DE GRUYTER OPEN

The Journal of Critical Care Medicine 2016;2(1):44-50

RESEARCH ARTICLE

DOI: 10.1515/jccm-2016-0008

Total Intravenous Versus Inhalation Anesthesia in Patients Undergoing Laparoscopic Cholecystectomies. Effects on Two Proinflammatory Cytokines Serum Levels: Il-32 and TNF-Alfa.

Adina Hadade[1*], Daniela Ionescu[1], Teodora Mocan[2], Alexandru Necula[3], Victor Cristea[4]

[1] Department of Anesthesia and Intensive Care, "IuliuHatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

[2] Department of Physiology, "IuliuHatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

[3] Department of Surgery, Regional Institute for Gastroenterology and Hepatology "Prof dr Octavian Fodor", Cluj-Napoca, Romania

[4] Department of Clinical Immunology, "IuliuHatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Abstract

Introduction: It has been reported that as compared with total intravenous anesthesia (TIVA), inhalation anesthesia is increasing the postoperative level of proinflammatory interleukins.

The **aim** of the study is to investigate if there is an in-vivo relationship between proinflammatory cytokines, Interleukin-32 (IL-32) and Tumour necrosis factor – α (TNF- α), in patients undergoing laparoscopic cholecystectomies with two different anesthetic techniques, TIVA or inhalation anesthesia.

Material and Methods: Twenty two consecutive patients undergoing laparoscopic cholecystectomies were prospectively randomized into two groups: Group 1: TIVA with target-controlled infusion (TIVA-TCI) (n=11) and Group 2: isoflurane anesthesia (ISO) (n=11). IL-32 and TNF-α were determined before the induction of anesthesia (T1), before incision (T2) and at 2h (T3) and 24h (T4) postoperatively. Our primary outcome was to compare plasma levels of IL-32 and TNF- α concentrations (expressed as area-under-the-curve) over 24 hours between study groups. Our secondary outcome was to establish whether there is a correlation between plasma levels of IL-32 and of TNF-α at each time point between the two groups.

Results: Area-under-the-curve (AUC) of IL-32 plasma concentration was 7.53 in Group 1 (TIVA) versus 3.80 in Group 2 (ISO), p= 1. For TNF-α, AUC of plasma concentration was 733.9 in Group 1 (TIVA) and 668.7 in Group 2 (ISO), p= 0.066. There were no significant differences in plasma concentrations of both IL-32 and TNF- α between the groups.

Conclusions: IL-32 expression in response to minor surgery is very low. There were no significant difference between plasma levels ofTNF- α and IL-32 after TIVA versus inhalation anesthesia during the first 24 hours postoperatively. Further studies are needed on larger groups to investigate whether there can be a correlation between these interleukins after 2 different anesthetic techniques and the impact of this correlation on postoperative outcome.

Keywords: total intravenous anesthesia with target-controlled infusion (TIVA-TCI), inhalation anesthesia, cytokines, laparoscopic cholecystectomy

Received: Received: 14 September 2015 / Accepted: 18 December 2015

* Correspondence to: Adina Hadade, Department of Anesthesia and Intensive Care, "Iuliu Hatieganu" University of Medicine and Pharmacy, 8, Victor Babes, 400012, Cluj-Napoca, Romania. E-mail: ahadade24@gmail.com

· Daniela Ionescu: Department of Anesthesia and Intensive Care, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, 8, Victor Babes, 400012, Cluj-Napoca, Romania

· Teodora Mocan: Department of Physiology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, 8, Victor Babes, 400012, Cluj-Napoca, Romania

· Alexandru Necula: Department of Surgery, Regional Institute for Gastroenterology and Hepatology "Prof dr Octavian Fodor", Cluj-Napoca, Romania, 5, Constanta, Cluj-Napoca, 400158, Romania.

· Victor Cristea: Department of Clinical Immunology, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, 8, Victor Babes, 400012, Cluj-Napoca, Romania

Introduction

Surgical patients exhibit changes in hemodynamic, metabolic and immune responses that are largely caused by an endocrine response to surgery and by cytokines. Unlike hormonal mediators, which are produced by specialized tissues and exert their influence predominantly by endocrine routes, cytokines are polypeptides or glycoproteins produced by diverse cell types.

Studies comparing the perioperative immunomodulation produced by inhalation and intravenous anesthesia have found that inhalation anesthesia has an enhanced suppressive effect on the immune system compared to total intravenous anesthesia (TIVA) [2].

In vitro and in vivo studies, involving human immune cells or animal models, investigating the effect of anesthetic drugs on the immune response, have illustrated diverse effects, including changes in immune cell count and functionality, and the secretion of cytokines. These changes affect the inflammatory response in the postoperative period [3].

In response to bacterial antigens, Tumor necrosis factor α (TNF-α) is produced by natural killer (NK) cells, T and B lymphocytes, mast cells, monocytes and macrophages. TNF-α is the main pro-inflammatory cytokine responsible for septic shock [3] and is involved in acute inflammatory processes, including acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) associated with sepsis [4].

It has been proposed that Intreleukin 32 (IL-32) plays an important role in modulating innate and adaptive responses to infection. IL-32 was recently described as a cytokine that demonstrates increased expression in endothelial, epithelial, monocytic, and other cell populations and that induces the production of proinflammatory cytokines, including TNF-α and IL-1β [4]. IL-32 has also been linked to other inflammatory diseases, including psoriasis, Crohn disease, and rheumatoid arthritis. Studies on transgenic mice also indicated that IL-32 has a role in vascular inflammation, causing the induction of inflammatory cytokines, such as IL-1 and TNF-α and cell adhesion molecules, with subsequent leukocyte adhesion [5]. IL-32 is also involved in the lipooxigenase pathway of arahidonic acid metabolism which leads to the production of leukotrienes (LTs) and lipoxins [6].

Other studies compared the effect of propofol and isoflurane anesthesia on the immune response to surgery [7], concluding that propofol anesthesia attenuated the surgical stress-induced adverse immune response better than isoflurane anesthesia, in patients undergoing craniotomy.

The present study was designed to compare the effect of TIVA and inhalation anesthesia on plasma concentrations of two potent proinflammatory cytokines (IL-32 and TNF-α), knowing that IL-32 is a potent inducer of TNF-α, in response to inflammation. At the same time, we tested the hypothesis that TIVA blunts the inflammatory response to laparoscopic cholecystectomy more than isoflurane anesthesia, and a correlation may be found between the two cytokines in response to minor surgery, similar to other studies.

Material and Methods

After approval by the Ethics Committee of our institution (Regional Institute of Gastroenterology and Hepatology "Prof dr Octavian Fodor", ClujNapoca) and after having written informed consent, 22 patients ASA I-II class according to American Society of Anesthesiology classification, scheduled for laparoscopic cholecystectomy, were included in the study. Patients were randomized by a computer-generated sequence in 2 study groups: group 1 (n=11) included patients undergoing TIVA and group 2 (n=11), included patients undergoing inhalation anesthesia. Exclusion criteria were patients with known inflammatory diseases, asthma, obesity (BMI ≥ 30 kg/m^2), diabetes, gastric ulcer, allergies, current steroid or anti-inflammatory medication or malignances.

All patients received oral premedication with 7.5 mg of midazolam, two hours prior to surgery. On arrival in the operating theatre, an intravenous cannula was inserted, blood was drawn for interleukin measurements, and 500 ml of crystalloid solution was administered. The cannula was used for subsequent fluid administration during the surgical procedures.

A second intravenous cannula was inserted for drug infusion, in the TIVA group.

TIVA was induced and maintained with a target-controlled infusion (TCI) of propofol, with an initial plasma concentration of 4 μg/ml, administered through TCI infusion pumps (Alaris PK, Cardinal Health, Rolle, Switzerland). The propofol plasma concentration was adjusted to target a bispectral index between 40-55 (Spacelabs Healthcare, Issaquah, WA,USA).

Isoflurane anesthesia was induced with propofol 1.5 to 2 mg/kg, and maintained with isoflurane 1 to 1.5 minimum alveolar concentration (MAC), titrated to a bispectral index of 40 to 55 and to hemodynamic parameters. In both groups, remifentanil was administered in a manually controlled infusion, with an initial dose, within the first minute, of 0.5 µg/kg/min and 0.25 µg/kg/min thereafter during induction and maintenance. The dose of remifentanil was adjusted in 0.05 µg/kg/minute steps according to analgesic needs and hemodynamic parameters : heart rate, blood pressure (more than 20% above baseline values), pupil size and lacrimation.

Atracurium, 0.5-0.6 mg/kg, was administered to facilitate tracheal intubation in both groups, and 10 mg boluses were injected during maintenance, if needed. During recovery, propofol and remifentanil infusions were stopped after the surgeon had completed the last suture, and neuromuscular blockade was antagonized with 2.5 mg neostigmine together with 1 mg atropine. Administration of isoflurane was stopped before the last two sutures were inserted.

Intraoperative monitoring included the measurement of mean arterial blood pressure, continuous ECG, oxygen saturation, end-tidal carbon dioxide (ET CO_2), end -tidal concentration of isoflurane and depth of anesthesia with BIS.

Hypotension, defined as a decrease in blood pressure >30% from baseline, was treated with additional fluids and/or ephedrine.

Postoperative analgesia was provided by oral paracetamol, 1 g every eight hours and intravenous meperidine, 0.3 to 0.4 mg/kg, according to the needs, or when the pain score, on a 10 - point visual analogue scale (VAS), was ≥ 3.

Serological parameters/Measurements

Blood samples (7 ml) were taken immediately after inserting the first peripheral cannula (T1), after intubation but before incision (T2), two hours after emergence from anesthesia (T3) and twenty four hours (T4) after anesthesia. The samples were centrifuged at 2500 rpm for 10 minutes and the plasma stored at less than - 20°C until assayed.

Interleukins plasma concentrations were measured using ELISA kits (Quantikine ; R&D Systems, Minneapolis, MN, USA). Laboratory staff were blinded to the study groups and were not involved in the anesthetic procedures.

Minimum detectable concentrations for interleukins as given by the manufacturer were as follows: for IL-32 less than 6 pg/mL and for TNF-α less than 0.5 pg/mL, with intra and inter-assay coefficients of variation less than 10%.

Normal plasma concentrations of interleukins measured with the Quantikine assays are in the following ranges: IL-32, 15.6-1.000 pg/mL and for TNF-α 15.6-1.000 pg /mL.

Statistical analysis

The Bartlett test was used to test for equal variances and the Kolmogorov-Smirnov test used to determine normal distribution.

For qualitative data, comparison between TIVA and Isoflurane was performed using Fisher's Exact Test or Chi Square test, as appropriate. Continuous data comparisons between the two groups were assessed using Student t test for independent samples or Mann-Whitney U Test, based on normality testing results. Correlations of continuous data were performed using non-parametric correlation analyzes (Spearman R calculus). Within- group comparisons were performed using Wilcoxon test. AUC calculations with AUC difference testing was performed using Delong Test. The level of statistical significance was set at $\alpha = 0.05$.

Based on presented results for TNF-alpha, it was calculated that for alpha=0.05 twenty-four patients per group would give a power threshold of 85%.

Results

All the enrolled patients completed the study.

The clinical characteristics of the patients are given in Table 1. Demographic and clinical data were similar in both groups, as were anesthetic and surgical management procedures (Table 1).

The mean plasma concentration of propofol in Group 1 (TIVA) was 2.92 ± 1.23 µg/mL. The mean end-tidal isoflurane level in Group 2 (ISO) was 0.89 ± 0.38 %. There were no significant differences between the remifentanil dose in Group 1 (0.25 ± 0.05 µg/kg/min) compared to Group 2 (0.24 ± 0.02 µg/kg/min).

Pre-induction plasma concentrations of interleukins were similar in both groups for IL-32 and TNF- α (Table 2).

Between-group comparisons of AUC of cytokine plasma concentrations are showed in Table 3. There

Table 1. Demografic data of the study groups

	Total intravenous anesthesia (n=11)	Isoflurane (n=11)	P value
Age (years)[a]	54 ± 13	47 ± 13	0.221
Weight (kg)[a]	79 ± 13	79 ± 14	1
Gender (M/F)	0/11	6/5	0.012
ASA status (I/II)	2/9	3/8	0.499
Anesthesia time (minutes)[a]	49 ± 9	53 ± 16	0.477
Intraoperative Bispectral index[a]	33 ± 13	43 ± 10	0.063
Intraoperative mean arterial pressure (mmHg)[a]	73 ± 22	69 ± 19	0.649
Intraoperative heart rate (beats/minute)[a]	71 ± 16	65 ± 14	0.354
Intraoperative intravenous fluids (L)[a]	1.1 ± 0.2	1 ± 0.4	0.481
Intraoperative remifentanil (µg/kg/minute)[a]	0.25 ± 0.05	0.24 ± 0.02	0.554

a Data expressed as mean±standard deviation; ASA I/II = American Society of Anesthesiologists physical status I/II; F=female; M=male

Table 2. Pre-induction interleukin plasma concentration in study groupsa

	TIVA group (n=11)	ISO group (n=11)	p-Value
TNF-α (pg/ml)	188.1	370.48	0.531
IL-32 (pg/ml)	15.07	7.6	0.752

a Data are expressed as median (minimum - maximum)
Concentrations less than the limit of detection were reported/considered as 0 pg/mL.

Table 3. Results of between-group longitudinal comparisons of plasma levels of interleukins in study groups

	TIVA group (n=11) AUC[a]	ISO group (n=11) AUC[a]	p-Value
IL-32	7.53 (0.6)	3.80 (0.5)	1.000
TNF-α	733.9 (20.2)	668.7 (27.5)	0.066

[a]AUC was calculated having baseline as common reference point of plasma levels of interleukins; between- group longitudinal comparisons were performed using Delong test. p<0.001 was considered statistically significant.
AUC = area under the plasma concentration time curve

were no significant differences in AUCs between the groups (p=1 for IL-32, and p=0.066 for TNF-α).

Plasma concentrations for IL-32 and TNF-α are shown in Figures 1 and 2, respectively.

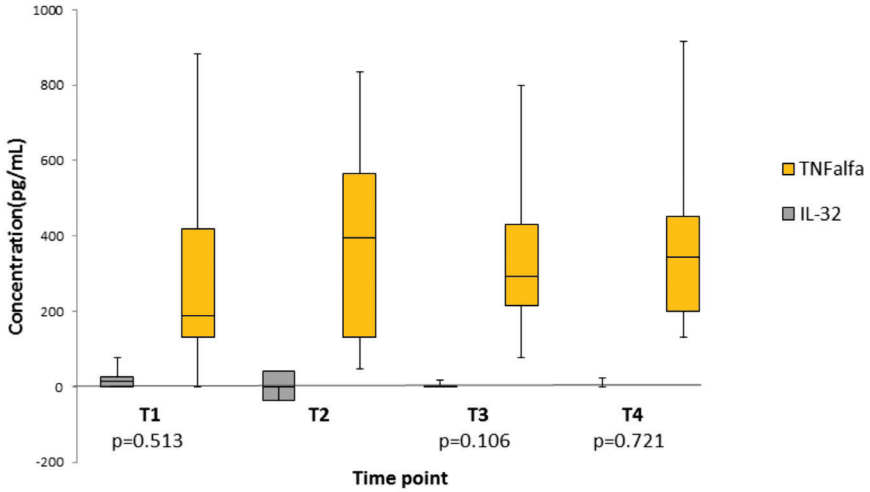

Fig. 1. Median plasma concentrations in pg/mL of interleukins at T1, T2, T3, T4. Data expressed as median (horizontal bar) with 25th-75th (boxes) and the 10th-90th (whiskers) percentiles. No difference were noted between interleukin plasma concentrations at different time points. T1 = before induction; T2 = after intubation; T3 = 2 hours after skin closure; T4 = 24 hours after skin closure; TIVA = total intravenous anesthesia

Table 4. Plasma levels of interleukins in study groups: T1-T3 within-group comparison of values at 2 hours postoperative with baseline values[a]

	TIVA group (n=11) T1	T3	P-Value (TIVA group)	ISO group (n=11) T1	T3	p-Value (ISO group)
IL-32	15.08 (ND-77.0)	ND (ND-18.0)	0.043	ND (ND-40.0)	ND (ND-35)	0.263
TNF-α	188.1 (ND-884.0)	294.3 (76.3-798.5)	0.594	370.5 (47.5-622.9)	215.2 (18.3-345.5)	0.091

[a] Data are expressed as median (minimum-maximum)
T1= before induction; T3= 2 hours after skin closure

Table 5. Plasma levels of interleukins in study groups: T1-T4 within-group comparison of values at 24 hours postperative with baseline values[a]

	TIVA group (n=11) T1	T4	P-Value (TIVA group)	ISO group (n=11) T1	T4	p-Value (ISO group)
IL-32	15.08 (ND-77.0)	ND (ND-24.0)	0.046	ND (ND-24.0)	ND (ND-23.0)	0.043
TNF-α	188.1 (ND-884.0)	345.5 (132.9-915.8)	0.477	370.5 (47.5-622.9)	268.3 (104.8-915.8)	0.424

[a] Data are expressed as median (minimum-maximum) T1 = before induction; T4 = 24 hours after skin closure

When comparing interleukin concentrations between groups at different time intervals, there was no significant difference in plasma concentration of IL-32 and TNF-α (Tables 4, 5). Consideration the larger variability in the TIVA group, a within-group analysis was used (Table 6). Intragroup comparisons revealed that in group 1, TNF-α concentrations increased by two hours postoperatively (T3), compared with IL-32 at the same anesthetic time, although with no significant difference (p=0.106), whereas at 24 hours postoperatively

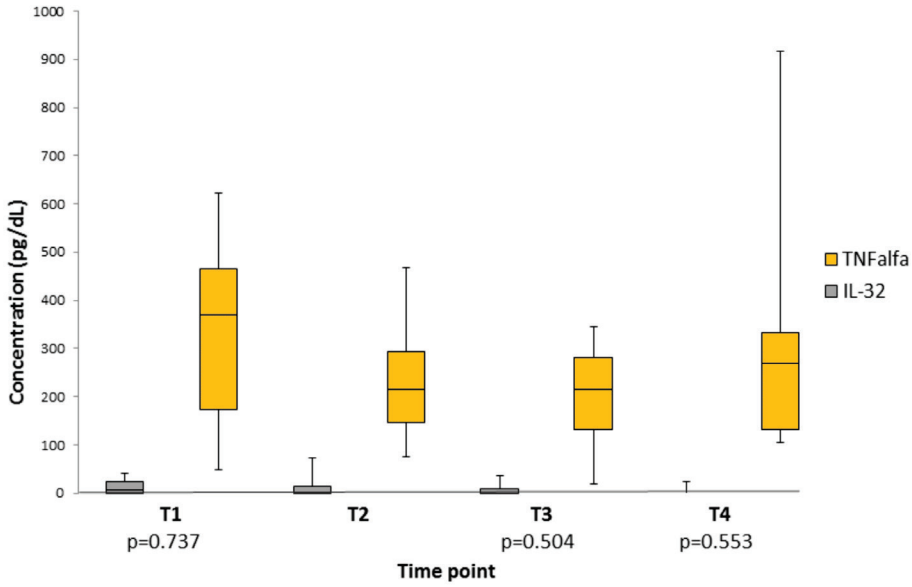

Fig. 2. Median plasma concentrations in pg/mL of interleukins at T1, T2, T3, T4. Data expressed as median (horizontal bar) with 25th-75th (boxes) and the 10th-90th (whiskers) percentiles. No difference were noted between interleukin plasma concentrations at different time points. T1 = before induction; T2 = after intubation; T3 = 2 hours after skin closure; T4 = 24 hours after skin closure; ISO= isoflurane

Table 6. Within-group correlation between IL-32 and TNF-alfa at different anesthetic times:

	IL-32	TNF-α	R	p-Value
TIVA group	T1 15.08 (ND-77.0)	T1 188.1 (ND-884.0)	-0.221	0.513
	T3 ND (ND-18.0)	T3 294.4 (76.3-798.5)	0.514	0.106[a]
	T4 ND (ND-23)	T4 345.5 (132.9-915.7)	0.122	0.721[b]
ISO group	T1ND (ND-40.0)	T1 370.5 (47.5-622.9)	0.115	0.737
	T3 ND (ND-35)	T3 215.2 (18.3-345.5)	-0.226	0.504
	T4 ND (ND-24)	T4 268.3 (104.7-915.7)	0.201	0.553

Data are expressed as median (minimum-maximum).
T1= before induction; T3= 2 hours after skin closure; T4= 24 hours after skin closure; [a] intragroup comparisons of IL-32 and TNF-α at T3; [b] intragroup comparisons of IL-32 and TNF- α at T4

our results didn't show a significant difference between the two interleukins (p=0.721). IL-32 at 24 hours postoperatively was slightly decreased in TIVA group as compared with baseline values, while TNF-α was still increased, but similar to baseline values.

Discussion

It is well known that any surgical procedure impairs homeostasis and triggers various metabolic and immunologic reactions. The intensity of postoperative response is correlated with the degree of the tissue trauma and the type of surgery. A postoperative immune response consists of an increased production of proinflammatory cytokines (IL-6 and TNF), followed by an increased production in anti-inflammatory cytokines [8]. Wang et al. demonstrated that the expression of mediator IL-6 in response to surgical stress has been statistically increased in open radical cystectomy compared with laparoscopic cystectomy, which reflected the lower tissue damage of laparoscopic cystectomy [8].

It has been demonstrated that the total intravenous anesthesia using propofol and remifentanil suppresses the inflammatory response caused by surgery, to a greater extent than a balanced inhalation technique [9]. Ihn et al. concluded that there are still controversies about which anesthetic technique best suppresses the inflammatory and immunological responses. The present study data suggests there is no significant difference in IL-6 levels between the two anesthetic regimens, but TIVA with propofol and remifentanil may have some advantages in terms of reducing stress-related responses to surgery.

TIVA with propofol-remifentanil, in patients undergoing open cholecystectomy, resulted in significantly lower concentrations of IL-6 and TNF-α, compared with the inhalation technique [10].

Schneemilch et al. reported that anesthetic management may influence the postoperative immune response in different ways and these may be relevant in patients with preexisting immune dysfunctions [11].

IL-32 and TNF-α are mostly involved in inflammatory diseases [12]. The current study found that the inflammatory cytokine response (IL-32, TNF-α) during a twenty four hour postoperative period did not differ in either the TIVA or ISO groups.

Ionescu et al reported [13] that TIVA significantly reduced the increase of IL-6 during the perioperative period compared to isoflurane, with a small though insignificant increase in IL-10.

The present results did not confirm a correlation between the expression of IL-32 and TNF-α as a response to different anesthetic regimens in laparoscopic cholecystectomies.

It is accepted that the sample size used in this study was small, although a large difference in cytokine concentrations were recorded. Laparoscopic cholecystectomies produce only a moderate amount of tissue injury and a smaller inflammatory response would be expected compared with major surgery. In a few patients, expression of IL-32 was undetectable, and was not considered to be influenced by the anesthetic regimen, probably due to the small surgical aggression. TNF-α showed a larger variability in the TIVA group, with a small increase at two hours postoperatively, (T3) as compared to isoflurane group, while at 24 postoperative hours (T4) TNF-α remained increased in both groups compared to baseline values.

Conclusions

There was no significant difference between the effects of TIVA and isoflurane anesthesia on plasma

concentrations of IL-32 and TNF-α after laparoscopic cholecystectomies, during the first twenty four hours postoperatively. There was no correlation between the two cytokines, in response to different anesthetic techniques.

Both regimens exhibit similar clinical effects, however further studies on larger groups of patients undergoing more extensive surgical interventions are needed for a better evaluation of their clinical implication.

Abbreviation list

AUC: area under the curve;

NK: natural killer;

TIVA: total intravenous anesthesia;

ISO -Isoflurane;

ELISA: Enzyme-linked immunosorbent assay;

TCI: target controlled infusion;

BMI: body mass index

Conflict of interest

Nothing to declare.

References

1. Lin E, Calvano SE, Lowry SF. Inflammatory cytokines and cell response in surgery. Surgery. 2000;127:117-26.
2. Kurosawa S, Kato M. Anesthetics, immune cells, and immune responses. J Anesth. 2008;22:263-77.
3. Colucci D, Puig N, Hernandez-Pando R. Influence of anaesthetic drugs on immune response: from inflammation to immunosuppression. OA Anaesthetics. 2013;1:21.
4. Arcaroli JJ, Liu N, Yi N, Abraham E. Association between IL-32 genotypes and outcome in infection-associated acute lung injury. Crit Care. 2011;15:R138.
5. Kobayashi H, Huang J, Ye F, Shyr Y, Blackwell TS, Lin PC. Interleukin-32 beta propagates vascular inflammation and exacerbates sepsis in a mouse model. PLoS One. 2010;5:e9458.
6. Conti P, Youinou P, Theoharides T. Modulation of autoimmunity by the latest interleukins (with special emphasis on IL-32). Autoimmun rev. 2007;6:131-7.
7. Inada T, Yamanouchi Y, Jomura S, et al. Effect of propofol and isofluraneanaesthesia on the immune response to surgery. Anaesthesia. 2004;59:954-9.
8. Wang S, Chen Y, Lin H, Chen L. Comparison of surgical stress response to laparoscopic and open radical cystectomy. World J Urol. 2010;28:451-5.
9. Ihn CH, Joo JD, Choi JW, et al. Comparison of stress hormone response, interleukin-6 and anaesthetic characteristics of two anaesthetic techniques: volatile induction and maintenance of anaesthesia using sevoflurane versus total intravenous anaesthesia using propofol and remifentanil. J Int Med Res. 2009;37:1760-71.
10. Ke J, Zhan J, Feng X, Wu Y, Rao Y, Wang Y. A comparison of the effect of total intravenous anaesthesia with propofol and remifentanil and inhalational anaesthesia with isoflurane on the release of pro-and anti-inflammatory cytokines in patients undergoing open cholecystectomy. Anaesth Intensive Care. 2008;36:74-8.
11. Schneemilch CE, Ittenson A, Ansorge S, Hachenberg T, Bank U. Effect of 2 anesthetic techniques on the postoperative proinflammatory and anti-inflammatory cytokine response and cellular immune function to minor surgery. J Clin Anesth. 2005;17:517-27.
12. Shoda H, Fujio K, Yamaguchi Y, et al. Interactions between IL-32 and tumor necrosis factor alpha contribute to the exacerbation of immune-inflammatory diseases. Arthritis Res Ther. 2006;8:R166.
13. Ionescu D, Margarit S, Hadade A, Mocan T, Cristea V, Szederjesi J. The effects of a small dose of dexamethasone on cell adhesion molecules during laparoscopic cholecystectomy. Drugs R D. 2011;11:309-16.