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. The modulating effects of propofol and its lipid carrier on canine neutrophil functions are unclear. Furthermore, whether lipid carrier itself has the biological activities 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$_2$O$_2$), through the phagocytosis. This process has an important role 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 barbituates, 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 activities 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...
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 ml 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 ml/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 ml/kg/hr). Blood samples were collected from jugular vein with 10 ml 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 Ca²⁺- and Mg²⁺-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% NH₄Cl solution containing 14.2 mM NaHCO₃ 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 × 10⁶ 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 percolation 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 ml of HBSS containing 5 × 10⁶ neutrophils and 100 ml of 10⁻⁴ mM luminol were incubated for 2 min at 37°C. Then, neutrophils were activated by adding 200 ml of opsonized zymosan (5 mg/ml) (Sigma-Aldrich Japan, Tokyo, Japan). Opsonized zymosan was prepared by suspending in canine sera at a concentration of 5 mg/ml 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/ml. 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-
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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 neutrophil 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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