Dose-dependent effects of propofol on expression 
ICAM-1 in rabbit aorta endothelial cells

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The dose-dependent effects of propofol on expression intracellular adhesion molecule (ICAM-1) in rabbit aorta endothelial cells was examined. Twenty adult New Zealand albino rabbits were used in this study. One control and three experimental groups were designed. In experimental groups; for group I propofol was not applied, group II 0.5, group III 4.0 and group IV 8.0 mg/kg propofol were applied to rabbits by marginal ear vein. One hour after applying propofol, control and experimental group rabbits were sacrificed and their aorta were removed. The sections were stained with APAAP immunohistochemical staining for evaluation using a light microscope. This marked ICAM-1 immunoreactivity was maintained in 8.0 mg/kg of propofol applied to rabbits of aorta endothelium, whereas mild stain was observed for other experimental groups. The present study indicated that there is parallel relationship between endothelial cells ICAM-1 expression and different doses of propofol.

Key words: ICAM-1, rabbit, aorta endothelium, propofol.

INTRODUCTION

Propofol is widely used for the induction and maintenance of anesthesia and as a sedative in intensive care units where it is given as a constant intravenous infusion for periods of many days. In addition to its clinical importance, propofol provides a valuable model for understanding the human pharmacokinetics of agents that are concentrated in fat. Propofol has an oil/water partition coefficient (K_{ow}) of about 4700 (Steward et al., 1973), one of the largest of any pharmacological agent. In comparison, the highly lipophilic volatile anaesthetics, such as halothane, have a K_{ow} of less than 300 (Weaver et al., 2001). Because of this large fat partition, propofol is highly concentrated in adipose tissue where it has slow uptake and release kinetics.

The endothelial cells that form the lining of all blood vessels perform a wide range of functions. In addition to providing a selective barrier between the bloodstream and tissues, vascular endothelial cells are critical for processes including thrombosis, angiogenesis, leukocyte trafficking, and the maintenance of vascular tone (Risau, 1995). The vascular endothelium also plays an important role in cancer metastasis and in the pathogenesis of non-neoplastic diseases such as rheumatoid arthritis and atherosclerosis (Folkman, 1995; Ross, 1993). To gain an understanding of the regulation of endothelium-specific gene expression and thus provide insights into these processes and conditions, a number of studies have focused on the characterization of endothelial cell-specific promoters (Pan and McEver, 1993; Schlaeger et al., 1997).

The FR (free radicals) and the products of the inflammatory reaction attract neutrophils, which adhere to the endothelium. Adhesion occurs through an interaction between proteins like the selectins (ELAM-1 and GMP-140) and immunoglobulin (ICAM-1 and VCAM-1), present in the endothelium, and proteins present in the neutrophil surface, known as leukocyte integrin (CD11, CD18). Endothelium may become activated by cytokines, especially the interleukin, and the tumor necrosis factor (TNF). The leukocyte integrins are modulated by leukotriens

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Antioxidants within cell membranes protect the phospholipids from free radical mediated lipid peroxidation. The best charactered of these is radical mediated lipid peroxidation. α-Tocopherol (Vitamine E) is used to protect lipid from oxidation. This compound contains a phenol group that donates hydrogen to free radicals, thus terminating lipid peroxidation. Propofol is an intravenous anesthetic with a chemical structure similar to phenol-based free radical scavengers such as vitamine E (Zhang et al., 2004).

Leucocytes are pivotal component of the inflammatory cascade that results in tissue injury in a large group of disorders. Free radical production and endothelial activation promote leucocyte-endothelium interactions via endothelial expression of vascular cell adhesion molecule 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1) which augments these processes, particularly in the setting of reperfusion injury (Corcoran et al., 2006).

In vivo expression patterns of ICAM-1 and ICAM-2 are distinct but overlapping. Overall tissue distribution of ICAM-2 is more restricted than that of ICAM-1. Both ICAMs are expressed at low levels on most leucocytes. ICAM-2 is constitutively expressed on all vascular endothelium, including high endothelial venules at much higher levels than ICAM-1 (De Fougerolles et al., 1991), ICAM-1 expression is strongly inducible by inflammatory cytokines, whereas ICAM-2 was reported to be down-regulated by inflammatory cytokines (McLaughlin et al., 1998). On the basis of the endothelial expression patterns of ICAM-1 and ICAM-2, it has been hypothesized that ICAM-2 mediates leucocyte traffic into noninflamed tissue, that is, lymphocyte recirculation during immunosurveillance, whereas up-regulated levels of ICAM-1 may increase leucocyte extravasation at sites of inflammation (Springer, 1994).

The aim of present study was to investigate the effects of different doses of propofol on expression intracellular adhesion molecule (ICAM-1) in rabbit aorta endothelial cells.

MATERIALS AND METHODS

Animals

Twenty adult New Zealand albino rabbits were used in this study (2000 - 2500 g in weight) were obtained from the Department of Medical Science Application and Research Centre of Dicle University (DUSAM). They were housed in individual cages in temperature-controlled environment (22°C) with a 12 : 12 h light-dark cycle. All rabbits were fed with standard pellet food and tap water ad libitum, which were performed according to the Declaration of Helsinki with the permission of the Governmental Animal Protection Committee. Group I (Control group): These rabbits served as control (n:5). Group II (0.5 mg/kg IV propofol applied): In this group 0.5 mg/kg propofol was applied to rabbits by marginal ear vein (n: 5). Group III: (4.0 mg/kg IV propofol applied): In this group 4 mg/kg propofol was applied by marginal ear vein (n: 5). Group IV: (8.0 mg/kg IV propofol applied). In this group 8 mg/kg propofol was applied by marginal ear vein (n: 5). One hour after applying propofol, control and experimental group rabbits were sacrificied and their aorta were removed.

**Immunohistochemical procedure**

The aorta tissues were fixed for 6 - 8 h in Bouin’s solution at 4°C. They were dehydrated though increasing concentrations of the ethanol series and the tissues were embedded in paraffin and cut into 4 - 5 μm transversal, dewaxed in xylene, and incubated for 20 min in 0.3% H₂O₂ to block endogenous peroxidase activity. Section then were microwaved for 4 min in 20% goat serum in PBS; in order to avoid undesired background staining, this was done for 20 min.

Monoclonal mouse anti-Human ICAM-1 (BioGenex San Ramon USA) primary antibody (dilution: 1/200) was applied to the sections for 3 hours at 37°C in a humidified staining chamber. Sections were then incubated with anti-mouse IgG secondary antibody (Lab Vision, dilution: 1/1000) for 1 h, and they were put into the APAAP complex for an hour. Sections were mounted with a glycerol-PBS mixture (1:1 glycerol: PBS). Following this step, sections were incubated in the fast red/TR naphtol mixture until the specific regions were stained red, and then the sections were either briefly put into Mayer’s hematoxilen in order to visualize the nuclei, or were not subjected to counterstaining. Sections were mounted with a glycerol-PBS mixture (1:1 glycerol: PBS). The control staining of some sections was performed without the primary antibody, and no ICAM-1 immunostaining was observed in these sections (Seidman, 1993).

The immunohistochemical expressions were evaluated in 3 categories such as mild, moderate, and intense. The microphotographs were taken by Nikon 400 Eclipse light microscope.

**RESULTS**

**Immunohistochemical changes**

The aim of present study was to investigate the effects of different doses of propofol on expression intracellular adhesion molecule (ICAM-1) in rabbit aorta endothelial cells. There was considerable changes in immunoreactivity among section evenwithin groups, the difference in the staining ICAM-1 between control and experimental groups was clear (Table 1). The control staining of some sections was performed without the primary antibody, and no ICAM-1 immunostaining was observed in these sections (Figure 1). Immunohistochemistry of ICAM-1 expression in the control groups showed mild marked staining in aorta endothelial cells (Figure 2).

In the 0.5 and 4.0 mg/kg applied propofol groups showed moderate staining with ICAM-1 (Figure 3 ). It was clearly observed that in 8.0 mg/kg propofol applied groups rabbit endothelial cells significantly intense stained (Figure 4).

**DISCUSSION**

Although there is now a considerable literature indicating the importance of endothelial cell activation and expression...
Table 1. Immunohistochemical expression of ICAM-1 in control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Propofol applied (I.V)</th>
<th>ICAM-1 Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I – Control group</td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>Group II – Experimental Group</td>
<td>0.5 mg/kg</td>
<td>++</td>
</tr>
<tr>
<td>Group III – Experimental Group</td>
<td>4.0 mg/kg</td>
<td>++</td>
</tr>
<tr>
<td>Group IV – Experimental Group</td>
<td>8.0 mg/kg</td>
<td>+++</td>
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+++: intense staining; +: moderate staining; ±: mild staining.

Figure 1. Histological appearance of Group I. The control staining was performed without the primary antibody. Immunogens show no positive staining (Original magnification X20).

of adhesion molecules for leukocyte-endothelial cell interactions (Greenwood et al., 1995; Oppenheimermarks et al., 1991), there is still relatively little detailed understanding of the precise dynamics of adhesion molecule expression during the course of inflammatory responses in vivo. A critical role for endothelial ICAM-1 and ICAM-2 in T-cell adhesion and transendothelial migration (TEM) has previously been demonstrated by several in vitro studies using monoclonal antibodies against ICAM-1 and ICAM-2 (Oppenheimermarks et al., 1991; Reiss et al., 1998). Using ICAM-1-deficient endothelioma lines, it was previously demonstrated that the important role of ICAM-1 in TEM could be dissociated from its role in T-cell adhesion to endothelium (Laschinger et al., 2002; Reiss and Engelhardt, 1999).

Expression of adhesion molecules in the endothelium and smooth muscle is a key component of the inflammatory response in atherosclerotic lesions (Davies et al., 1993). Increased endothelial inflammatory activity reflected by enhanced expression of intercellular adhesion molecule-1 (ICAM-1) and tumor necrosis factor-α is common in human carotid atherosclerotic plaques, and its role in the occurrence of neurological symptoms is under active research (Ameriso et al., 1999; Davies et al., 1993;...
Figure 2. Histological appearance of control groups. ICAM-1 mild staining was seen in aorta endothelium (arrows), capiller (k). Immunostaining was performed using secondary antibodies (Original magnification X 20).

Figure 3. Histological appearance of Group II. ICAM-1 moderate staining was seen in aorta endothelium (arrows), capiller (k). Immunostaining was performed using secondary antibodies (Original magnification X 20).
Degraba et al., 1998; Endres et al., 1997).
Free radicals may play an important role in the pathogenesis of myocardium and lung injury. Reduction of free radical may improve outcomes of patients undergoing on surgeries. Common antioxidants such as vitamin E and butylated hydroxytoluene cannot be used routinely. Propofol may be the first candidate because of its anaesthetic properties, rapid acting and recovering. Therefore, it may have a protective role in disorders and surgeries where free radical mediated injury promotes leucocytes-endothelium adhesive interactions (Corcoran et al., 2006; Zhang et al., 2004).

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