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The effect of repeated sevoflurane and nitrous oxide exposure on immunity in rabbits

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ABSTRACT

The aim of this study was to investigate the effect of repeated/prolonged exposure to sevoflurane and nitrous oxide on the immune response in rabbits. Venous blood samples were obtained from twenty New Zealand rabbits, randomly allocated to four groups of five rabbits each. Group A rabbits served as controls, breathing 100% oxygen, group B rabbits inhaled a mixture of oxygen and nitrous oxide, group C inhaled a combination of oxygen and sevoflurane, and group D inhaled a combination of oxygen, nitrous oxide and sevoflurane. Flow cytometry was used to determine the proportions of $CD45^+$, $CD4^+$, $CD8^+$ and $CD21^+$ leukocytes subpopulations. Peripheral lymph nodes, lungs and spleen were sampled and subjected to histologic examination. The greatest changes in the proportion of $CD21^+$, $CD4^+$, $CD8^+$ and $CD45^+$ cells were recorded in groups C and D on days 7, 14 and 21. Comparison of the $CD21^+$, $CD4^+$, $CD8^+$ and $CD45^+$ cell counts on day 0 and day 21 showed a 30% decrease. This study demonstrated that sevoflurane alone and in combination with nitrous oxide exerts a strong immunosuppressive effect, modifying the physiological values of the hematologic parameters, and inducing histopathological alterations in peripheral lymph node, lung and spleen tissues.

Key words: immunity; sevoflurane; rabbit

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Introduction

Inhalant anesthetics are a major component of modern, balanced anesthesia. Besides their effects on the central nervous system, effects may also be observed in other organ systems. Inhalant anesthetics induce dose-dependent respiratory depression, lead to arterial pressure reduction and potential cardiac arrhythmias, and are known to influence the immune system as well. Volatile anesthetics appear to suppress the effectors' functions of both innate and adaptive immunity, assist tumor growth in animal models, and facilitate aggregation of certain neurodegenerative disease proteins (HOMBURGER and MEILER, 2006). It has been demonstrated that anesthetic agents given during any procedure may lead to immunosuppression (KELBEL and WEISS, 2001). Most clinical studies have been focused on the combined effects of surgery and anesthesia on the immune function (MORISAKI et al., 1998, DELOGU et al., 2000; SCHNEEMILCH and BANK, 2001; HORI et al., 2003; COCELLI et al., 2012), with only a few studies dealing specifically with anesthetic agents (DAGAN et al., 1989; MOUDGIL and SINGAL, 1997; PUIG et al., 2002; ELENA et al., 2003; MÜLLER-EDENBORN et al., 2015).

The effect of sevoflurane on immune cells is well known. Isoflurane (TYTHER et al., 2001) and sevoflurane (TYTHER et al., 2003) were found to induce dose-dependent inhibition of polymorphonuclear neutrophil apoptosis *in vivo* and *in vitro*. As reported by MORISAKI et al. (1998), total lymphocyte count did not change following sevoflurane anesthesia, but neutrophil count was observed to decrease while lymphocyte count increased. Data on repeated sevoflurane exposure are scarce and no major differences have been described between the adverse effects caused by initial and repeated sevoflurane exposure. Changes in leukocyte populations in peripheral blood and in antibody-producing capacity occur after either a single exposure or repeated exposures to sevofluran (COLUCCI et al., 2003)

Nitrous oxide may also modify immune anti-inflammatory response by reducing the neutrophil and monocyte chemotactic activity (MOUDGIL et al., 1984; SCHNEEMILCH et al., 2005) and cause dose-dependent inhibition of polymorphonuclear leukocytes (MORGAN et al., 2006). KAYE et al., (2014) also reported that nitrous oxide administration is associated with both depressed neutrophil function and reduced mononuclear cell production. Adverse effects of nitrous oxide may occur after both acute (surgical) or long term (occupational) exposure to the gas. Bone marrow depression depends on the dose of nitrous oxide and the length of exposure. Reports such as these have led to concern that nitrous oxide may detrimentally affect immune function.

Although anesthesia is generally administered just once for a surgical procedure, there are conditions that require multiple anesthesia administration (e.g., frequent dressing changes in burn patients).

The aim of the present study was to monitor the effect of sevoflurane and nitrous oxide on the immune system of rabbits exposed to prolonged/repeated administration of these inhalant anesthetics.

Materials and methods

The study was conducted in accordance with the Act on Animal Welfare, and European Union recommendations, with approval from the Ethics Committee of Faculty of Veterinary Medicine, and the Ethics Committee of the Ministry of Agriculture.

Animals. White New Zealand rabbits were used as experimental animals. The experiments were carried out respecting the principles of animal welfare, using standardized protocols of experimental animal preparation. The study included 20 rabbits, with mean body mass of 2.8 kg, divided into 4 study groups (n = 5). Initially, each animal was allocated a number 1-20 and then they were randomly divided into groups, and each group was caged separately. The rabbits were anesthetized once daily for 20 minutes over a 21-day period. No surgical procedures were performed.

Protocol of the experimentally study. Group A (control group) rabbits inhaled 100% oxygen; group B inhaled a combination of 40% oxygen and 60% nitrous oxide (Messer, Croatia); group C inhaled a combination of 100% oxygen and sevoflurane (Sevorane, Abbott, Great Britain) (at 3% concentration for the first 5 minutes, then at 2% concentration); and group D inhaled a combination of 40% oxygen, 60% nitrous oxide and sevoflurane (at 2% concentration for the first 5 minutes, then at 1.3% concentration). In the last group, the concentration of sevoflurane had to be reduced compared with the previous group because of the effect of nitrous oxide (reduction of the minimum alveolar concentration of inhalant anesthetics).

Prior to the study, on day 0, the animals underwent a thorough examination and blood sampling for complete blood count and flow cytometry determination of leukocyte subpopulations ($CD21^+$, $CD4^+$, $CD8^+$ and $CD45^+$). Subsequently, blood samples were obtained on study days 3, 7, 14 and 21. Hematological and immunologic parameters were determined from venous blood samples of the study animals. Hematological parameters, including red blood cell (RBC) count, hemoglobin concentration (Hb), platelet count (PLT), leukocyte count (LC) and the number of particular leukocyte cells were determined in peripheral blood samples; neutrophil (Neu) and lymphocyte (Lym) counts. Immunological parameters were determined by immunophenotyping and quantification of rabbit CD^+ subpopulations of T- and B-lymphocytes and total leukocyte count in peripheral blood on a Coulter EPICS-XL (Coulter, USA) flow cytometer.

The animals were euthanized by an intravenous injection of T-61 (mixture of Embutramide/Tetracaine hydrochloride/Mebezonium iodide), in a dose of 0.3 mL/kg during sevoflurane anesthesia on the 21st day of the experiment. Immediately after sacrifice,

peripheral lymph node, lung and spleen samples were obtained and embedded in 10% buffered formalin solution, followed by hematoxylin-eosin staining for histopathologic analysis.

Statistical analysis of experimental results. The data obtained were processed using Statistica 8.0 (StatSoft Inc., 2008) software, and tested by the Kolmogorov-Smirnov test. Student's *t*-test was employed to assess the difference between the groups with normal distribution. Data with non-normal distribution were analyzed by the Mann-Whitney test. The level of statistical significance was set at $P<0.05$.

Results

The values of the hematological parameters measured in rabbit peripheral blood during the study are shown in Table 1. In all four study groups, the platelet count was statistically significantly lower on day 14 as compared with day 0 ($P<0.05$), followed by an increase recorded on day 21. On day 14, a statistically significant decrease in leukocyte count as compared with day 0 was only observed in group A. The proportion of lymphocytes in the population of leukocytes increased in all study groups on 21 days. However, a statistically significant ($p<0.05$) increase on days 3 and 14 as compared with day 0 was only recorded in group D. In the other groups, this increase did not reach statistical significance. In all study groups, neuthropil count was statistically significantly lower on day 21 as compared with day 0 ($P<0.05$).

The proportions of $CD45^+$, $CD4^+$, $CD8^+$ and $CD21^+$ leukocytes in the peripheral blood of rabbits treated daily with the study agents for 21 days are shown in Table 2. In group B rabbits, no changes were recorded in the proportion of $CD45^+$ cells as compared with control group A on days 3, 7 and 14, but statistically significantly lower proportion of $CD45^+$ cells *versus* group A was recorded on day 21. In group C rabbits, a statistically significantly lower proportion of $CD45^+$ cells *versus* group A was recorded on day 7 and this continued to the end of the study. In group D rabbits, a statistically significantly lower proportion of $CD45^+$ cells *versus* group A was recorded on day 3 and this continued to the end of the study. In comparison to control group A, changes in the proportion of $CD4^+$ cells were observed in group B rabbits on days 0, 14 and 21. In group C rabbits, a statistically significantly lower proportion of $CD4^+$ cells *versus* group A was found on days 7, 14 and 21; however, it should be noted that this proportion increased from day 14 to day 21. In group D rabbits, a statistically significantly lower proportion of $CD4^+$ cells *versus* group A was found on days 7, 14 and 21, the last being the lowest proportion of these cells recorded in the study. In group B rabbits, no changes were recorded in the proportion of $CD8^+$ cells as compared with control group A on days 3, 7 and 14.

Table 1. Peripheral blood parameters (mean ± SEM) according to rabbit groups and days of experiment

Rabbit groups	Day	Blood parameters					
		RBC $\times 10^6$	Hb g/dL	PLT $\times 10^9$	LC $\times 10^3$	Lym %	Neu %
A -inhalation 100% O_2	0	4.56 ± 0.49	100.20 ± 9.96	504.00 ± 46.00	16.42 ± 1.12	57.20 ± 3.06	42.60 ± 2.40
	3	5.04 ± 0.32	115.80 ± 6.74	509.80 ± 120.2	12.94 ± 5.00	54.00 ± 6.46	45.00 ± 6.45
	7	4.80 ± 0.14	116.80 ± 3.7	395.80 ± 59.56	12.86 ± 1.54	60.20 ± 4.08	39.00 ± 3.91
	14	5.28 ± 0.23	119.20 ± 3.87	381.40 ± 82.44*	11.80 ± 1.56*	66.80 ± 3.85	32.80 ± 5.12
	21	6.30 ± 0.48	137.60 ± 5.90*	461.00 ± 42.09	14.12 ± 1.41	68.40 ± 4.95	29.60 ± 4.62*
B - inhalation 40% O_2 + 60% N_2O	0	5.40 ± 0.32	116.40 ± 6.74	563.80 ± 120.2	16.52 ± 5.00	57.20 ± 6.46	41.80 ± 6.45
	3	5.55 ± 0.14	120.20 ± 3.70	579.20 ± 59.56	15.74 ± 1.54	54.40 ± 4.08	44.60 ± 3.91
	7	5.49 ± 0.23	126.00 ± 3.87	320.00 ± 82.44*	14.60 ± 1.56	53.60 ± 3.85	44.40 ± 5.12
	14	5.83 ± 0.48	127.20 ± 5.90	366.80 ± 42.09*	15.72 ± 1.41	63.00 ± 4.95	35.80 ± 4.62
	21	5.89 ± 0.40	125.80 ± 7.56	458.80 ± 60.40	15.36 ± 1.10	67.20 ± 6.43	32.80 ± 6.60*
C - inhalation 100% O_2 + sevofluran	0	4.94 ± 0.14	110.40 ± 3.70	565.80 ± 59.56	13.56 ± 1.54	60.40 ± 4.08	41.80 ± 6.45
	3	5.13 ± 0.23	110.40 ± 3.87	540.20 ± 82.44	12.88 ± 1.56	63.20 ± 3.85	44.60 ± 3.91
	7	5.54 ± 0.48	121.40 ± 5.90	510.80 ± 42.09	13.26 ± 1.41	68.00 ± 4.95	44.40 ± 5.12
	14	5.57 ± 0.40	124.40 ± 7.56	397.60 ± 60.40*	10.82 ± 1.10	72.20 ± 6.43	35.80 ± 4.62
	21	5.30 ± 0.30	116.60 ± 5.44	428.60 ± 112.60	11.36 ± 4.09	63.40 ± 3.74	32.80 ± 6.60*
D - inhalation 40% O_2 + 60% N_2O + sevofluran	0	5.39 ± 0.23	119.20 ± 3.87	493.20 ± 82.44	13.08 ± 1.56	58.60 ± 3.85	36.20 ± 5.12
	3	5.21 ± 0.48	111.80 ± 5.90	474.40 ± 42.09	10.42 ± 1.41	76.80 ± 4.95*	22.40 ± 4.62
	7	5.90 ± 0.40	128.20 ± 7.56	431.60 ± 60.40	13.46 ± 1.10	69.20 ± 6.43	30.40 ± 6.60
	14	6.39 ± 0.30	138.00 ± 5.44	386.60 ± 112.60*	12.48 ± 4.09	78.40 ± 3.74*	26.00 ± 3.61
	21	6.22 ± 0.35	133.40 ± 5.63	422.40 ± 24.17	14.64 ± 0.50	74.00 ± 3.72	19.20 ± 3.61*

* parameter mean value within the same rabbit group is significantly different ($P < 0.05$) in comparison to the day 0

Table 2. Mean ± SEM proportions of $CD45^+$, $CD4^+$, $CD8^+$ and $CD21^+$ leukocytes subpopulations in peripheral blood of rabbit groups treated with different inhalation agents for 21 days

Rabbit groups	Day	Leukocytes subpopulations			
		$CD45^+$	$CD4^+$	$CD8^+$	$CD21^+$
A - inhalation 100% O_2	0	58.57 ± 0.89	18.68 ± 0.32	10.11 ± 0.18	29.78 ± 0.18
	3	59.96 ± 0.74	18.67 ± 0.38	11.79 ± 0.15	29.50 ± 0.15
	7	59.87 ± 0.85	19.94 ± 0.13	11.97 ± 0.07	27.96 ± 0.07
	14	60.82 ± 0.49	20.02 ± 0.16	11.16 ± 0.10	29.64 ± 0.10
	21	59.45 ± 0.35	21.30 ± 0.12	11.89 ± 0.07	26.26 ± 0.07
B - inhalation 40% O_2 + 60% N_2O	0	58.79 ± 0.64	17.00 ± 0.22*	11.15 ± 0.18*	30.63 ± 0.12
	3	59.74 ± 0.75	18.90 ± 0.31	10.73 ± 0.15	30.11 ± 0.15
	7	58.84 ± 0.24	19.55 ± 0.09	11.77 ± 0.05	27.52 ± 0.05
	14	57.67 ± 0.43	20.45 ± 0.18*	10.73 ± 0.09	26.49 ± 0.09
	21	58.16 ± 0.41*	20.45 ± 0.16*	10.23 ± 0.08	27.48 ± 0.08
C - inhalation 100% O_2 + sevofluran	0	58.40 ± 0.55	18.74 ± 0.32	11.15 ± 0.18*	28.51 ± 0.18
	3	52.56 ± 0.49	17.82 ± 0.35	10.59 ± 0.18	24.15 ± 0.18
	7	52.19 ± 0.36*	17.61 ± 0.26*	10.16 ± 0.08*	24.15 ± 0.18
	14	48.13 ± 0.48*	15.21 ± 0.30*	8.06 ± 0.17*	24.86 ± 0.17*
	21	47.20 ± 0.28*	15.99 ± 1.40*	8.15 ± 0.08*	23.06 ± 0.08*
D - inhalation 40% O_2 + 60% N_2O + sevofluran	0	59.90 ± 0.44	18.99 ± 0.19	11.27 ± 0.28*	29.64 ± 0.28
	3	52.23 ± 0.46*	15.74 ± 0.17	9.71 ± 0.21	26.78 ± 0.21
	7	47.76 ± 0.22*	14.60 ± 0.17*	8.46 ± 0.08*	24.70 ± 0.08*
	14	46.09 ± 0.45*	14.91 ± 0.16*	7.30 ± 0.10*	23.88 ± 0.10*
	21	42.41 ± 0.48*	13.27 ± 0.12*	7.53 ± 0.06*	21.61 ± 0.06*

*within the same leukocytes subpopulatin mean proportion is significantly ($P< 0.05$) different in comparison to the mean proportion on the same day in rabbit group A

In group C and D rabbits, a statistically significantly lower proportion of $CD8^+$ cells as compared with group A was found on days 7, 14 and 21; however, it should be noted that the lowest proportion of $CD8^+$ cells was recorded on day 14 and a slightly increased level on day 21. In group B rabbits, there were no changes in the proportion of $CD21^+$ cells as compared with control group A. In group C and D rabbits, a statistically significantly lower proportion of $CD21^+$ cells *versus* group A was found on days 7, 14 and 21, with the lowest proportion of $CD21^+$ cells recorded on day 21. The combinations of 100% oxygen + sevoflurane and 40% oxygen + 60% nitrous oxide + sevoflurane proved to be potent immunosuppressants for $CD45^+$, $CD4^+$, $CD8^+$ and $CD21^+$ cells; on day 21, the 40% oxygen + 60% nitrous oxide + sevoflurane combination exerted the statistically significantly most potent immunosuppressive effect, manifesting as a decrease in the proportions of $CD45^+$, $CD4^+$, $CD8^+$ and $CD21^+$ cells from day 0 to day 21 by 29%, 30%, 33% and 27%, respectively.

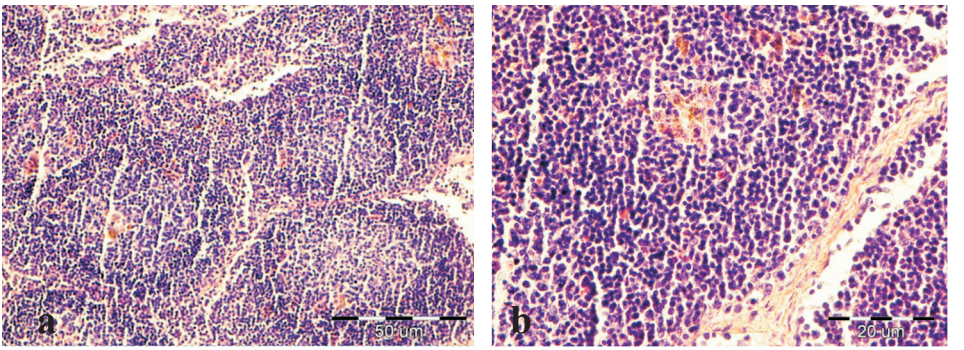

Fig. 1. Histopathological changes in peripheral lymph node tissue. 1a. Hyperplastic lymphadenitis in rabbit from group C - inhalation 100% O_2 + sevofluran; 1b. Hemosiderosis and hyperplasia in rabbit from group D - inhalation 40% O_2+ 60% N_2O + sevofluran

Histopathological alterations in peripheral lymph node, lung and spleen tissues of rabbits administered different anesthetics for 21 days are illustrated in Figs 1-3. No histopathological alterations were observed in the samples of peripheral lymph nodes, lungs and spleens in group A and B rabbits. In group C rabbits, marked histopathological alterations were found in peripheral lymph node, lung and spleen samples. In lymph node samples, reactive hyperplasia was observed in group C (Fig. 1a), and hemosiderosis and follicular in group D (Fig. 1b). Lung samples revealed interstitial pneumonia with an interalveolar amorphous mass in group C (Fig. 2a), and interstitial pneumonia with interalveolar amorphous mass and predominantly heterophilic infiltration in group D (Fig. 2b).

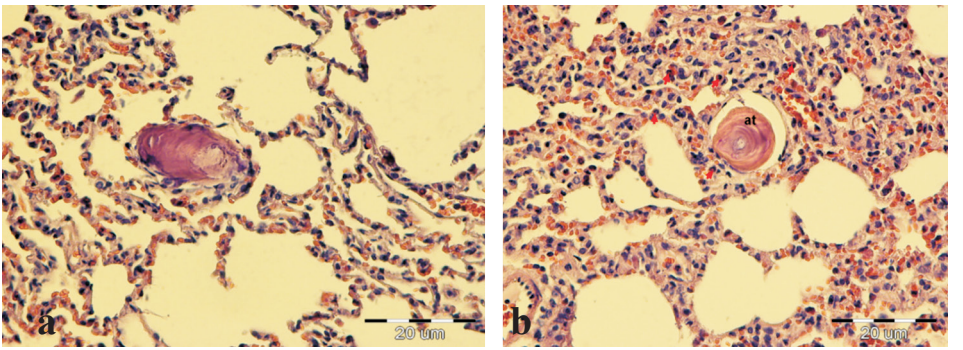

Fig. 2. Histopathological changes in lung tissue. 2a. Interstitial pneumonia with interalveolar amorphous mass in rabbit from group C - inhalation 100% O_2 + sevofluran; 2b. Pneumonia with interalveolar amorphous mass and predominantly heterophilic infiltration in rabbit from group D - inhalation 40% O_2+ 60% N_2O + sevofluran.

In spleen samples, hyperemia, lymphoid follicle histiocytosis and individual lymphocyte apoptosis were detected in group C (Fig. 3a), and hyperemia, lymphoid follicle histiocytosis and individual lymphocyte apoptosis, hemosiderosis and infiltration of heterophils in red pulp in group D (Fig. 3b).

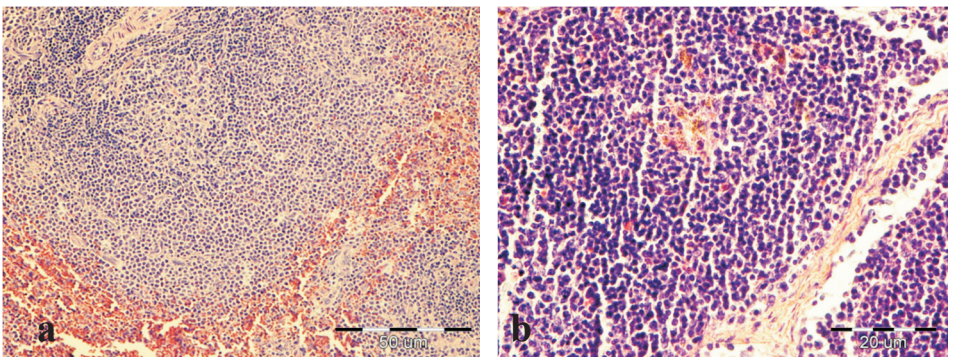

Fig. 3. Histopathological changes in spleen tissue. 3a. Hyperemia, lymph follicular histiocytosis and individual lymphocyte apoptosis in rabbit from group C - inhalation inhalation 100% O_2 + sevofluran 100% O_2 + sevofluran; 3b. Hyperemia, lymph follicular histiocytosis and individual lymphocyte apoptosis, hemosiderosis and red pulp heterophilic infiltration in rabbit from group D - inhalation 40% O_2+ 60% N_2O + sevofluran

Discussion

The formation of volatile anesthetic metabolites that bind to tissue macromolecules or produce free radicals can cause quite severe cell lesions, which is explained by the high reactivity of these compounds. These lesions are then additionally worsened due to the insufficiency of cellular antioxidant mechanisms (e.g., glutathione deficiency). Considering the lipophilicity of inhalant anesthetics, the above mechanisms will have the greatest effect on the cell membrane lipid components. The inhibitory effect of volatile inhalant anesthetics on lymphocyte proliferation and their suppressive effect on cytokine release from mononuclear cells in peripheral blood have been demonstrated in a number of studies (MITSUHATA et al., 1995; STOLLINGS et al., 2016). Rat spleen T-lymphocytes, following 5-hour anesthesia with 1% halothane *in vivo,* showed a reduced proliferative capacity and $CD25^+$ (interleukin 2 (IL-2)) receptor expression in response to mitogens. In the case of neutrophil cells, inhalant anesthetic does not only reduce their microbicidal capacity but also the information needed for triggering inflammatory response. Sevoflurane, isoflurane and enflurane also inhibit IL-1β and tumor necrosis factor alpha (TNF-α) release from human peripheral blood mononuclear cells (including lymphocytes and natural killer (NK) cells) in response to tumor cells. Isoflurane and sevoflurane directly induce apoptosis of human peripheral lymphocytes *in vitro,* depending on the dose and length of exposure. In the present study, the use of flow cytometry clearly demonstrated the immunosuppressive effect of sevoflurane on $CD45^+$, $CD4^+$, $CD8^+$ and $CD21^+$ cells as early as day 7 of exposure. TYTHER et al. (2001; 2003) found isoflurane and sevoflurane to cause dose-dependent inhibition of polymorphonuclear neutrophil apoptosis *in vivo* and *in vitro*. MOUDGIL and SINGAL (1997) showed that equipotent concentrations of isoflurane, enflurane, halothane and methoxyflurane in a gas mixture with 70% nitrous oxide influenced the chemotactic activity of neutrophils and monocytes. Consistent to this, our results also showed an increased neutrophil count in sevoflurane treated rabbits as compared with control group A rabbits. Furthermore, it is known from the literature that inhalant anesthetics (nitrous oxide in particular), with the exception of isoflurane, reduce the chemotactic migration of these cells (MOUDGIL et al., 1984). There are numerous *in vitro* studies investigating the effects of inhalant anesthetics on peripheral blood lymphocytes, and to a lesser extent on other cell types, while *in vivo* studies in animals and humans are very rare. According to MORISAKI et al. (1998), total leukocyte count did not change following sevoflurane anesthesia, but the number of neutrophils decreased and the number of lymphocytes increased, which is in contrast to the results obtained in our study. Unlike the study cited above, the results reported by PUIG et al. (2002) are consistent with our study. As mentioned above, they used 3% sevoflurane anesthesia in mice and demonstrated that it induced changes to the peripheral blood total leukocyte count, B-lymphocyte count, $CD4^+$ cell count and immune response to sheep RBCs, while observing no hepatotoxic or nephrotoxic effect. Namely, literature reports

quite frequently state that major operations performed under general anesthesia modify the patient immune response, i.e. the number and function of leukocytes, lymphocytes and neutrophils. This in turn increases the likelihood of postoperative complications, including infections, inadequate response to stress, hyperkatabolism, increased tumor growth, etc. This is supported by our results obtained by histopathological analysis of lung tissue, suggesting interstitial pneumonia with predominantly heterophilic infiltration and the presence of interalveolar amorphous mass in rabbits anesthetized for 21 days with sevoflurane or sevoflurane in combination with nitrous oxide. Histopathology of the spleen tissue from the same group of rabbits revealed hyperemia and hemosiderosis, marked histiocytosis of lymph follicles and individual lymphocyte apoptosis. The lymph nodes of sevoflurane anesthetized animals showed marked hyperplastic lymphadenitis and hyperplasia of lymph follicles. In our experiment, the immunosuppressive effect of sevoflurane was additionally enhanced when combined with nitrous oxide, which did not affect the immune system of treated animals when applied alone.

Based on the clinical, biochemical and hematologic parameters, the results obtained in the present study are consistent with the descriptions elsewhere of the toxic effect on peripheral blood lymphocytes, spleen and lung cells (BROZOVIĆ, 2007).

Conclusions

The results of the present study indicated the marked immunosuppressive effect of sevoflurane alone and in combination with nitrous oxide. These inhalant anesthetics also induced changes in the physiological values of hematologic parameters and histopathological alterations in the peripheral lymph node, lung and spleen tissues. Nitrous oxide exerted no major immunosuppressive effect when administered alone, but did so when used in combination with sevoflurane. Accordingly, the results obtained warn the clinicians to evaluate carefully the justifiability of using volatile anesthetics in patients where their prolonged administration can be expected, and justify additional research into the effects of sevoflurane and nitrous oxide on cellular immunity.

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SAŽETAK

Kirurški zahvati i anestezija mogu imati znatan učinak na imunosni sustav. S obzirom na to da određeni kirurški postupci zahtijevaju opetovanu anesteziju, u ovom smo istraživanju pratili učinak višekratnog izlaganja sevoflurana i dušikova oksida na imunosni sustav kunića. Dvadeset novozelandskih kunića podijeljeno je slučajnim izborom u četiri skupine. U svakoj je skupini bilo 5 životinja. Istraživanje je provedeno u trajanju od 20 minuta dnevno tijekom 21 dana. Kunići skupine A, udišući 100 %-tni kisik, služili su kao kontrolna skupina. U skupini B kunići su udisali mješavinu kisika i dušikova oksida, u skupini C primijenjena je kombinacija kisika i sevoflurana, dok je u skupini D korištena kombinacija kisika, dušikova oksida i sevoflurana. Uzorci krvi prikupljeni su 3., 7., 14. i 21. dan za kompletnu krvnu sliku i za određivanje subpopulacije leukocita na protočnom citometru ($CD21^+$, $CD4^+$, $CD8^+$, $CD45^+$). Nakon završetka istraživanja sve su životinje žrtvovane. Uzorci perifernog limfnog čvora, pluća i slezene uzeti su za histološku analizu. Najočitije promjene uočene su u promjeni udjela $CD21^+$, $CD4^+$, $CD8^+$, $CD45^+$ stanica u skupinama C i D 7., 14. i 21. dan istraživanja. Ako se usporedi vrijednost udjela $CD21^+$, $CD4^+$, $CD8^+$, $CD45^+$ stanica nulti dan i 21. dan istraživanja, uočava se pad udjela za približno 30 %. U ovom je istraživanju dokazano da sevofluran, te sevofluran u kombinaciji s dušikovim oksidulom, ima izrazit imunosupresijski učinak te utječe na promjenu fizioloških vrijednosti hematoloških pokazatelja i pojavu histopatoloških promjena na tkivima perifernog limfnog čvora, plućiju i slezene.

Ključne riječi: stanična imunost; sevofluran; kunić
