原 著

Effect of Ovariohysterectomy under the Halothane Anesthesia on Canine Neutrophil Nitroblue Tetrazolium (NBT) Reduction Ability

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ハロタン麻酔下の卵巣子宮全摘出術が犬好中球の Nitroblue
Tetrazolium (NBT) 還元能に及ぼす影響

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SUMMARY

The effect of ovariohysterectomy under the halothane anesthesia on canine neutrophil function was investigated. The ovariohysterectomy was performed in 13 clinically healthy female dogs. Nitroblue tetrazolium (NBT) reduction test was performed before and after operation. The percent positive cells in NBT reduction test without stimulation (resting NBT reduction test) was significantly decreased immediately after operation ($p < 0.01$) and at 1 day postoperation ($p < 0.01$). The NBT reduction test with stimulation with endotoxin (stimulated NBT reduction test) was significantly decreased immediately after operation ($p < 0.001$) and at 1 day postoperation ($p < 0.001$). The difference in percent positive cells between stimulated and resting NBT test at each sampling time was also significantly decreased immediately after operation and at 1 day postoperation ($p < 0.01$, respectively). The decrease in the percent positive cells of NBT reduction tests and the difference between stimulated and resting NBT test recovered to the preoperative values at 7 days postoperation. The results suggest that NBT reduction ability in canine neutrophils is suppressed after ovariohysterectomy under the halothane anesthesia.

Key Words: canine neutrophil (犬好中球), nitroblue tetrazolium reduction test (NBT 還元試験), halothane anesthesia (ハロタン麻酔), ovariohysterectomy (卵巣子宮全摘出術)

Introduction

The suppressed blastogenesis of peripheral blood lymphocytes is reported as one of the effects of

general anesthesia and/or surgery on cell-mediated immunity in dogs.^{5,17,19-21} Although the neutrophils also play important roles in nonspecific defense mechanisms,^{25,27} the effect of ovariohysterectomy under the halothane anesthesia on their functions is not clearly understood in dogs. Recently, the authors suggested that nitroblue tetrazolium (NBT) reduction test was available for assessment of the canine neutrophil function.^{8,9}

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Nitroblue tetrazolium is a water soluble dye, which is reduced by superoxide anion in stimulated neutrophils and converted to insoluble formazan deposits.^{1,10)} Therefore, the NBT reduction test is considered to represent the oxidative sterilizing ability related to phagocytosis in neutrophils.^{1,6,26)} The present study was undertaken to investigate the effect of ovariectomy under the halothane anesthesia on canine peripheral neutrophil function as determined by the NBT reduction test.

Materials and Methods

Thirteen clinically healthy female dogs (11 mongrel and two Shiba dogs), aging 7 months to 5 years old and weighing from 7.4 to 11.0 kg were used in this study. All dogs were normal in blood and fecal examinations.

The ovariectomy was performed as the surgery. After atropine sulfate (0.05 mg/kg) and xylazine hydrochloride (1.0 mg/kg) were administered subcutaneously, anesthesia was introduced by an intravenous injection of thiopental sodium (12.5 mg/kg) and maintained with a mixture of oxygen (1 l/min) and halothane (1–2%).

Blood samples were collected before, immediately after, 1 day after and 7 days after operation. Total and differential leukocyte counts were determined by the routine methods. Nitroblue tetrazolium reduction test was performed by the method previously reported.^{8,9)} In brief, blood was collected by heparinized disposable syringe (containing 20 units of heparin sodium/ml of blood) and the blood of 200 μ l was transferred to a plastic tube. The NBT (Kanto Chemical Co., Inc., Tokyo, Japan) solution was prepared at a concentration of 2.0 mg/ml in 0.13 M phosphate buffer (pH 7.2). Endotoxin (lipopolysaccharide from *E. coli*, 0111-B4, Sigma Chemical Co., St. Louis, USA) solution was prepared at a concentration of 100 μ g/ml of physiological saline. The NBT solution (25 μ l) was mixed with an equal volume of physiological saline in a plastic tube, and the tube was used in resting NBT reduction test. The endotoxin solution of (25 μ l) was mixed with an equal volume of NBT solution in a plastic tube, and this tube was used in stimulated NBT reduction test. The blood sample tubes, the resting NBT test tube and stimulated NBT test tube, were preincubated at 37°C for 3 minutes.

Fifty microliter of the stirred blood sample was respectively transferred to resting and stimulated NBT test tube. Each test tube content was briefly mixed and incubated at 37°C for 15 minutes. At the end of incubation period, each test tube was gently shaken and blood smear was rapidly made. The smears were stained by Wright-Giemsa stain and 100 neutrophils were examined microscopically to determine the percentage of cells showing intracytoplasmic deposits of formazan (positive cells). Student's t-test was used for statistical comparison.

Results

Total leukocyte, absolute neutrophil and monocyte counts increased significantly at 1 day postoperation as compared with the preoperative values ($p < 0.001$, $p < 0.001$, $p < 0.01$, respectively). On the contrary, the absolute eosinophil count decreased significantly immediately after operation ($p < 0.05$) and at 1 day postoperation ($p < 0.01$). The absolute lymphocyte count also decreased significantly at 1 day postoperation ($p < 0.05$). All these parameters returned to the respective preoperative values at 7 days postoperation (Table 1).

The percent positive cells of resting and stimulated NBT reduction tests decreased significantly at immediately and 1 day after operation (resting: $p < 0.01$, stimulated: $p < 0.001$). The decreased percent positive cells recovered to the respective preoperative values at 7 days postoperation (Figs. 1 and 2).

The percent positive cells in stimulated NBT test were significantly greater than those in resting NBT test before and 7 days after operation ($p < 0.01$, respectively), but this tendency was not observed at the sampling immediately and 1 day after operation. Thus, the differences between the stimulated and resting NBT tests with these postoperative samplings were reduced significantly ($p < 0.01$, Fig. 3).

Discussion

The NBT reduction in neutrophil is related to phagocytosis^{1,6,16,26)} and intracellular metabolic changes following phagocytosis.^{2,7,15,16,28)} Endotoxin is one of the stimulants for neutro-

Table 1 Total leukocyte, eosinophil, neutrophil, lymphocyte and monocyte counts before and after operation ($\times 10^3/\mu\text{l}$)

		Preoperative	Postoperative		
			Immediately after	1 day	7 days
Total	mean	11.8	11.0	20.6***a)	11.0
Leukocyte	S.D.	3.5	2.8	5.4	2.9
Eosinophil	mean	0.7	0.4*	0.2**	0.9
	S.D.	0.3	0.3	0.4	0.4
Neutrophil	mean	7.7	8.1	18.1***	6.4
	S.D.	2.8	2.7	4.9	2.6
Lymphocyte	mean	3.0	2.2	1.5*	3.3
	S.D.	1.8	1.3	0.8	1.5
Monocyte	mean	0.3	0.4	0.8**	0.4
	S.D.	0.1	0.2	0.5	0.2

a) Significantly different from the preoperative value: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

a); Mean $\pm$ S.D.

b); Significantly different from the preoperative value, ** $p < 0.01$

Fig. 1 Percent positive cells in resting NBT reduction test in canine neutrophils before and after operation.

phils.^{15,16,22,23,28}) By adding a stimulant, there is usually a significant increase in the number of positive cells associated with increases of phagocytosis and intracellular metabolism, and thus the stimulated NBT test represents the activated function of neutrophils.^{15,16,22,28}) The NBT reduction ability of neutrophils becomes clearer when the results of the stimulated NBT test is evaluated in comparison to those by resting test.^{15,16,23,28,20}) The difference between the stimulated and resting NBT tests therefore indicates the ability of neutrophil NBT reduction for the stimulation.¹⁵⁾

a); Mean $\pm$ S.D.

b); Significantly different from the preoperative value, *** $p < 0.001$

c); Significantly different from the value of each resting NBT test at same sampling times. ## $p < 0.01$

Fig. 2 Percent positive cells in stimulated NBT reduction test in canine neutrophils before and after operation.

The results of the present study suggest that the neutrophil NBT reduction ability is significantly decreased immediately and 1 day after ovariohysterectomy under the halothane anesthesia, though the absolute neutrophil count was significantly increased at 1 day after operation. It is unclear that the factors responsible for suppressed NBT reduction ability in neutrophil, however, the disturbances of neutrophil phagocytosis and/or intracellular metabolism, which are not in

a); Mean ± S.D.

b); Significantly different from the preoperative value, ** $p < 0.01$

Fig. 3 The difference in percent positive cells between resting and stimulated NBT reduction test in canine neutrophils before and after operation.

response to even endotoxin stimulation, are suspected from the principle of NBT reduction test.

The suppression of phagocytosis in neutrophils is introduced by glucocorticoid,¹¹⁾ and the decreased NBT reduction ability is demonstrated in patients with steroid therapy.^{2,18,29)} Furthermore, the glucocorticoid inhibits the egress of granulocytes from the blood stream, thereby prolonging the intravascular lifespan.⁴⁾ The prolonged neutrophil lifespan in patients receiving glucocorticoid therapy results in significant inhibition of neutrophil adherence.¹²⁻¹⁴⁾ Thus, the decreased NBT reduction ability in this study may also be related to the prolonged neutrophil lifespan induced by glucocorticoid. The increased neutrophils and monocytes, decreased eosinophils and lymphocytes after the operation are also considered to reflect the effect of glucocorticoid.^{21,25,27)}

The direct effect of general anesthesia on neutrophil function is unknown. The halothane, however, directly suppresses blastogenesis of lymphocytes via a depression of intracellular metabolism.²⁴⁾ The depression of intracellular metabolism probably occurs in neutrophils under the halothane anesthesia, and the fact perhaps brings about the suppressed NBT reduction ability. Further studies *in vivo* and *in vitro* are required to elucidate the effects of general anesthesia and surgery on the NBT reduction ability in canine neutrophils.

要 約

ハロタン麻酔下の卵巣子宮全摘出手術が犬好中球の機能に及ぼす影響について明らかにするため、臨床的に健康な雌犬 13 頭を用いてキシラジンおよびチオペンタールを投与後、OF 麻酔下で手術を行い、手術前後における犬好中球の Nitro-blue Tetrazolium (NBT) 還元能を検索した。その結果、好中球数は、手術前の $7,700 \pm 2,800/\mu\text{l}$ から有意に増加して手術後 1 日目には $18,100 \pm 4,900/\mu\text{l}$ となった ($p < 0.001$)。一方、非刺激 (resting) NBT 還元試験の陽性率は、手術前の $49.8 \pm 23.4\%$ から有意に減少して手術終了直後には $26.5 \pm 17.5\%$ ($p < 0.01$) となり、手術後 1 日目には $26.3 \pm 17.7\%$ ($p < 0.01$) となった。また、エンドトキシンを用いた刺激 (stimulated) NBT 還元試験の陽性率も手術前の $74.4 \pm 19.5\%$ から有意に減少して手術終了直後には $39.8 \pm 19.0\%$ ($p < 0.001$)、手術後 1 日目には $39.3 \pm 21.7\%$ ($p < 0.001$) となった。さらに、stimulated NBT 還元試験と resting NBT 還元試験の陽性率の差も手術前の $24.6 \pm 7.6\%$ から手術終了直後と手術後 1 日目に有意に縮小してそれぞれ $13.4 \pm 8.3\%$ 、 $13.0 \pm 8.5\%$ となった (それぞれ $p < 0.01$)。これらの減少ないしは縮小した NBT 還元試験の陽性率および陽性率の差は、手術後 7 日目には手術前の値に回復した。

以上のことから、犬好中球の NBT 還元能はハロタン麻酔下の卵巣子宮全摘出術により低下することが示唆された。

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