Original Article

MedInform

DOI: 10.18044/MedInform.201522.184

ISSUE 2, 2015

Effect of three anesthetic protocols on the haematological indices in cats during ovariohysterectomy

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Abstract

The investigation was performed on 21 healthy adult cats in order to evaluate the effects of three anesthetic protocols on haematological indices during the anesthesia, surgery and for a 24-hour post-surgery period. The animals were divided in to three experimental groups (n=7 in each). The first group (X/K group) was premedicated with 2 mg/kg xylazine i.m., the second group (In group) – with 0.025 mg/kg acepromazine i.m., and the third group (MM group) – with 0.025 mg/kg acepromazine i.m., 0.4 mg/kg butorphanol i.m. and 0.3 mg/kg metacam s.c. The induction and maintenance of anesthesia in group X/K was done with 10 mg/kg ketamine i.v., while in the other two groups, induction with done with 5 mg/kg propofol i.v. After intubation, anesthesia was maintained with isoflurane 2.5 vol% in group In and 1.8 vol% in group MM in oxygen flow 2.0 l/min. Blood samples were collected prior to anesthesia - 0 min and at min 30, 60, 120 and hour 24 after the inception of anesthesia.

A statistically significant reduction in erythrocyte counts, haemoglobin and haematocrit was observed in the three experimental groups immediately after the beginning of anesthesia. Total white blood cells count was also substantially changed, but marked decrease in leukocyte, granulocyte and lymphocyte counts in groups In and X/K were established on min 120, while in group MM – by the 60th min. The three tested anesthetic protocols decreased the studied blood parameters after the beginning of anesthesia, most probably as a result of neuro-hormonal interactions of the anesthetic drugs and the related blood volume redistribution.

Keywords: anesthesia, cats, complete blood count, hematological indices

Introduction

Dental materials and products are widely used, and some of their ingredients may cause problems both for dental personnel because of occupational exposure and for patients undergoing dental treatment. Photoallergic contact dermatitis is a variant of allergic contact dermatitis. In the photoallergic variant, subsequent to the exposure to exogenous hapten irradiation, typically with ultraviolet (UV) light, is required to initiate the pathologic processes. The photons deliver energy for either creating covalent bonds between hapten and endogenous protein (formation of antigenic photo adducts), or converting a prohapten into the actual sensitizing hapten (1,2).

Photosensitization reactions are a continuously growing area of research which deals with the desirable and undesirable processes induced in biological systems by the absorption of UV. The common photosensitizing agents include chemicals used in dental composites, antiseptic agents, and non-steroidal anti-inflammatory agents widely used in dental practice. Photopatch testing is an effective approach for the diagnosis of photodermatitis or unclear photoreactions and helps in determining the sensitizing potentials of commonly used agents (3,4). Quite a few studies are available in regard to the incidence of reactions of photosensitization among dental professionals.

Materials and methods

Animals

Fourteen female cats at the age between 2 and 4 years, weighing 2.8 – 3.9 kg, mixed breed, were included in the study. Two weeks before the experiment, the animals were kept at 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 experiment, the animals were examined and determined to be clinically healthy on the basis of the physical examination.

Anesthetic protocols

The cats were randomly allocated in three experimental groups (n=7 in each group). The premedication in the first group was made with xylazine hydrochloride 2 mg/kg (Xylasin®, Alfazan-Turkey) intramuscularly; in the second - group (group In) the premedication was made with acepromazine maleate 0.025 mg/kg (Vetranquil® Ceva) intramuscularly, and the third group (group MM) was given acepromazine maleate 0.025 mg/kg (Vetranquil®, Ceva Santé Animale), butorphanol (Butomidor®, Richter Parma)–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. In the group X/K induction and maintenance of anesthesia were made with ketamine hydrochloride, (Ketamin, Intervet-Holand) 10 mg/kg body weight intravenously. For group In and MM 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 a 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 and it was made by the same surgeon.

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 the samples were put into a sterile micro vacutainer, containing EDTA (ethylene diamine tetraacetic acid) for haematological analysis.

Hematological and biochemical analysis

A complete blood count (CBC) was performed by automated hematology analyzer BC - 2800 Vet, MINDRAY, China. Hematological values provided by the hematology analyzer included: red blood cell (RBC) count; hemoglobin (Hb) concentration; hematocrit (HCT); mean red blood cell volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); red blood cell distribution width (RDW); total white blood cell (WBC) count; lymphocyte (Ly) count; monocyte (Mo) count; granulocytes (Gran) count.

Statistical analysis

All data were recorded with taking into account mean and standard deviation (mean±SD). Differences in the groups were analyzed using one-way analysis of variance (ANOVA) and the least-significant difference (LSD) in a post hoc test is at a level of significance 0.05.
The study was approved by the Committee on Animal Ethics of the National Veterinary Service in Bulgaria.

Aim

The purpose of the present study was to monitor the changes in complete blood count in ovariohysterectomised cats submitted to intravenous, inhalational and multimodal anesthesia.

Results

When comparing the obtained values of the examined analytes, no significant differences in the initial concentrations (0 min) between the groups were determined.
Statistically significant reduction in Er, Hb and Hc vs. baseline values was established on all time intervals in all groups (Table 1), and the values of these parameters remained considerably lower by the 24th hour.

The lowest values were detected on the 60th min, except for Hb content in cats from group X/K, where the lowest haemoglobin occurred by min 30 – 108.6±11.6 g/L ($p<0.001$).

Table 1. Changes in the values of RBC count, Hb, Hc, MCV, MCH, MCHC, RDW during intravenous, inhalational and multimodal anesthesia.

t (min) group	index	0 min	30 min	60 min	120 min	24 h
x/к (intravenous)	**rbc** *x10^{12}/l*	8.88±0.43	6.93±0.68 ***	6.78±0.53 ***	7.48±0.97 **	7.29±0.20 ***
	hb *g/l*	131.4±9.6	108.6±11.6 **	110.4±16.9 **	112.0±11.6 *	111.2±2.6 *
	hc *%*	45.1±2.7	32.9±4.0 **	32.2±3.9 **	35.8±5.3 *	34.6±0.5 *
	mcv fl	48.37±1.49	47.38±1.42	47.57±2.07	48.15±1.94	46.38±2.08
	mch, pg	15.83±0.67	15.71±0.50	15.60±0.63	15.78±0.67	15.43±0.80
	mchc, g/l	327.5±7.66	332.1±2.86	328.5±7.92	328.3±4.54	326.5±4.14
	rdw %	15.4±0.4	15.5±0.5	15.8±0.5	15.6±0.7	15.7±0.7
in (inhalational)	**rbc** *x10^{12}/l*	9.35±1.86	6.41±0.51 ***	5.00±0.74 ***	7.06±0.47 **	7.64±1.06 *
	hb *g/l*	140.4±16.8	103.6±6.3 ***	96.4±4.9 ***	107.4±16.8 ***	118.2±20.4 *
	hc *%*	47.8±6.4	30.8±2.1 ***	28.6±1.2 ***	33.2±0.5 ***	38.3±2.9 ***
	mcv fl	47.10±1.86	46.05±1.70	45.61±1.51	46.31±1.42	46.95±1.64
	mch, pg	15.67±0.77	15.76±0.60	15.37±0.82	15.05±0.58	15.36±1.07
	mchc, g/l	334.3±8.94	346.5±7.89 *	338.1±12.60	329.1±4.99	325.6±12.81
	rdw %	15.5±0.7	15.3±0.6	14.9±0.9	14.9±0.7	15.0±0.7
мм (multimodal)	**rbc** *x10^{12}/l*	9.49±0.54	6.40±0.37 ***	5.40±0.04 ***	7.36±0.53 ***	8.14±0.35 ***
	hb *g/l*	140.8±22.0	105.4±1.8 ***	95.2±3.4 ***	118.4±9.3 **	135.2±8.4
	hc *%*	46.9±1.2	31.7±1.5 ***	28.0±1.6 ***	35.7±2.4 ***	40.5±0.6 ***

	mcv fl	48.32±2.06	47.51±2.21	46.68±2.51	47.55±2.49	48.90±2.41
	mch, pg	16.05±0.99	16.21±0.75	15.75±0.92	15.65±0.98	16.25±0.76
	mchc, g/l	332.6±4.63	343.8±4.45 ***	340.5±5.43 **	331.1±3.71	337.6±7.81
	rdw %	15.0±0.3	14.9±0.5	14.9±0.5	14.8±0.4	15.3±1.2

*** p<0.001; ** p<0.01; * p<0.05 vs min 0

The changes in red blood cell indices consisted in statistically significant reduction of MCHC in group In – 346.5±7.89 g/L (p<0.05) and group MM – 343.8±4.45 g/L (p<0.001) by min 30 and in the latter group by the 60th min - 340.5±5.43 g/L (p<0.01). At the end of the study period (24th hour) RBC indices regained their initial levels in all three groups.

The total white blood cell counts decreased substantially in all groups (tabl. 2) immediately after the beginning of anesthesia with lowest counts on min 120 in group X/K - 6.90±1.41 x 109/L (p<0.001) and group In - 9.50±4.04 x 109/L (p<0.001), while in group MM the lowest counts were observed earlier – by the 60th min - 7.86±0.81 x 109/L (p<0.001).

Table 2. Changes in WBC, Gran, Ly and Mo counts during intravenous, inhalational and multimodal anesthesia.

т (min) group	index	0 min	30 min	60 min	120 min	24 h
х/к (intravenous)	**wbc** *x 10^9/l*	13.05±2.20	8.30±1.68 ***	7.80±1.48 ***	6.90±1.41 ***	12.3±2.16
	gran *x10^9/l*	7.95± 0.68	4.25±0.37 *	4.64±0.35 *	3.32±0.25	8.16±0.29
	ly *x10^9/l*	4.35±1.58	3.57±1.08	2.22±0.69 *	3.42±0.39	4.12±0.53
	мо x10^9/l	0.78±0.02	0.48±0.04 **	0.34±0.03 **	0.35±0.02 **	0.51±0.02 ***
in (inhalational)	**wbc** *x 10^9/l*	16.90±1.73	12.20±4.91 *	10.82±3.10 *	9.50±4.04 **	17.37±2.30
	gran *x10^9/l*	9.90±2.65	7.92±3.52	6.66±2.85 *	6.56±3.29 *	13.44±1.82 **
	ly *x10^9/l*	4.75±1.10	3.41±0.69 *	3.55±1.24 *	2.47±0.72 ***	3.13±0.47 **
	мо x10^9/l	0.85±0.03	0.50±0.04 **	0.55±0.01 **	0.50±0.01 **	0.75±0.04
мм (multimodal)	**wbc** *x 10^9/l*	15.66±2.54	9.73±0.31 ***	7.86±0.81 ***	12.63±1.42 **	16.04±2.08
	gran *x10^9/l*	9.82±2.20	6.22±0.76 **	5.58±0.74 **	8.36±1.35	12.08±1.92
	ly *x10^9/l*	4.51±1.02	3.01±0.53 ***	1.82±0.11 ***	3.53±1.13 **	3.20±0.33 ***
	мо x10^9/l	0.80±0.04	0.50±0.02 **	0.43±0.02 ***	0.66±0.06	0.73±0.04

*** p<0.001; ** p<0.01; * p<0.05 vs min 0

The individual leukocyte subpopulations exhibited statistically significantly lower counts immediately after the beginning of anesthesia except for the insignificant decline in Gran counts in group In. By the 24th h, the counts of monocytes remained considerably lower - 0.51±0.02 x109/L (p<0.001) in group X/K, as did lymphocyte counts in groups In (3.13±0.47 x109/L; p<0.01) and MM (3.20±0.33 x109/L; p<0.001), while the Gran counts in the inhalational anesthesia group remained higher than baseline – 13.44±1.82 x109/L (p<0.01).

Discussion

The body reacts to altered environment through activation of various adaptive and defense mechanisms under the control and via the interaction of nervous, immune, endocrine, reticuloendothelial and other systems.

Our study has shown that the total erythrocyte counts as well as haemoglobin content and haematocrit of cats submitted to all three anesthetic protocols were significantly reduced immediately after drug applications and before the surgery.

The changes in these parameters are due on the one hand, to direct effects of anesthetic drugs on organs – both vasodilation of smooth vascular muscles and hormone-mediated effects – and suppression of catecholamine release (6). Previous studies of ours of both principal stress hormones (adrenaline and cortisol) in cats showed correlation between periods of hormonal levels decline and lower content of Hb and Hc, and reduced erythrocytes counts (9).

The reduction of blood hormone concentrations under the influence of anesthetic drugs is accompanied by altered vascular tone and blood volume redistribution. Previous studies in cats showed that the intravenous or intramuscular application of ketamine hydrochloride resulted in substantial repeated decline in hematocrit values and red blood cell counts (1,3). Dissimilar to that, the subcutaneous application which is not the usual route of application, caused increase in hematocrit values (2). It is demonstrated that ketamine acts directly towards smooth muscle vasodilation. Apart from ketamine, propofol and acepromazine also cause vasodilation, altering the vascular wall tone (6). These effects of anesthetics explain the vasodilation of splenic blood vessels, respectively the changes in CBC parameters during anesthesia (10,11).

On the other hand, Wilson D. et al. (2004) observed no direct correlation between changed splenic volume and the extent of reduction in haematological parameters, demonstrating that the sequestration of erythrocytes is not only splenic, but specific also for other organs, such as liver, skin and skeletal muscles. The established statistically significant increase in MCHC in groups In and MM was most probably due to the reduced red blood cell volume. According to Biermann et al., (2012) these changes are directly related to the redistribution and deposition of a large proportion of the blood volume. Another probable reason for the observed change in red blood cell indices is the direct effect of propofol on red blood cells. The application of propofol in cats resulted in the formation of the so-called Heinz bodies – aggregates of denatured precipitated haemoglobin within the erythrocytes that cause increase in MCHC. The frequency and dose of administered propofol did not have any effect on the extent of Heinz bodies' appearance in feline red blood cells (13).

Unlike the similar changes in red blood cell parameters observed in the three groups, the alterations in total leukocyte, granulocyte and lymphocyte counts occurred at various time intervals depending on the used anesthetic protocol. The WBC, Gran, Ly and Mo counts are in direct relation with the total circulating blood volume on the one hand, and are also influenced by changes in autonomous nervous system, endocrine and immune system on the other (7,14).

The blood adrenaline and cortisol dynamics established in previous studies, largely explain the leukopenia occurring in the three groups at various time intervals, corresponding to hormonal changes (9,15). Contrary to this, other authors did not observe changes in granulocytes and lymphocytes count in cats,

anesthetized with diazepam and ketamine (7). The application of α2-agonists suppresses the circulating catecholamines by exerting a modulating effect on leukocyte subpopulations (15). Dissociation agents also reduce leukocyte counts (16).

The marked and rapidly occurring leukopenia, granulocytopenia and lymphopenia in the group receiving multimodal anesthesia were most probably due to the applied drug combination and the resulting modulations in stress hormone levels. The preoperative application of meloxicam reduces the systemic stress response, by decreasing the plasma cortisol and adrenaline (17,18). The peak of the analgesic effect of butorphanol is between the 30th and 60th min of intramuscular application (19), which corresponded to the lowest detected WBC, Ly, Gran in group MM, in agreement with data from earlier research of ours regarding the blood hormonal levels in the same periods (9).

By the end of the study period, the total leukocyte and granulocyte blood counts in the three groups were comparable or higher than baseline values, indicating recovery of animals from the inhibiting effect of anesthetics on main stress hormones. The total lymphocyte counts were lower by the 24th hour of anesthesia in the three groups, most pronounced in inhalational and multimodal anesthesia groups. One of the possible reasons is that lymphocyte counts are mediated via β-adrenoceptor activation, while the increase in granulocytes results from α-adrenoceptor stimulation (20). Postoperative granulocytosis and lymphopenia could result from a non-specific stress response associated with postoperative and postanesthetic sympathetic and adrenocortical stimulation (15).
Another probable cause with particular influence on lymphocyte counts is apoptosis. Numerous studies provide evidence that the depression of leukocytes and lymphocyte subpopulation are at the background of the immunosuppressive effects of anesthesia and that they are mediated via apoptosis (8, 21).
The analysis and monitoring of complete blood count changes and their effects on the different organs and systems during anesthesia and surgery broaden the opportunities for control of these responses and the intermediate events responsible for their triggering.

Conclusion

The three tested anesthetic protocols decreased the studied blood parameters after the beginning of anesthesia, most probably as a result of neuro-hormonal interactions of anesthetic drugs and the related blood volume redistribution.

Total leukocyte, granulocyte and lymphocyte counts decreased under the influence of tested anesthetic protocols immediately after application of the drugs.
The use of butorphanol and meloxicam in the anesthetic schedule of cats resulted in more rapid and more intense reduction of white blood cell counts. The lower CBC values should be taken into consideration during blood analysis in anesthetised animals, as well as the immunomodulatory effect of anesthetics, especially in immunocompromised cats.

Acknowledgements

This study is a part of Project No 140/2012 funded by the University of Forestry – Sofia.
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