FULL PAPER *Surgery*

The modulating effects of propofol and its lipid carrier on canine neutrophil functions

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ABSTRACT. Propofol (2,6-diisopropylphenol), being used as an intravenous sedative and anesthetic agent, influences not only upon nervous system but also for host inflammatory response through modulating neutrophil functions. This study is designed to evaluate the modulating effects of propofol and its lipid carrier administration at clinically relevant rate on canine neutrophil functions. Clinically healthy beagle dogs were received propofol (8.8 mg/kg) from cephalic vein and maintained with propofol dropping infusion (26.4 mg/kg/hr). Blood samples were collected from the dogs before infusion and 30 min after the start of propofol administration, and neutrophil functions were evaluated. The dogs were also administered lipid carrier, and neutrophil functions were evaluated in the same manner as propofol administration. Peripheral white blood cell and neutrophil counts decreased after the propofol or lipid carrier administration. The administration of propofol or lipid carrier significantly reduced neutrophil adherence ability. The superoxide production of neutrophils was measured by luminol-dependent chemiluminescence response using with opsonized zymosan. Peak height of neutrophil chemiluminescence curve was reduced by propofol and lipid carrier administration, on the contrary, peak time of neutrophil chemiluminescence curve was delayed. Administration of propofol or lipid carrier also reduced neutrophil adherence ability to nylon fibers. In the present study, we showed the modulating effects of propofol and its lipid carrier on canine neutrophil functions. However, there was no significant difference in the modulating effects between propofol group and lipid carrier group. Therefore, the modulating effects observed here were deeply concerned in lipid carrier administration.

KEY WORDS: canine neutrophil function, lipid carrier, propofol

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It is well known that neutrophils play an important role against the primary host defense system. Neutrophils are the initial responder to extracellular pathogens. Activation of neutrophils leads to sequent reactions including the chemotaxis, adherence and phagocytosis. Neutrophils generate cytotoxic reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), through the phagocytosis. This process has an importance in killing pathogens and simultaneously involves in inflammatory response.

On the other hand, there are many subjects modulating neutrophil functions, such as some of anesthetics. Propofol (2,6-diisopropylphenol), being one of such anesthetics, formulated as an emulsion in soybean oil, glycerol and egg lecithin, is an intravenous sedative and anesthetic agent used for induction and maintenance of anesthesia both in human and veterinary medicine. Propofol has been also used for sedative of patients in the intensive care unit (ICU) [6]. Upon intravenous administration, the pharmacokinetics of propofol is characterized by a rapid initial distribution half-life (from 2~8 min) with slow distribution half-life ranging from 30 to 70 min and terminal elimination half-life ranging from 4 to 24 hr [15]. The effect of propofol against brain system is similar with barbitulates, such as decreasing cerebral blood flow and reducing the cerebral metabolic rate dose-dependently [15]. Propofol influences not only upon nervous system but also for host inflammatory response through modulating neutrophil functions; decreasing production of proinflammatory cytokines, altering expression of nitric oxide (NO) and inhibiting neutrophil functions [10]. Therefore, propofol is administered to surgical patients of brain, heart and lung bearing the risk of free-radical-mediated injury in anticipation of anti-oxidant effects [1, 5, 13, 19].

Since propofol is not soluble in water, it is formulated in an emulsion that contains soybean oil, glycerol and egg lecithin, and lipid carrier itself has the biological activities [10]. There are some reports referring the anti-oxidant abilities of its lipid carrier [4, 7]. Clinical effects of propofol on alleviation of free-radical-mediated injury seem to owe a lot both to propofol and its lipid carrier. Lee [9] evaluated the anti-oxidant effects of propofol in dogs undergoing anesthesia and surgery. This report was designed to evaluate anti-oxidant effects of propofol on a condition of surgical operation, and propofol was used with other drugs simultaneously. Therefore, modulating effects of propofol itself on canine neutrophil function are unclear. Furthermore, whether lipid

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carrier also has an influence to canine neutrophil function is not fully understood. In veterinary medicine, there are a few informations about modulating ability of propofol and its lipid carrier on neutrophil functions, especially generation of reactive oxygen species. Therefore, this study is designed to evaluate the modulating effects of propofol and its lipid carrier on canine neutrophil functions.

MATERIALS AND METHODS

Experimental dogs: Ten healthy beagle dogs (4 males and 6 females, 1–6 years old and 9.4–14.7 kg) were used. All dogs were kept in our experimental animal room, and fed commercially-available dry dog food twice a day and given water *ad libitum*. All dogs were received clinical examinations for confirmation of healthy condition before using experiments. First, the dogs were administered propofol intravenously, and blood samples were collected before and later treatment. At least two weeks after, the dogs were administered lipid carrier in the same manner, and also blood samples were collected. This study was approved by the Iwate University Laboratory Animal Care and Use Committee.

Propofol administration: The dogs were intravenously administered propofol at clinically relevant rate (8.8 mg/kg, Rapinovet®, Schering-Plough Animal Health Co., Osaka, Japan) from cephalic vein. Propofol was administered slowly taking 90 sec. Rapinovet® contains 1% propofol, 10% soybean oil, 2.25% glycerol, 1.2% egg lecithin and 0.025% sodium hydroxide. Next, the dogs were maintained with propofol dropping injection (26.4 mg/kg/hr), and blood samples were collected from jugular vein with 10 m*l* heparinized syringe before administration and 30 min after the start of propofol administration. Propofol dosage was decided according to the method described by Plumb [14], and sampling time of blood samples was decided according to the results of preliminary test (data not shown).

Lipid carrier administration: The dogs were intravenously administered a lipid carrier (0.88 m*l*/kg, Intrafat® injection 10%, Nihon Pharmaceutical Co., Tokyo, Japan) from cephalic vein in the same manner as the propofol administration and then infused the lipid carrier (2.64 m*l*/kg/hr). Blood samples were collected from jugular vein with 10 m*l* heparinized syringe before administration and 30 min after the start of lipid carrier administration. The lipid carrier used in the present study also contains 10% soybean oil, 2.25% glycerol, 1.2% egg lecithin and a little of sodium hydroxide and is almost the same as Rapinovet® both in the kind and the ratio of contents.

Isolation of canine peripheral neutrophils: Collected heparinized-blood samples were used for blood cell count and neutrophil isolation. Peripheral neutrophils were isolated using dextran sedimentation and Ficoll-conray density-gradient separation followed by hemolysis as described previously [8]. Briefly described, collected whole blood was suspended in an equal volume of Ca^{2-} and Mg^{2+}-free Hanks' balanced salt solution (HBBS). The leukocytes were separated by dextran (6%, w/v) sedimentation with half its volume of whole blood for 30 min at room temperature. After the supernatant was washed, neutrophils were isolated by Ficoll density (1.077) gradient centrifugation. The contaminating erythrocytes were lysed at 4°C by 0.83% NH_4Cl solution containing 14.2 mM $NaHCO_3$ and 120 mM ethylenediaminetetraacetic acid (EDTA) 2Na. The viability of isolated neutrophils was determined by 0.2% trypan blue staining (>95%).

Neutrophil function tests: Neutrophil adherence to nylon fibers was examined according to the method of Nagahata *et al*. [12] with some modification as described previously [8]. Neutrophil suspension (5×10^6 cells) containing 10% autologous plasma was incubated for 10 min at 37°C and then was applied to a preincubated nylon wool fiber column (50 mg, Polysciences, Inc., Warrington, PA, U.S.A.). After percolating through the nylon fiber at room temperature, neutrophil counts were performed. Neutrophil adherence was calculated from the formula: Percentage of neutrophil adherence = (1− counts of effluent neutrophil / counts of initial neutrophil) × 100. The production of superoxide was measured by luminol-dependent chemiluminescence response as described in our previous study with little modification [8]. Briefly, 600 m*l* of HBSS containing 5×10^7 neutrophils and 100 m*l* of 10^{-4} mM luminol were incubated for 2 min at 37°C. Then, neutrophils were activated by adding 200 m*l* of opsonized zymosan (5 mg/m*l*) (Sigma-Aldrich Japan, Tokyo, Japan). Opsonized zymosan was prepared by suspending in canine sera at a concentration of 5 mg/m*l* and incubating for 30 min at 37°C. After incubation, the suspension was washed twice with HBSS and resuspended in HBSS at the concentration of 5 mg/m*l*. The chemiluminescence was measured with a luminometer (LKB-Wallac Co., Turku, Finland) at intervals of 30 sec for total 30 min at 37°C.

Statistical analysis: All values were shown as the mean and two sided 95% confidence interval (CI). Statistical comparison between pre-treatment value and post-treatment value was followed by paired *t*-test. The difference of the results in propofol group and lipid carrier group was analyzed by Welch's *t*-test. The reduction rate (post-treatment value / pre-treatment value) in both groups was also compared by Welch's *t*-test. The results were considered significant when *P* value was less than 0.05.

RESULTS

Peripheral white blood cell and neutrophil counts were decreased after propofol or lipid carrier administration (Fig.1). After propofol administration, the mean value of peripheral white blood cell count was decreased from 12,500 (CI: 10,921–14,079)/μl to 10,683 (CI: 8,974–12,392)/μl, and neutrophil count was significantly ($P<0.05$) decreased from 6,623 (CI: 5,756–7,490) μl to 5,282 (CI: 3,835–6,729)/μl. After lipid carrier treatment, the mean value of peripheral white blood cell count was significantly ($P<0.05$) decreased from 12,950 (CI: 10,500–15,400)/μl to 11,925 (CI: 8,963–14,887)/μl, and neutrophil count was also decreased from 7,542 (CI: 5,309–9,779)/μl to 6,918 (CI: 4,100–9,736)/μl. The decreasing rate of peripheral white blood cell and neutrophil counts was greater in propofol group than that in lipid carrier group. However, there was no significant differ-

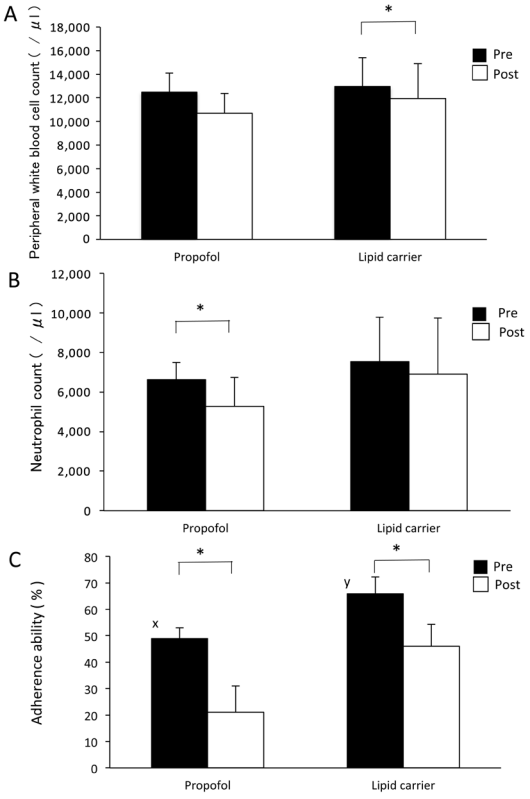

Fig. 1. The change of peripheral white blood cell and neutrophil counts and adherence ability after propofol or lipid carrier treatments. After propofol or lipid carrier administration, peripheral white blood cell count (A) and neutrophil count (B) were decreased. The adherence ability was also suppressed (C). The asterisk indicates a significant difference between values of pre-treatment and post-treatment (P<0.05). The letters (x, y) also indicate a significant difference between values of pre-treatment (P<0.05).

ence between two groups.

Administration of propofol or lipid carrier reduced neutrophil adherence ability to nylon fibers (Fig.1). After propofol administration, the mean value of neutrophil adherence ability was significantly (P<0.05) decreased from 49.0 (CI: 45.0–53.0)% to 21.0 (CI: 11.0–31.0)%. The pre-treatment value of neutrophil adherence in lipid carrier group was significantly (P<0.05) higher than that in propofol group. The mean value of neutrophil adherence ability to nylon fibers was also significantly (P<0.05) decreased from 65.9 (CI: 59.5–72.3)% to 46.0 (CI: 37.7–54.3)% after lipid carrier administration. The degree of reduction in propofol treatment was bigger than that in lipid carrier treatment. However, there was no significant difference in reduction rate between groups.

The superoxide production of neutrophils was measured by luminol-dependent chemiluminescence response. Canine neutrophils activated with opsonized zymosan released superoxide, and chemiluminescence curve elevated gradually to make a peak at 14 to 23 min after opsonized-zymosan stimulation in both groups. Propofol administration showed inhibitory effect on superoxide production in canine neutrophils (Fig.2). The mean value of peak height of neutrophil chemiluminescence curve was reduced from 1,316.8 (CI: 904.6–1,729.0) mV to 1,029.2 (CI: 589.2–1,469.2) mV. On the contrary, the mean value of peak time of neutrophil chemiluminescence curve was delayed from 19.5 (CI: 16.1–22.9) min to 22.4 (CI: 18.7–26.1) min. Lipid carrier administration also led to the suppression of superoxide production in canine neutrophils. The mean value of peak height of neutrophil chemiluminescence curve was reduced from 991.8 (CI: 822.4–1,161.2) mV to 878.9 (CI: 471.7–1,286.1) mV. The mean value of peak time of neutrophil chemiluminescence curve was significantly (P<0.05) delayed from 22.2 (CI: 21.7–22.7) min to 25.1 (CI: 24.0–26.2) min. The reduction rate of peak height was greater in propofol group than that in lipid carrier group. However, there was no significant difference between two groups.

DISCUSSION

This study was designed to evaluate the modulating effects of propofol and its lipid carrier on canine neutrophil functions. The ROS generation of neutrophils is an important process in killing pathogens. The luminol-dependent chemiluminescence response used in the present study evaluates the abilities of phagocytosis and superoxide production in canine neutrophils. First, neutrophils attach to opsonized zymosan with Fc receptor and take zymosan inside [17]. This process stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and then, the generation of superoxide starts. In the present study, after the injection of propofol or its lipid carrier, there was a decreasing tendency of the peak height of luminol-dependent chemiluminescence curve.

After administration of lipid carrier, the peak time of neutrophil chemiluminescence curve was significantly delayed. Lipid carrier used in this study was containing long chain triglycerides (LCTs) extracted from soybean oil and reported to show the modulating effects similar to propofol on human neutrophils [2, 4]. In the present study, intravenous administration of lipid carrier inhibited the adherence and the superoxide production in canine neutrophils. These modulating effects observed here seemed to be caused by lipid carrier. Furthermore, Heine *et al*. [4] suggested that lipid carrier like LCTs can be rapidly incorporated into cell membranes, thereby increasing membrane rigidity. The increasing membrane rigidity seemed to lead the difficulty of expression of adhesion molecules and combination in NADPH oxidase components, and cause the inhibition of adherence, phagocytosis and superoxide generation in canine neutrophils. In the present study, the suppressed effect on neutrophil adherence activity observed after lipid carrier administration was bigger than that observed in chemiluminescence assay. The rigidity of membrane may directly affect adherence-related receptor expression.

On the other hand, Mikawa *et al*. [11] reported that propofol itself inhibited the production of ROS in human neutrophils and suggested that the decrease of intracellular calcium

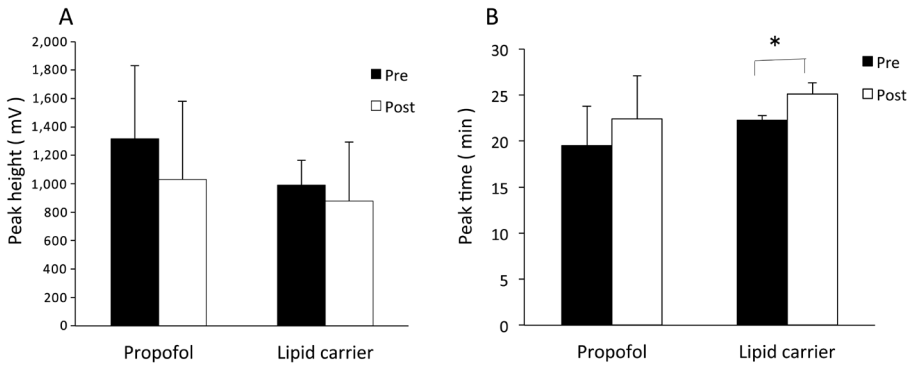

Fig. 2. The change of neutrophil chemiluminescence in propofol or lipid carrier treatment. After propofol or lipid carrier administration, the peak height of chemiluminescence curve was reduced (A), and the peak time was extended (B). There was a significant difference between values of pre-treatment and post-treatment in lipid carrier group ($P<0.05$).

concentration seemed to represent one of the mechanisms responsible for the inhibition of neutrophil functions by propofol. There are also some reports that propofol reduced the activity of cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and hypoxia inducible factor (HIF)-1α, in surgical patients and experimental animals [3, 20]. Some of these cytokines are reported to lead the neutrophil priming [16]. Furthermore, Galley *et al*. [2] reported that the release of IL-8 strengthen adherence ability of neutrophils was inhibited by propofol. On the other hand, adhesion molecule-expression on the surface of leukocytes was also decreased by propofol administration [18]. Neutrophil functions, such as chemotaxis, adherence, phagocytosis and superoxide production, are strongly concerned with neutrophil priming by cytokines and intercellular action through adherence molecules. In the present study, canine neutrohil adherence ability was reduced after propofol administration. In the assay of neutrophil adherence ability, we used nylon wool fiber column with autologous plasma for priming of neutrophils. Therefore, these results suggested that propofol administration also reduced the cytokine activation and decreased canine neutrophil-priming, that might lead the down-regulation of adherence molecule-expression on the surface of canine neutrophils. However, neutrophil adherence also decreased after lipid carrier injection, and there is no significant difference in suppressive effects between propofol group and lipid carrier group. Furthermore, the limitation of this study is a significant difference between pre-administration values of two groups. Therefore, the modulating effects observed in propofol group seem to be deeply concerned in lipid carrier.

In conclusion, we clearly demonstrated the modulating effects of propofol and lipid carrier on canine neutrophil functions with clinically relevant rate. The modulating effects observed here were deeply concerned in lipid carrier.

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