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EFFECT OF OVARIOHYSTERECTOMY ON CANINE POSTSURGICAL LEUKOCYTE FUNCTION

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The effect of surgery on phagocytic activity of blood leukocytes and mitogen-induced blastogenesis of lymphocytes was studied in fourteen dogs. Simple ovariohysterectomy with anaesthesia induced by ketamine and xylazine or by ketamine, xylazine and halothane caused a short nonsignificant depression of phagocytic activity that persisted for four hours after surgery. Ingestion capacity of leukocytes decreased significantly immediately after surgery. Mitogen-induced blastogenesis of lymphocytes was depressed significantly in the first 48 hours and despite partial recovery this parameter did not reach the value of the control groups until the end of observation (7 days). A more conspicuous decrease of blastogenic response of blood lymphocytes to mitogens was found after the use of ketamine and xylazine in a dose maintaining anaesthesia. Anaesthesia with ketamine and xylazine in the lower dose and maintained with halothane resulted in a later improvement of the blastogenic response of lymphocytes.

Key words: Ovariohysterectomy, dogs, lymphocyte proliferation, phagocytosis

Surgery together with anaesthetics is believed to have an adverse effect on the immune function. Optimal immune system responsiveness plays an important role in the control of postoperative infection and successful recovery. Impaired immunity is often observed after surgery and is multifactorial (Procopio et al., 2001). Surgical stress and anaesthetic agents can induce alteration of various aspects of polymorphonuclear leukocyte and lymphocyte functions. Postoperative immunosuppression is well documented in the human population (Cullen and Van Belle, 1975; Tonnesen and Wahlgreen, 1988; Nishina et al., 1999). The depression of cell-mediated immunity can develop in various degrees and is usually dependent on the kind of anaesthetic, its dose and on the extent of surgical trauma. Various anaesthetics (propofol, thiopentone, midazolam, ketamine) are known to depress human neutrophil functions (Salo, 1989; Davidson et al., 1995; Mikawa et al., 1998; Nishina et al., 1998). Depression of lymphocyte function was studied after the use of thiopentone, methohexitone, etomidate, and pheno-

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barbitone (Puppo et al., 1980; Devlin et al., 1994). Surgical stress also significantly reduces lymphocyte proliferation in human patients (Eskola et al., 1984; Sacerdote et al., 2000). There are only limited data concerning immunosuppression associated with anaesthesia and surgery in animals. Lymphocyte blastogenesis was depressed in dogs after simple laparotomy (Kelly, 1980) and ovariohysterectomy (Medleau et al., 1983) and in cattle after skin papilloma excision (Paulík et al., 1999). The effect of surgery on postsurgical immunocompetence was also described by Taura et al. (1995) in puppies vaccinated before surgery.

The aim of the present study was to determine the effect of the surgical procedure using two kinds of anaesthetic techniques in dogs on the total leukocyte count, phagocytosis and ingestion capacity of leukocytes and on the *in vitro* lymphocyte blastogenesis induced by Con A and PHA-P.

Materials and methods

Animals

Fourteen healthy female dogs of different breeds (average age: 4.7 years, age range: 3.2 to 6 years) undergoing ovariohysterectomy were included in the study. For premedication i.m. injection of diazepam and atropine was used. In eight dogs (Group A) anaesthesia was induced and maintained by i.m. injection of xylazine (2 mg/kg) and ketamine (10 mg/kg) In six dogs (Group B) i.v. injection of ketamine and xylazine was used in the lower dose (one-third of the dose commonly used for i.m. administration) and anaesthesia was maintained by a mixture of halothane, nitrous oxide and oxygen. The duration of anaesthesia ranged from 2 to 3 hours while the duration of surgery from 1 to 2 hours. There were no major operative and postoperative complications in any of the dogs.

Control group (Group C): Six healthy female dogs at the average age of 3.1 years, different breeds, not operated, kept and fed under the same conditions as the experimental animals served as controls.

All dogs came from a home for stray dogs and ovariohysterectomy was indicated because of the control of reproduction. The experiment was carried out respecting all legal requirements and a high standard of ethics was applied. Pain and suffering of animals were minimised.

Blood collection

Peripheral blood samples were obtained by puncture of the cephalic vein and placed into a tube containing heparin. Blood samples were collected before the anaesthesia (sampling 1), immediately after the end of surgery (sampling 2), 4 h after surgery (sampling 3), 24 h after surgery (sampling 4), 48 h after surgery (sampling 5), and 7 days after surgery (sampling 6).

Blastogenic response of blood lymphocytes to mitogens. Lymphocytes were separated from venous blood on the Ficoll density gradient (Pharmacia Biotech AB, Sweden). The viability of the isolated cells was determined by trypan blue exclusion and exceeded 97%. Most (> 95%) isolated cells were mononuclear cells. The cultivation (culture medium contained 10% of autologous serum), mitogen stimulation and measurement of the blastogenic response of lymphocytes by the fluorescence method were performed according to Nakanishi et al. (1986). Concanavalin A (Con A, Sigma Chemical Co., USA) and phytohaemagglutinin (PHA-P Sigma Chemical Co., USA) were used for stimulation in a concentration of 25 μg/ml and 20 μg/ml, respectively (Tajima et al., 1990). The level of blastogenic response of lymphocytes was expressed as the stimulation index (SI). The SI was calculated according to the formula SI = (A–C)/(B–C), A = mean fluorescence intensity (FI) with mitogen, B = mean FI without mitogen, C = background. The FI was measured by a spectrofluorometer (Jasco FP–550, Japan) at Ex = 525 nm and Em = 600 nm.

The phagocytic ability of blood leukocytes was examined as described by Větvička et al. (1982). Briefly, 0.1 ml of fresh heparinised blood (5 U of heparin · 1 ml^{-1} of blood) was mixed with 0.05 ml of 2-hydroxyethylmetacrylate particles (MSHP, diameter 1.2 μm, ARTIM Prague, Czech Republic) and incubated for one hour at 37 °C with occasional shaking. The phagocytic activity of leukocytes was expressed as the percentage of the cells phagocytosing 3 or more MSHP, and as the phagocytic index representing the ingestion capacity of leukocytes (the ratio of the number of phagocytosed MSHP to the number of all potentially phagocytosing leukocytes).

Total leukocyte count was determined with blood cells analyser (SERONO, 150 plus, USA).

Statistical analyses. The immunological parameters of surgical patients with different anaesthetic regimens were compared between them and with those in healthy dogs and analysed by Mann-Whitney U test.

Results

As shown in Fig. 1, total leukocyte count significantly increased at different times after surgery in Groups A and B (Group A – 24, 48 hours, Group B – 4, 24, 48 hours, 7 days) when compared with the control group and the presurgical value. Forty-eight hours after ovariohysterectomy leukocyte count was significantly higher in the dogs of Group A than in Group B. Then by day 7 the number of leukocytes had partially recovered in Group A but was still higher, although not significantly, than the presurgical value and the value in the control group. In Group B the total leukocyte count was significantly higher until the end of the observation period. In Group A the percentage of the phagocytosing leu-

kocytes (Fig. 2) slightly decreased immediately and 4 hours after the end of surgery but without statistical significance. Then a significant elevation of this parameter was found as compared to the control group and the presurgical value (24 hours after surgery) and only as compared to the control group (48 hours after surgery). In Group B of surgical patients there was no decrease in the percentage of phagocytosing leukocytes. In relation to the increase of leukocyte count, a significant increase in the percentage of phagocytosing leukocytes was observed 24, 48 hours and on 7 day after surgery in Group B as compared to the control group and to the presurgical value. No significant differences were found between the two groups of dogs undergoing ovariohysterectomy. The ingestion capacity of leukocytes expressed as phagocytic index (Fig. 3) decreased in both groups of surgical patients significantly immediately after surgery and nonsignificantly 4 hours after surgery. Then a gradual increase to values comparable with those of healthy dogs was observed. No difference was present between Groups A and B. The blastogenic response of lymphocytes stimulated with PHA-P expressed as stimulation index (Fig. 4) was significantly lower immediately after surgery and 4 hours after surgery in both surgical groups in comparison with the control group and the presurgical values. Twenty-four hours after surgery the stimulation index of lymphocytes started to increase in the dogs of Group A, although this parameter was significantly lower than in the healthy dogs until the end of the observation period. A similar course was present in Group B, but 24 hours later (48 hours after surgery) a significant difference was found between the two groups at sampling 4. The stimulation index of lymphocytes stimulated with Con A (Fig. 5) significantly decreased after surgery until the end of observation in all surgical patients when compared with the control group and the presurgical values. A significantly bigger decrease was found immediately after surgery in Group A as compared to Group B.

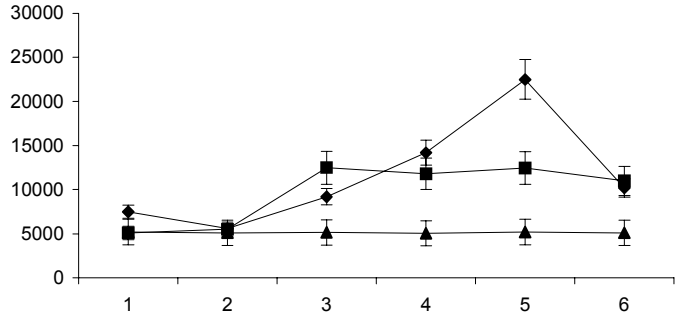

Fig. 1. Total leukocyte count. Sampling 1 (presurgery), 2 (immediately after surgery), 3 (4 hours after surgery), 4 (24 hours after surgery), 5 (48 hours after surgery), 6 (7 days after surgery); ◆ Group A: significant differences ($p < 0.05$) on samplings 4, 5 versus presurgical value and Group C; ■ Group B: significant differences ($p < 0.05$) on samplings 3, 4, 5, 6 versus presurgical value and Group C; Group A versus Group B: significant difference ($p < 0.05$) on sampling 5; ▲ Group C

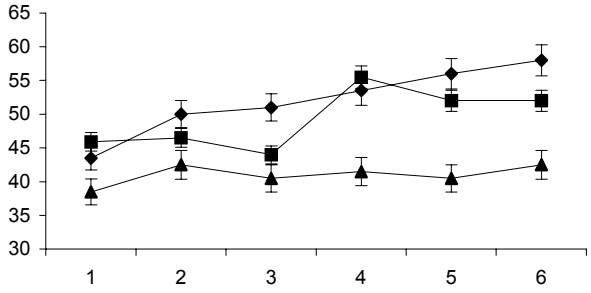

Fig. 2. Percentage of phagocytosing leukocytes. For samplings see Fig. 1. ◆ Group A: significant differences ($p < 0.05$) on sampling 4 versus Group C and presurgical value, 5 versus Group C; ■ Group B: significant differences ($p < 0.05$) on samplings 4, 5, 6 versus Group C and presurgical value; Group A versus Group B: NS; ▲ Group C

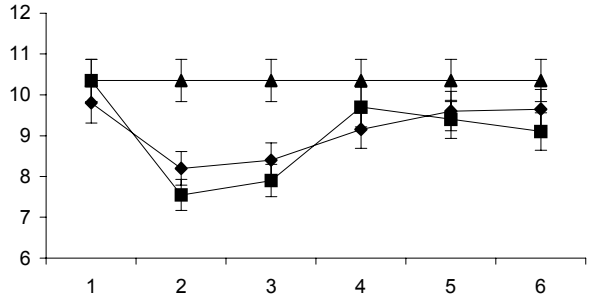

Fig. 3. Phagocytic index of leukocytes. For samplings see Fig. 1. ◆ Group A: significant decrease ($p < 0.001$) on sampling 2 versus Group C and presurgical value; ■ Group B: significant decrease ($p < 0.05$) on sampling 2 versus Group C and presurgical value; Group A versus Group B: NS; ▲ Group C

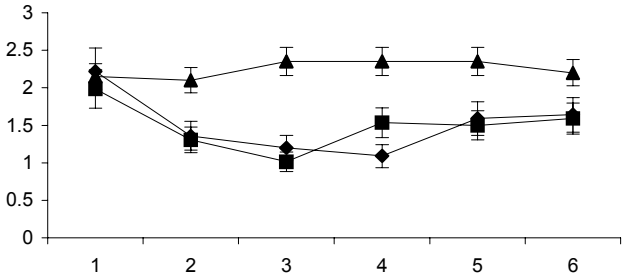

Fig. 4. Stimulation index of lymphocytes stimulated with PHA-P. For samplings see Fig. 1. ◆ Group A: significant decrease ($p < 0.05$) on samplings 2, 3, 4, 5, 6 versus group C and presurgical value; ■ Group B: significant decrease ($p < 0.05$) on samplings 2, 3, 4, 5, 6 versus Group C and presurgical value; Group A versus Group B: ($p < 0.05$) on sampling 4; ▲ Group C

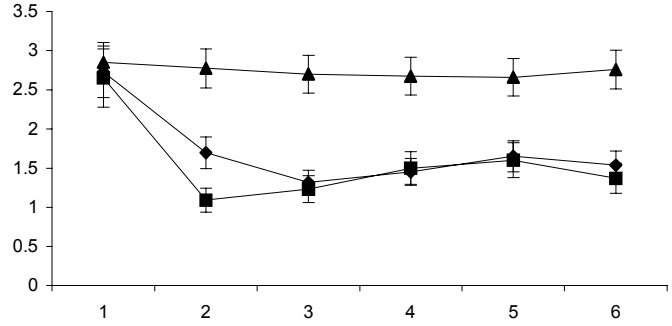

Fig. 5. Stimulation index of lymphocytes stimulated with Con A. For samplings see Fig. 1. ◆ Group A: significant decrease ($p < 0.05$) on sampling 2, 3, 4, 5, 6 versus Group C and presurgical value; ■ Group B: significant decrease ($p < 0.05$) on samplings 2, 3, 4, 5, 6 versus Group C and presurgical value; Group A versus Group B: ($p < 0.05$) on sampling 2; ▲ Group C

Discussion

Trauma, pain and anaesthesia itself may induce a series of processes known as stress response including immunological changes with an impact on the postoperative course. Higher leukocyte count was found in our patients undergoing ovariohysterectomy, similarly as described by Taura et al. (1995). The phagocytic ability of leukocytes in our study was altered within 4 hours after the end of surgery, but during the recovery period it even exceeded the value found in the control group. Alteration of any function of phagocytes can result in postoperative infection. Phagocytosis is known to be inhibited by various anaesthetics. Nishina et al. (1998) found that thiopental, midazolam and ketamine impaired chemotaxis, phagocytosis and oxygen radicals production. The results of Duncan and Cullen (1977) and Davidson et al. (1995) suggest that halothane, propofol, thiopentone, midazolam and ketamine have minimal effects on phagocytosis, only extremely high and clinically irrelevant concentrations of thiopentone and ketamine may affect phagocytic function. Alteration of phagocytic ability was found in animals in the study by Paulík et al. (1999) after excision of skin papillomas in calves using anaesthesia with xylazine and procaine. Our findings indicate that alteration of the function of phagocytes was of short duration and only minor, nonsignificant differences were found in this regard between the anaesthetic procedures. Increase of phagocytic activity later during recovery is beneficial from the point of view of postsurgical complication control.

Proliferation activity of lymphocytes stimulated by nonspecific antigens is a test widely used for determining the functional status of lymphocytes (Barta and Oyekan, 1981). In human surgical patients there are many data that confirm the postoperative depression of lymphocyte function. The degree of postsurgical inhibition of lymphocytic proliferation was reported to be proportional to the se-

verity of the surgery (Berenbaum et al., 1973; Cullen and Van Belle, 1975; Salo, 1978). Depressed lymphocyte transformation was found in association with major surgical stress but not in association with anaesthesia alone before surgery (Eskola et al., 1984), as well as during minimally stressful surgical procedures using inhalation halothane anaesthesia (Mattila-Vuori et al., 1999). Similarly, Puppo et al. (1980) and Devlin et al. (1994) confirmed that thiopentone, methohexitone, etomidate and barbiturate caused a decrease in lymphocyte blastogenesis. These findings indicate that besides surgical stress also the type and dose of anaesthetic agents may influence postsurgical lymphocyte depression.

Postoperative suppression of lymphocyte functional activity is not so well documented in animals. Surgery-induced depression of lymphocytic proliferative response to T-cell mitogens after skin papilloma excision in calves has been reported by Paulík et al. (1999). Studies of the immunosuppressive effect of ovariohysterectomy and laparotomy in dogs showed various degrees of alteration in lymphocyte function. In a study by Medleau et al. (1983) a transient depression of T lymphocyte function was found in healthy dogs after ovariohysterectomy, but this effect disappeared within 24 hours. Kelly (1980) reported that 24 hours after laparotomy there was only a partial recovery of lymphocyte responsiveness in dogs. Our results showed a similar course of lymphocyte function alteration immediately and 4 hours after surgery. Partial improvement of lymphocyte reactivity was found 24 hours after surgery in dogs that had received only ketamine and xylazine, whereas in groups where anaesthesia was maintained by halothane a partial increase appeared 48 hours after surgery. However, the depression of lymphocyte activity persisted until the end of observation (7 days). These findings are consistent with the results of Taura et al. (1995) who described a significant depression of lymphocyte blastogenic response after halothane anaesthesia in healthy puppies, persisting for 6 days after surgery.

On the basis of the present results we can conclude that simple ovariohysterectomy with anaesthesia induced by ketamine and xylazine or by ketamine, xylazine and halothane has a transient effect on phagocytosis, but both anaesthetic techniques caused a severe depression of the blastogenic response of lymphocytes. Minor differences in the phagocytic ability of leukocytes were found between the two anaesthetic techniques. Although the degree of surgical stress was minimised and the animals were healthy, trauma followed by pain, in combination with the effect of anaesthetics, could influence the changes of leukocyte function as a complex of stress factors.

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