ORIGINAL RESEARCH

IN VITRO EFFECTS OF INTRAVENOUS PROPOFOL INFUSION ON CYTOKINE GENE EXPRESSION IN RATS

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ABSTRACT

Objective: The aim of this study was to evaluate the effects of propofol infusion on cytokine gene expression levels of bone marrow and mesentheric lymph nodes in rats after surgical trauma.

Methods: Twenty male Sprague-Dawley rats weighing between 300-320 g were divided into two groups (n=10). Midline laparotomy incision was performed on both groups. Group I received 5 ml/kg/hr iv serum physiologic infusion. Group II received 5 mg/kg/hr iv propofol infusion. After infusion, mesentheric lymph node and bone marrow samples were obtained for the measurement of IL-1, IL-6 and TNFα gene expression levels by real time polymerase chain reaction (RT-PCR) method.

Results: IL-1 ($p<0.002$) and TNFα ($p<0.018$) levels of the mesentheric lymph node were significantly lower in group II compared to group I. TNFα gene expression level in the bone marrow was also significantly lower in group II compared to group I ($p<0.0015$).

Conclusion: Propofol infusion during surgery caused significantly more proinflammatory cytokine gene expression supression in the mesentheric lymph nodes compared to bone marrow in rats. We concluded that propofol infusion may have a limited and especially local immune supressive effect.

Keywords: Anesthesia, Propofol, Immune system, Cytokine, Gene expression

SIÇANLARDA İNTRAVENÖZ PROPOFOL İNFÜZYONUNUN SİTOKİN GEN EKSPRESYONUNA ETKİSİNİN İNVİTRO ARAŞTIRILMASI

ÖZET

Amaç: Bu çalışmanın amacı; sıçanlarda intravenöz propofol infüzyonunun sitokin gen ekspresyonuna etkisinin invitro araştırılmasıdır.

Yöntem: 300-320 g ağırlığında, 20 adet, erkek, Spraque-Dawley cinsi sıçan iki gruba ayrıldı (n:10). Her iki gruba orta hat laparotomi insizyonu uygulandıktan sonra 90 dakika süre ile Grup I'e 5ml/kg/s serum fizyolojik infüzyonu, Grup II'ye 5ml/kg/s propofol infüzyonu verildi. İnfüzyon bitiminde mezenter lenf nodu kemik iliği aspiratları IL-1, IL-6 and TNFα ölçümleri için alındı. Ölçümler; real time polimeraz zincir reaksiyonu (RT-PCR) yöntemi ile yapıldı.

Bulgular: Mezenter lenf nodlarında IL-1 ($p<0.002$) ve TNFα ($p<0.018$) seviyeleri grup II'de grup I'den anlamlı olarak düşük saptandı. Kemik ikliğindeki TNFα gen ekspresyon değerleri grup II'de grup I'den anlamı olarak düşük saptandı($p<0.0015$).

Sonuç: Cerrahi uygulanan sıçanlarda propofol infüzyonunun mezenter lenf nodunda kemik iliğine göre daha belirgin proinflamatuarsitokin gen ekspresyon baskılanmasına neden olduğu saptandı. Propofol infüzyonunun immün sistemi baskılayıcı etkisinin sınırlı ve özellikle bölgesel bir etki olduğu kanısına varılmıştır.

Anahtar Kelimeler: Anestezi, Propofol, Immün sistem, Sitokin, Gen ekspresyonu

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INTRODUCTION

Although the direct effect of anesthesia over the immune system could not be clearly identified, it has been shown that some anesthetic agents and interventions suppress immune functions by reducing the number of circulating immune cells and inhibiting the synthesis of interleukins and proteins during the acute phase[1].

Propofol (2,6- diisopropilphenol) is a chemically inert phenol derivate. It is a short-acting anesthetic drug with a very fast induction period. In 30 seconds (approximately one 'arm and brain circulation' period) propofol may induce loss of consciousness. It is used for target-controlled intravenous infusions and patient-controlled sedation. After a single dose of propofol administration, awakening is rapid[2]. This makes propofol a suitable agent for anesthesia and the sedation of ambulatory surgeries. The basic success of propofol as an intravenous anesthetic agent is the awakening period of 10 to 20 minutes after cessation of long-term and high dose administration[3].

Gene expression is a process which starts with DNA transcription and ends with mRNA production. After that, gene products are formed by posttranscriptional modification and translation methods[4].

Suppression of the immune system functions in physiological conditions or in the presence of local or systemic infections is not a desired property of the anesthetic agents. As cytokine accumulation frequently does not reach significant levels in the peripheral blood during local immune response, in this study; we aimed to evaluate the effects of propofol on proinflammatory cytokine "interleukin 1 (IL-1), interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α)" levels in mesentheric lymph nodes for local response and in bone marrow for systemic response.

MATERIAL AND METHOD

The study protocol was approved by the InstitutionalAnimal Care and Use Committee. The animals were housed individually in a temperature (20±1 ºC) controlled environment, maintained on a 12h light/ dark cycle, and provided with food and water ad libitum.

Twenty adult, male Sprague-Dawley rats weighing 300-320 g were divided into two groups (n=10). Anesthesia was induced with 0.2 mg/kg urethane administered intraperitoneally. An intratracheal cannula was inserted from a midline incision for a tracheostomy to facilitate breathing. A polyethylene catheter was inserted into the right vena jugularis for intravenous infusions.

After midline laparotomy, the abdomen was covered by sterile gauze and hypothermia was prevented by heating pads. Group I received 1 ml/kg/hr serum physiologic infusion. Group II received 5 mg/kg/hr propofol infusion for 90 min. All rats were sacrified and mesentheric lymph node dissection was performed. Lymph node samples were obtained and bone marrow was aspirated from both femurs. The samples were collected in sterile tubes including ringer lactate and were kept in -80ºC.

Cytokine gene expression measurements from the mesentheric lymph node and blood marrow aspirates were carried out real time polymerase chain reaction method (RT-PCR).

RT- PCR Method: Total RNA was separated from bone marrow and lymph node samples by the trizol method. Reverse transcription was performed by randomized hexamers and 1.0µg total RNA in a solution produced from RNAse inhibitor which is in a total of 20 µl (RNAsin 1.0 U), 1 mM of each deoxynucleotide triphophate, 2.5 U reverse transcriptase (Promega), 1x PCR buffer and 5.0 mM MgCl2. The samples were initially incubated for 30 minutes in 42 ºC and then were re-incubated for 5 minutes in 99 ºC to inactivate reverse transcriptase. Then, amplification was obtained by cDNA, "Light-cycler RNA Master Sybergreen Kit". Sybergreen I is a double spiral DNA specific dye whose florescence significantly increases when binding to DNA. During DNA synthesis of PCR reaction, "Sybergreen" binds to reproducing PCR product DNA and produces

a florescence, and the measurement of DNA is automatically evaluated by this device. Specific primers were used for IL-1, IL-6 and TNF-α during the process. GAPDH "House-keeping gene" amplification was used as control.

Gene expression levels were determined according to their ratio to 'House keeping gene' GAPDH expression.

Specific primary gene line for each cytokine is as follows:

IL-1 β: 5'-ATGGCAACTGTTCCTGAACTCAACT-3'
3'-TTTCCTTTCTTAGATATGGACAGGAC-5'
IL-6 : 5'-ATGAAGTTCCTCTCTGCAAGAGACT-3'
3'- CACTAGGTTTGCCGAGTAGATCTC-5'
TNF α: 5'-ATGAGCACAGAAAGCATGATCCGC-3'
3'- CTCAGGCCCGTCCAGATGAAACC-5'

Statistical analysis: The values were represented as mean±SD. Statistical differences were analysed by using 'Student unpaired *t-test*' and ' Mann-Whitney's U-test'. A p value <0.05 was considered significant.

RESULTS

There was a significant decrease in the IL-1 ($p< 0.002$) and TNFα ($p<0.018$) levels of the lymph node samples in the propofol group compared with the serum physiologic group. IL-6 levels were the same between the groups ($p>0.05$) (Table I).

There was a significant decrease in the TNFα levels of the propofol group in the bone marrow samples compared with the serum physiologic group ($p<0.0015$). However; the IL-1 ve IL-6 levels of the bone marrow samples of the two groups were similar ($p>0.05$) (Table II). .

Table I: Cytokine gene expression levels in the mesenteric lymph node samples (mean±SD)

	IL-1	*IL-6*	*TNF α*
Group I (N:10)	*0.0345802±0.01631*	*0.002131±0.001427*	*0.0443326±0.01323*
Group II (N:10)	*0.0096836±0.005675**	*0.0014698±0.001226*	*0.0178593±0.01865***

* p<0.05; ** p<0.01

Table II: Cytokine gene expression levels in the bone marrow samples (mean±SD)

	IL-1	IL-6	TNF α
Group I (N:10)	0.0736004±0.02041	0.0304346±0.01497	0.1699774±0.03773
Group II (N:10)	0.1021464±0.07861**	0.060545±0.1031**	0.103821625±0.04105*

* p<0.05; ** p<0.01

DISCUSSION

Anesthesic drugs and surgical trauma may have suppressive effects on the immune system leading to infections[1]. The choice of anesthetic agent becomes critical for anesthesiologists especially in immune-compromised patients. It has been shown that all of the anesthetic methods (inhalational, intravenous or regional anesthesia) may influence perioperative immune functions[5]. Propofol is a popular intravenous anesthetic agent with a rapid onset and short duration of effect. However the results of the studies evaluating the effects of propofol on the immune system are conflicting. The antiproliferative effects of propofol is observed only in the polymorphonuclear cells of the immune-suppressed patients[6]. In animal studies, propofol has been shown to have antiinflammatory effects during endotoxic inflammation[7,8]. However, in the blood culture media of healthy volunteers, propofol did not affect the lymphocyte proliferation and cytokine release which are the primary responses to endotoxins[9]. In the study of Crozier et al. [10,] IL-6 levels of the patients who received propofol and alfentanil anesthesia were found to be lower than those who received an inhalational anesthetic agent. Taylor et al[11] in their study evaluating the effect of opioids on interleukins concluded that the IL-6 response is not affected when opioids are added to inhalational anesthetics during surgery.

In clinical trials, it is difficult to interpret the effects of anesthesic agents on immune cells by measuring only the plasma cytokine levels during anesthesia and surgery, as the immune system is under the influence of neuroendocrine stress response which alters the effects of the anesthesic agents on organ systems. From that point of view, we aimed to evaluate the effects of propofol on the local and systemic cytokine gene expression in rats under surgical stress, without stimulating the immune system by an endotoxin.

Cytokine bioactivity is measured by the mrna expression level in the plasma or tissues. However, the plasma cytokine levels do not always reflect the cytokine bioactivity in the inflammed region[12]. It should be kept in mind that, cytokines have different half lives and rapid use-up or excretion periods with urine, so the plasma levels may differ from time to time[13]. Tnfα, which has a short plasma half life, is known to have high plasma levels in the first hours of the reaction, after which plasma levels rapidly decrease and disappear[13]. In contrast, ıl-6 which has a longer half-life, is released in the plasma as a response to tnfα and peak plasma levels are reached several hours after the release of tnfα [13].

Cytokines have a local effect in mild to medium immune and inflammatory responses. Activation of the immune system generally occurs as a local response and that local site is frequently an infectious site which is drained by a lymph node. Cytokine concentration usually does not reach significant levels in the peripheral blood during local responses. Moreover, the alterations in the gene expression are not always correlated and occur simultaneously with the changes in plasma levels of the related protein. In our opinion, evaluation of cytokine levels by the gene expression levels in the mesenteric lymph node and bone marrow, particularly during the initial periods of inflammation gave us the possibility to obtain more dependable results.

Badia et al.[14] have shown that cytokine levels increase in the peritoneal fluid after abdominal surgery. In our study, we observed that the highest tnfα levels of the peritoneal fluid were reached between the 6th and 10th hours, ıl-6 levels at the 24th hour and ıl-1 levels between the12th and 24th hours, while the plasma cytokine levels were very low, almost unmeasurable during the first days. This condition suggests that the cytokine response of the inflammatory cells is a local entity. We suggested that we could not observe a significant increase in the ıl-6 levels throughout the study as ıl-6 is released in response to tnfα. Significant increases of the

ıl-6 levels are observed particularly following major surgery such as joint prothesis implantations, vascular and colorectal surgeries[15]. It has been reported that the degree of trauma and blood loss during the operation significantly influences postoperative ıl-6 levels[16]. A major limitation of our study was that the surgical trauma was minor and could cause the statistically significant but clinically minor difference between the propofol infusion and control groups.

We concluded that propofol infusion may have a limited and especially local immune supressive effect during laparotomy in rats and further studies are needed to clarify the effect of propofol on cytokine gene expression.

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