The Effect of Propofol on Oxidative Stress in Platelets from Surgical Patients

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We investigated the changes in oxidative stress in platelets from surgical patients anesthetized with propofol. We studied 60 surgical patients (ASA physical status I and II) and 12 healthy volunteers. The patients were divided into three groups: anesthesia induced with an IV bolus dose of 4 mg/kg thiopental; anesthesia induced with an IV bolus dose of 2 mg/kg propofol; and total IV anesthesia (induction with propofol 2 mg/kg, infusion with propofol 10 mg/kg during the first 10 min, then 8 mg/kg for 10 min, and 6 mg/kg during the rest of the operation). Healthy volunteers were given an IV bolus dose of 10% fat emulsion (Intralipid).

We measured the following variables in platelets: thiobarbituric acid reactive substances content, glutathione content, and glutathione peroxidase, reductase, and transferase activities. Thiopental did not modify any of the variables. Propofol decreased thiobarbituric acid reactive substances production by 25.7% and increased total glutathione content by 24.6%. The percentage of glutathione in oxidized form was 29.5% smaller in patients anesthetized with propofol. Glutathione peroxidase activity was 28.3% less, glutathione transferase was 44.5% more, and glutathione reductase was not significantly different. Intralipid had no effect on any of the variables. After infusion of propofol for 1 h, the effects were, in qualitative terms, the same as those seen after an initial bolus dose. In conclusion, our findings show that propofol has an antioxidant effect in humans. This effect may be beneficial in patients who have diseases in which free radicals play an important role. Implications: This study demonstrates that propofol inhibits cellular oxidative damage, measured in platelets from surgical patients. Neither thiopental nor the fat emulsion (Intralipid) showed any effect. Moreover, propofol increased the antioxidant defense of glutathione. This could be applied in the protection of tissues from ischemic damage.

Propofol has a structure (2,6-diisopropylphenol) similar to that of known antioxidants, such as tocopherol and butylhydroxytoluene. The ability of propofol to inhibit the formation of lipid peroxides has been found in several media in which free radicals are produced, e.g., liver and brain microsomes in the rat (1), liver mitochondria in the rat (2,3), and chemical media enriched in arachidonic acid or linoleic acid (4,5). Using normal rat tissues (6) and an in vitro model of cerebral anoxia in the rat, we found that the antioxidant effect of propofol is manifested not only as an inhibition of lipid peroxidation, but also as a decrease in tissue consumption of glutathione (7).

Free radicals increase the formation of lipid peroxides in the cell membrane, and thus participate in many pathological processes such as ischemia, tissue anoxia, and diabetes (8). In such diseases, antioxidant drugs can protect tissues by inhibiting lipid peroxide formation or increasing the activity of the glutathione antioxidant system, among other mechanisms (9,10).

During surgery with general anesthesia, changes in tissue and organ perfusion and the degree of oxygenation can affect oxidative stress, defined as the equilibrium between oxidizing factors (lipid peroxidation) and antioxidizing factors (mainly the glutathione system) (11). Studies in animals show that propofol, indeed, reduces the formation of lipid peroxides (1–3,6,7).

In humans, Khinev et al. (12) found no effect on plasma lipid peroxide levels in patients given propofol. Stratford and Murphy (13) and Hans et al. (14) show an increase in plasma antioxidant capacity in patients anesthetized with propofol. However, the highest levels of peroxides occur in cell membranes, rather than in plasma, and the antioxidant glutathione pathway is an important intracellular system. The
present study was, therefore, designed to study components of oxidative stress (lipid peroxide production and glutathione system activity) in platelets obtained from surgical patients who were given propofol anesthesia.

**Methods**

We studied 60 patients who underwent surgery (17 men, 43 women, mean age 36 ± 2.45 yr) and 12 healthy volunteers (5 men, 7 women, mean age 30 ± 3 yr) (Table 1).

Patients scheduled for surgery with general anesthesia, who were ASA physical status I or II, were selected (15). Patients with any of the following were excluded from study: diabetes mellitus, arterial hypertension, use of nonsteroid antiinflammatory drugs or antioxidants during the 7 days before inclusion, or patients who underwent open abdominal surgery (to avoid excessive oxidative stress that might have masked the effects of the anesthesia).

The patients were divided into three groups according to the type of anesthesia used for the surgical procedure. In Group I, anesthesia was induced with an IV bolus dose of thiopental 4 mg/kg. In Group II, propofol 2 mg/kg was used. In Group III, total IV anesthesia was used for operations of at least 1 h. An IV bolus dose of propofol 2 mg/kg was followed by an IV infusion of propofol at a dose of 10 mg/kg during the first 10 min, 8 mg/kg during the next 10 min, and 6 mg/kg during the rest of the operation. The assignment of patients to Group I and II was randomized aleatory; the assignment to Group II was according to the predetermined duration of the surgery (prevision of more than 1 h).

Healthy volunteers were given an IV bolus dose of 10% fat emulsion (Intralipid), the commercial excipient for use with propofol, in a volume equivalent to that used to give propofol 2 mg/kg.

Surgical patients received premedication with 3 mg bromazepam orally, 2 h before the operation. After the vein was cannulated, physiological saline solution was infused, and 2 mg of midazolam, 2 μg/kg pentanyll, and 0.5 mg/kg atracurium were given IV. Then, anesthetic was administered IV.

In all groups, blood was collected before thiopental, propofol, or Intralipid was given, and 5 min after the anesthetic or excipient was infused. In Group III, a third blood sample was obtained 60 min after the first bolus dose of propofol. Between the second and the third blood sample, a dose of phentanyll 1 μg/kg and atracurium 0.1 mg/kg were administered IV.

The study procedure was approved by the ethics committee of our hospital. Each participant was informed as to the purpose of the study and gave his or her verbal consent.

Sodium citrate at 3.8% was used at a proportion of 1:10 to prevent blood coagulation. Samples were centrifuged at 190 g for 10 min at 18°C to obtain platelet-rich plasma (PRP); this fraction was used for platelet counts. An aliquot of the PRP was centrifuged at 1500 g for 20 min at 18°C to separate the plasma and concentrate the platelets; both fractions were frozen at −80°C until analysis, which was completed within 5 days.

Platelet oxidative stress was quantified by measuring lipid peroxide formation in the membranes. We also determined glutathione system activity and the activities of enzymes related to the maintenance of glutathione levels in the platelet cytoplasm.

We measured thiobarbituric acid reactive substances as an index of lipid peroxidation without induction. All assays were done in platelet membrane-enriched fractions, as described by Bossman and Hemsworth (16). Briefly, the platelet pellet was diluted (1:10 wt/vol) in a buffer consisting of 0.1 M NaCl, 5 × 10⁻⁴ M KCL, 3.1 × 10⁻³ M CaCl₂, 1 × 10⁻³ M MgSO₄, 4.9 × 10⁻³ M glucose, 2.4 × 10⁻² M Na₂CO₃, 5.5 × 10⁻³ M PO₄H₂K, and 0.32 M sucrose. PRP was obtained by centrifugation of whole blood at 180 g for 10 min, then the PRP was centrifuged at 1800 g for 15 min. The pellet (platelet concentrate) was homogenized and centrifuged at 10,000 g for 15 min at 4°C, and the supernatant was collected and centrifuged again at 12,000 g for 20 min at 4°C. The resulting pellet was resuspended in the same buffer without sucrose at a proportion appropriate for the determination of lipid peroxide production.

Lipid peroxides were determined (17) by dividing the sample into 850-μL aliquots and adding 100 μL dilution buffer per tube (basal lipid peroxidation). The tubes were shaken and incubated at 37°C for 45 min, then 500 μL of 0.5% thiobarbituric acid in 20% trichloroacetic acid was added. The samples were shaken and incubated at 100°C for 15 min, then centrifuged at 2000 g for 15 min at 4°C. Absorbance of the resulting supernatant was determined spectrophotometrically at 532 nm (Perkin Elmer C-532001 spectrophotometer; Brook Instrument Division, Oak Brook, IL). Blank samples were prepared in an identical manner, except that they were incubated at 4°C. The results were expressed as μmole of thiobarbituric acid reactive substances per 10⁸ platelets, according to the values in PRP.

Total glutathione was measured spectrofluorometrically according to the technique described by Hissin and Hill (18). Briefly, platelet pellets were diluted and homogenized in 1 mL 0.1 M sodium phosphate buffer (pH 8.0) with 25% phosphoric acid at a proportion of 1:20, then centrifuged at 13,000 g for 15 min at 4°C to obtain the supernatant. Duplicate cuvettes were prepared for spectrofluorometry with the following components: 1.8 mL sodium phosphate buffer, 100 μL supernatant for each sample, and 100 μL o-pthalaldehyde. The cuvettes were shaken and incubated for 15 min at
duced glutathione, we incubated 200 mM and were expressed as glutathione that was processed in an identical manner compared with those of a standard curve for commercial an emission wavelength of 440 nm. The results were 4°C, then read at an excitation wavelength of 350 nm and an emission wavelength of 440 nm. The results were compared with those of a standard curve for commercial glutathione that was processed in an identical manner and were expressed as μmol glutathione/g of tissue.

To determine the proportions of oxidized and reduced glutathione, we incubated 200 μL of supernatant from each sample with 8 μL 4-vinylpyridine for 1 h at room temperature, then proceeded as described above for total glutathione. The resulting figure represented oxidized glutathione (GSSG); reduced glutathione (GSH) was considered the difference between total glutathione and GSSG.

Enzyme activities were measured with a spectrophotometric method. Platelet pellets were diluted in 4 mL 0.1 M potassium phosphate buffer (pH 7.0) with 1 mL 25% phosphoric acid. The samples were homogenized and centrifuged at 13,000 g for 15 min at 4°C, and proteins were analyzed in the supernatant.

Enzyme activities were determined as described below:

- Glutathione peroxidase activity (GSHpx) was measured using the method of Flohe and Gunzel (19). Briefly, to a volume of each supernatant equivalent to 25 μg protein, we added 0.1 M potassium phosphate buffer to obtain a volume of 880 μL, 53 μL glutathione reductase, 133 μL GSH, and 100 μL nicotinamide adenine di-nucleotide phosphate, reduced form (NADPH). The microcuvette was shaken by inversion and incubated at 37°C for 3 min. Then, 100 μL 1-chloro-2,4-dinitrobenzene was added, and the signal was read at 340 nm for 5 min, recording the decrease in absorbance every 30 s.

- Glutathione reductase activity (GSSGrd) was determined using the method of Flohe and Gunzel (19). The volumes of sample and buffer were the same as for GSHpx assays. After 100 μL NADPH was added, the microcuvette was shaken by inversion and incubated as described above. Then 100 μL GSSGrd was added, and the sample was shaken and read spectrophotometrically at 340 nm, recording the decrease in absorbance every 30 s.

Table 1. Main Characteristics of Patients and Healthy Volunteers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>18</td>
<td>18</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>5/13</td>
<td>6/12</td>
<td>6/18</td>
<td>5/7</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>34.3 ± 2.2</td>
<td>36.2 ± 3.0</td>
<td>37.6 ± 2.3</td>
<td>30.0 ± 3.1</td>
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<tr>
<td>Weight (kg)</td>
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<td>65.4 ± 2.2</td>
<td>68.1 ± 2.7</td>
<td>65.5 ± 3.56</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Curetage</td>
<td>9</td>
<td>9</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Ligation of fallopian tubes</td>
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</tr>
<tr>
<td>Ingual hernia</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Cholecystectomy</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Red blood cell count (×10^{12}/L)</td>
<td>4.6 ± 0.09</td>
<td>4.6 ± 0.1</td>
<td>4.7 ± 0.07</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Leukocyte count (×10^{6}/L)</td>
<td>7.4 ± 0.3</td>
<td>8.0 ± 0.5</td>
<td>7.8 ± 0.4</td>
<td>7.5 ± 0.3</td>
</tr>
<tr>
<td>Platelet count (×10^{6}/L)</td>
<td>224 ± 15</td>
<td>248 ± 14</td>
<td>226 ± 8</td>
<td>264 ± 15</td>
</tr>
</tbody>
</table>

Values are mean ± sem or n.

Results

There were no statistical differences among groups in any of the variables (P < 0.1). However, at the postinduction point, all the values measured in Groups II and III were statistically different from Groups I and IV (P < 0.05). There were no statistical differences between the postinduction values in Groups I and IV.

Thiopental did not significantly modify any of the variables of platelet oxidative stress (Table 2). Propofol significantly inhibited (-25.7%) lipid peroxide production in platelets, whereas the administration of
Intralipid to healthy volunteers had no significant effect on this value (Fig. 1).

Total platelet glutathione was significantly higher in patients given the bolus of propofol, who had a mean postinduction value that was 24.6% higher than the preinduction value (Fig. 2). In volunteers who were given Intralipid, there was no significant change in total platelet glutathione values (Fig. 2).

The percentage of GSSG was significantly lower after propofol was given (29.5% inhibition), whereas Intralipid had no effect on this value (Fig. 2).

Enzymes related with maintenance of glutathione levels showed changes in activity after propofol administration: platelet GSGpx was 28.3% lower, and GSHtf was 44.5% higher. The 9.05% increase in GSSGrd activity was not statistically significant. Intralipid had no effect on the activity of any of the enzyme activities studied here (Fig. 3).

After the initial bolus injection of 2 mg/kg, the effects on platelet oxidative stress in Group III patients were the same as those found in patients in whom anesthesia was induced with a single IV bolus dose (Table 3).

After infusion of propofol for 1 h, the effects were qualitatively the same as those seen after the initial IV bolus. However, there were some quantitative differences: the percentage of GSSG showed a further significant decrease ($P < 0.05$), whereas the values for other measures of oxidative stress were similar to those found after the initial bolus (Table 3).

**Table 2. Platelet Oxidative Stress Variables in Patients Treated with 4 mg/kg IV of Sodium Thiopental**

<table>
<thead>
<tr>
<th></th>
<th>Preinduction</th>
<th>Postinduction</th>
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<tbody>
<tr>
<td>TBARS (nmol/10^8 platelets)</td>
<td>0.69 ± 0.05</td>
<td>0.65 ± 0.034</td>
</tr>
<tr>
<td>Total glutathione (GSH + GSSG)</td>
<td>2.39 ± 0.12</td>
<td>2.42 ± 0.13</td>
</tr>
<tr>
<td>% oxidized glutathione</td>
<td>7.42 ± 0.84</td>
<td>8.09 ± 0.45</td>
</tr>
<tr>
<td>GSHpx activity (µmol/min)</td>
<td>33.75 ± 0.23</td>
<td>34.16 ± 0.28</td>
</tr>
<tr>
<td>GSSGrd activity (µmol/min)</td>
<td>52.33 ± 3.54</td>
<td>52.68 ± 4.65</td>
</tr>
<tr>
<td>GSHtf activity (µmol/min)</td>
<td>40.98 ± 3.59</td>
<td>43.60 ± 2.68</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

TBARS = thiobarbituric acid reactive substances, GSH = reduced glutathione, GSSG = oxidized glutathione, GSHpx = glutathione peroxidase, GSSGrd = glutathione reductase, GSHtf = glutathione transferase.

Differences between pre- and postinduction values were not statistically significant.

**Figure 1.** Thiobarbituric acid reactive substances (TBARS) in platelets from patients induced with an IV bolus of 2 mg/kg of propofol (hatched bars) or from healthy volunteers given Intralipid (open bars), before and 5 min after administration. *$P < 0.05$ versus preinduction value.

**Figure 2.** Total glutathione content (GSH + GSSG) (upper panel) and percent oxidized glutathione (GSSG) (lower panel) in platelets from patients induced with an IV bolus of 2 mg/kg of propofol (hatched bars) or from healthy volunteers given Intralipid (open bars), before and 5 min after administration. *$P < 0.05$ versus preinduction value.

**Discussion**

Our results show that propofol, at the doses usually used for surgical anesthesia, had antioxidant effects as evidenced by the inhibition of lipid peroxidase production in the platelet membrane and changes in the glutathione antioxidant system. The ability of propofol to reduce lipid peroxide production has been documented in animal tissues, particularly in liver and
The antioxidant effects of propofol have been attributed to its chemical similarity with other known antioxidants such as butylhydroxytoluene and α-tocopherol (3,5). These substances bind to cell membrane phospholipids and capture free radicals, thus disrupting the chain of transmission through membrane fatty acid molecules (11). However, Aarts et al. (2) found that in the mitochondria of rats deficient in vitamin E (where the GSH was unable to impede the production of lipid peroxides), GSH in the presence of propofol effectively inhibited lipid peroxide formation. In addition, we have reported that, in several animal tissues (6) and in a model of anoxia-hyperoxia in rat brain tissue (7), propofol not only inhibits lipid peroxide formation but also increases the activity of the glutathione antioxidant system. This latter effect may be a result of the fact that the lower level of lipid peroxidation does not oblige these tissues to exhaust their GSH reserves. The effect of propofol, in the glutathione-related enzyme activities, reinforces the antioxidant effect of this drug, because propofol increases the cellular ability to recovery GSH from GSSG, through GSSGrd activity, and from other proteins with sulphydril groups, by the GSHtf activity. The inhibition in GSHpx activity may be a consequence of the lower level of the oxidative stress, which makes it unnecessary to consume GSH.

We show that propofol also has antioxidant effects in humans. Our findings are similar to earlier observations that showed that propofol inhibited human platelet functioning (21), a process in which lipid peroxide production plays an important role.

In their studies in humans, Khinev et al. (12) found no changes in plasma levels of lipid peroxides after the administration of propofol. The discrepancy between their results and ours may be because lipid peroxidation occurs mainly in the cell membrane, where we concentrated our search for changes in peroxidation.

Three important features of the antioxidant effect of propofol should be emphasized: the effect is immediate, is not dependent on the solvent Intralipid, and is maintained after IV infusion. Nearly the entire effect of propofol was detectable five minutes after a single bolus dose. Our results show that Intralipid had no effect on platelet oxidative stress. After continuous IV infusion, the antioxidant effect of propofol was nearly the same as the effect seen immediately after a single dose.

The changes we found in platelet oxidative stress may have been caused by the operation itself rather than by the anesthetic. However, this possibility can be ruled out, as we found no such effect in the group of patients in whom anesthesia was induced with thiopental sodium.
References


Table 3. Platelet Oxidative Stress Variables in Patients Treated with 2 mg/kg iv Plus Continuous Infusion of Propofol

<table>
<thead>
<tr>
<th>Variable</th>
<th>Preinduction</th>
<th>Postinduction</th>
<th>Postinfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/10⁶ platelets)</td>
<td>0.69 ± 0.08</td>
<td>0.33 ± 0.03*</td>
<td>0.38 ± 0.017*</td>
</tr>
<tr>
<td>Total glutathione (GSH + GSSG)</td>
<td>2.08 ± 0.07</td>
<td>2.84 ± 0.09*</td>
<td>2.64 ± 0.07*</td>
</tr>
<tr>
<td>% oxidized glutathione</td>
<td>7.13 ± 0.71</td>
<td>4.30 ± 0.21*</td>
<td>2.84 ± 0.19*†</td>
</tr>
<tr>
<td>GSHpx activity (μmol/min)</td>
<td>37.14 ± 0.39</td>
<td>28.00 ± 0.31*</td>
<td>30.09 ± 2.08*</td>
</tr>
<tr>
<td>GSSGrd activity (μmol/min)</td>
<td>55.95 ± 3.09</td>
<td>66.55 ± 3.60*</td>
<td>68.25 ± 4.71*</td>
</tr>
<tr>
<td>GSHtf activity (μmol/min)</td>
<td>44.89 ± 3.54</td>
<td>65.99 ± 5.88*</td>
<td>68.85 ± 4.83*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

TBARS = thiobarbituric acid reactive substances, GSH = reduced glutathione, GSSG = oxidized glutathione, GSHpx = glutathione peroxidase, GSSGrd = glutathione reductase, GSHtf = glutathione transferase.

* P < 0.05 with respect to preinduction values.
† P < 0.05 with respect to postinduction values.

It is important to determine whether the modifications we found in platelets can be extrapolated to other tissues, particularly the brain. In animal models, the effects of propofol are identical to those found in human platelets (6,7). In addition, some authors have shown that, in patients with cerebrovascular stroke, the changes in oxidative stress in peripheral blood were very similar to those reported in animal models of cerebral ischemia (22).

The main limitation of our study is that we determined the oxidative variables in platelets, not in organs (e.g., brain, liver). According to experimental in vitro studies (6,7), propofol exerts an antioxidant effect in several tissues (brain, liver, vessel wall, lung, kidney, and heart); moreover, propofol easily crosses bio-logical membranes (23). For these reasons, it may be possible that an effect similar to that found in platelets could be present in organs.

In conclusion, our results show that propofol has antioxidant effects in humans. The effects may be beneficial for patients in whom free radicals play an important role, such as those with ischemic processes. Further studies will be needed to determine whether these antioxidant effects of anesthetic are of clinical value.

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