The Anti-Oxidant Effects of Sevoflurane Anaesthesia and Surgery: A Preliminary Study


This study was designed to assess the influence of sevoflurane and surgery on the anti-oxidant balance in humans. Fifteen adult Class I/II of the American Society of Anaesthesiologists (ASA) patients scheduled for inguinal hernia repair under sevoflurane induction followed by sufentanil-vecuronium supplementation were recruited. Anti-oxidant biomarker malondialdehyde (MDA) and anti-oxidant biomarkers [superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and total anti-oxidant status (TAO)] in plasma were measured. Mean values at various sampling times were compared to baseline values and showed a significant reduction in mean SOD 60 min post-induction, while at 24 h post-operatively showed a significant rise. Following induction of anaesthesia and throughout surgery, mean GSHPx increased significantly at 10 min and at end of anaesthesia. To the contrary, by 24 h post-operatively, it dropped significantly. Mean TAO showed a significant elevation at all times except at end of surgery. MDA was significantly reduced at end of surgery and at 24 h. The results of this study support the anti-oxidant property of sevoflurane anaesthesia in humans and describe the impact of anaesthesia and surgery on the measured biomarkers. Further studies are required to confirm the anti-oxidant properties of sevoflurane anaesthesia with and without surgery for a longer period of time and at different sevoflurane concentrations during various types of surgery with more emphasis on the time course of these changes during the post-operative period.

Key words: Sevoflurane, oxidative status, malondialdehyde, superoxide dismutase, glutathione peroxidase, total anti-oxidant status
INTRODUCTION

Oxidative stress is a condition in which oxidants overwhelm anti-oxidant defences (Minghetti et al., 2006). Indeed, tissue injury, however caused, almost certainly leads to oxidative stress which if unopposed may then contribute significantly to worsening of the tissue injury (Halliwell, 2007). The potential of volatile anaesthetics to induce, augment or protect against oxidant mediated cell damage has gained a lot of interest lately. These effects are of particular importance in certain surgical procedures where ischemia-reperfusion injury may occur, as in cardiopulmonary bypass (CPB), organ transplantation or in patients with severely compromised liver, kidney or heart function.

Free radicals, induced by several diseases, trigger oxidative stress, leading to the production of malondialdehyde (MDA) and protein carbonyl content (PCC) (Sivaci et al., 2006). Because of the serious damaging potential of Reactive Oxygen Species (ROS), cells depend on a wide spectrum of enzymatic and non-enzymatic anti-oxidant protective mechanisms (Jaeschke, 1995). The primary intracellular defence mechanisms against oxidative injury include superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPx) (Remacle et al., 1992).

Laboratory assessment of oxygen-derived free radicals is very difficult, because they are highly reactive and hence their short half-life. Thus, most researchers have relied on indirect measurements of free radical reactions by measuring their end products, principally lipid peroxidation products such as malondialdehyde (MDA) and protein carbonyl content (PCC), as indicators of the occurrence of free radicals reactions (Sivaci et al., 2006; Krinsky, 1992).

On the other hand, the evaluation of the total reducing (anti-oxidant) capacity of biological fluids such as serum or plasma may provide a better estimation of the peripheral resistance to oxidant injury than the measurement of a set of individual anti-oxidant species at various tissues. The anti-oxidant activity measured by the total anti-oxidant (TAO) assay in plasma, which has been made available recently, is a reflection of the net result of the contribution of all the individual anti-oxidants defence mechanisms and their interactions against oxidative load (Minghetti et al., 2006).

Since anti-oxidant capacity is the end result of a dynamic multiple enzyme activities, evaluation of variations in blood levels of an individual anti-oxidant biomarker may not fully reflect the overall capacity of the body to handle oxidant conditions. Therefore, no single measurement of anti-oxidant agent is sufficient to assess the anti-oxidant status, but a battery of measurements, including MDA, SOD, GSHPx and TAO, is necessary to adequately assess oxidative stress in biological systems (Prior and Cao, 1999).

While isoflurane and halothane were shown to increase the susceptibility of cells to oxidant damage (Shayevitz et al., 1991), desflurane was shown to increase the extent of oxidative status, i.e., it is considered as pro-oxidant agent (Sivaci et al., 2006). The impact of sevoflurane anaesthesia on the oxidative-anti-oxidative balance is controversial. While some investigators reported lack of influence of sevoflurane on oxidative stress and anti-oxidant mechanisms (Allaouchiche et al., 2001; Sivaci et al., 2006), others demonstrated that sevoflurane can cause ROS formation (Kevin et al., 2003; Yoshida and Okabe, 1992) and induce oxidative stress in cardiac tissue (Özer and Kaman, 2007), whereas others have demonstrated the anti-oxidant properties of sevoflurane (Kudo et al., 2001; De Ruiter et al., 2003; De Hert et al., 2005; Bouwman et al., 2006). Most, if not all of these investigations, were performed on tissue preparations (Kudo et al., 2001) or anaesthetized animals (Allaouchiche et al., 2001). They mostly examined outcome measures such as organ function, or measurement of indirect indicators of oxidative reactions at a specific tissue. Reports on the effect of sevoflurane on the oxidative status in anaesthetized human subjects are scarce. To present knowledge, there is only one report on the effect of sevoflurane anaesthesia on the human blood levels of biomarkers of oxidative reactions and anti-oxidant defence systems (Sivaci et al., 2006).

This prospective study was designed to assess the effect of sevoflurane anaesthesia with and without surgery the oxidative status in humans, by measuring the plasma levels of MDA (oxidant biomarker), SOD, GSHPx and TAO (anti-oxidant biomarkers) during the intra-operative and immediate post-operative period.

MATERIALS AND METHODS

After Ethics Committee approval and informed patients' consent were obtained, this study was conducted on 15 adult patients (ASA I-II) scheduled for body surface surgery, e.g., inguinal herniorrhaphy at the Theodor Bilharz Research Institute between March 2007 to November 2007. Patients were not included if they were pregnant, lactating, menstruating, underweight, obese, at extremes of age, smokers, drug abusers, ASA III or more, on medications or multivitamins supplementation, or if they had malignancy, fever, sepsis or a known systemic disease.
Standard monitors including electrocardiogram, non-invasive blood pressure monitor and pulse oximeter using a modular monitor (Athena 9050, S and W Medico Teknik A/S, Denmark) were instituted prior to anaesthesia induction. Peripheral nerve stimulator (TOF-Guard INMT Organon Teknika NV- Belgium), end-tidal carbon dioxide concentration (Ohmeda-520 Capnography Monitor, Ohmeda BOC Health Care, Louisville CO, USA) were instituted after induction of anaesthesia. Baseline readings of HR, NIBP and SpO₂ were recorded before induction, at intubation, every 5 min onwards during the surgical procedure and at 24 h post-operatively.

Sedative premedication was omitted. Two 18-gauge Teflon venous cannulae were inserted under local anaesthetic in either arm. One was used for venous sampling and the other for drug and fluid administration.

Prior to induction, 500 mL of Lactated Ringer’s solution was infused to compensate for overnight fluid deficit. Pre-oxygenation was started for 3 min before the induction of anaesthesia.

Volatile Induction and Maintenance of Anaesthesia (VIMA) using sevoflurane (Sevoflurane, Abbot Laboratories Ltd. Queen borough) was used. Patients were induced using a face mask and a circle system with Sevoflurane (8%) at 6 L min⁻¹ initially. After loss of lash reflex, the fresh gas flow was reduced to 3 L min⁻¹ and sevoflurane concentration was reduced to 3% in 40% oxygen in air for 15 min. Then, vecuronium (Norcuron, N.V. Organon OSS, Holland), (0.1 mg kg⁻¹) was administered to all patients intravenously. Three minutes later tracheal intubation was performed. Controlled ventilation was started using an electronic ventilator (Fabius GS, Dräger Medical Corporation-Germany) and adjusted to maintain normocapnia.

Sevoflurane concentration was kept 2-3% in oxygen-air mixture (FiO₂ 40%) throughout the whole procedure. Increments of Vecuronium (0.03 mg kg⁻¹) were administered as required. Lactated Ringer solution was infused to maintain haemodynamic stability. Residual curarization was reversed with prostigmine (40 μg kg⁻¹) and atropine sulphate (20 μg kg⁻¹) at the end of surgery followed by suctioning and extubation. Post-operative analgesia consisted of morphine 10 mg IM/4 h as required.

**Sampling and preparation of samples:** Five venous blood samples (10 mL each) were taken from each patient before induction, 10 min post-induction, 1 h after induction, at the end of surgery and 24 h post-operatively. Five milliliter from each sample were added to a heparinized glass tube while the remaining amount was added to an ordinary tube. The heparinized samples were later centrifuged and the separated plasma samples were used for the measurement of SOD and GSPHx. The non-heparinized samples were divided into two portions. The first portion was centrifuged at 3000xG at 4°C for 10 min and the separated serum samples were immediately frozen at -70°C until the time of measurement of MDA. The second portion of the non-heparinized samples was centrifuged and the separated sera were stored at 2-8°C until the time of measurement of TAO.

**Determination of superoxide dismutase (SOD):** Heparinized plasma samples prepared as mentioned above. SOD measurements in the prepared plasma were measured using RANSOD SD-125 reagent (Randox Laboratories Ltd.). Reference range: 164-240 U mL⁻¹.

**Determination of glutathione peroxidase (GSPHx):** Heparinized plasma samples prepared as mentioned above. The method used is that recommended by RANSEL RS-505 (Randox Laboratories Ltd.) and double strength Drabkin's reagents (Randox Laboratories Ltd. Cat. No: MS-181). Reference range: 4171-10881 U L⁻¹.

**Determination of total anti-oxidants (TAO):** Non-heparinized sera prepared as mentioned above were used. Measurement of TAO was performed by testing sera using RANDOX Total Anti-oxidant Status Kits (TAS) NX-2332 Reagent (Randox Laboratories Ltd. ABTS®). Reference range: 1.30-1.77 mmol L⁻¹.

**Determination of malondialdehyde (MDA):** Sample preparation: non-heparinized sera prepared as mentioned above were used.

**Assay principle:** 0.2 mL of the serum is tested using the Colorimetric Assay for Lipid Peroxidation (OxisResearch TM BIOXYTECH® USA-LPO-586™). Reference range: 0.2 μmol L⁻¹ human plasma.

**Statistical analysis:** Data are expressed as Mean±SD and confidence interval CI 95%. Blood levels of the measured biomarkers were compared to baseline using ANOVA for repeated measures using the commercial SPSS statistical program (Version 10). A p-value<0.05 was considered significant.

**RESULTS**

Demographic data of the patients, ASA status and duration of surgery are shown in Table 1.

The mean SOD 10 min following induction did not change significantly compared to the baseline value (Fig. 1). However, at 60 min from the start of anaesthesia,
Table 1: Demographic data, ASA classification of the patients and duration of surgery

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>32.73±3.37</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.67±5.07</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.9±5.31</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>7/8</td>
</tr>
<tr>
<td>ASA I/II ratio</td>
<td>14/1</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>112.0±10.6</td>
</tr>
</tbody>
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Data are expressed as Mean±SD, M = Male, F = Female.

Fig. 1: Effects of sevoflurane anaesthesia on mean SOD (U mL⁻¹). Data are presented as Mean±SD. *p<0.05 as compared to baseline. **p<0.001 compared to baseline.

Fig. 2: Effects of sevoflurane anaesthesia on mean GSHPx (U mL⁻¹). Values are expressed as Mean±SD. *p<0.05 compared to baseline.

The mean SOD level was significantly reduced compared to baseline value only to return to baseline value by the end of surgery. At 24 h post-operatively, the mean SOD showed a significant rise compared to baseline value (Fig. 1).

Following induction of anaesthesia and throughout surgery, the GSHPx level increased significantly compared to baseline value, except at 60 min into surgery when the increase did not reach statistical significance (Fig. 2). To the contrary, by 24 h post-operatively, the mean GSHPx dropped significantly as compared to baseline value (Fig. 2).

During the first hour of surgery, the mean TAO values were consistently elevated compared to baseline value. By the end of surgery, the TAO level started to decline insignificantly. However, after 24 h post-operatively, the TAO level was significantly increased compared to baseline (Fig. 3).

Fig. 3: Effects of sevoflurane anaesthesia on mean total anti-oxidant TAO (mmol L⁻¹). Values are expressed as Mean±SD. **p<0.01, *p<0.05 compared to baseline.

Fig. 4: Effects of sevoflurane anaesthesia on MDA (μmol L⁻¹). Values are expressed as Mean±SD. *p<0.05 compared to baseline.

Following induction of anaesthesia and during the first hour of surgery, the mean MDA did not change significantly (Fig. 4). By the end of anaesthesia and surgery, it dropped significantly compared to baseline value. By 24 h post-operatively, it increased towards baseline value but the rise was not statistically significant compared to the end of surgery value instead, it was still significantly below baseline value.
Fig. 5: Changes in the heart rate oxygen saturation (SpO2) arterial blood pressure. Values are expressed as Mean±SD. *p<0.05 compared to baseline value

The heart rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP) followed the same pattern where they both significantly dropped (p<0.05) following anaesthesia induction and remained significantly reduced throughout the surgical procedures (Fig. 5). They all returned to baseline values at the end of anaesthesia and surgery without significant changes at 24 h post-operatively. None of the patients developed cardiovascular instability requiring pharmacological interference. There were no statistically significant changes in the oxygen saturation throughout the study period.

**DISCUSSION**

The results of this study show an overall positive anti-oxidant effect for sevoflurane anaesthesia as denoted by alterations in plasma level of oxidant/anti-oxidant status biomarkers.

During the sevoflurane anaesthesia phase of this study, the GSHPx and TAO levels increased while the SOD and MDA levels remained the same. This show the overall anti-oxidant properties of sevoflurane anaesthesia.

When surgery started and analgesia and muscle relaxants were administered during the operative phase of this study, the initial reduction in SOD level may represent the increasing depth of anaesthesia and analgesia associated with analgesia and muscle relaxants administration. In the meantime, GSHPx and TAO remained high suggesting that the surgical trauma balanced and prevented further increases in their levels due to continuing sevoflurane anaesthesia. As sevoflurane and analgesia lightens towards the end of the surgical phase, the SOD level increased, while the GSHPx and TAO remained higher than baseline values suggesting that the response to the surgical trauma is taking the upper hand in the body's oxidative balance status. In the meantime, the reduction in MDA towards the end of anaesthesia and surgery is probably a delayed response to the increased anti-oxidant status in the earlier 2 h.

During the immediate post-operative (Surgical trauma perse) phase, the very high increases in SOD levels explains the consumption of the elevated GSHPx to below baseline value and the reduction of the elevated TAO levels towards baseline. The end result is gradual rise of reduced MDA towards baseline value.

Although both SOD and GSHPx are members of the anti-oxidant system, this study revealed that they moved in a reciprocal manner rather than parallel direction as would probably be expected (Fig. 1, 2). This observation could be explained by the fact that the SOD enzyme transforms superoxide anion radical (O2−) into hydrogen peroxide, which in turn is broken down into harmless products by catalase and GSHPx enzymes (Allaouchiche et al., 2001; Özer and Kaman, 2007). This implies that the rise in SOD level leads to an increased load on the GSHPx system leading to consumption of the GSHPx enzyme. This observation show the cascade nature of the anti-oxidant defence reactions (Allaouchiche et al., 2001; Özer and Kaman, 2007).

Present results agree with Kudo et al. (2001), who on examining the anti-oxidant effect of several volatile anaesthetics, including sevoflurane, on neuronal glial tissue cultures, concluded that they exhibit anti-oxidant protective effect and reduce oxidative stress induced cell death by inhibition of iron uptake.

Present results are also in agreement with results of several other investigators who have demonstrated the anti-oxidant properties of sevoflurane in the form of protective effects against ischemia-reperfusion injury during cardiac surgery due to anaesthetic preconditioning effect (De Ruijter et al., 2003; De Hert et al., 2005; Bouwman et al., 2006).

Present results seem to differ from those reported by Sivaci et al. (2006), who investigated the effect of sevoflurane on malondialdehyde (MDA) and protein carbonyl content (PCC) production during laparoscopic abdominal surgery and concluded a lack of significant influence on oxidative stress and anti-oxidant mechanics with sevoflurane anaesthesia. This discrepancy could be explained by the timing of sampling since they looked at the biomarkers levels at 6 and 24 h post-operatively rather than during sevoflurane anaesthesia. Moreover, they only assessed PCC and MDA which are indirect indicators of oxidative reactions without measuring concomitant
changes in any anti-oxidant biomarkers. Another possible explanation for such discrepancy is the difference in the type of surgery performed (open versus laparoscopic surgery) since open surgery, as in present case, may cause more oxidative load which in turn may stimulate the body's anti-oxidant defence activity.

Present findings also differ from some of the previously performed animal in vivo and in vitro studies that reported either lack of effect on the oxidative status (Allaouchiche et al., 2001) or actually inducing a state of oxidative stress on the myocardium (Özer and Kaman, 2007), by sevoflurane anaesthesia. These discrepancies could be explained by the differences in species (swine and rats versus human) or study design, i.e., use of nitrous oxide (Özer and Kaman, 2007) and lack of surgical stimulus (Allaouchiche et al., 2001; Özer and Kaman, 2007). Moreover, Özer and Kaman (2007) measured biomarkers only in the myocardial tissue rather than the blood.

It is noteworthy, that sedative premedication and intravenous induction agents were avoided in this study, to eliminate their potential influence on the oxidative status (Tsuchiya et al., 2001; Almas et al., 2000). Similarly, this study was confined to body surface surgeries, in order to circumvent major events such as blood loss and hypothermia associated with major surgery that may affect the oxidative status and would make interpretation of the results more difficult.

Although some of the cardiovascular changes observed during this study were statistically significant, none of the patients required pharmacological treatment apart from adjustment of the inhaled sevoflurane concentration and increasing the rate of fluid administration.

CONCLUSION

Present results imply an anti-oxidant property of sevoflurane anaesthesia, in the clinical context, as shown by alterations in plasma level of antioxidant/anti-oxidant status biomarkers, SOD, GSHPx, TAO and MDA in blood especially at the anaesthesia perse phase of this study. Further studies are required to confirm the anti-oxidant properties of sevoflurane anaesthesia with and without various types of surgery, for longer periods of time, at different sevoflurane concentrations and with more emphasis on its time course during the post-operative period. This anti-oxidant property of sevoflurane anaesthesia may influence the choice of anaesthetic agents for patients suffering from, or susceptible to, perioperative oxidative stress or reperfusion injury.

REFERENCES


