

Human and Animal Physiology

Permeability of Air-Blood Lung Barrier for Surfactant Protein D (SP-D) in Healthy Dogs and those Suffering from Dirofilariasis

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ABSTRACT. The goal of this study was to evaluate the permeability of the air-blood lung barrier for the SP-D protein in healthy dogs and those suffering from dirofilariasis. We have found that in 31.1% of dogs suffering from dirofilariasis there are air-blood barrier disorders for the surfactant protein D of lungs. Also, we have found that in case of invasion and chronic stage of dirofilariasis, protein concentration in the blood plasma of dogs up to 2 years old far exceeds similar indicators of healthy dogs, and after 2 years of age, the level of protein is considerably reduced compared with a population of healthy dogs.
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Key words: *air-blood barrier, protein D, dirofilariasis, lungs, dogs, surfactant.*

Positive trends regarding the spread of dirofilariasis have been observed throughout the Russian territory, especially in its southern regions, causing an urgent need to study the disease comprehensively. The recent years have revealed an increased number of cases of dirofilariasis in the Russian Federation. Currently, cases of dirofilariasis have been observed not only in the residences of endemic areas in the southern territories of the Russian Federation (Volgograd, Astrakhan, Rostov region), but also in the residences of other regions not endemic for the disease [1-3]. The greatest risk for animals

contracting this disease is the possibility of multiple organ failure.

It is known that dirofilaria is mainly found in the pulmonary circulation of adult animals. The pulmonary artery and the right ventricle are the structures of particular interest [4]. Thus, it is necessary to assume that the circulatory system is not the only system that can be affected by the disease [5], but also the lung surfactant system [6]. According to the existing reports, type 2 alveolocytes and Clara cells are the cells responsible for synthesis and secretion of surfactant. Newly synthesized surfactant is removed

by alveolar macrophages from the respiratory tract by phagocytosis, or it is captured by type 2 alveolocytes for its subsequent re-synthesis [7, 8]. Under ideal conditions degradation products from the normal functioning of the air-blood lung barrier (ABLB) don't appear in the general blood circulation. Their detection may indicate an increased permeability of the ABLB for a particular component of surfactant.

Currently a detailed study of surfactant protein fraction is being done. There are four surfactant-associated proteins: SP-A, SP-B, SP-C, SP-D, two of which (SP-B and SP-C) have hydrophobic properties, and facilitate the distribution of phospholipids in the form of a monomolecular layer at the alveolar level [7, 8]. It was established that SP-A and SP-D proteins enhance phagocytosis of bacteria and viruses by alveolar macrophages [7], while the exchange of oxygen radicals regulates the production of cytokines and immunoglobulin by alveolar macrophages and type II pneumocytes [8-10]. However, the role of these proteins in dirofilariasis has not been studied. In this regard, the aim of our study was to evaluate the permeability of the air-blood lung barrier for SP-D protein in healthy dogs and those suffering from dirofilariasis.

Materials and Methods

Our sample included 82 German Shepherd dogs from special breeding kennels belonging to the security forces from the cities of Rostov-on-Don and Krasnodar, (41 males and 41 females), 37 of which were healthy, and 45 were infected with dirofilariasis. The dogs ranged from 1.5 to 12 years in age. A clinical examination of the healthy and infected canines was carried out using standard procedures, followed by entering results in corresponding reports and medical charts. Under fasting conditions, blood was drawn from each dog from the cephalic vein of the forearm and the lateral saphenous vein. Then, the presence of microfilaria was investigated using the modified Knot test. To detect specific *Dirofilaria immitis* antigens, the blood was studied using the

immunochromatographic assay (Canine SNAP 4Dx, IDEXX). Also, general and biochemical blood tests were performed. The SP-D concentration was determined by an immunoenzyme stain method using canine test kits from the Chinese company Cusabio (PCR) (ELISA Kit). The protein concentration is expressed in ng/ml. The statistical processing was conducted by variation analysis, Fisher's exact test, and the Chi-square analysis using the standard computer program Biostat. The differences were considered valid at $p < 0.05$.

Results

Detectability analysis of the SP-D in male dogs showed positive readings in 13.5% of the entire healthy population. In the total population of healthy male dogs this figure rose to 31.25%.

Fluctuations in the concentration of the SP-D were significant, ranging from 0.72 ng/ml to 200 ng/ml.

An analysis of the results from testing the female canines demonstrated that 24.3% of the entire pool of healthy dogs was SP-D positive. This figure rose to 42.8% in the population of healthy females. In quantitative terms, the fluctuations in protein concentrations were close to the results seen in males and ranged from 0.05ng/ml to 200ng/ml. A comparison of the incidence rate of the SP-D for males and females using the chi-square analysis revealed no significant differences. The investigation of the same results in the dogs with dirofilariasis was of great interest.

The incidence rate of positive dogs in the pool of infected dogs was 17.8% for the male dogs, which is slightly higher than the incidence observed in healthy specimens, comprising 32.0% of the infected male dogs. It is close to the percentage of incidence in the healthy population of males (31.2%). In numerical terms, fluctuations of the protein concentration ranged from 11.33ng/ml to 90.87ng/ml.

A comparison of the serum SP-D detection frequency between healthy and infected males did not reveal significant differences ($p < 0.07$).

The detection of the protein in infected female dogs was 13.3% of the entire population of infected

dogs, and 30.0% of the total population of infected female dogs. This result on average was slightly higher than the rate for the pool of healthy dogs (24.3%). However, significant differences were not observed ($p < 0.5$). Fluctuations in the serum levels of SP-D of infected female dogs ranged from 0.02ng/ml to 200.0ng/ml. Differences in the incidence rate of the serum protein of infected females and infected males were not significant ($p > 0.8$).

Thus, it is established that in the healthy dogs the SP-D was detected in 37.8% of cases at different concentrations (11.33ng/ml to 200.0ng/ml). Moreover, the incidence rate was similar between the male and female test groups.

In infected dogs the ratio did not significantly change, and in 31.1% of the cases various concentrations of the protein were observed (from 0.02ng/ml to 200.0ng/ml). Likewise, in the sample of healthy dogs, differences between males and females in the incidence rate of the protein were not observed.

Discussion

The analysis of the SP-D detection frequency in the various age groups indicated that, in the dogs between the ages of 1-2, the incidence rate of the marker in the blood plasma did not differ significantly across the pool of infected dogs.

Regardless of the evident tendency to a direct correlation between the diagnosed *Dirofilariasis* and positive test results (in sick dogs protein has been detected in 3 of 8 animals), significant differences in the detection frequency have not been established (in 2 out of 15, $p < 0.81$).

A similar pattern was observed in the 2 to 8 year-old age group. In healthy dogs positive readings were determined for 10 of 20 cases, where in the sick population of dogs the positive readings were found only in 8 out of 35. Analyzing these results it was established that the differences were also insignificant ($p < 0.11$).

The 8 to 12 year-old group consisted of two subgroups: infected and healthy canines, 2 in each subgroup. In each of these subgroups the marker for the

Table 1. Comparative analysis of the serum SP-D concentration in healthy dogs and dogs with *dirofilariasis*

Age groups, Years	Subgroups of dogs	
	Healthy	Infected
	Concentrations, ng/ml	Concentrations, ng/ml
Complete group	26.89±9.33 (n=37)	11.61±5.32 (n=45) $p < 0,02$
Less than 2-years	0.01±0.008 (n=15)	16.16±10.37 (n=8) $p < 0.05$
>2-8 years	34.66±14.35 (n=35)	3.89±2.66 (n=20) $p < 0,01$
>8-12 years	67.5 (n=2)	36.63 (n=2)

Note: Accuracy is calculated in relation to the results for healthy dogs.

SP-D protein was detected in 1 of the dogs.

Thus, the biochemical marker for the surfactant system -protein-D (SP-D) was detected in blood plasma of 37.8% of the healthy dogs, and of 31.1% of dogs infected with *Dirofilaria*.

The presence of the marker in the blood plasma is independent of both the sex and the age of the animal. The investigation of the absolute values of the concentration of protein in the blood plasma was of specific interest. The results of the research are presented in Table 1.

In *healthy* dogs (Table 1) the average concentration of SP-D in blood amounted to 26.89±9.33 ng/ml. However, in some of the dogs the concentration of protein was lower than test-sensitivity (in 23 of 37) and, hence, the concentration was defined as zero. In the remaining cases the fluctuations ranged from 0.12ng/ml to 200.0ng/ml. This explains the considerably significant standard deviation values.

In the group of 2-year-old dogs the marker was detected only in isolated cases (in three of the dogs) and the concentration was within the range of 0.05-0.12ng/ml. In the remaining cases the concentration

of protein was lower than in the exact test.

In the group aged 2-8 years, the concentration was 34.66 ± 14.35 ng/ml with significant fluctuations ranging from 0.72 ng/ml to 200.0 ng/ml. In 10 out of 20 dogs the protein was not detected in the blood plasma.

In the 8-12 year-old group, the marker was detected in only 1 dog in significant concentrations (135.9 ng/ml).

Across the pool of infected dogs the SP-D value was determined to be in the range of 0.02 ng/ml to 90.87 ng/ml. The statistical mean for this group had a high degree of accuracy ($p < 0.02$) and it turned out to be lower than the mean values found for the healthy dogs.

It is worth noting that in the group of dirofilariasis infected dogs under 2 years of age the SP-D values were found to be significantly higher ($p < 0.05$) than in the pool of healthy dogs of the same age, where the fluctuations of concentration ranged from 8.37 ng/ml - 79.59 ng/ml.

In the group aged 2-8 years, the serum SP-D was detected in 8 out of 35 of infected dogs. Ranges of concentration variation were found between 0.02 ng/ml and 90.87 ng/ml. In comparison with the results found in healthy dogs of the same age the statistical mean was significantly lower ($p < 0.01$).

In the group of infected dogs aged 8-12 years, as in the group of healthy dogs, in 1 canine a high SP-D value (73.27 ng/ml) was detected, where in the other dog the marker was not found in the blood. The correlation analysis between the age and the concentration of the marker in healthy dogs revealed a significant direct correlation ($r = 0.60$; $p < 0.046$). An analogous link, but with lower protein concentration values, was found in the infected dogs ($r = 0.74$; $p < 0.008$).

Thus, changes in the level of surfactant protein-

D in the blood plasma in the development and progression of dirofilariasis can be regarded as a natural phenomenon. The presence of significant concentrations of the protein in the blood plasma of conditionally healthy dogs aged over 2 years is an ontogenetic feature of the reaction by the surfactant system. This "rupture" in the aero-hematic barrier could be the result of previous respiratory infections that have migrated. Current research permits us to draw the solid conclusion that the SP-D might play a role in the endogenic factor of reprogramming markers and, in the process, in determining the nature of the immune response and its expression of the inflammatory lung diseases.

Conclusions

1. In healthy dogs in 37.8% of the cases there is a rupture of the AHB in relation to the SP-D, where the ratio of positive tests is not statistically significant between males and females. In dogs with dirofilariasis, in 31.1% of the cases there was a rupture of the AHB in relation to the SP-D. This rupture is unrelated to the gender of the dogs.
2. There is a good resistance to the protein-D in healthy dogs under the age of 2. However, a significant weakening of the protein in the dogs with dirofilariasis of the same age is an ontogenetic reaction of the aero-hematic barrier. In the groups of dogs over 2 years of age, the resistance of the AHB is diminished both in the case of healthy and infected dogs.
3. Since the onset and, further on, in the circumstances of chronic development of dirofilariasis, the concentration of the protein in the blood plasma of dogs up to 2 years in age significantly exceeds the analogous values of healthy dogs, and in dogs over 2 years in age the protein is significantly lower in comparison with the population of healthy dogs.

ადამიანისა და ცხოველთა ფიზიოლოგია

ჯანმრთელ და დიროფილარიოზიან ძაღლებში ფილტვის ალვეოლარულ-კაპილარული ბარიერის გამავლობა სურფაქტანტ D (SP-D) ცილისთვის

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სტატიის მიზანია ჯანმრთელ და დიროფილარიოზიან ძაღლებში სურფაქტანტ SP-D ცილისთვის ალვეოლარულ-კაპილარული ბარიერის გამავლობის შეფასება. როგორც აღმოჩნდა, დიროფილარიოზიანი ძაღლების 31,1%-ში აღინიშნება ალვეოლარულ-კაპილარული ბარიერის დარღვევა ფილტვების სურფაქტანტ D ცილისთვის. აგრეთვე, ჩვენ დავადგინეთ, რომ დიროფილარიოზის დასაწყისსა (ინფაზიის) და ქრონიკული სტადიის შემთხვევაში 2 წლამდე ასაკის ძაღლების სისხლის პლაზმაში ცილის შემცველობა ბევრად აჭარბებს ჯანმრთელ ძაღლებში არსებულ მსგავს მაჩვენებლებს და 2 წლის ასაკის ზემოთ ცილის დონე მნიშვნელოვნად მცირდება ჯანმრთელ ძაღლებთან შედარებით.

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