Background: We previously demonstrated that propofol had a protective effect on the intestine at risk after an ischemic injury in a rat model. The current study tested the hypothesis that such beneficial effects, so far mainly seen in the laboratory, are reproducible in humans.

Methods: Seventy patients undergoing elective open infrarenal abdominal aortic aneurysm repair were randomized to receive general anesthesia with either propofol or sevoflurane. General anesthesia was induced with 3 µg/kg fentanyl, 0.2 mg/kg cisatracurium, and target-controlled infusion of propofol, set at a plasma target concentration of 4-6 µg/ml, or sevoflurane initially started at 8%. Anesthesia was maintained with target-controlled infusion of propofol (2-4 µg/ml) or sevoflurane (0.8-1.0 minimum alveolar concentration). Except for this, anesthetic and surgical management was the same in both groups. Serum intestinal fatty acid binding protein was taken as the primary outcome for evaluating intestinal injury. The secondary outcomes included other intestinal injury variables (serum endotoxin levels, serum diamine oxidase activity and the score of intestinal injury severity) as well as markers reflecting oxidative stress and systemic inflammatory response.

Results: The levels of biomarkers reflecting intestinal injury were higher than baseline in both groups (all P<0.05). However, in sevoflurane-anesthetized patients, these variables increased significantly more than those in patients anesthetized with propofol (all P<0.05), whereas the scores of the intestinal injury severity didn’t differ between two groups (P=0.53).

Conclusions: Patients receiving propofol for abdominal aortic aneurysm repair surgery had less intestinal injury than patients receiving sevoflurane, which may be related to its better anti-oxidative property.
Intestinal ischemia/reperfusion (I/R) injury may occur after acute mesenteric ischemia, traumatic or septic shock, hemorrhagic or some surgical procedures including small bowel transplantation and abdominal aortic surgery (1). It not only leads to injury of the intestine itself, but also subsequently results in the translocations of bacteria and endotoxin across intestinal mucosal barrier, leading to the systemic releases of reactive oxygen species (ROS) and inflammatory cytokines with subsequent multiple organ dysfunction syndrome (MODS) (2, 3).

Propofol is an intravenous anesthetic with antioxidant properties that is commonly used for the induction and maintenance of anesthesia during surgery and for postoperative sedation in intensive care unit (ICU) (4-8). We have recently demonstrated that pretreatment with propofol attenuates intestinal mucosal injury induced by intestinal I/R in rats, which is attributable to its antioxidant properties and the effects in inhibiting over-production of nitric oxide and decreasing endothelin-1 level (9). Further, we found that the intestinal protective effect of propofol involves the attenuation of intestinal I/R-induced intestinal epithelial apoptosis via modulating the ceramide signaling pathway (10). However, whether such beneficial effects, so far mainly seen in the laboratory, are reproducible in human remains unclear.

Open abdominal aortic aneurysm (AAA) repair still is a good alternative for some special patients. However, it is known that open AAA repair causes significant morbidity and mortality and postoperative MODS is a major cause (11, 12). Since the procedure usually involves clamping and unclamping of aorta and inferior mesenteric artery, it is postulated that I/R injury of the intestine could be the leading cause for MODS (13, 14).

Thus, we hypothesized that the intestinal protective effect of propofol seen in the laboratory is reproducible in humans and thereby selected patients undergoing elective open infrarenal AAA repair as a clinical model of intestinal I/R injury to investigate the intestinal protective potential of propofol. Meanwhile, sevoflurane, a commonly used halogenated volatile anesthetic which has been demonstrated to induce myocardial protection (15, 16), was taken as a control to determine which anesthetic regimen would lead to a better protection against intestinal I/R injury induced by open infrarenal AAA repair.

Intestinal protection was assessed by the serum level of intestinal fatty acid binding protein (I-FABP), a sensitive marker of early intestinal ischemia (17), which was considered the primary outcome variable, endotoxin level and the diamine oxidase (DAO) activity in serum as well as a modified intestinal dysfunction score based on previously described methods (18). Markers of systemic inflammation and oxidative stress were determined as well.

MATERIALS AND METHODS

Protocols and Ethics

The study protocol was conducted in accordance with the Helsinki declaration (2008) and approved by the Research Ethics Committee of the First Affiliated Hospital, Sun Yat-sen University, China.

Before the trial, enrolled patients were randomized into a sevoflurane group or a propofol group. Randomized treatment allocations with no further stratification were generated using a computer random number generator with a 1:1 allocation using blocks of varying sizes. Allocation details were placed in sequentially numbered sealed envelopes. To standardize technical skills and experience, every case was performed by the same anesthesiologists and surgeons. Treatment allocation was revealed by anesthesiologists on the morning of surgery and supervised by an independent statistician. None of the anesthesiologists participated in the data assessment or analysis. Patients, investigators, surgeons, critical care teams and individuals participating in data analysis were all blind to group allocation. The trial was monitored by an independent data and safety monitoring board. Group allocation was not revealed until the final statistical analysis was completed.

Patients

A prospective, single-blind, randomized controlled trial following the CONSORT statement was conducted on patients undergoing elective open infrarenal AAA repair. Written informed consent was obtained from each participant. In-
clusion criteria were age greater than 18 years, elective open infrarenal AAA repair surgery and American Society of Anesthesiologists (ASA) physical status III or less. Exclusion criteria included patients older than 80 years, a history of allergy to propofol, previous cardiac surgery, combined surgery, myocardial infarction within the previous 6 weeks, severe chronic obstructive pulmonary disease (forced expiratory volume in 1 second, FEV1<50%), severe hepatic disease (alanine aminotransferase or aspartate aminotransferase >150 U/L), renal failure (creatinine concentration >150 µmol/L), preoperative antioxidant therapy and pregnancy, history of inflammatory bowel disease, history of diarrhea (≥ 2 liquid stools/day for ≥ 2 days) within 1 week of surgery and intestinal chronic inflammatory disease. Additionally, Glasgow Aneurysm Score was assessed for each patient before operation in order to ensure that operative risk between groups was similar (19).

Anesthesia and Surgical Procedure
No premedication was given before anesthesia. Routine monitoring for open infrarenal AAA repair surgery included electrocardiograph (ECG), pulse oximetry, capnography, temperature, arterial blood pressure, central venous pressure (CVP) and urine output.

All patients underwent general anesthesia combined with epidural anesthesia. Before induction of general anesthesia, a thoracic epidural catheter was inserted between the T12-L1 or L1-L2 level using a midline or paramedian approach in all patients, through which a test dose of 5 ml of 1% lidocaine was given. After 15 minutes, the quality of the epidural analgesia was assessed using cold discrimination. If pain relief was inadequate, the position of the epidural catheter was adjusted or a new catheter was placed if necessary. The anesthesia staff was instructed not to use the epidural catheters intraoperatively.

In the sevoflurane group, anesthesia was induced with sevoflurane (Sevorane®, Abbott Scandinavia AB, Solna, Sweden) initially started at 8% by deep breathing, followed by 0.2 mg/kg cisatracurium and 3 µg/kg fentanyl. Anesthesia was maintained with sevoflurane 0.8-1.0 minimum alveolar concentration and remifentanil 0.2-0.4 µg/kg/minute. In the propofol group, general anesthesia was induced with a target-controlled infusion of propofol (Diprifusor; AstraZeneca S.p.A, Milan, Italy), set at a plasma target concentration of 3-5 µg/ml, followed by 0.2 mg/kg cisatracurium and 3 µg/kg fentanyl. Anesthesia was maintained with propofol at target concentrations of 2-4 µg/ml and remifentanil 0.2-0.4 µg/kg/minute. Depth of anesthesia was determined with the bispectral index (BIS) (BIS Vista, Aspect Medical Systems, Norwood, MA, USA) with a target range between 40 and 50 during surgery. Cisatracurium was administered for further muscle relaxation, as clinically indicated in two groups. Patients were intubated with a 7.5 mm cuffed endotracheal tube and the ventilation parameters were standardized (respiratory rate 12-15 breaths/minute, tidal volume 8-10 ml/kg, fraction of inspired oxygen 1.0) to achieve 35-45 mm Hg of end-tidal carbon dioxide concentration (ETCO₂). Standardized fluid replacement consisted of 10 ml/kg lactated Ringer’s solution applied preoperatively and 6 ml/kg/hour of the solution applied perioperatively. Colloid was given to obtain a stable heart rate, CVP of 8-10 cm H₂O, a steady mean arterial pressure (MAP) and a urine output >1 ml/kg/hour. Packed red cells were transfused as necessary to maintain a circulating hemoglobin level approximately 8 g/dl. Hemodynamic management was standardized to maintain a steady MAP by inotropic and vasodilator therapy.

Postoperative Management
At the end of surgery, the patients were routine-ly transferred to ICU, and all patients received the same routine postoperative care. The epidural catheter infusions were begun immediately upon entry to ICU. The loading dose was 6 ml of 0.25% bupivacaine with 2 mg morphine and during the first 3 postoperative days, all patients received epidural analgesia with a mixture of bupivacaine 0.125% and 0.1 mg/ml morphine with a basal rate of 2 ml/hour, bolus doses of 3-5 ml, and a lockout interval of 20 minutes. To make sure that those patients had a working epidural analgesia, postoperative pain was assessed at rest and movement during postoperative days 1, 2, and 3 by using the visual analog scale rating from 0 (no pain at all) to 10 (worst possible
pain). In ICU, crystalloid fluid replacement was infused at 2 ml/kg/hour to maintain a stable heart rate, CVP of 8-10 cm H2O and a steady MAP. Also, blood was given to maintain hemoglobin more than 8 g/dl. Extubation was managed according to the standard ICU protocols by the ICU staff.

The Preparation of Blood Sampling
Blood samples were collected for analysis at the following time points: before the start of surgery (baseline), 30 minutes, 4, 8, 12, and 24 hours after cross-clamping release (reperfusion). Venous blood was sampled from the jugular venous line and centrifuged at 2700 rpm for 15 minutes. Serum samples were stored at -70°C for subsequent analysis. Radial arterial blood was analyzed using a GEM premier 3000 blood gas system (GEM Premier 3000, Instrumentation Laboratory, Bedford, MA, USA).

Assessment of Intestinal Injury
Intestinal injury was assessed by measuring the serum concentrations of I-FABP and endotoxin, as well as the activity of DAO at predetermined time points. The concentration of I-FABP was measured by enzyme-linked immunosorbent assay according to the instruction manual (ADL Co, Mukwonago, WI, USA). The concentration of endotoxin was determined using a quantitative Limulus amoebocyte lysate (LAL) chromogenic assay (Ruicheng Bio-engineering Research Institute, Shanghai, China). DAO activity was assessed using a sandwich enzyme-linked immunoassay with a commercially available kit (HuiJia Bio-engineering Research Institute, Xiamen, China).

To evaluate the patients’ intestinal function after aneurysm repair, a modified intestinal dysfunction score based on previously described methods (19) was used. Since patients usually start enteral feeding from the third day after AAA surgery in our center, the recording of intestinal injury score was initiated from 72 hours after operation.

Evaluation of Inflammatory Response and Oxidative Stress
The levels of the inflammatory cytokines including tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) were measured using a sandwich enzyme-linked immunoassay with a commercially available kit (jiancheng Bio-engineering Research Institute, Nanjing, China). The variables reflecting oxidative stress including malondialdehyde level and superoxide dismutase (SOD) activity in serum were analyzed using methods of thiobarbituric acid reaction and the generation of an artificial chromophore, respectively.

Statistical Analysis
Serum I-FABP concentration served as the primary outcome variable. Sample size was calculated based on differences in I-FABP concentration measured at 30 minutes after cross-clamping release in a pilot study of 10 patients who received either propofol anesthesia (204.0 ± 21.1 pg/ml) and sevoflurane anesthesia (230.1 ± 36.3 pg/ml). The formula \( N = \frac{15.7}{ES^2} + 1 \), wherein ES is the effect size = (difference between groups) / (mean of the standard deviation [SD] between groups), with \( \alpha = 0.05 \) and power=0.8 was used to determine that the study required N of 33 per group (20). However, in order to compensate for a 10% of drop-out rate during study period, 70 patients were recruited in this study.

Continuous data were expressed as means ± SD, or median (25% percentile, 75% percentile) of patients and compared with independent t test or Mann-Whitney U test, respectively. Categorical data were expressed as frequency or percentage and compared with Fisher's exact test or the chi-square test where appropriate. The severity of intestinal injury was compared by the Mann-Whitney U test. The hemodynamic data and biochemical serum markers were analyzed using two-way repeated-measures Analysis of Variance (ANOVA) with Bonferroni correction for both within-groups and between group comparisons. A P-value of less than 0.05 was considered statistically significant. All P values were 2-sided and the statistical significant level was 0.05. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS
Between April 2010 and August 2012, 77 patients were assessed for eligibility, of whom 70 were actually recruited and randomized to propofol group (N=35) or sevoflurane group (N=...
There were no statistically significant differences between groups on either variable listed. Continuous data were reported as means (SD) or median (25% percentile, 75% percentile). Categorical data were given as counts (percentages). ACE, angiotensin-converting enzyme.

Figure 1. CONSORT Diagram Showing the Flow of Participants through Each Stage of the Randomized Trial.

Table 1. Preoperative and Intraoperative Characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Sevoflurane group (N=35)</th>
<th>Propofol group (N=35)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62 (7)</td>
<td>65 (8)</td>
<td>0.33</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68 (65, 72)</td>
<td>61 (58, 68)</td>
<td>0.27</td>
</tr>
<tr>
<td>Sex, males</td>
<td>31 (89%)</td>
<td>33 (94%)</td>
<td>0.67</td>
</tr>
<tr>
<td>Smoking, N (%)</td>
<td></td>
<td></td>
<td>0.47</td>
</tr>
<tr>
<td>Current smokers</td>
<td>9 (26%)</td>
<td>6 (17%)</td>
<td></td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>15 (43%)</td>
<td>20 (57%)</td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>11 (31%)</td>
<td>9 (26%)</td>
<td></td>
</tr>
<tr>
<td>Associated illness, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>26 (74%)</td>
<td>21 (60%)</td>
<td>0.32</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>12 (34%)</td>
<td>14 (40%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>6 (17%)</td>
<td>10 (29%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Glasgow Aneurysm score</td>
<td>64 (55, 72)</td>
<td>65 (54, 70)</td>
<td>0.88</td>
</tr>
<tr>
<td>Preoperative medications, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-blockers</td>
<td>17 (49%)</td>
<td>21 (60%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Calcium channel antagonist</td>
<td>9 (26%)</td>
<td>7 (20%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Statin</td>
<td>16 (46%)</td>
<td>18 (51%)</td>
<td>0.81</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>11 (31%)</td>
<td>8 (23%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Duration of anesthetic (minute)</td>
<td>287 (32)</td>
<td>269 (35)</td>
<td>0.64</td>
</tr>
<tr>
<td>Cross-Clamp time (minute)</td>
<td>60 (8)</td>
<td>55 (11)</td>
<td>0.23</td>
</tr>
<tr>
<td>Aneurysm diameter (mm)</td>
<td>73 (9)</td>
<td>68 (11)</td>
<td>0.59</td>
</tr>
<tr>
<td>Median operating time (min)</td>
<td>145 (21)</td>
<td>139 (19)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Intestinal Injury

As shown in figure 2A, I-FABP levels increased significantly in both groups 30 minutes after cross-clamp release, and remained high until the first postoperative day. The sevoflurane group had a higher serum I-FABP concentration at 30 minutes, 4 hours after cross-clamp release than the propofol group (all P<0.05). Likewise, serum endotoxin levels and DAO activity progressively and significantly increased in both groups, returning to basal values 24 hours after surgery. In propofol group, endotoxin levels and DAO activity remained constant and were significantly lower than those in the sevoflurane group (all P<0.01).

In addition, no difference of the scores of intestinal injury severity was observed between groups at 72 hours after surgery (P=0.53, Table 2).

Evaluation of Systemic Inflammatory Response and Lipid Peroxidation

Serum markers of systemic inflammatory in-
creased postoperatively with both anesthetics from comparable baseline values. As shown in figure 2D, the IL-6 levels peaked 12 hours after the release of cross-clamp in both groups. However, the level in sevoflurane group was higher than that in propofol group (P=0.02). Different from the changes of IL-6 levels, TNF-α level gradually increased over the whole observation period and sharply peaked 24 hours after the release of the cross-clamp. Likewise, TNF-α level in sevoflurane group was higher than that in propofol group (P=0.04, Figure 2E).

As shown in figure 2F, the serum malondialdehyde levels in both groups increased transiently in
Figure 2. Biomarkers of Intestinal Injury as well as Variables Reflecting Oxidative Stress and Inflammatory Response at Various Time Points in Patients Undergoing Open Infrarenal AAA Repair (N=35 for Each Group).

A. Serum I-FABP concentration; B. Serum endotoxin level; C. Serum DAO activity; D. IL-6 concentration; E. TNF-α concentration; F. Serum malondialdehyde concentration; G. SOD activity. Data were represented as means (SD). *P<0.05 versus baseline; #P<0.05 versus sevoflurane group.

AAA, abdominal aortic aneurysm; DAO, diamine oxidase; I-FABP, intestinal fatty acid binding protein; IL-6, interleukin-6; Rep, reperfusion; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α.

DISCUSSION

In this prospective, randomized, controlled trial, significant occurrence of intestinal injury in patients undergoing open infrarenal AAA repair was demonstrated by marked increases in the serum I-FABP and endotoxin levels and the serum DAO activity. Meanwhile, the results from this study also showed that the first 12 hours after the release of cross-clamp and returned to the baseline values 24 hours after reperfusion. Sevoflurane group demonstrated a significant increase in malondialdehyde level after reperfusion, whereas propofol significantly reduced the malondialdehyde levels in comparison to the sevoflurane group (P<0.01).

Figure 2G showed that in the propofol group, SOD activity increased significantly 8 hours after cross-clamp release (68.8 ± 12.2 U/ml vs. 149.9 ± 30.6 U/ml, P<0.05). However, SOD activity in sevoflurane group remained constant after the release of cross-clamp, and the activity of SOD was significantly different between groups (P<0.01).
investigation indicated that patients receiving propofol for elective open infrarenal AAA repair had less intestinal injury than patients receiving sevoflurane for the same intervention. This is inferred from significantly lower levels of the serum I-FABP, endotoxin and the DAO activity after cross-clamping release. Of note, the current data also showed that patients receiving propofol demonstrated a significantly shorter ventilator support time and ICU stay than those receiving sevoflurane. Nevertheless, the data presented herein were in good agreement with our recent studies in the laboratory which also reported intestinal protection by the application of propofol in rats undergoing superior mesenteric artery occlusion-induced intestinal I/R injury.

Previous studies have found that intestinal injury occurred following open AAA repair (21-23). The urine excretion ratio of lactulose and mannitol has previously been used to assess intestinal mucosal permeability of intestinal permeability in research, but it is not sensitive enough in critically ill patients (24). Different from the previous studies, three different markers which respectively reflect intestinal injury from different aspects, together with the intestinal dysfunction score were used in the present study to evaluate intestinal injury. I-FABP, being uniquely located at the tips of intestinal mucosal villi, is generally undetectable in the peripheral circulation, whereas it will increase rapidly when intestinal mucosal injury occurs. Therefore, I-FABP has been taken as a sensitive marker of early intestinal ischemia (17, 25). The serum endotoxin level was used to reflect the intestinal permeability in the present study. In addition, the marker for reflecting intestinal injury was serum DAO activity, which was also used as an index of small intestinal mucosal injury (26). In our study, the above three biomarkers increased significantly after the release of cross-clamp, which implied that the intestinal barrier function was disrupted after open AAA repair.

Inflammatory responses in the intestine were involved in the pathogenesis of gastrointestinal dysfunction. Cytokines and inflammatory mediators such as TNF-α and IL-6 could induce damages of microvilli, tight junction between enterocytes and paracellular junction, which would lead to increased intestinal permeability (34). In this study, propofol had better inhibitory effects on the releases of IL-6 and TNF-α, indicating that propofol has more protective effect on intestinal injury by inhibiting the proinflammatory mediators than sevoflurane.

As a commonly used halogenated volatile anesthetic, sevoflurane was documented to induce better organ protection than propofol (16, 35-37). But to our knowledge, the impact of commonly applied anesthetics such as sevoflurane and propofol on the intestinal I/R has not been measured in patients undergoing open AAA repair. In contrast, the findings herein showed
that patients receiving propofol for elective open AAA repair had less intestinal injury than patients receiving sevoflurane. That might be attributable to different clinical settings in which propofol and sevoflurane could have different performances. In addition, the pathogenesis between organs could exist due to different pathophysiological process.

Several potential limitations existed in the present study. Firstly, both propofol and sevoflurane were used as a part of a multidrug anesthetistic regimen. All patients received a continuous infusion of remifentanil throughout the operation. We cannot exclude the effect of remifentanil on intestinal injury, even if no studies previously showed its intestinal protective effect. Secondly, despite the power analysis to calculate the sample size, the effective group size was much smaller due to detect a significant difference in the scores of intestinal injury severity adverse between groups. Thirdly, propofol conferring better intestinal protection than sevoflurane could be related to the dosage of propofol or the concentration of sevoflurane we chose in this study.

Further studies will need to evaluate the question of a dose dependency of intestinal protective effects.

In conclusion, patients receiving propofol for elective open infrarenal AAA repair had significantly lower levels of biomarker reflecting intestinal injury than patients receiving sevoflurane for the same procedure. This study supported intestinal protective effects of propofol so far only documented in the laboratory and suggested that the choice of an anesthetic with respect to intestinal reperfusion injury might be of importance in the surgical patient population. The underlying mechanisms and the clinical implications of these beneficial effects remain subject to further investigation.

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The authors declare no other competing interests.

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