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COMPARATIVE STUDY ON INTERLEUKIN 1, INTERLEUKIN 6 AND TUMOR NECROSIS FACTOR A IN OVARIOHYSTERECTOMIZED CATS ANESTHETIZED WITH DIFFERENT ANESTHETIC SCHEMES

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Abstract

The use of anesthetics is an integral part of any surgery. Despite their widespread use, their mechanisms and interactions with the nervous-endocrine and immune systems are insufficiently studied.The study was conducted with healthy, adult cats subjected to anesthesia and surgery (ovariohysterectomy) in order to trace the effect of anesthesia and surgery on the secretion of proinflammatory cytokines IL1, IL6 and TNFα. The ovariohysterectomy was performed when a deep plan of anesthesia occurred. Blood samples were obtained at 0 min (prior to anesthetic administration), 30, 60, 120 min and 24 h.The chosen anesthetic schemes modulate the immune response and the response depends on the type of anesthetics used.

Key words: *cats, anesthesia, Interleukin 1, Interleukin 6 and Tumor necrosis factor α*

INTRODUCTION

The use of anesthetics is an integral part of any surgery. Despite their widespread use, their mechanisms and interactions with the nervous-endocrine and immune systems are insufficiently studied.

Possible effects of anesthesia on the immune system have been a subject of discussion since the last century. The immune system is a complex of interactions between cells, molecules and organs in order to protect the body and preserve its homeostasis. Graham E. (1911) and Gaylord H. and B. Simpson (1916) reported at the beginning of the last century about the influence of ethereal anesthesia on bacteriolisis and phagocytosis in humans and the effect of anesthetics on tumor growth in experimental animals. Recent studies have shown that anesthesia can alter the immune response by modulating the stress-response of the body and associated stress-chromosomes (Schnnemilch C., 2005).

Cytokines are key regulatory molecules for the immune response to stress, including surgical and anesthetic. They represent a heterogeneous group of proteins that act on cell-surface receptors and regulate the amplitude and duration of response by short-term secretion and self-limiting.(Sheeran P. and G. Hall, 1997).

Contemporary human studies have shown that this area of medicine has great potential for development in the direction of cytokine response control and reduction of proinflammatory activity in order to successfully recover after surgery and anesthesia (Dinarello C., 2000).

The aim of the present study was to monitor the response changes by investigating **interleukin 1, interleukin 6 and tumor necrosis factor α** in two anesthesia schemes during ovariohysterectomy in female cats.

MATERIALS AND METHODS

Animals

Fourteen mixed breed female cats at the age between 2 and 4 years, weighing 2.8-3.4 kg, were included in the study. The animals were presented from the animal protection organization. One week before the examination, the animals were kept in the University Clinic for Small Animals at the Faculty of Veterinary Medicine, University of Forestry, Sofia. They were fed commercial dry food without limitation except for the 12-hour fasting period before the anesthesia and surgery. The water was restricted two hours before surgery. Immediately prior to the operation and anesthesia, the animals were examined and determined to be clinically healthy on the basis of the physical and blood laboratory examinations. All values were within normal physiological ranges. The cats were randomly allocated in two groups (n = 7 in each group).

Anesthetic protocol

The cats were randomly allocated in two experimental groups (n=7 in each group). The

premedication in the first group (group In) was made with acepromazine maleate 0.025 mg/kg (Vetranquil®, Ceva Sante Animale) intramuscularly, and the second group (group MM) was given acepromazine maleate 0.025 mg/kg (Vetranquil®, Ceva Santé Animale), butorphanol (Butomidor®, Richter Pharma)–0.4 mg/kg, intramuscularly and meloxicam (Loxicom®, Norbrook) - 0.3 mg/kg, subcutaneously.
All animals were submitted to fluid therapy with sodium chloride 0.9 %, 10 ml/kg/h (Natrii chloridum®, Actavis) through a venous catheter 22 gauge (B.Braun) applied in v. cephalica antebrachii. Induction of anesthesia was made with propofol (Propofol®, B Braun) at 5 mg/kg body weight intravenously, fifteen minutes after the premedication.
Immediately after the application of the general anesthesia, the animals were intubated with a tube of a suitable size. The anesthesia was maintained with isoflurane (Forane®, Abbott) 2.5 vol. % in group In and 1.8 vol.% in group MM in 2.5 l/min oxygen flow by using semi-opened breathing circuit system type T/Y detail, Kuhn modification. The extubation was made 60 min later at manifestation of swallowing reflex.

Surgery protocol
Ovariohysterectomy was performed through caudal median laparotomy. The average duration of the operation was between 8 and 10 min. Surgery started 30 minutes after the initiation of anesthesia at the surgical plane of anesthesia.

Collection of blood specimens
Blood specimens were obtained from the jugular vein in sterile 2.0 ml syringes by 23 G needles at strictly determined intervals - at 0 min (before the application of the anesthetics) 30, 60, 120 min and 24 h from the beginning of the anesthesia.
Immediately after collection of the specimens, 1.5 ml of each sample was put into a sterile micro vacutainer, containing heparin and centrifuged for 15 min at room temperature for interleukin analysis.
The plasma specimens were stored at -22 °C for 27 days, prior to determination of the interleukin`s concentration.

Analytical methods of study
Interleukin 1 – (IL-1) and Interleukin 6 (IL-6) was studied using a specific Feline IL-1L и Feline IL-6L VetSet TM ELISA Development Kit, KINGFISHER BIFTECH, Inc., USA;
Interleukin studies were performed by an apparatus TRITURUS ANALYSER, USA;

Statistical analysis
All data were expressed as median and range. Differences between the two groups were analyzed using one way analysis of variance (ANOVA) and the least-significant difference (LSD) post hoc test at a level of significance 0.05. The study was approved by the Committee on Animal Ethics of the National Veterinary Service in Bulgaria.

RESULTS AND DISCUSSIONS

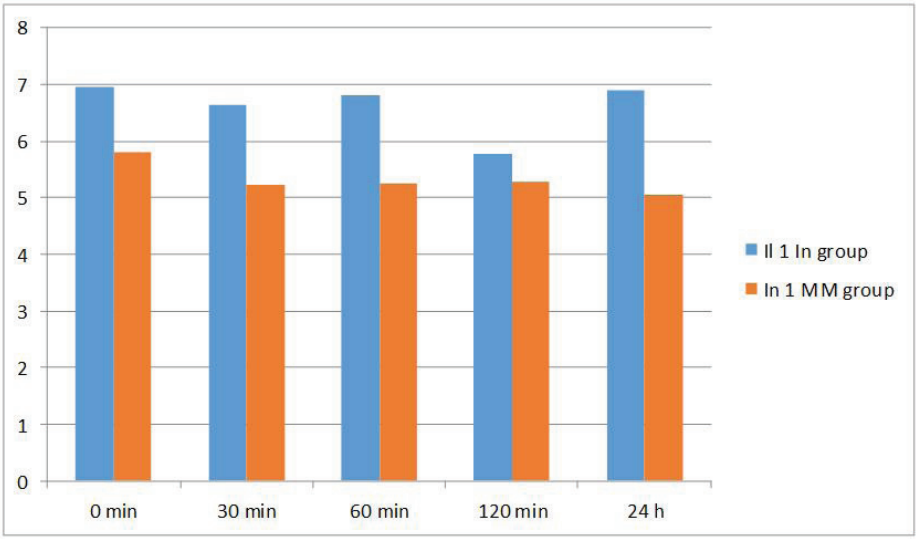
The statistical significance of the results obtained for the MM group is $p < 0.001$ and for the In group is $p < 0.05$. There were no statistically significant differences in 0 min between the two groups.
A reliable difference in In1 levels between the two groups was observed at 30 min ($p < 0.05$), at 60 min ($p < 0.01$) and at 24 h ($p < 0.001$). The IL6 concentrations were reliably elevated in the In group at 120 min ($p < 0.05$) and 24 h compared to the MM group. TNF-α is significantly lower in the MM group at 24 h compared to its established levels in the In group (Table 1).

Table 1. Changes in the levels of IL-1, IL-2 and TNF-α in both two groups

	Interleukin	0 min	30 min	60 min	120 min	24 h
In (inhalational)	**IL-1** pg/ml	6.95±1.64	6.64±1.07	6.81±0.79 *	5.78±0.37	6.91±1.36
MM (multimodal)	**IL-1** pg/ml	5.81±0.33	5.22±0.4 *** #	5.24±0.32 *** ##	5.28±0.18 ***	5.06±0.17 ***###
In (inhalational)	**IL-6** pg/ml	3.92±0.92	3.52±0.50 **	3.68±0.73 *	4.06±0.80	5.21±1.39 *
MM (multimodal)	**IL-6** pg/ml	3.82±0.47	3.95±0.41	3.39±0.16 *	3.17±0.09 ** #	3.24±0.43 ** ##
In (inhalational)	**TNF-α** pg/ml	6.41±1.45	5.59±1.20	5.81±1.93	5.29±1.12	6.62±1.33
MM (multimodal)	**TNF-α** pg/ml	5.84±0.31	5.47±0.31	5.41±0.19 *	5.81±0.19	4.94±0.55 *** ##

IL-1 in the In group was reliably reduced at 60 min (6.81 ± 0.79 pg / ml, p <0.05), but no significant changes in the concentration were observed at the end of the study period. Expressed changes in the concentrations of the studied interleukins were detected in the multimodal anesthesia group. IL-1 was reliably reduced in all study periods - 30 min (5.22 ± 0.4 pg / ml, p <0.001), 60 min (5.24 ± 0.32 pg / ml, p <0.001), 120 min (5.28 ± 0.18 pg / ml, p <0.001), 24 h (5.06 ± 0.17 pg / ml, p <0.001) compared to the initially established concentrations (5.81 ± 0.33 pg / ml) (Table 2).

Table 2. Changes in IL1 levels in In group and MM group



Significantly lower IL-6 concentrations in the In group were observed at 30 min (3.52 ± 0.50 pg / ml, p <0.01), followed by an upward trend (3.68 ± 0.73 pg / ml, p <0.05) and at 24 h were significantly higher (5.21 ± 1.39 pg / ml, p <0.05) from the original levels (3.92 ± 0.92 pg / ml).

IL-6 is unreliably increased in the MM group at 30 min, but the values found in subsequent periods are significantly lower - 60 min (3.39 ± 0.16 pg / ml, p <0.05), 120 min (3.17 ± 0.09 pg / p <0.01), 24 h (3.24 ± 0.43 pg / ml, p <0.01) from the baseline levels (3.82 ± 0.47 pg / ml) (Table 3).

Table 3. Changes in IL6 levels in In group and MM group

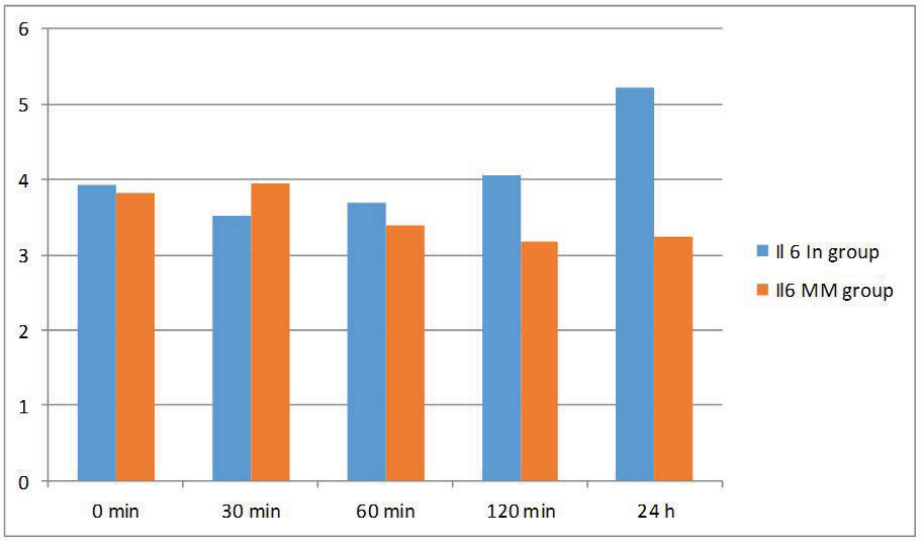
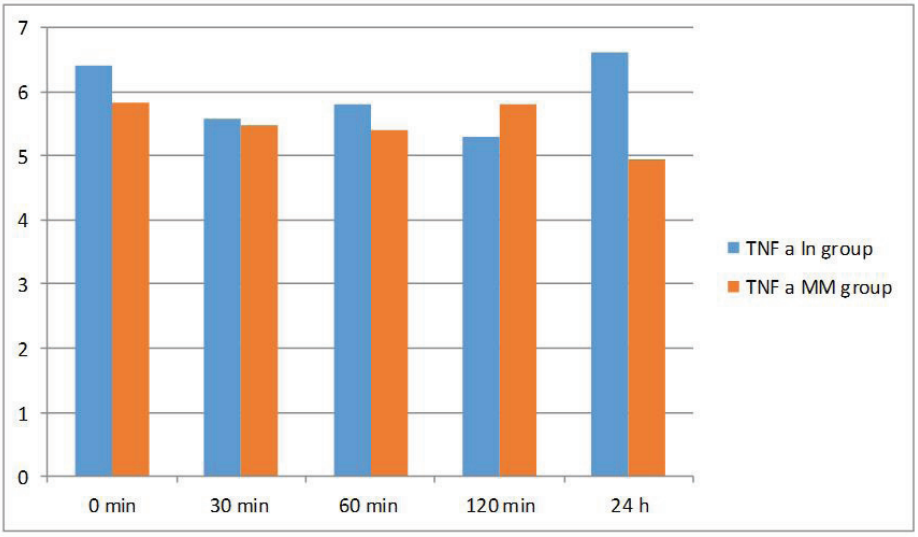


Table 4. Changes in TNFα levels in In group and MM group



An analysis of the data obtained for changes in TNF-α concentrations in group In revealed minor changes in the first three study periods, but at 24 h they were unreliably higher (6.62 ± 1.33 pg / ml) compared to baseline values (6.41 ± 1.45 pg / ml).

TNF-α concentrations in the MM group were also declined reliably to 60 minutes (5.41 ± 0.19 pg / ml, p <0.01), and the lowest reliable values compared to the baseline values (5.84 ± 0.31 pg / ml) were established to be at 24 h (4.94 ± 0.55 μg / ml, p <0.001) (Table 4).

The post-operative immune response is multifactorial and chronological. It is believed that the cytokine response changes in the first hour after anesthesia and surgery. The early pro-inflammatory response includes increased concentrations of pro-inflammatory Th1 cytokines (IL-2, IL-12, INF-γ). As a result of the surgical trauma and elevated levels of

cortisol and catecholamines in the acute phase, the release of anti-inflammatory Th2 cytokines (IL-4, IL-5, IL-6, IL-10 and IL-13) is stimulated together with suppression of cellular immunity (Ahmed W. et al., 2012). In our study, IL-6 and TNFα concentrations were reduced at the first hour in the inhalational anesthesia group, while in the MM group the two interleukins decreased reliably at 120 min. Suppression of IL-6 and TNFα in the immediate perioperative period indicates suppression of the pro-inflammatory immune response. At 24 h, the IL-6 and TNFα concentrations in MM group were significantly lower than the initial values.

Suppression of pro-inflammatory cytokines limits the possibility of infection, promotes the recovery of tissues and the organism. One of the reasons for the decrease in IL-6 and TNFα levels in our studies is probably due to the depression of adrenaline concentrations (Zlateva N. et al., 2014). The impact of multimodal anesthesia on stress-response is limited in the time of the operation and immediately after it. Increased levels of adrenaline and cortisol 24 hours after surgery are not able to suppress IL-6 and TNFα expression to such an extent. In multimodal anesthesia, interleukin concentrations are also dependent on the administered meloxicam. Blocking the synthesis of prostaglandins by influencing cyclooxygenase activity leads to a decrease in cAMP responsible for the regulation of IL-6 and IL-10. (Mahdy A. et al., 2002). On the other hand, the studies show the direct modulating action of isoflurane on cytokine secretion. According to Xu Wu et al. (2012), isoflurane anesthesia is directly responsible for the increase in IL-6, IL-1 and TNFα in rats. Stimulation of IL-6 production in isoflurane and sevoflurane anesthesia has also been proved in humans (Zhang L. et al., 2013). These results are supported by Schneemilch C. et al. (2001), which found sevoflurane anesthesia to increase IL-6 and TNF-α levels as compared to TIVA anesthesia with propofol and remifentanil.

The lack of significant change in IL-1 levels in both groups is indicative of the minor activation of the cytokine response in the direction of postoperative inflammation. High concentrations of IL-1, as a result of activation of the immune response, cause fever and mediate the response of the host to infection and inflammation. In stimulation of IL-1 production, decreased appetite, low motor activity and behavior associated with a disease state is observed (Kent S, et al., 1992), which we did not observe in the postoperative period. IL-1 concentrations at 24 h were slightly changed in the inhalational anesthesia group and were reliably reduced in the MM group.

Relatively new studies have shown that significant increase of interleukin concentrations in the post-operative period and especially of IL-1, IL-6 and TNFα, may be the cause of their crossing through the blood-brain barrier (Sanders R. and M. Maze, 2010). In response to these cytokines, microglial cells release additional cytokines that lead to inflammation of the CNS (Wan Y. et al., 2007). Pro-inflammatory cytokines play a key role in regulating immune responses in both cellular and humoral immune responses. Through their influence on T-helper lymphocytes and antigen-presenting cells, including B-lymphocytes, they induce secondary cytokine secretion and, in cooperation with them, promote the activation and proliferation of B-lymphocytes and the production of different classes of immunoglobulins.

Our study of certain factors of the immune response shows that different anesthetic agents have indirect and direct effects on the immune system. On the one hand, anesthetics alter the hormonal balance in the body that affects immune functions and, on the other hand, they directly influence the factors of non-specific and adaptive immunity. Anesthesia can both suppress and activate the immune system, which is essential for survival of patients after surgery.

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REFFERENCES

Ahmed W., N. Hamdi, 2012. Effects of different nesthetic techniques on inflammatory cytokines response and T-lymphocyte subsets in patients undergoing lower abdominal surgery. Ain shams journal of anesthesiology, 5, 1, 38-44.

Dinarello C., 2000. Proinflammatory cytokines. Chest,118, 2, 503-508.

Gaylord H., B. Simpson, 1916. The effect of certain anestheticsand loss of blood upon the growth of transplanted mouse cancer. Cancer Res. 1, 379-386.

Graham E., 1911. The Influence of Ether and Ether Anesthesia on Bacteriolysis, Agglutination, and Phagocytosis The Journal of Infectious Diseases, 8, 2, 147-175

Kent S., R. Bluthe, R. Dantzer, A. Hardwick, K. Kelley, N. Rothwell, J. Vannice, 1992. Different receptor mechanisms mediate the pyrogenic and behavioral effects of interleukin 1.PNAS,89, 19, 9117–9120.

Mahdy A., H. Galley, M. Abdel‐Wahed, K. El‐Korny, S. Sheta, N. Webster, 2002. Differential modulation of interleukin‐6 and interleukin‐10 by diclofenac in patients undergoing major surgery.Br. J. Anaesth., 88, 6, 797-802.

Sanders R., M. Maze, 2010. Neuroinflammation and postoperative cognitive dysfunction: can anaesthesia be therapeutic? European journal of anaesthesiology, 27, 3-5.

Schneemilch C., A. Ittenson, S. Ansorge, T. Hachemberg, U. Bank, 2005. Effect of 2 anesthetic techniques on the postoperative proinflammatory and anti-inflammatory cytokine response and cellular immune function to minor surgery. Journal of clinical anaesthesia, 17, 7, 517-27.

Schneemilch C., U. Bank, 2001. Pro and anti-inflammatory cytokine release in plasma of patients under different anesthetic techniques. Anesthesia ranimat, 26,1, 4-10.

Sheeran P., G. Hall, 1997. Cytokines in anaesthesia. Br j anaesthesia, 78, 201–19.

Wan Y., J. Xu, D. Ma, Y. Zeng, M. Cibelli, M. Maze, 2007. Postoperative impairment of cognitive function in rats - a possible role for cytokine-mediated inflammation in the hippocampus. Anesthesiology, 106, 436-443.

Xu Wu, Yan Lu, Zhongcong Xie, 2012. The inhalation anesthetic isoflurane increases levels of proinflammatory cytokine TNF-α, IL-6 and IL-1β. Neurobiol. Aging, 33,7, 1364-1378.

Zhang L., J. Zhang, L. Yang, Y. Dong, Y. Zhang, Z. Xie, 2013. Isoflurane and sevoflurane increase interleukin-6 levels through the nuclear factor-kappa B pathway in neuroglioma cells. Br janaesth., 110, 1, 82-91.

Zlateva N., B. Aminkov, 2014. Comparative studies on blood ACTH, cortisol, adrenaline, insulin and glucose in ovariohysterectomized cats anesthetized with isoflurane alone or combined with butorphanol and meloxicam. Revue Méd. Vét., 165, 5-6, 183-187.