Serum Erythropoietin Level in Normal Dogs
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Erythropoietin (EPO) is a glycoprotein hormone which functions as a growth factor for stimulating the proliferation and differentiation of erythroid precursor cells. EPO is thought to be essential as a differentiation factor to trigger the transformation of the erythroid stem cell (CFU-E) into a proerythroblast, the first morphologically recognizable erythroid cell. The serum EPO level is thus an important index of the erythropoietic activity in vivo. Although the EPO activity has been found in many species, few studies have been directed to the estimation of the normal levels of EPO in the sera. In the present study, we examined the serum EPO levels in normal dogs and some other species, using the mouse spleen cell method [5].

Thirty-four normal healthy mongrel dogs were used to determine the serum EPO level. These dogs were clinically healthy. The EPO concentration were also examined for 3 horses aged 3–19 years (2 Thoroughbred and one Arab), 4 cows aged 4–8 years (Holstein), 7 cats aged 1–5 years (Domestic) and 3 goats aged 1–3 years (Saanen). Serum was immediately separated after blood collection and stored at −20°C. Just before the assay, the samples were heated for 30 min at 56°C followed by filtration through 0.2 μm pore membranes (Schleicher & Schuell). Serum EPO was measured using the mouse spleen cell method [5]. For the preparation of spleen cells, a female B6C3F1 (C57BL/6×C3H/He, F1 hybrid) mouse, more than 6 weeks old was used. Freshly prepared phenylhydrazine hydrochloride (60 mg/kg) in α-minimum essential medium (α-MEM, Flow Laboratories) at a concentration of 6 mg/ml was given intraperitoneally to the mouse on two consecutive days to produce hemolytic anemia. At the third day after the last injection, the mouse was euthanized and the spleen was removed. The spleen was teased into α-MEM and clumps were disrupted gently by several passages through a 21-gauge needle. The cell suspensions were then filtered through gauze-mesh, counted and suspended in the α-MEM containing 40% fetal calf serum (Flow Labs) and 0.2 mM 2-mercaptoethanol at a concentration of 8×10⁶ cells/ml. Fifty microliters of the spleen cell suspension was aliquoted into each well of flat-bottomed, 96-well plates (Coster), and 50 μl of test sample or standard EPO in α-MEM containing 0.1% bovine serum albumin was added to give final volumes of 100 μl/well. Each test sample contained 5 or 10 μl of sample serum. Recombinant human EPO (>160,000 units/A₂₈₀; AMGen) in the range from 0.2 to 25 mU/well was used as the standard. The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂ in air. After 19 hours, 1 μCi of [6-³H]-thymidine (15.7 mCi/mmol, New England Nuclear) with a concentration of 100 μCi/ml in α-MEM was added to each well. After an additional incubation for 2 hours, the cells were harvested onto the glass fiber filters and the radioactivity was measured by a liquid scintillation counter.

Figure 1 shows the serum EPO level in normal dogs. In 25 normal adult dogs, the concentration of serum EPO ranged from 38.5 to 135.0 mU/ml, with a mean of 88.2±30.7 (S.D.). There was no significant difference between the serum EPO level in male and female subjects. However, the mean level of serum EPO in 4 old dogs aged 8 to 13 years was significantly lower (56.5±11.6 mU/ml) than that in 21 dogs aged 1 to 7 years (94.9±28.7 mU/ml) (p<0.05). Conversely, 9 puppies (1–2 months) showed a significantly higher level (182.7±56.2 mU/ml) than that in adult dogs aged 1 to 7 years (p<0.01). The serum EPO levels in other animals are also determined. The mean level of the serum EPO was as follows; in horse, 55.2±8.9 mU/ml; in cow, 41.7±10.3 mU/ml; in cat, 39.4±5.4 mU/ml. These EPO levels were significantly different from that in normal adult dogs (p<0.05). However, in some sample samples from cow and cat, and all samples from goat, EPO could not be detected (< 30 mU/ml) (These data were not included for statistic analysis). Thus, it was shown that dogs
most sensitive, because more than 90% of the spleen cells consist of erythroid cells which are highly sensitive to EPO and not responsive to other growth factors [6]. The serum EPO level in puppies was significantly higher than that in adult dogs, which might be related to their increasing red cell mass during the growth period as seen in mouse [7] and rat [3]. The low EPO level seen in old dogs indicates that the production of EPO in vivo may decrease in proportion to age. In other animals, the normal EPO level was reported to be 0.01±0.0 U/ml in cat by fetal mouse liver cell assay [4] and 36.4±6.7 mU/ml in sheep by a radioimmunoassay [2]. However, normal EPO levels in the other animals examined, horse, cattle and goat, have not been reported. In the present study, serum EPO levels in some animals, horse, cattle, cat and goat, were considerably lower than that in dogs. However, the present study was unable to determine whether this high EPO level seen in dogs was due to the presence of more EPO molecules in their serum than in the other animals'. However, it is possible to consider that some factors presented in sera of normal dogs might stimulate erythroid cell proliferation in vitro.

REFERENCES


要約

正常イヌの血清エリスロポエチン（短報）：池田富夫・稲葉 睦・前出吉光（北海道大学獣医学部家畜内科学講座）——臨床的に健康なイヌの血清エリスロポエチン（EPO）をマウス腫細胞法により測定した。1-7歳のイヌ21頭では94.9±28.7 mU/ml（平均±標準偏差）であり、性差は認められなかった。一方1-2カ月齢のイヌは182.7±56.2 mU/mlと高値を、8-13歳では56.5±11.6 mU/mlと低値を示した。同様に測定したウマ、ウシ、ネコ及びヤギの血清EPOは、いずれもイヌに比べ低値を示した。