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The Impact of Blood Cells Component to The Drug Binding of Enrofloxacin in Dogs

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Abstract

The research had deducted to evaluate the others components of blood cells that may contribute in drug interaction besides the protein plasma. As known that albumin or globulin of plasma played the majority role of functions in drug binding, some results of many studies still need explanation about the impact of other cells components of blood that may involved in the drug binding. These research used the whole blood of 10 patients of dogs that severed from deaseases and 5 samples of healthy dogs. The therapeutic doses of enrofloxacine (10 mg/kg body weight) were injected an hour before the blood sampling through cephalica vein. The whole blood were analyzed to measure the count of red blood cell (RBC), haemoglobin (Hb), white blood cell (WBC), and platelet by impedance, fluorescent flow and hydrodynamic focusing methods, respectively. Enrofloxacin level was analyzed with high performance liquid chromatography (HPLC) method. The calculation results with regression analysis showed that the values of RBC, Hb, WBC, and platelet of sick dogs had no significant impact to the enrofloxacin level ($P>0.05$). Except the platelet, the blood components of sick dogs were significantly different compare to the healthy dogs ($P<0.1$ and $P<0.05$). The enrofloxacin level of all sick dogs were in therapeutic level (1.22±0.11µg/mL) for some pathogenic bacterias.

Key words; enrofloxacin; blood components; drug binding

Introduction

The blood cell counts such as RBC, WBC, Hb and platelet were take important roles in diagnostic establishment of a specific disease. These cells may also alienating as targets of some infectious agents such as bacteria, virus, parasites, or hazardous chemicals. The drug binding evaluation is important because it affects closely to the effectiveness of drug. Albumin and globulin are the protein plasma that bind to the drugs so this will affects the rate of distribution, bioavailability, and effectiveness of drug. Some of studies mention the involving of RBC and Hb as delivery systems of drug in the body (Muzykantov, 2010). To cope with the function, drug molecules were temporary bond to spesific site of the cells. Biagiotti *et al*. (2011) reported that RBC can be engeeneered and had the capability to bind and played as transporter to spesific of drugs. According to Mintzer *et al.* (2009) review, some of drugs may induced the hematologic syndrome with varies of mechanisms. They can induce almost the entire spectrum of hematologic disorders, affecting white cells, red cells, platelets, and the coagulation system. These involvement of cells in circulation either in therapeutic or deleterious effects of drugs, will bring to a question of the others component of blood that may have the role in drug binding. The drug binding to macromoleculs (mainly to albumin or globulins) is well explained by many studies for decades. The binding of protein and cells to drug in circulation will limited drug's penetration to next compartement e.g. extravascular and

intracellular spaces. This will alter the fate of drug in the body, especially the drug bioavailability and distribution.

Enrofloxacin is a fluoroquinolone group of antibiotics, wide spectrum of activity, and good effectiveness to combat the gastrointestinal and systemic infections in animals. In companion animals, especially dog, the drugs is now widely used, and reported has in range of 27-34% of protein plasma binding (Plumb, 2008). These study was conducted to know the relation of RBC, Hb, WBC and platelet values to enrofloxacin level, along with measurement of albumin.

Materials and Methods

The materials used in the research were whole bloods of healthy (n=5) and sick dogs (n=10) from animal hospital and clinics in Yogyakarta. All the animals used were identified and the owners had signed the informed concents. The bloods were taken from cephalica vein with syring, then divided into two different sterile tubes contained heparin and EDTA. The plasma were collected by centrifugation of whole blood from all tubes at 2500 G for 5 minutes, and then analyzed for the RBC, WBC, Hb and platelet counts by Sysmex XT 1800i with empedance method and by bromcresol green method for albumin (blood in EDTA tube) and enrofloxacin concentration (heparinized tube). The level of enrofloxacin was analyzed with high performance liquid chromatography (HPLC) method. The HPLC system used were Shimadzu version 1, isocratic, reversed phase, equipped with column Shimpack C18 150 mm length and diameter 5 µm, detector UV-Vis at wavelength 350 nm, pumps, degasser and controller system, and using the Class VP software version 1. Mobile phase used were oxalatic acid solution of 0.126 % : acetonitrile: methanol (6:3:1, v:v:v), with fow rate set at 1mL/minute, and temperature 30º Celcius. This HPLC validated method was established at prior study and gained the good linearity, precision, accuration and high spesificity to detect the enrofloxacin from plasma. The results of Hb, RBC, WBC, and platelet counts analyzed with Student T-Test and linear regression to know the relation of all blood components with enrofloxacin concentration and albumin-enrofloxacin concentrations.

Results and Discussion

The results of blood cell counts are showed in Tables 1 and 2. The single symbols shows the significantly difference between averages of each paramater value of two tables, and double symbols shows the difference in table 2 only. Table 1 shows the results of blood components counts of healthy dogs that no received the enrofloxacin injection. The count results of blood components shows the relatively normal range according to Weiss & Wardrop (2010).

Table 2 shows the results of Hb, RBC, WBC, platelet values, and albumin and enrofloxacin concentration in plasma of sick dogs. Counts of cells, except the platelet showed the significant difference with healthy dogs. Instead of Hb and RBC that had less significant of difference ($P<0.1$), albumin and the white blood count of sick dogs were significantly increase compare to the healthy ($P<0.05$). The increasing of WBC associated with the drug binding was lack of explanation, but the relation of platelet with albumin concentration has the influence and strong correlation (double symbols in Table 2) .

Table 1. The count of blood components of healthy dogs.

Dog codes	Hb (g/dL)	RBC (10^6/µL)	WBC (10^3/µL)	Platelet (10^3/µL)	Albumin (g/dL)
DH1	14.5	6.86	8.39	133	3.00
DH2	13.6	6.21	11.04	95	2.61
DH3	13.2	5.94	7.47	178	2.47
DH4	13.7	6.14	10.56	250	4.88
DH5	13.1	6.18	8.80	234	2.97
Averages	13.62	2.26	9.25	178	3.20
Standars of deviation	0.55	0.34	1.50	65.63	0.97

Table 2. The results of blood components count and enrofloxacin concentrations of sick dogs.

Dog codes	Hb (g/dL)	RBC (10^6/µL)	WBC (10^3/µL)	Platelet (10^3/µL)	Albumin (g/dL)	Enrofloxacin (µg/mL)
DS1	11.40	6.50	10.99	70	1.90	1.31
DS2	11.30	6.42	10.41	47	1.84	1.24
DS3	10.80	5.43	9.19	4	1.72	1.36
DS4	13.70	6.51	13.29	38	2.66	0.96
DS5	13.80	6.04	13.89	382	2.82	1.31
DS6	15.60	7.59	16.81	230	2.69	1.29
DS7	7.20	3.15	4.56	12	1.57	1.13
DS8	14.70	6.05	12.27	173	2.79	1.17
DS9	15.20	7.61	12.14	205	1.93	1.22
DS10	7.70	3.24	30.10	6	2.47	1.21
Averages of	12.14#	5.85#	13.36*	116.70##	2.24*/##	1.22
Standard Deviation	2.89	1.55	6.70	126.20	0.49	0.11

#significant compare to healthy dog (P<0.1)
*significant compare to healthy dog (P<0.05)
##significant influence and strong correlation of sick dog (P<0.1, R<0.5)

The results of blood components count including albumin concentration were evaluated to know the impact on drug-binding. The albumin and globulin take the most important role in drug-protein plasma binding. Albumin compiling 50-60% of total protein plasma, and maintaining the level of drugs in the circulation (Naveen *et al*., 2016). Table 2 shows the lower results of Hb and RBC counts (P<0.1) or albumin (P<0.05) compare to the count of same cells of healthy dogs (Table 1). These explained that Hb and RBC counts functioned as the routine analysis to determine the status of physical condition, which influenced by decreasing of body conditions caused by nutrition imbalance or disease. Muzykantov (2010), studied the RBC as drug delivery agent, and found that eritrocyte can be encapsulated with drug then carrying to the spesific target such as phagocytic cells. RBC are been tested for drug delivery in numerous animal (Ktavtzoff *et al*.,1992) and human studies (Rossi *et al.,*2001). Result of study by Mazoit & Samii (1999) showed that fifty percent of propofol, an anaesthetic agent, was bound to erythrocytes and 48% to serum proteins, almost exclusively to human serum albumin. In the clinical range of concentrations (0.5–16 µg/mL) 40% of the molecules bound to erythrocytes are on the red blood cells membranes. Certain challenges and limitations of using RBC as drug carriers have been identified in these studies. It is immediately evident that the encapsulation of such a kind of molecules would be of no added value because, even in case of a successful entrapment, they would immediately escape from the red cell container without any practical pharmacokinetic advantage over conventional delivery. According to Mikhailidis and Ganotakis reviews (1996) plasma albumin concentrations can influence platelet function, with epidemiological evidence showing that low plasma concentrations of albumin predict mortality from some cardiovascular diseases. Platelet produced in the bone marrow of spinal cord, and played as the main source of e-granule protein, including albumin. However, in this study resulted that platelet value has no impact to the enrofloxacin concentration.

The protein plasma binding of enrofloxacin in dog is 27% (Wiebe, 2015). The variations of the binding were found interspecies in range 15-40% (Nites *et al*.,2011). The important thing resulted from this study was the concentration of enrofloxacin that given intramuscularly to the sick dogs with dose of 10 mg/kg body weight was achieving the therapeutic level. The enrofloxacin concentrations of sick dogs were ranged and above in minimun inhibitory concentration (MIC) of some pathogenic bacteria. The mean of enrofloxacin concentration (1.22±0.11 µg/mL) is effective to *Bordetella bronchiseptica* (0.5-2.0 µg/mL), *Staphylococcus intermedius* (0.12-0.5 µg/mL), *Staphylococcus pseudintermedius* (0.25 µg/mL), *Staphylococcus aureus* (0.12-0.25 µg/mL), *Enterococcus sp.* (1.0-2.0 µg/mL), *Escherichia coli* (0.03-0.125 µg/mL) , *Klebsiella*

pneumoniae (0.06-0.12 µg/mL), *Pasteurella multocida* (0.03 µg/mL), *Proteus spp.* (0.12-0.5 µg/mL) and *Pseudomonas spp.* (1.0-8.0 µg/mL) (Walker & Dowling, 2006). Albumin arranges at least 50% of total protein plasma, and it usually decrease in hampered condition of patients, which can reducing the 40-50% of total albumin. This condition would alter the percentage of albumin-drug binding, especially the drug with high binding percentages. Ulldemolins *et al.* (2011), reported the alteration of pharmacodynamic and pharmacokinetic profiles of drug caused by the shifting of albumin level in some chronic diseases. Zeitlinger *et al.* (2004) also reported the eficacy some of drugs were decreased by the addition of albumin.

The results of this study described that the alteration of blood cells component in sick dogs have no impact to the effectiveness of enrofloxacin injected in therapeutic dose. The albumin that mostly might affects to the drug binding percentage was not alter the level of enrofloxacin to subtherapeutic or toxic levels, eventhough the albumin concentration was significantly lower in sick dogs compare to the healthy dogs

Conclusion

It concluded that the study showed the alteration of counts of RBC, Hb, WBC, and albumin concentration in sick dogs compare to the healthy, but the changes has no impact to the drug binding and therapeutic level of enrofloxacin.

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