Acta Anaesthesiologica Taiwanica 54 (2016) 6–10

Contents lists available at ScienceDirect

Acta Anaesthesiologica Taiwanica

journal homepage: www.e-aat.com

Research Paper

The effect of propofol and sevoflurane on antioxidants and proinflammatory cytokines in a porcine ischemia–reperfusion model

Hung-Tsung Hsiao, Hung Wu, Pei-Chi Huang, Yu-Chuang Tsai, Yen-Chin Liu*

Department of Anesthesiology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan

ARTICLE INFO

Article history:
Received 10 February 2015
Received in revised form
14 September 2015
Accepted 2 November 2015

Keywords:
free radicals;
ischemia–reperfusion injury;
propofol;
sevoflurane;
tumor necrosis factor-α

ABSTRACT

Objectives: Ischemia–reperfusion (IR) features massive oxidative stress of tissues and cytokine response. Propofol and sevoflurane, both of which are commonly used anesthetics, are thought to have different antioxidant activities. The aim of this study is to delineate the influence of these two drugs on the production of free radicals and proinflammatory cytokines in IR conditions via *in vitro* and *in vivo* models.
Methods: An *in vitro* IR model was performed by incubating porcine cells (including mononuclear cells, and coronary and aortic smooth muscle cells) with either propofol 25 μM or sevoflurane 2% in the hypoxia chamber (1% O_2, 37°C) for 1 hour, followed by room temperature air for 2 hours. Reactive oxygen species (ROS) and tumor necrosis factor-α (TNF-α) were also measured via flow cytometry and enzyme-linked immunosorbent assay methods, respectively. Ten pigs were used for the *in vivo* study. After anesthesia with either propofol (10–15 mg/kg/h) or sevoflurane (2%), internal carotid and femoral arterial catheters were inserted for direct blood pressure monitoring and blood sampling. The IR models were produced via descending thoracic aorta clamping for 1 hour and declamping for 2 hours during the procedure for left ventricular assist device implantation. Blood serum was sampled from upper and lower body vessels for ROS and TNF-α evaluation via thiobarbituric acid reacting substances method and enzyme-linked immunosorbent assay, respectively.
Results: The results showed significant reduction of both ROS and TNF-α levels in the propofol group *in vitro* IR model. However, there was no difference in lipid peroxidation and TNF-α level between propofol and sevoflurane for the *in vivo* IR model.
Conclusion: We concluded that propofol, compared with sevoflurane, can significantly inhibit ROS formation on a cell level. In addition, propofol can significantly inhibit TNF-α formation of monocytes and coronary smooth muscle cells but not aortic smooth muscle cells.

1. Introduction

Ischemia–reperfusion (IR) produces a lot of free radicals and causes tissue damage.[1] In clinical practice, there are lots of conditions associated with IR, such as tourniquet-induced IR in peripheral or major vascular surgery or organ transplantation. When IR occurs, overproductive reactive oxygen species (ROS) and inflammatory cytokines such as tumor necrosis factor-α (TNF-α) may cause severe problems owing to associated tissue damage. It has been said that vascular smooth muscle cells are the possible target.[2] Meanwhile, modern anesthesia uses rapid recovery anesthetic drugs to reduce the postanesthesia effect. Propofol and sevoflurane are two of the commonly used drugs. However, it has been reported that propofol has a more antioxidant effect and reduces free radical-induced injury.[3,4] Propofol was also reported for its proinflammatory cytokine response suppression in sepsis.[4,5] However, there are little data available that indicate the difference of these two drugs in an IR model. This study investigated the antioxidant and anti-inflammatory effects of propofol and sevoflurane via *in vitro* and *in vivo* IR models with porcine cells and animal methods. As we know, porcine cells are even closer to human cells than rodents. The results should provide better clinical implications.

Conflicts of interest: The authors have nothing to disclose and no conflicts of interest.

* Corresponding author. Department of Anesthesiology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan.
E-mail address: inp1965@mail.ncku.edu.tw (Y.-C. Liu).

http://dx.doi.org/10.1016/j.aat.2015.11.002

2. Methods

2.1. Porcine polymorphonuclear cells and smooth muscle cells preparation

Peripheral porcine blood polymorphonuclear (PMN) cells were purified with Ficoll-Paque density gradient centrifugation. Whole blood (50 mL) was diluted with an equal volume of Hank's Balanced Salt Solution (HBSS) with 5% bovine serum albumin and then carefully layered over Ficoll-Paque. After centrifugation was performed (40 minutes, 400g), the PMN layer was carefully aspirated, washed in HBSS three times, and incubated in 60 mm plastic culture dishes in HBSS with 5% bovine serum albumin. The nonadherent cells were aspirated and the adherent cells (PMN) were incubation for 3 days for stability before experimentation. Porcine aorta smooth muscle cells (PAOSMC, Cell Application Inc., San Diego, CA, USA) and coronary smooth muscle cells (PCASMC, Cell Application Inc.) were all cultured in 37°C 5% CO_2/95% air, and maintained with specific growth medium (P311-500) without fetal bovine serum added. Cells were stored in liquid nitrogen if not used. Medium was changed every other day until the culture was > 60% confluent and the subculture was 80% confluent.

2.2. Experimental protocol in cells and hypoxic cytotoxicity assay

Cells were coincubated with either propofol 25 μM or sevoflurane 2% condition and then incubated in a hypoxia culture chamber (1% O_2, 37°C) for 1 hour and reoxygenated (21% O_2, 37°C) for 2 hours. Cells were then collected and ROS and proinflammatory cytokines were measured. Cell damage evaluation after hypoxia was evaluated by 2-(4-iodo-phenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-1) colorimetric method.[6] WST-1, a water-soluble formazan enzyme, is thought to reflect a sensitive respiratory enzyme activity after injury. Cells (5 × 103/well) were in microplates (96 wells, flat bottom) with 100 μL medium and 10 μL WST-1 reagent (Roche Molecular Biochemicals, Indianapolis, IN, USA) was added and then incubated for 4 hours. Optical density was measured at 450 nm on a microplate reader.

2.3. Superoxide measurement in cells

Cellular accumulation of superoxide was determined with the dihydroethidium method. To measure ROS levels, treated cells were incubated with 1M of dihydroethidium for 30 minutes at 37°C, followed by cell washing and resuspension in ice-cold phosphate-buffered saline. Cell Quest software was applied for flow cytometric analysis (FACS Calibur, BD Biosciences, San Jose, CA, USA). The mean fluorescence intensity of white cell, separated with electronic gating in the forward scatter/side scatter dot plot, was measured and quantified in arbitrary units of 10,000 events.

2.4. Animal preparation

The Institutional Animal Ethic Committee in our university (College of Medicine, National Cheng Kung University, Tainan, Taiwan) approved all procedures in this study. Pigs (55–65 kg, Duroc), purchased from Livestock Research Institute (Council of Agriculture, Hsinhua, Taiwan), were randomly assigned to propofol or sevoflurane groups (n = 5 in each groups). Xylazine (0.2 mg/kg, intramuscular) was used for sedation. Thiopental (25 mg/kg) and succinylcholine (1 mg/kg) were administrated intravenously via the ear vein for anesthetic induction and tracheal intubation. Carotid and femoral artery catheters were then inserted for blood sampling and represent upper and lower body's conditions, respectively. Arterial blood pressure, large bore intravenous catheter, electrocardiography, end-tidal CO_2, and pulmonary artery catheter were also used for intraoperative monitor.

2.5. Animal experimental protocol

Animals were maintained with propofol (10–15 mg/kg/h) or sevoflurane (1.5–2.5%) according blood pressure change. Serial blood serum sampling (10 mL for each time point) including basal level, 30 minutes, and 60 minutes after aortic clamp and 60 minutes and 120 minutes after aortic declamp were also harvested when surgical procedure for special designed ventricular assist device implantation was performed in the descending thoracic aorta. During aorta clamping or declamping, vessel control drug (nitroglycerin and epinephrine) plus fluid infusion were administered to maintain blood pressure within ±25% of the basal level. The total clamp time (clamp over descending thoracic aorta) was 1 hour and blood pressure was also recorded. Serum, centrifuged from whole blood (14,000 rpm, 4°C, 15 minutes), was then stored at −80°C for analysis.

2.6. Lipid oxidation measurement

The antioxidant effect of propofol on lipid peroxidation was evaluated by determining malondialdehyde (MDA) level in the blood via the thiobarbituric acid reacting substances method.[7] Serum mixed with trichloracetic acid and thiobarbituric acid was boiled. Butanol was then added to the tube and results were obtained at 532 nm after centrifugation.

2.7. Proinflammatory cytokine analysis

The level of proinflammatory cytokines (TNF-α) in serum and culture medium were analyzed with a commercial enzyme-linked immunosorbent assay kit (R&D Systems, UK) and obtained with an enzyme-linked immunosorbent assay reader at 570 nm. Briefly, after centrifugation, supernatant were collected and stored at −80°C. Cytokine levels were determined by interpolation with standard curves assayed on individual plates and normalized to protein content in each sample.

2.8. Statistical analysis

The results are expressed as means ± standard error mean. One-way analysis of variance and Student *t* test or multiple comparisons were used for the statistical evaluation of differences among means. A value of $p < 0.05$ was considered to be statistically significant.

3. Results

The hypoxia injury evaluations for the *in vitro* IR model with different anesthetics within different porcine cells were shown in Figure 1. Cellular IR model damaged all cell types regardless of the presence of anesthetics [all cell data were presented in a serial of monocytes, PAOSMC, and PCASMC; control (C), 1.08 ± 0.03, 1.07 ± 0.02, and 1.00 ± 0.11; hypoxia (H), 0.62 ± 0.05, 0.37 ± 0.06, and 0.70 ± 0.06; hypoxia plus propofol (H + P), 0.62 ± 0.04, 0.35 ± 0.06, and 0.62 ± 0.09; and hypoxia plus sevoflurane (H + S), 0.59 ± 0.05, 0.45 ± 0.04, and 0.66 ± 0.08; $n = 4$ in each group, * showed $t < 0.05$ compared with C]. Sevoflurane showed a little increase than propofol but the difference is not statistically insignificant. The production ratio of ROS in different hypoxia conditions is shown in Figure 2 (C, 0.56 C,0.03, 0.54 .00.04, and 0.57 .50.06; H, 1.03, 0.02, 1.00 .00.10, and 1.00 .00.04; H + P, 0.65, 0.02, 0.62 .00.02, and 0.60 .60.08; and H + S, 1.10 ± 0.07, 0.88 ± 0.02, and 0.88 ± 0.06;