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An Influence of a Combined Administration of Propofol and Isoflurane on Antioxidative Enzyme Activities in Growing Swine Erythrocytes

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Abstract : The present study was aimed to evaluate and compare the oxidative stress status of isoflurane and propofol in pigs undergoing surgery with measuring the activities of antioxidant enzymes. The pigs were divided into 2 groups according to the type of anesthesia used for the surgical procedure. In the isoflurane group (group 1), anesthesia was induced and maintained with 2-2.5% isoflurane under 100% oxygen. The propofol group (group 2) received 8 mg/kg/h of IV propofol with 0.5-1% isoflurane under 100% oxygen. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities of isoflurane group were significantly lower at the end of surgery than at induction of anesthesia, while that of the propofol group maintained their baseline values. There were significant differences in all enzymes activities between groups at the end of surgery. These results indicate that propofol is capable of preserving the antioxidant capacity in pigs anesthetized with the combination of isoflurane and propofol infusion.

Key words **:** antioxidant effects, isoflurane, oxidative stress, pigs, propofol.

Introduction

General anesthesia, either with inhalation or nonvolatile anesthetics, is known to affect many organ systems, such as the cardiovascular and the bronchoalveolar system (1). Besides several anesthetic agents produce free radicals and decrease the serum antioxidant levels in patients. Anesthesia with halothane, under leading to liver damage, resulted in the formation of free radicals in the liver (5).

In vivo studies were undertaken to determine whether free radical formation in the liver during administration of various halogenated anesthetics is associated with hepatotoxicity of these agents in an animal model. Free radicals were detected after administration of halothane or carbon tetrachloride, compounds which were hepatotoxic under the conditions of the experiment in animal models (10). On the other hand, there was increasing evidence of anesthetic agent-induced protection. Isoflurane, sevoflurane and morphine appear to be most promising as preconditioning-inducing agents. After the onset of ischemia, propofol could be selected to reduce ischemia-reperfusion injury (6).

Previous studies in animals suggest that exposure to desflurane or sevoflurane can suppress the cytotoxic or phagocytosis response of alveolar macrophages (1). However, propofol is a contrast to volatile anesthetics, because it is chemically similar to the endogenous antioxidant α-tocopheral (vitamin E) and, theoretically, should have similar properties (2). The possibility of potential side effects or antioxidant effects of anesthetics has been the subject of various studies. Nevertheless, the oxidant and antioxidant status of anesthetics in animals has not been fully evaluated and the oxidative stress issue remains controversial.

The aim of the present study was to evaluate and to compare the oxidative stress status of isoflurane and propofol in pigs undergoing surgery with measurements of the activities of antioxidant enzymes.

Materials and Methods

Eighteen male Landrace and Yorkshire mixed pigs (39.6 ± 1.6 kg, 3 to 4 month old) were used in the experiments. All pigs were obtained from the experimental livestock farm of the College of Agriculture, Chungnam National University (CNU). These experimental and housing protocols were approved by the CNU Animal Care and Use Committee (approval no. 2010-2-10). Food was withheld for 12 h prior to anesthesia, but access to water was allowed. The animals were premedicated with intramuscular injections (IM) of atropine sulfate (Atropine Sulfate®, Houns Co., Ltd., Seoul, Korea, 0.04 mg/kg) and xylazine hydrochloride (Rompun®, Bayer Co., Ltd., Seoul, Korea, 4.4 mg/kg) for immobilization. Prophylactic antibiotics, ampicillin sodium (Penbrrok®, Chong Kun Dang Co., Ltd., Seoul, Korea, 20 mg/kg IM) and analgesic agent, butorphanol (Butophan Injection®, Myungmoon Pharm Co., LTD., Seoul, Korea, 0.2 mg/kg IM) were

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administered before surgical operation.

The pigs were divided into 2 groups according to the type of anesthesia used for the surgical procedure. In isoflurane group (group 1, n = 9), anesthesia was induced and maintained with 2-2.5% isoflurane (Forane Sol®, Choongwae Pharm Co., LTD., Seoul, Korea) under pure oxygen. After endotracheal intubation, anesthesia was maintained with isoflurane, at an end-tidal concentration of 2-2.5%, using a Datex ohmeda ADU anesthesia system (Helsinki, Finland), with an oxygen flow rate of 2 L/min. The other group received constant rate infusion (CRI) of propofol (Aanepol Injection®, Hana Pharm Co., LTD., Seoul, Korea) at a rate of 8 mg/kg/h with 0.5-1% isoflurane under 100% oxygen (group 2).

Laparotomy and gastrotomy were performed to create surgical trauma in this study. Laparotomy and gastrotomy could be an important cause of oxidative stress by reduced abdominal visceral perfusion. A ventral midline celiotomy was performed. The body of the stomach was grasped with atraumatic forceps or fingers and delivered into the ventral abdominal wall incision. Then, the incision was made in the center of the body (approximately 15-25 mm in length). The resultant incision was closed in an appositional, simple continuous pattern, and the second inverting layer was applied over this initial layer. The abdomen was closed in 3 layers. During the surgical operation, the pigs were given intravenous fluid (Hartmann's Solution, 10 mL/kg/hr).

Postoperative care was provided to the pigs. Pigs were given butorphanol 0.2 mg/kg intramuscularly at the end of the surgical procedure for postoperative analgesia. A second dose of butorphanol 0.1 mg/kg was given intramuscularly 6 hours after surgery. Antibiotics (ampicillin sodium, 20 mg/kg, IM, bid) and ranitidine (1 mg/kg, IM, bid) were administrated for 7 days, and antibiotics cream was applied to the middle line area, once daily for 13 days. Oxidative stress parameters were determined in venous blood samples before induction of anesthesia, before gastrotomy; immediately before the gastrotomy, after gastrotomy; immediately after the gastrotomy and at the end of the surgery; immediately after the end of abdominal closure.

Anesthesia time and operation time were determined. Anesthesia time was the time interval between complete immobilisation and the first attempt made by the animal to lift its head a few centimeters. Operation time was the time interval between start of abdominal incision and complete closure of abdomen.

Heparinized blood was obtained on the day of experiment from pigs. After separation of erythrocytes from plasma and buffy coat by centrifugation for 5 min at 4,000 rpm, the erythrocytes were lysed in ice-cold high performance liquid chromatography-grade water at 4 times the volume. This sample was centrifuged at 10,000 × g for 15 min at 4°C and supernatant was immediately collected and stored at −80°C until measured. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) concentrations were measured with a commercial kit (Cayman Chemical Company, Ann Arbor, MI, USA) using a spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA).

Data were expressed as median (inter-quartile range), and the Mann-Whitney U-test was used for detection of differences between blood samples. A p-value < 0.05 was considered significant. All statistics were performed using a computer statistical package (Statistics Package for the Social Sciences, version 18.0; SPSS, Chicago, IL, USA).

Results

Mean anesthesia time was 109 minutes (ranging from 88 minutes to 128 minutes). Mean operation times of group 1 and group 2 were 38 minutes (ranging from 28 minutes to 50

Table 1. Heart rate (HR), mean arterial pressure (MAP), rectal temperature (RT), respiratory rate (RR), saturation of peripheral oxygen (SpO_2) and end-tidal CO_2 ($ETCO_2$) in pigs

parameter	Group	Pre	15 min	30 min	45 min	60 min	90 min
HR (breath/min)	Group 1	146 (14)	133 (16)	115 (15)	114 (15)	111 (16)	115 (20)
	Group 2	157(11)	139 (15)	119 (17)	113 (10)	118 (14)	125 (16)
MAP (mmHg)	Group 1	87 (9)	88 (5)	85 (10)	76 (12)	80 (8)	91 (15)
	Group 2	88 (6)	88 (9)	86 (11)	77 (17)	82 (6)	88 (9)
RT (°C)	Group 1	39.2 (0.4)	39.2 (0.4)	39.1 (0.4)	39.1 (0.3)	38.9 (0.4)	38.7 (0.3)
	Group 2	39.5 (0.4)	39.4 (0.2)	39.4 (0.4)	38.9 (0.2)	38.3 (1.0)	38.4 (1.2)
RR (breath/min)	Group 1	50 (14)	43 (6)	35 (15)	34 (12)	37 (10)	40 (11)
	Group 2	53 (11)	46 (11)	40 (15)	41 11)	48 (10)	44 (12)
$ETCO_2$ (mmHg)	Group 1	52.3 (10.14)	58.0 (11.35)	46.8 (7.34)	49.8 (8.88)	44.7 (15.86)	56.7 (6.21)
	Group 2	51.5 (12.33)	51.2 (12.13)	50.5 (9.03)	49.5 (9.64)	48.6 (9.74)	55.5 (6.34)
SpO_2 (%)	Group 1	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)
	Group 2	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)

The values represent the median (inter-quartile range) (n = 9).
Variables were measured before induction of anesthesia (pre) and at 15, 30, 45, 60, and 90 minutes after induction of anesthesia.

Table 2. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) data

	Group	Before induction of anesthesia	Before gastrotomy	After gastrotomy	End of the surgery
SOD(U/ml)	Group 1	2.6 (0.4)	2.5 (0.6)	1.2 (0.8)*	1.1 (0.3)*
	Group 2	2.5 (0.5)	2.5 (0.6)	2.3 (0.5)**	2.3 (0.3)**
CAT (U/ml)	Group 1	63.5 (8.2)	63.0 (7.2)	36.8(6.7)*	37.6 (5.7)*
	Group 2	62.3 (6.4)	62.1 (6.6)	62.1 (6.6)**	57.8 (7.03)**
GPx (nmol/min/ml)	Group 1	72.7 (8.4)	72.5 (8.3)	53.6 (8.8)*	53.5 (9.5)*
	Group 2	71.4 (7.6)	71.2 (7.0)	71.2 (6.0)**	70.9 (8.5)**

The values represent the median (inter-quartile range).
*Statistically difference compared to "before induction of anesthesia." (n = 9).
**Statistically difference compared to group 1 (n = 9).

minutes) and 46 minutes (ranging from 32 minutes to 53 minutes), respectively. The differences in the respective mean operation times of the 2 groups were not statistically significant. All pigs were hemodynamically stable during the experiments. There were no significant worsening in heart rate, mean arterial pressure, end-tidal carbon dioxide, peripheral oxygen saturation, and respiratory rates (Table 1).

SOD, CAT, and GPx data are summarized in Table 2. The SOD, CAT, and GPx activities of the group 1 were significantly lower at the end of surgery than at induction of anesthesia, while that of the group 2 maintained their baseline values. In addition, there were significant differences in all enzymes activities between groups at the end of surgery.

Discussion

Trauma and surgical injury are associated with increased production of reactive oxygen species (ROS), and the use of antioxidant system, in particular when associated with relative tissue ischemia followed by reperfusion, may inhibit ROS production. In the present study, oxidative stress was multifactorial in origin; the main impacts were from the surgical trauma of both laparotomy and gastrotomy, and from ischemia-reperfusion events due to visceral organ manipulation and anesthesia. A recent study reported that surgical trauma alone raises oxidative stress (8). In a similar manner, in this study, the activities of antioxidant enzyme were decreased after surgery.

Blood contains many antioxidant molecules that prevent and inhibit harmful effects of ROS. The 3 main enzymes that control the biological effects of ROS are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (9). These enzymes are important antioxidant defenses. They are involved in the clearance of superoxide and H_2O_2 to maintain the structure and function of biological membranes. SOD causes the dismutation of superoxide into oxygen and hydrogen peroxide, and these compounds are catabolized by catalase and GPx. In higher organisms, GPx appears to have largely supplanted the need for catalase membranes (9). The activities of these enzymes increased to eliminate free radicals as a result of the elevation in oxidative stress. Under oxidative stress conditions, the consumption of these enzymes is increased. In this study, lower activities of SOD, CAT, and Gpx at the end of surgery may be responsible for wasting SOD, CAT, and GPx to eliminate free radicals produced during surgery and anesthesia. Therefore, the measurement of antioxidant enzymes activities reflects the antioxidative status on biological systems.

In the current study, the antioxidant enzyme activities were significantly lower than preoperative value after surgery in group 1. Although, volatile agents are frequently used in general anesthesia practice without complications, the result of this study showed that isoflurane are negative effects on oxidative stress and antioxidant system during surgery. On the other hand, systemic antioxidant activities were not altered in group 2. Thus, our findings support that anesthesia conducted with propofol attenuated the oxidative stress caused by anesthesia or surgical trauma and enhanced antioxidant defense against ROS. However, there are a few limitations to this study that deserve consideration. The lack of effects of anesthesia only, correlation with surgery or anesthesia, and the ability of the antioxidant effects of a single agent means that we cannot be certain oxidative stress by anesthesia and surgery.

Propofol is an excellent free radical scavenger that has been shown to enhance the antioxidative ability of various tissues in vivo studies (7). In addition, other studies have found that propofol increased glutathione activity in male rats and in platelets from surgical patients (3,4). The mechanism for these propofol effects is not clear, but the protective effect of propofol on oxidative stress could be mainly attributable to this antioxidant property. Our findings suggest that propofol at 8 mg/kg/h CRI is a promising anesthetic for the prevention of oxidative stress due to surgical trauma in pigs. However, it should be noted that this study may have limitations in the dose response of propofol. Further studies should be conducted to investigate the propofol dose response relationship with oxidative stress on this and other animals models, which will provide more convincing evidence of a cause-effect relationship.

In conclusion, the present results indicate that administra-

tion of propofol attenuates decrease of SOD, CAT and GPx activities and this may be attributable to its antioxidant property in the antioxidant defense pathway. This finding may be worthy of further clinical study in situations where propofol is an option for anesthesia and surgery in patients with ischemic processes.

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성장 돼지 적혈구에서의 항산화 효소 활성도에 대한 propofol 과 isoflurane 병용 투여의 영향

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요 약 : 본 연구에서는 돼지에서 수술 시 propofol 및 isoflurane 투여가 생체내 항산화효소 활성도에 미치는 영향을 연구하였다. 실험동물은 수술에 사용되는 마취 종류에 따라 isoflurane 그룹 (group 1; 100% 산소 및 2-2.5% isoflurane 투여)과 isoflurane-propofol 그룹 (group 2; 8 mg/kg/h propofol 정맥 투여, 100% 산소 및 0.5-1% isoflurane 투여)으로 나누었다. 그룹 1에서는 마취 전과 비교 시 수술 후 생체내 Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) 활성도가 유의적으로 낮아졌으나 그룹 2에서는 마취 전 수준을 유지하였다. 또한 모든 효소 수치에서 군간 비교 시 유의성 있는 변화가 관찰되었다. 본 연구 결과를 통해 propofol의 투여가 돼지에서 마취 및 수술 중 항산화 능력을 유지 할 수 있음을 확인 할 수 있었다.

주요어 : 항산화 효과, isoflurane, 산화 스트레스, 돼지, propofol