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EXPERIMENTAL STUDY

Protective effects of propofol on peritoneal adhesions in cecal ligation and puncture model

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Abstract: *Aim:* We assumed that one of the most widely used anesthetic agents, propofol, which is the most widely used anesthetic for sedation, may reduce inflammatory processes and organ injury induced by cecal ligation and puncture.
Study design: Bacterial peritonitis was induced in 18 rats by cecal ligation and puncture. The rats were randomly assigned to three groups. Group 1 (n=6) received propofol, group 2 (n=6) received intralipid, group 3 (n=6) was a control group, which did not receive any injection. All animals were killed 14 days later so we could assess the adhesion score. Tissue antioxidant levels were measured in 1-g tissue samples taken from the abdominal wall.
Results: The adhesion score was significantly lower in the propofol group than in the control group ($p<0.05$). The catalase levels were higher in the intralipid and control groups than the propofol groups.
Conclusions: Intraperitoneal propofol reduced the formation of postoperative intra-abdominal adhesions without compromising wound healing in this bacterial peritonitis rat model. Propofol also decreased the oxidative stress during peritonitis *(Tab. 1, Fig. 5, Ref. 28)*. Full Text (Free, PDF) *www.bmj.sk.*
Key words: protective effects, propofol, peritoneal adhesions, cecal ligation, puncture model.

Patients with intraabdominal infections due to intestinal perforation before the operation are at increased risk for prolonged recovery and complications. Propofol is the most widely used anesthetic during surgery and in postoperative sedation at the intensive care units (1). The direct effects of several anesthetics on immune function have been described (2). The release of inflammatory mediators and free radicals has been clearly demonstrated in generalized inflammatory reactions involving the production of leucocytes (3). Propofol has been shown to have free radical scavenging properties in vitro (4) and to supress neutrophil chemotaxis and phagocytosis.

Therefore, in this study, we assumed that one of the most widely used anesthetic agents, propofol, may reduce inflammatory processes and peritoneal adhesions, organ injury induced by cecal ligation and puncture (CLP).

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Materials and methods

Animals

The experimental protocol used for this study was approved by the Animal Ethics Review Committee of the Faculty of Medicine, University of Kahramanmaras Sutcuimam and adhered to National Institutes of Health guidelines for the use of experimental animals. Animals were housed in individual cages in a temperature-controlled room with alternating 12-h light-dark cycles, and acclimatized for a week before the study. Food was removed 8 h prior to the study, but all animals had free access to water and rat chow diet after the CLP procedure. Eighteen Wistar Albino rats weighing between 200 to 220 grams were randomly assigned into the three groups of 6 rats per group.

Operative procedure

The animals were anesthetized with a mixture of 40 mg/kg ketamine and 5 mg/kg xylazine hydrochloride. In all rats, adhesions was induced by performing a CLP procedure, using the methods of Wichterman et al (5). The abdomen was shaved and swabbed with a povidone iodine solution preoperatively. The same researcher performed all surgical procedures. A 3 cm midline incision was made and the abdomen was opened under clean surgical conditions. The caecum was dissected without damaging the vascularization and was filled backwards with faeces. Thereafter, the caecum was ligated just proximal to the ileocecal valve, with a 3/0 silk suture, and at the anti-mesenterial site the caecum was punctured twice with a 22-gauge needle (Fig. 1),

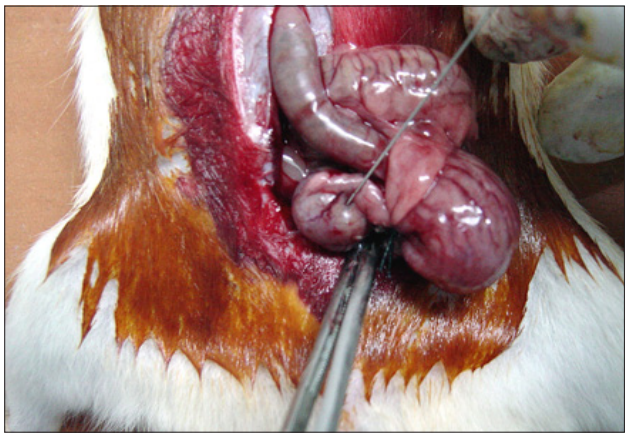

Fig. 1. The caecum was ligated just proximal to the ileocecal valve and at the anti-mesenterial site the caecum was punctured twice with a 22-gauge needle.

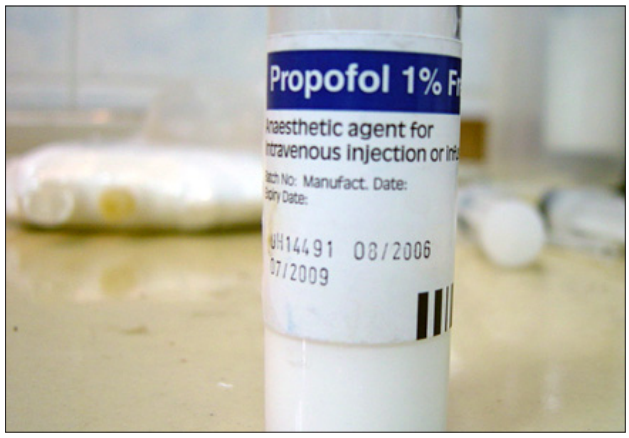

Fig. 2. Propofol.

squeezed gently to force out a small amount of faeces, and then returned to the abdominal cavity. Then the midline incision was closed in one layer with a 3/0 silk suture. On day 1, all animals were operated on again under anaesthesia, and peritoneal fluid samples were taken for microbiological examination. The cecal ligated stump was isolated and resected. Before closure of the abdomen, the rats were randomly assigned to three groups: Group 1 (n=6) received single dose of propofol (25 mg/kg, Propofol 1 % Fresenius, Fresenius Kabi AB, Uppsala/Sweden (Fig. 2)), group 2 (n=6) received single dose of intralipid (250 mg/kg, intralipid 10 % 500 ml Fresenius, Fresenius Kabi AB, Uppsala/Sweden) as vehicle solution and group 3 (n=6) was the control group, which did not receive any injection. All animals were given water only on the first postoperative day; standard rat chow and water were provided on the second postoperative day. Microbiological examination was performed for a sepsis indicator. Samples of peritoneal fluid were cultured in aerobic and anaerobic conditions.

Tab. 1. Cumulative adhesion scoring scale (6).

Points	
0	No adhesions
+1	One adhesion band from the omentum to the target organ
+1	One adhesion band from the omentum to the abdominal scar
+1	One adhesion band from the omentum to another place
+1	One adhesion band from the adnexa/epididymal fat bodies to the target organ
+1	One adhesion band from the adnexa/epididymal fat bodies to the abdominal scar
+1	One adhesion band from the adnexa/epididymal fat bodies to another place
+1	Any adhesive band other than described above
+1	Target organ adherent to the abdominal wall
+1	Target organ adherent to the abdominal scar
+1	Target organ adherent to the bowel
+1	Target organ adherent to the liver or the spleen
+1	Any other organ adherent

* Target organ was ligated and resected cecum.

Evaluation of adhesions

All rats were sacrificed on the 14th day after being anaesthetized with an overdose of ethyl ether before relaparotomy. The abdomen was opened with an inverted U incision. One point was given for each adhesion and a cumulative adhesion score was calculated. Adhesions were scored in a blinded manner according to the method of Bothin et al (Tab. 1) (6). One point was given for each adhesion and a cumulative adhesion score was calculated.

Antioxidant study

In order to determine the tissue antioxidant levels, 1 x 1 cm^2 tissue samples were taken from the left lateral part of the incision line on the abdominal wall. The samples were preserved in a deep freezer until examination. The tissues were homogenized with three volumes of ice-cold 1.15 % KCI. The activities of antioxidant enzymes and the levels of lipid peroxidation were measured in the supernatant obtained from centrifugation at 14 000 r.p.m. Superoxide dismutase activity was measured according to the method described by Fridovich (7). Catalase activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Beutler (8). Lipid peroxidation level in the tissue samples was expressed in malondialdehyde and measured according to procedure of Ohkawa et al (9). Protein concentration was determined according to the method of Lowry (11).

Statistical analysis

All variables were expressed as mean and standart deviation. Differences between the groups were evaluated by Kruskal-Wallis variance analysis followed by a post-hoc Mann-Whitney U test. p-values <0.05 were considered statistically significant. All data were processed by the SPSS 9.05 for Windows statistical package.

Results

Figure 3 shows the adhesion scores of the three groups. By Kruskal-Wallis, the differences in adhesion scores between all

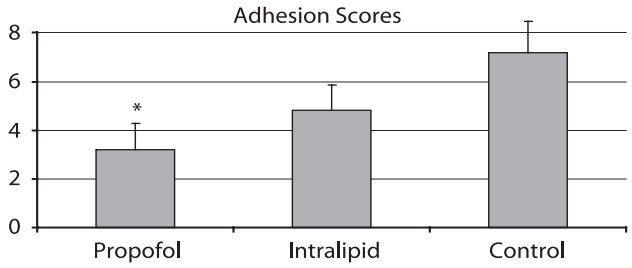

Fig. 3. Adhesion scores. The differences of adhesion scores among all the groups were significant (p<0.05, Kruskal-Wallis test). * The adhesion score of the propofol group was significantly lower than in the control group. (p<0.05, post hoc Mann-Withney-U).

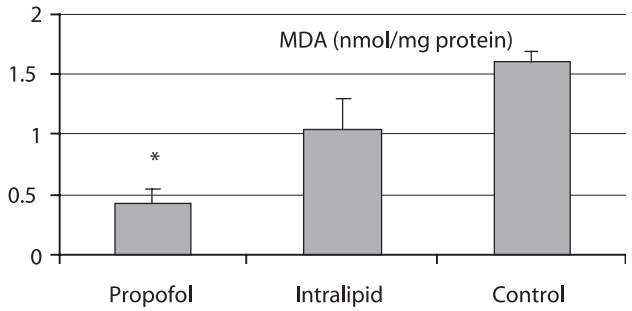

Fig. 4. MDA levels (nmol/mg protein). The differences of MDA levels among all the groups were significant (p<0.05, Kruskal-Wallis test). * The MDA levels in the propofol group were significantly lower than in the intralipid group (p<0.05, post hoc Mann-Withney-U).

groups were statitically significant (p<0.05). Each couple of groups were compared individually by the Mann-Whitney U test, which showed a significant difference between the groups receiving propofol and the control group (p=0.003). There were no significant differences between the groups propofol-intralipid and the groups intralipid-control.

When the antioxidant levels of the lateral wall of the abdomen were evaluated on day 14, the MDA values of propofol group was low from the control group but statistically not significant (p>0.05). Levels in the intralipid group was higher than in the propofol group (p<0.05) and statistically significant (Fig. 4). CAT levels were high in the control and intralipid groups, but the difference between each other was not statistically significant. The differences between the propofol and control groups were significant. If the SOD levels were taken into account, the control group was higher than the propofol group (statistically siginificant). It was detected that the results of the intralipid group for SOD were higher compared to the propofol group, but it did not reach statistical significance when the intralipid group was compared to propofol (Fig. 5).

Bacterial cultures of the peritoneal fluid, taken at the day of cecal resection, revealed a mixed aerobic and anaerobic flora of Escherichia coli, Enterobacter aerogenes, Proteus mirabilis, Proteus vulgaris, group D Streptococcus, Enterococcus, Staphylococcus aureus, Clostridium difficile and Bacteroides fragilis.

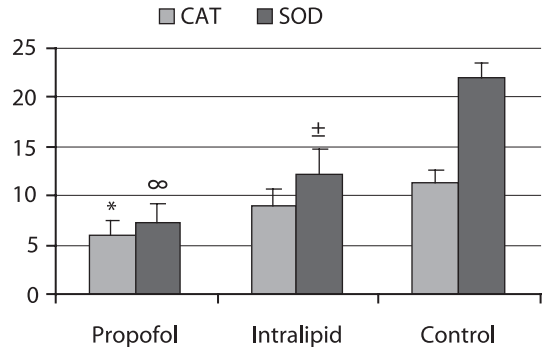

Fig. 5. SOD and CAT levels (U/mg protein). The differences in CAT and SOD levels among all the groups were significant (p<0.05, Kruskal-Wallis test). * The CAT levels in the propofol group were significantly lower than in the control group (p<0.05, post hoc Mann--Withney-U). • The SOD levels in the propofol group were significantly lower than in the control group (p<0.05, post hoc Mann--Withney-U). ± The differences between intralipid and control groups were significant (p<0.05, post hoc Mann-Withney-U).

Discussion

Bacterial peritonitis is characterized by a neutrophil migration into the peritoneum because these cells are the first lines of defense for clearing the microorganisms (11, 12). Because early sepsis is characterized by an uncontrolled overactive immune system activation leading to organ injury.

In the current study, we demonstrated that propofol treatment significantly reduced intraperitoneal adhesions (p<0.05) and peritoneal MDA levels (p>0.05). Oxygen-derived free radicals have been proposed as important mediators of tissue injury and are known to be released during early sepsis and may possibly be responsible for the neutrophil activation (13). The production of free radicals and subsequent lipid peroxidation plays a key role in injury (14).] Lipid peroxidation is a free-radical initiated chain reaction resulting in sequential abstraction of hydrogen ions from polyunsaturated fatty acids. Lipid peroxides, such as MDA, are markers and intermediate products of lipid peroxidation and are used for the assessment of tissue injury attributable to the free radicals produced by ischemia and reperfusion (15). The highest MDA values were observed in the control group. Consequently, according to our results, propofol seems to have some advantages for the prevention of oxidative stress and peritoneal adhesions when compared to other groups.

Propofol has an antioxidant property, and the majority of studies attributed this capacity to the phenolic structure of propofol. Propofol appears to inhibit lipid peroxidation in two ways: it reacts with lipid peroxyl radicals to form the relatively stable propofol phenoxyl radical (16, 17), and it scavenges peroxynitrite, as shown in Kahraman S et al study (18). The free radical scavenging properties of propofol resemble those of the endogenous antioxidant alpha-tocopherol that is chemically similar to propofol (4). Propofol also suppresses chemotaxis (19, 20) and the killing of bacteria by neuthropil with inhibition of phagocy-

tosis (21). This suppression could affect the patient's ability to fight infection and could decrease the body's defense against infection. Since peroxynitrite is a potent bactericidal agent (22), this suppression may be tolerated by this activity of propofol.

Free radicals such as superoxide radical or hydroxyl radical are constantly produced as a normal consequence of aerobic metabolism (23). Oxidative stress results from the imbalance between radicalgenerating and radical-scavenging systems leading to cell membrane impairment (23). MDA is a reflection of lipid peroxidation, whereas SOD and CAT are important antioxidant defenses. These enzymes are involved in the clearance of superoxide and H_2O_2 to maintain the structure and function of biological membranes (23). SOD dismutases superoxide H_2O_2 and this compound is catabolized by catalase. Thus, our findings support the propofol scavenging properties. Moreover, anesthesia conducted with propofol reduced oxidative stress and enhanced antioxidant defense mechanisms expressed by larger concentrations of free radical scavengers.

Propofol is supplied as intralipid, which was included in the groups. Previous in vitro studies have suggested that intralipid itself may cause immunosuppression in terms of neutrophil respiratory burst activity (24), and neutrophil chemotaxis (21). Although intralipid has been shown to have antioxidant properties, these are very weak and substantially less potent than propofol (25). Furthermore, propofol's beneficial properties have been clearly shown to be independent of intralipid (26, 27) and Galley HF et al (28) study showed that intralipid caused a small decrease in extracellular IL-8 accumulation, but the effect of propofol exceeded that of intralipid alone. In our present study we observed the same effects as in previous studies.

The findings of this study suggest that propofol administration after bacterial peritonitis may have beneficial effects. Propofol administration prevented a marked increase of MDA production in the sepsis conditions. It also decreased peritoneal adhesions and prevented the increase of antioxidant levels that were observed in the propofol group.

The most important finding in this study was that the administration of propofol after bacterial peritonitis prevented the marked intraperitoneal adhesions that were detected in the control group. Finally, our data indicate that exposure to propofol reduces lipid peroxidation and enhances antioxidant defenses. Further experiments are necessary to elucidate the mechanisms of action of propofol in bacterial peritonitis.

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