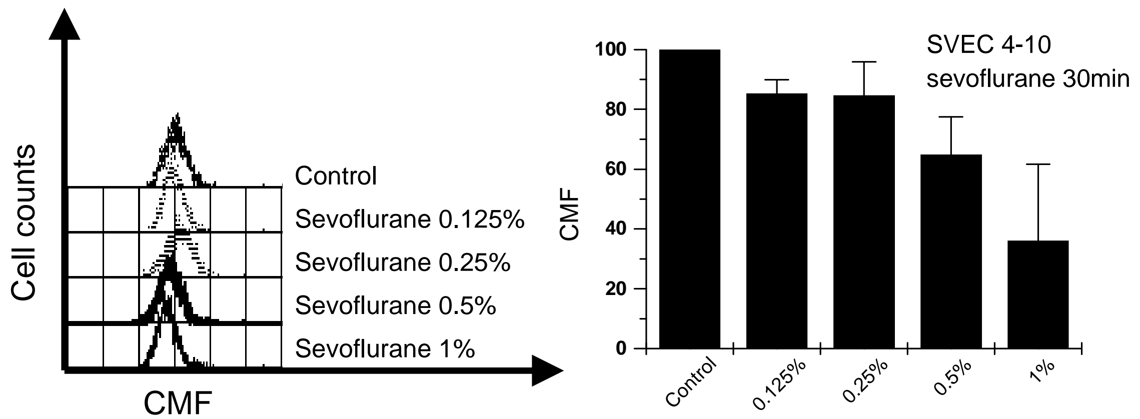

Fig 4. Effects of sevoflurane on GSH level in SVEC4-10 cells.

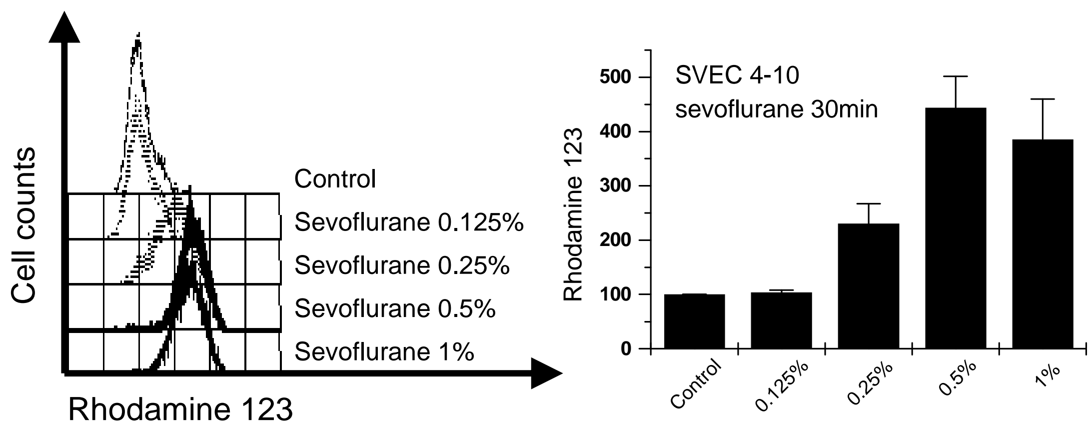

Fig 5. Effects of sevoflurane on $\Delta\Psi m$ in SVEC4-10 cells.

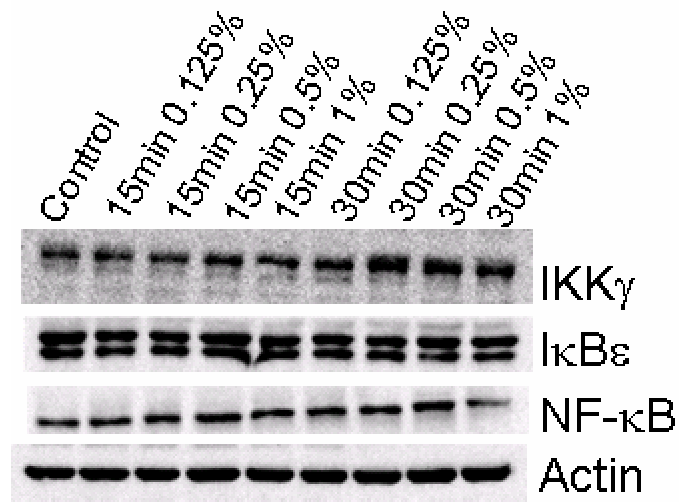

Fig 6. The relative amounts of NF-κB, IKKγ, IκBε after SVEC4-10 cells were treatment with sevoflurane for both 15 and 30minutes.

計畫成果自評

研究內容與原計畫相符程度…相符

達成預期目標情況…均已達成

研究成果之學術或應用價值…可做為臨床使用之參考

是否適合在學術期刊發表…是

行政院國家科學委員會補助國內專家學者出席國際學術會議報告

98 年 10 月 5 日

附件三

報告人姓名	張雯惠	服務機構及職稱	中山醫學大學應用化學系 副教授
時間 會議地點	2008/09/15 — 2008/09/19 Athens, Greece	本會核定補助文號	96-2320-B-040-008-MY2
會議名稱	（中文）2008 國際微米暨奈米工程研討會 （英文）International Conference on Micro & Nano Engineering, MNE 2008		
發表論文題目	（中文）EUV 相位偏移光罩之光罩倍率對於繞射及成像結果之影響 （英文）Influence of mask magnification effects on the diffracted light and lithographic imaging for EUV phase shifting mask.		

報告內容應包括下列各項：

一、參加會議經過

國際微米暨奈米工程研討會(International Conference on Micro & Nano Engineering, MNE)始於 1975 年，每年輪流在歐洲各國定期舉辦、是一個具悠久歷史之研討會，主要邀請世界各地在微奈米結構、半導體製程技術、元件製造與相關應用之科學家及工程人員參與、提供發表討論微奈米工程及技術之最新進展與發明之平台，歷年平均與會人數、發表口頭及壁報論文達五～六百之量，每年會後更進一步收錄論文專刊發表於 SCI 國際期刊 Microelectronic Engineering；本研討會與每年在美國舉辦之 the Electron-, Ion-, and Photon-Beam and Nanotechnology Conference (EIPBN)及日本舉辦之 the Microprocesses and the Nanotechnology Conference (MNC)為姊妹研討會,是半導體及電子元件相關微奈米製程技術非常重要之會議，每年這三個研討會之最佳論文作者，也必是 MNE 邀請演講之學者。今年研討會涵括三大主題：1. Micro & Nano lithography; 2. Micro & Nano Fabrication, Nano Engineering, MEMS, NEMS; 3. Micro & Nano Engineering for Life Sciences；其中第三主題為**因應人類未來在預防醫學、醫療檢測與治療之設備需求及相關應用所新增的主題，故近年也吸引許多生物醫學領域的研究人員及設備廠商與會，以進行跨領域的合作及交流**。今年的主辦單位為希臘雅典之國際科學研究中心(National Center for Scientific Research (NCSR) "Demokritos")的微電子研究所(Institute of Microelectronics – IMEL)，是歐洲著名之微電子研究單位，成立迄今已有二十餘年，近年亦執行多項歐盟支持的研究計畫，這個在奈米半導體技術研究執牛耳的機構目前積極投入生醫領域的研究，研發具有可遙控、體積小、成本低等功能的微電子感測器，今年 IMEL 爭取主辦 MNE2008，除了專業學術演講之安排及邀稿審查外，更集合全世界著名的奈米工程及技術相關廠商參與，國際上各學術研究機構亦派員與會並發表論文，以展示其技術及學術的水準，並進行跨領域的交流，因此研討會的學術地位及重要性由其主辦單位 IMEL 在奈米電子、生醫電子與製程技術是全球先進的研究機構，前瞻技術領先全球 3～5 年，部分技術甚至可達 10 年、委員會成員組織、涵蓋主題內容包羅萬象、贊助廠商規模之龐大與完整，窺其究竟。

二、與會心得

這次前往希臘雅典參加一年一度的 MNE 會議，透過聆聽演講、閱讀研究成果壁報，以及與國外專家的交流中，瞭解目前國際上有關奈米科學與技術，尤其是在半導體、電子元件相關微奈米製程技術與生物醫學研究之跨領域的結合之最新進展，期望藉由與會能進一步尋求國際合作伙伴，提升台灣在奈米科技及生物醫學跨領域研究的競爭力。

三、考察參觀活動(無是項活動者省略) …無參訪活動

四、建議

國際微米暨奈米工程研討會(International Conference on Micro & Nano Engineering, MNE) 始於 1975 年，每年輪流在歐洲各國定期舉辦、是一個具悠久歷史之研討會，主要邀請世界各地在微奈米結構、半導體製程技術、元件製造與相關應用之科學家及工程人員參與、提供發表討論微奈米工程及技術之最新進展與發明之平台，歷年平均與會人數、發表口頭及壁報論文達五～六百之量，每年會後更進一步收錄研討會論文專刊發表於 SCI 國際期刊 Microelectronic Engineering；由於此研討會具有相當的代表性及重要性，本次參加此研討會帶回的相關資訊，將可提供同事掌握微奈米科技的最新研究方向及趨勢。同時藉由與會時各學界及業界的討論，將可作為本校在制定研發計畫或整合學校發展方向時之參考。

五、攜回資料名稱及內容

2008 國際微米暨奈米工程研討會(International Conference on Micro & Nano Engineering, MNE)論文集、研討會大會議程手冊及參展科學儀器廠商之相關資訊。

六、其他

因參與本次研討會，有機會認識微奈米工程相關領域之專家學者，本人因此於 2008 年底獲推薦為第三十五屆國際微米暨奈米工程研討會（the 35th International Conference on Micro & Nano Engineering (MNE 2009)）之國際議程委員會（international programme committee）委員。