Trakia Journal of Sciences, Vol. 7, No. 4, pp 63-68, 2009

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Original Contribution

COMPARATIVE STUDY ON PHAGOCYTIC ACTIVITY OF NEUTROPHILS AFTER EXPERIMENTAL SUBCUTANEOUS *STAPHYLOCOCCUS INTERMEDIUS* INFECTION IN OBESE AND NONOBESE DOGS

E. Slavov*, P. Dzhelebov, M. Andonova, D. Girginov

Faculty of Veterinary Medicine, Trakia University, Stara Zagora 6000, Bulgaria

ABSTRACT

The aim of the present study is to investigate the effects of obesity in dogs on one of the main elements of innate immunity – phagocytosis. The experiment was conducted on 20 healthy, male, mongrel dogs at the age of 4 to 7 years. Dogs were divided into three groups – two experimental (I and II) and one control group. Dogs of group I (obese dogs) were submitted to a high – fat hypercaloric diet for three months in order to cause experimental obesity. Dogs of group II (non-obese dogs) were fed a standard maintenance diet and had normal body condition. Infection was induced in dogs of group I and group II by subcutaneous application of bacterial suspension of *Staphylococcus intermedius*. Percentage of phagocyting neutrophils and phagocytic index were estimated in the following dynamics: initial levels (before infection-0 hour), 3rd, 24th, 48th hour, and 7th and 14th day after infection. In non-obese dogs both percentage of phagocyting neutrophils and phagocytic index increased on 48th hour after infection. In obese dogs both parameters did not show any statistically significant changes within the whole period of experiment.

Key words: dog, obesity, *Staphylococcus intermedius*, infection, phgocytosis

INTRODUCTION

Staphylococcus intermedius was first described in 1976 [1] and was found in various animal species – horses, cats, pigeons and others, but studies show it is most pathogenic for the dog [2]. In this animal species it causes different pyogenic infections and most often pyoderma. About 80% of the dogs with skin allergic reactions develop a secondary bacterial infection, in almost 100% of the cases caused by *Staphylococcus intermedius* [3]. Although it is a part of the resident microflora, in some conditions *Staphylococcus intermedius* can reproduce very quickly, colonizing extensive parts of the skin and becoming pathogenic. Its pathogenic features are due to certain structures of bacterial cell wall and also some secretory proteins, acting as exotoxins. These components provoke activation of defense mechanisms of organism and mostly activation of elements of innate immunity, most important of which is phagocytosis. Exotoxins, parts of which are super antigens, activate not only T – lymphocytes but also B – lymphocytes, increase secretion of proinflammatory cytokines and induce acute – phase response and influence phagocytosis [4, 5, 6, 7].

In the recent years, in industrially developed countries, obesity has become a problem of epidemic importance not only for humans but also for their pets. Thorough investigations of white adipose tissue show that it is not only a depot of tryacylglycerols, but it is also an important endocrine organ secreting a large number of biologically active substances, which are called adipokines – leptin, adiponektin, resistin [8, 9]. They play role not only in metabolism, but also influence defense mechanisms of organism. Adipose tissue in obese individuals is a source of various proinflammatory cytokines and factors – TNF-

* **Correspondence to**: *Evgeny Slavov, Faculty of Veterinary Medicine, Trakia University, Stara Zagora 6000, Bulgaria, e-mail: pathoz@abv.bg*

-α, IL-1, IL-6, iNOS /NOS2/, TGF-β1, soluble ICAM, IL-8, IL-18 and others [10]. Part of these molecules (TNF-α, IL-1, IL-6) induce production of acute – phase proteins by hepatocytes. Increased levels of cytokines and acute – phase proteins lead to activation of inflammatory signal pathways, and are associated with low – grade, but chronic systemic inflammation [11]. Data concerning effects of obesity related production of proinflammatory factors on innate defense mechanism and especially phagocytosis in humans and animal species are scarce and to some extent controversial [12, 13, 14].

The aim of the present study is to evaluate comparatively and establish features and differences in phagocytic activity of neutrophils in dogs with normal body condition and dogs with obesity (induced by high-fat hypercaloric diet), which are experimentally infected with *Staphylococcus intermedius*.

MATERIAL AND METHODS

Experimental animals

Experimental animals

We used 20 male, healthy, mongrel dogs at the age of 4 to 7 years. Period of adaptation continued one month. The dogs were treated against parasites with Prazimec – D (Biovet, Peshtera, Bulgaria) at a dose of 1 tablet/10 kg. Also, they were treated against ectoparasites with antiparasitic shampoo, Ectomin and Tapilan (Dorvet, Israel). Animals were kept in individual cages situated indoors, providing constant room temperature. Dogs went for walks twice a day; half an hour in the morning and another walk in the evening. Members of the team conducting the experiment took care of the dogs in order to avoid stress.

Dogs were divided into three groups:

Group I (obese dogs) – animals of this group (n=6) had initial body weight 12.86 ± 1.43 kg. They were submitted to a high-fat overfeeding diet (standard maintenance diet plus lard supplement-10g/kg body weight) for a period of three months, in order to induce experimental obesity.

Group II (non-obese dogs) – animals of this group (n=8) had initial body weight of 13.65 ± 3.31 kg. They were fed only a standard maintenance diet ("Jumbo Dog", Gallisman S. A., Bulgaria; contents: protein-17%, fats-8%, fiber-4%, vitamin D_3-3000 IU/kg, vitamin E-200 mg/kg, vitamin A-11000 IU/kg, Zn-35 mg/kg, Na-0.4%, Mg-50 mg/kg, Ca-0.95-1.3%, Cl-0.95%, Cu-9 mg/kg, humidity-9%).

Experimental staphylococcal infection was induced in animals of group I and group II.

Control group – it consisted of animals (n=6), which were fed the same standard maintenance diet and were submitted to no other treatment. Initial body weight was 12.43 ± 1.4 kg.

Experimental infection

Local *Staphylococcus intermedius* infection was induced by subcutaneous application, in the lumbal region, of 5 ml bacterial suspension ($1x10^9$ CFE/ml). Suspension was prepared from bacterial culture after 24-hour growth. A terrain strain isolated from a dog with a clinical infection, was used. Microbiological identification was done by BD BBL Crystal Gram Positive ID System. Strain was determined as catalase-positive, oxidase-negative, producing plasmocoagulase and desoxiribonuclease.

Sample collection

Blood samples were collected in sterile glass tubes by punction of vena cephalica in the following dynamics- right before infection (0 hour), and on 3rd, 24th, 48th hour, 7th and 14th day after infection. As anticoagulant we used for each sample 0,2 ml heparin (50 units/ml).

Method for evaluating phagocytic activiy of neutrohpils

Phagocytic activity was evaluated by measuring the following parameters:

- Percentage of phagocyting neutrophils in whole blood samples – defined by the immunofluorescent method of Samnaliev [15]. On the smear 150 neurophils are counted. Then the parameter is defined by the formula:

% phagocyting cells = (count of phagocyting cells) / 150 x 100

- Phagocytic index – shows the mean number of engulfed bacteria by a phagocyting cell.

Phagocytic index = total count of engulfed bacteria / 150

Statistical analysis

Results are presented as means±SD and submitted to standard F- and t-tests (StatMost, version 2.5), provided by DataMost corporation. Differences were considered statistically significant at the $p<0.05$ level.

RESULTS

Body weight

Body weight

In group I body weight increased significantly after 90 days of high-fat overfeeding diet (16.54±1.67 kg, $p<0.01$), as compared to initial levels before diet (12.86±1.43 kg).

In group II body weight at the end of the 90 days period (13.83±2.3 kg) showed no statistically significant differences, as compared to initial levels (13.65±3.31 kg).

Body weight of control animals also had no statistically significant changes at the end of the 90 days period (12.98±1.7 kg), as compared to initial values (12.43±1.4 kg).

Comparison of body weight between groups after the 90 days period showed statistically significant difference – it was higher in group I, as compared to control group ($p<0.01$).

Percentage of phagocyting neutrophils

Percentage of phagocyting neutrophils in group I had no statistically significant changes within the whole period of experiment, as compared to initial levels. On the contrary, in group II percentage of phagocyting neutrophils rose on 48th hour after infection (18.7±5.7%, $p<0.05$), as compared to initial level (12.23±5%), (Table 1). On 7th day after infection percentage of phagocyting neutrophils decreased to its minimal value (8.9±2.3%), which is significantly lower, as compared to the values on 3rd hour (16±6%, $p<0.05$), 24th hour (19.6±7.1%, $p<0.01$) and 48th hour ($p<0.01$).

Comparison between groups in dynamics reveals significantly higher values in control group on 3rd hour, vs. group I (group I – 11.8±6.4%, controls – 26.2±8.2%, $p<0.01$), and vs. group II ($p<0.05$). Percentage of phagocyting neutrophils in group I was significantly higher vs. group II on 7th day after infection (group I – 14.6±5%, $p<0.05$).

Phagocytic index

In obese dogs (group I) phagocytic index showed no statistically significant differences, as compared to its initial values.

In group II phagocytic index rose on 48th hour after infection (0.5±0.06, $p<0.05$), as compared to its initial value (0.34±0.15), (Table 1). On 7th day phagocytic index decreased to its minimal value (0.21±0.06), which is significantly lower, as compared to the values on 3rd hour (0.55±0.15, $p<0.01$), 24th hour (0.4±0.12, $p<0.01$) and 48th hour ($p<0.001$).

Comparison between groups in dynamics reveals significantly higher values of phagocytic index in group II vs. group I on 3rd hour (group I – 0.28±0.14, $p<0.05$). Phagocytic index in group II was also higher as compared to controls on 48th hour (controls – 0.36±0.13, $p<0.05$).

DISCUSSION

Phagocytosis, as a response to infections, is the main component of innate defense mechanisms. Neutrophils are the most active phagocyting cells from all leucocytes, and adequate production of oxide radicals (their main microbicide factor) is crucial for their function [16]. Opsonisation of bacteria is an important stage of phgocytosis. Shearer [17] in his in vitro experiments discovers that opsonins of canine serum,obtained from dogs with pyoderma, stimulate phagocytosis of *Staphylococcus intermedius* by neutrophils, but capability for intracellular killing does not change significantly. Andonova et al. [18] found that after experimental subcutaneous infection with *Staphylococcus aureus* percentage of phgocyting neutrophils increases on 24th hour after infecting, which is accompanied by an increase of phagocyting index. Chaprazov et al. [19] found increase in percentage of phagocyting neutrophils on 24th hour, and increased production of oxide radicals on 24th and 48th hour after intravenous application of suspension of *Staphylococcus aureus*.

Analysis of data, from the experiment conducted, showed that on 3rd hour after infection in dogs of both experimental groups percentage of phgocyting neutrophils is decreased and later on 48th hour it increases in group II, which is accompanied by an increase of phagocytic index at the same time. In dogs of group I there is no increase in the parameters. All these data indicate lower

phagocytic activity of neutrophils in the early stages of activation of innate defense mechanisms in obese dogs.

Results of our experiment are similar to those of studies of Debczinski [12] in humans and Gottschlich [13] in obese dogs.

One of the possible reasons for decreased phagocytic activity of neutrophils, together with cytokine disbalance, is impaired glucose tolerance and insulin resistance found in the obese dogs (unpublished data), which have been described to cause insufficient energy delivery to phagocyting cells [20, 21, 22, 23].

Table 1. *Changes in percentage of phagocyting neutrophils (%) and phagocytic index in control group (n=6), experimental group I (n=6, obese dogs submitted to infection) and experimental group II (n=8, non-obese dogs submitted to infection). Results are expressed as mean±SD.*

Dynamics / Parameter	0 hour			3 hour			24 hour			48 hour			7 day			14 day		
	Control group	Group I	Group II	Control group	Group I	Group II	Control group	Group I	Group II	Control group	Group I	Group II	Control group	Group I	Group II	Control group	Group I	Group II
Percentage of phagocyting cells (%)	19.05 ± 7.6	14.2 ± 4.2	12.23 ± 5	26.2 ± 8.2	11.8 ± 6.4	**d*** 16 ± 6	18.32 ± 3.9	15.6 ± 7.9	19.6 ± 7.1	23.37 ± 12.5	14.2 ± 6.8	**c** 18.7 ± 5.7	18.8 ± 9.9	14.6 ± 5	**d*** 8.9 ± 2.3	22.6 ± 11.6	12.5 ± 4.9	15.3 ± 7.7
Phagocytic index	0.35 ± 0.1	0.4 ± 0.12	0.34 ± 0.15	0.36 ± 0.17	**d*** 0.27 ± 0.13	0.55 ± 0.15	0.39 ± 0.13	0.4 ± 0.23	0.44 ± 0.12	0.36 ± 0.12	0.46 ± 0.2	**c a** 0.5 ± 0.06	0.29 ± 0.08	0.45 ± 0.24	0.21 ± 0.06	0.32 ± 0.15	0.4 ± 0.25	0.41 ± 0.2

Statistically significant differences in group I and group II vs. control group: **a** - p<0.05, **a*** - p<0.01, **a**** - p<0.001
Statistically significant differences within group I vs. 0 hour: **b** - p<0.05, **b*** - p<0.01, **b**** - p<0.001
Statistically significant differences within group II vs. 0 hour: **c** - p<0.05, **c*** - p<0.01, **c**** - p<0.001
Statistically significant differences between group I and group II: **d** - p<0.05, **d*** - p<0.01, **d**** - p<0.001

CONCLUSION

Analyzing the results we can conclude, that in obese dogs with experimental subcutaneous *Staphylococcus intermedius* infection, reaction of neutrophils is inadequate in the early stages of activation of innate mechanisms of defense. Thus innate immunity is impaired and can not ensure enough time and provide proper conditions for timely and effective triggering of adaptive immunity.

REFERENCES

1. Hajek, V. 1976. *Staphylococcus intermedius*, a New Species Isolated from Animals., International Journal of Systematic Bacteriology, 26(4), 401-408.
2. Futagawa-Saito, K., Sugiyama, T., Karube, S., Sakurai, N., Ba-Thein, W. and Fukuyasu, T. 2004. Prevalence and characterization of leukotoxin-producing *Staphylococcus intermedius* isolates from dogs and pigeons. J. Clin. Microbiol., 42, 5324–5326.
3. Greene R. T., Lämmler C. 1993. Staphylococcus intermedius: current knowledge on a pathogen of veterinary importance. Zentralbl. Veterinarmed. B., 40(3):206–214.
4. Cleveland, M., Gorham, J., Murphy, T., Tuomanen, E., and Murphy, K. 1996. Lipoteichoic acid preparations of gram-positive bacteria induce interleukin-12 through a CD14-dependent pathway. Infect. Immun., 64, 1906–1912.
5. De Kimpe, S., Kengatharan, M., Thiemermann, C., and Vane, J. 1995. The cell wall components peptidoglycan and lipoteichoic acid from *Staphylococcus aureus* act in synergy to cause shock and multiple organ failure. Proc. Natl. Acad. Sci. USA, 92, 10359–10363.
6. von Aulock, S., Schroder, N., Traub, S., Gueinzius, K., Lorenz, E., Hartung, T., Schumann R, and Hermann C. 2004. Heterozygous Toll-like receptor 2 polymorphism does not affect lipoteichoic acid-induced chemokine and inflammatory responses. Infect. Immun., 72, 1828–1831.
7. Zouali, M. 1995. B-cell superantigens: implications for selection of the human antibody repertoire., Immunol. Today, 16, 399–405.
8. Combs, T., Berg A., Obici, S., Sherer, P., Rosetti, L. 2001. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30., J. Clin. Invest., 108, 1875-81.
9. Steppan C.M. 2002. The hormone resistin links obesity to diabetes. Nature., 409, 307-312.
10. Weyer, C. 2002. Humoral markers of inflammation and edothelial dysfunction in relation to adiposity and in vivo insulin action in Pima Indians. Atherosclerosis, 161, 233-142.
11. Xu, H, Barnes, GT, Yang, Q., Tan, G., Yang, D., Chou, C., Sole, J., Nichols, A., Ross J., Tartaglia, L., Chen, H. 2003. Chronic Inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. Journal of Clinical Investigation, 112(12),1821-30.
12. Debczynski, W., Pietruska Z., Kuzma A., 1996. Examination of human neutrophil activation in hyperlipidemia. Pol. Tyg. Lek., 51(19-22), 269-271.
13. Gottschlich, M. M., Mayes T., Khoury J. C., Warden G. C., 1993. Significance of obesity on nutritional, immunologic, hormonal, and clinical outcome parameters in burns. J. Am. Diet. Assoc., 93(11), 1261-1268.
14. Nieman, D., Henson, D., Nehlsen-Cannarella, S., Ekkens, M., Utter, A., Butterworth, D., Fagoaga, O. 1999. Influence of obesity on immune function. J. Am. Diet. Assoc., 99, 294 – 299.
15. Samnaliev M., Mladenow K., Draskova T., Radinov A. 1995. Development and clinical assessment of some nonspecific factors of immunity. Processing First National

Congress of immunity., PV31, 135-137.
16. Sawyer, D.W., Donowitz G. R., Mandell G.L. 1989. Polymorphonuclear neutrophils: An effective antimicrobial force. Rev. Infect. Dis., 11, 1532-1544.
17. Shearer D.H., Day M.J. 1997. Aspects of the humoral immune response to Staphylococcus intermedius in dogs with superficial pyoderma, deep pyoderma and anal furunculosis. Vet. Immunol. Immunopathol. 58(2):107-20.
18. Andonova M., Borissov I., Dimitrova D. 2002. Experimental staphylococcal infection in dogs and its effects upon the functional activity of phagocytes. First International Conference "Bacterial, Viral and Parasitic infections in human and veterinary medicine", Varna, 2002.
19. Chaprazov Ch., Borissov I., Slavov E., Dzhelebov P. 2007. Phagocytosis in *Staphilococcus aureus* bacteriaemia in dogs. Vet. Glaznik, vol., 61, 1-2, 43-52.
20. Chandra R. K. 1981. Immune response in overnutrition. Cancer Research, vol. 41, pp. 3795–3796.
21. Hofmann, C., Lorenz, K., Braithwaite, S., Colca, J., Palazuc, B., Hotamisligil, G., Spiegelman, B. 1994. Altered gene expression for tumor necrosis factor and its receptors during drug and dietari modulation of insulin resistance. Endocrinology, 134, 264-270.
22. Marhoffer, M. Stein, E. Maeser, and K. Federlin. Impairment of polymorphonuclear leukocyte function and metabolic control of diabetes. Diabetes Care, vol. 15, no. 2, pp. 256–260, 1992.
23. Schmidt M. I. and Duncan B. B. Diabesity: an inflammatory metabolic condition. Clinical Chemistryand Laboratory Medicine, vol. 41, no. 9, pp. 1120–1130, 2003.