

Acute Effect Of Propofol On Polymorphonuclear Neutrophils Burst Respiration

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Citation

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Abstract

INTRODUCTION

Surgical stress is followed by profound endocrine and metabolic changes, which have been demonstrated to influence the host defence response by direct effect on the immune system or activating the hypothalamic–pituitary-adrenal axis and the sympathetic nervous system [1,23]. Several investigators have studied the effect of stressful events on immune function. They were able to show that a variety of stresses, including surgery and pain, induced alterations of immune function in vivo, thus resulting in increased susceptibility to infection and tumour progression [4,5]. Concern about the impact of anaesthetics and sedatives used for general anaesthesia and in intensive care medicine is growing because of an increasing number of immunosuppressed patients require anaesthesia and intensive care treatment. The autonomic nervous system and the hypothalamic- pituitary-adrenal axis provide an interface between stress and other organ systems [1]. Thus, anaesthetics may influence immune function by merely reducing the stress response. Phagocytosis and burst activity of polymorphonuclear neutrophils (PMNs) are essential to the bacterial and fungal killing capacity of the host. In surgical patients, an attenuated up regulation of cytokine secretion was observed together with a decrease in total lymphocyte counts and function. [6,7]. These alterations were positively correlated with the duration of surgery and the blood loss volume [6]. Surgical stress depressed monocyte mCD14 expression and human leukocyte antigen-DR expression. The concentration of the anti-inflammatory cytokine IL-10 increased, whereas the endotoxin-induced TNF- α production was suppressed [8]. Treatment with anti-IL-10 antibodies partially restored the endotoxin-induced TNF- α production, indicating that IL-10 participates in this hyporesponsiveness[9].

The aim of this study is to investigate the acute effect of propofol on the respiratory burst of PMNs.

PATIENTS AND METHODS

After obtaining approval from the hospital ethics committee and patient consent, 16 patients ASA classification I and II aged between 18 and 40 yr were enrolled in the study using a computer generated randomization from a list of patients posted for different surgeries in the operating room on the day of surgery. Patients with any type of infection, trauma, allergic reaction to any drugs, immunocompromised, and patients on regular antibiotics were excluded from the study. Patients were monitored routinely and an intravenous access was established with large bore 16G cannula (BD Insyte). Anaesthesia was induced with 1-2 mic/kg of fentanyl (Jassen Pharmaceuticals), propofol (Fernisius Kabi) 2-3mg/kg followed by 0.6 mg/kg of rocuronium bromide(Organon) and airway was secured by an endotracheal tube.

Blood samples were extracted at 0 sec (before injection), 30 and 120 sec after injection of propofol. The blood samples were refrigerated, labelled and immediately dispatched to the laboratory within a few min after collection.

PREPARATION OF PHORBOL MYRISTATE ACETATE (PMA) SOLUTION

Phorbol myristate acetate (PMA Sigma Chemical Co., St. Louis, MO, USA) was dissolved in DMSO to give a stock solution of 2mg/ml. This stock solution was stored at -20°C till used. This stock solution was further diluted to 20 ng/ml with PBS prior to use.

OPSONIZATION OF ZYMOBAN

Zymosan (Sigma Chemical Co., St. Louis, MO, USA) was opsonized by suspending 50mg in 6 ml human serum and 1ml PBS. The suspension was incubated for 30 minutes at

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37°C and then centrifuged at 300g for 10 minutes. The supernatant was then removed and the pellet washed twice with 4ml buffer. After the last washing, the pellet was resuspended in PBS at a concentration of 6.25 mg/ml and stored in the freezer until use. The concentration of opsonized zymosan used was 1.25 mg/ml.

PREPARATION OF LUMINOL

Luminol (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in DMSO to give a concentration of 10^{-2} M and this stock solution was further diluted in PBS to 10^{-5} M prior to use.

POLYMORPHONUCLEAR LEUKOCYTE (PMN) SEPARATION

PMN was separated by using neutrophil isolation medium (NIM) (Cardinal Associates Inc., PO Box 5220, Santa Fe, NM 87502). Fresh blood was collected by venipuncture in sterile containers with heparin (10IU/ml, Fischer Scientific Co., NJ). Five to seven milliliters of heparinized blood was layered over 4ml of NIM in a 15 ml tube and then centrifuged at 400xg for 30 min at room temperature. The leukocyte-rich plasma was carefully removed with a Pasteur pipet and transferred to a 15 ml conical centrifuge tube. Tube was filled with phosphate buffered saline (PBS) and centrifuged at 350xg for 10 min in a Heraeus centrifuge (Model GmbH, Osterode). Two milliliters of lysing buffer (E-Lyse) from the same company was added to lyse the residual erythrocytes, vortex to resuspend the pellets and centrifuged at 250xg for 10 min. The supernatant was discarded and the sediment suspended in 1 ml of 5% FCS. The cells was then counted and adjusted to the desired final concentration.

POLYMORPHONUCLEAR NEUTROPHIL VIABILITY

The percentage of viable PMNs was estimated by trypan blue exclusion test which was carried out by a microscopic count of cells not stained by 0.2% trypan blue and was expressed as percent of unstained cells to total cell numbers.

LUMINOL-ENHANCED CHEMILUMINESCENCE

A Berthold (AutoLumatPlus LB 953) luminometer with a constant temperature (37°C) controller (Berthold Technologies GmbH & Co. KG, Calmbacher Straße 22, D-75323 Bad Wildbad-Germany) connected to a computer was used. The reaction mixture consist of 100µl of whole blood or PMN suspension and 900µl medium containing 10^{-5} M luminol (5-amino-2,3-dihydro,1,4-phthalazinedione

Sigma Chemical Co., St. Louis, MO, USA) , 2ng/ml phorbol myristate acetate (PMA) Sigma Chemical Co., St. Louis, MO, USA , 1.25 mg/ml opsonized zymosan (OPZ) Sigma Chemical Co., St. Louis, MO, USA and phosphate buffered saline (PBS). Light emission was recorded in millivolts (mV) and the readings were recorded at 1 minute intervals for 30 minutes. CL emission was quantified as the peak height in mV.

STATISTICAL ANALYSIS

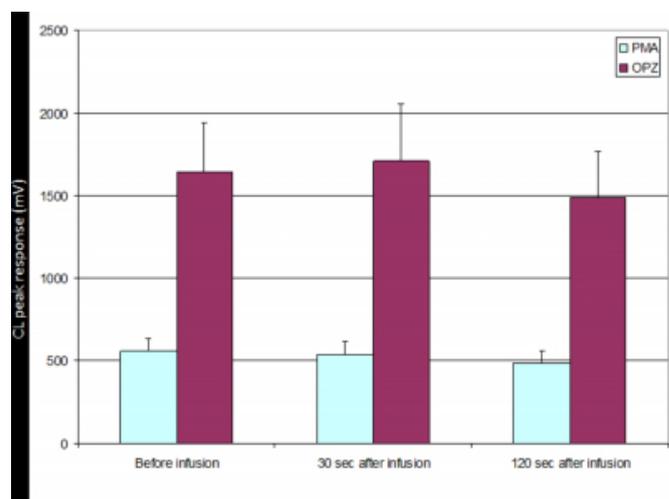
Data are presented as mean (SD) , number (percentage) or ratio as appropriate. After testing of normal distribution, data were compared using a Mann-Whitney U test. P value<0.05 was considered significant.

RESULTS

Results showed that there is no significant difference between respiratory activity of PMNs before injecting propofol and 30 and 120 sec after injection. The mean value of PMA was 556.725 before propofol injection and dropped to 534.793 at 30 sec following propofol injection. Further it was decreased to 485.206 at 120sec afterward. The mean values of OPZ were 1644.881 before propofol injection and 1711.993 and 1485.775 at 30 and 120 sec consequently after propofol injection (Table , Figure 1).

Figure 1

Figure 1: PMA and OPZ peak responses



PMA concentration = 2ng/ml

OPZ concentration = 1.25mg/ml

Figure 2

Table 1: Mean values of PMA and OPZ

	PMA	OPZ
	Mean	Mean
Before infusion	556.725±76.1618	1644.881±295.6576
30 sec after infusion	534.793±85.3187	1711.993±343.6449
120 sec after infusion	485.206±70.7658	1485.775±278.981

DISCUSSION

Intravenous anesthetics may cause transient changes in the immune systems of animals and humans [10,11,12]. In present study there was no significant suppression of PMN respiratory burst by acute administration of propofol .

Many in vitro or in vivo studies were conducted to demonstrate the effects of anesthetics on PMN function [14,15,16,17] . Those studies showed a reduction in the phagocytotic capacity of alveolar macrophages during anesthesia using propofol [18]. In another study it was showed that isoflurane and propofol anesthesia in humans undergoing cataract surgery reduced the phagocytic capacity of PMNs [14].

While in another studies propofol did not depress T-lymphocyte proliferation or leukocyte function compared with thiopental and etomidate and maintained the microbicidal function of alveolar macrophage during anesthesia comparing with inhaled anesthetic, such as isoflurane [19,20,21].

In another study propofol was able to suppress oxidative burst formation in TNF-a-primed neutrophils [22]. The discrepancies between in-vivo and in-vitro effects in general may be due to the great pool of extravascular PMNs in the organism [23]. Multiple causes were postulated for such inhibition.

The reduction of the intracellular calcium concentration in neutrophils represents one of propofol's pivotal mechanisms of functional inhibition [24]. Moreover, propofol, because of its phenolic hydroxyl group, chemically resembles the chain-breaking antioxidant a-tocopherol. This phenolic hydroxyl group is responsible for the antioxidant properties of propofol [25].

In conclusion our study failed to demonstrate acute inhibition of propofol on PMN burst. That might be due to

small sample size of the present study. Therefore we do recommend further studies on large sample size to prove or disprove our results.

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