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Original article

INFLUENCE OF EXHAUSTIVE EXERCISE ON OSMOTIC RESISTANCE OF ERYTHROCYTES AND SOME CLINICAL PARAMETERS IN DOGS

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Summary

Gundasheva, D., M. Andonova, P. Dzhelebov & E. Slavov, 2014. Influence of exhaustive exercise on osmotic resistance of erythrocytes and some clinical parameters in dogs. *Bulg. J. Vet. Med.,* **17**, No 2, 96–103.

The aim of the experiment was to study the *in vivo* effect of exhaustive aerobic exercise on osmotic resistance of erythrocytes, neutrophil/lymphocyte ratio and some clinical parameters in dogs. We used 12 male, mongrel dogs divided into two groups – animals from experimental group were submitted to exhaustive exercise; animals from control group did no exercise. Minimum osmotic resistance, 5 % haemolysis, 50 % haemolysis, 90 % haemolysis and maximum osmotic resistance were measured using an osmotic fragility test in the following dynamics: before exercise (BE), right after exercise (0 h), on 2nd hour, 4th hour, 24th hour, 48th hour, 72nd hour and on 7th and 14th day after exercise. Neutrophil to lymphocyte ratio (N/L), body temperature (BT), heart rate (HR) and breathing rate (BR) were measured in the same dynamics. We found a decrease of red blood cell osmotic resistance in experimental dogs (measured by 50 % haemolysis) on 24th hour, 48th hour and 72nd hour and on 7th and 14th day after exercise, as compared to control group ($P<0.05$). Surprisingly in the experimental group maximum osmotic resistance (100 % haemolysis) increased on 24th hour after exercise, compared both to initial level and control group ($P<0.05$). N/L ratio increased significantly in the experimental group on 2nd hour compared to BE level ($P<0.05$). In conclusion, exhaustive exercise acts as a stressor and affects adversely the fragility of red blood cells.

Key words: dog, exhaustive exercise, osmotic haemolysis

INTRODUCTION

Exercise has been in the focus of scientific studies for many years. Various experiments involving humans and different animal species have been conducted and many aspects of exercise have been studied. Endurance horse racing and sled dog racing have been popular for many centuries in some parts of the world. Recently endurance exercise, like marathon running, triathlons and ultramarathons, are becoming very popular among humans. This popularity of endurance sports is

Influence of exhaustive aerobic exercise on some cytokines and serum iron parameters in canine experimental model

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- Abstract
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Abstract

The aim of the experiment was to study the effect of exhaustive exercise on some cytokines and iron status parameters. We used 12 male, mongrel dogs divided into two groups – animals from experimental group were submitted to exercise at moderate intensity with exhaustion as the end-point; animals from control group did no exercise. Serum levels of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), hepcidin prohormone, serum iron (SI), total iron binding capacity (TIBC) and transferrin saturation (TS) were measured before exercise (BE), right after exercise (0 hour) and on 2, 4, 24, 48 and 72 hours after exercise. SI, TIBC and TS were measured also on day 7 and 14 after exercise. Serum levels of TNF-α increased after the exhaustive exercise. Serum levels of IL-6 demonstrated an increase at 0 hour, but increase was not statistically significant compared to BE level. Serum levels of hepcidin prohormone marked a slight increase 48 hours after the exercise, but change was insignificant. Levels of SI decreased on hour 72 ($P<0.01$) and on day 7 ($P<0.01$) and 14 ($P<0.05$) after the exercise, as compared to BE level. Similar were changes in TS. TIBC decreased on 4, 24 and 72 hours ($P<0.05$) after exercise, but only compared to control group. In conclusion, exhaustive exercise causes inflammatory response and a significant decrease in SI levels.

Keywords: exhaustive exercise, dog, iron parameters, IL-6, TNF-α

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Original article

CHANGES IN SERUM CORTISOL AND SOME INNATE IMMUNITY PARAMETERS AFTER EXHAUSTIVE EXERCISE IN MALE DOGS

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Summary

Dzhelebov, P. V., D. I. Gundasheva, M. Y. Andonova & E. P. Slavov, 2018. Changes in serum cortisol and some innate immunity parameters after exhaustive exercise in male dogs. *Bulg. J. Vet. Med.* (online first).

The aim of the present study was to investigate the influence of exhaustive exercise on some innate immunity parameters and cortisol levels. Twelve male, mongrel dogs were divided into an experimental group, submitted to prolonged, strenuous exercise with exhaustion as the end-point, and a control group without any exposure to exercise. Serum cortisol levels were measured before exercise (BE), right after (0 h) and on 2nd hour and 4th hour after exercise. The neutrophil function (phagocytosis, phagocytic index, hydrogen peroxide production-H_2O_2) and classical pathway of complement activation (CPCA) were measured as followed: BE, right after exercise and on 2, 4, 24, 48, 72 h, and 7, 14 day after exercise. In experimental animals cortisol decreased on hour 4 after exercise ($P<0.05$), compared to BE level, and on hour 2, compared to controls ($P<0.05$). Percentage of H_2O_2 producing neutrophils in experimental animals dropped significantly on hour 4 and day 7 after exercise ($P<0.05$), compared to BE level and on hour 4 ($P<0.01$) vs control group. Percentage of phagocytising neutrophils decreased slightly on hour 48 ($P<0.05$), compared to BE level. Phagocytic index and CPCA had an insignificant increase after exercise. Inappropriate changes in cortisol levels could indicate inadequate adaptive response to exercise. Overtraining could make animals more susceptible to infection.

Key words: complement system, cortisol, dog, exercise, phagocytosis

INTRODUCTION

Recently exercise has become a field of intense scientific studies. Many of the studies concerning effects of exercise on endocrine and immune system are highly controversial. Results from such studies are often influenced by the animal species involved in the study, individual inherent factors (especially in humans), type of exercise and exercise protocol, nutrition, climate and other experimental conditions. Glucocorticoids are the most often investigated hormones during physical exercise. Some studies have found increase in cortisol levels after training in hunting

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Original article

HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS CHARACTERISING THE PROGRESSION OF EXPERIMENTAL *PSEUDOMONAS AERUGINOSA* SKIN INFECTION IN DOGS

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Summary

Andonova, M., V. Urumova, B. Petkova, E. Slavov, P. Dzhelebov, Ts. Chaprazov, R. Roydev & I. Borissov, 2014. Haematological and biochemical parameters characterising the progression of experimental *Pseudomonas aeruginosa* skin infection in dogs. *Bulg. J. Vet. Med.*, **17**, No 1, 32–41.

The aim of the study was to investigate the changes in some rapid, indicative clinical laboratory parameters – white blood cells (WBC), leukogram, erythrocyte sedimentation rate (ESR), total protein (TP), albumin (A), globulins (G), albumin/globulin ratio (A/G) during experimental *Pseudomonas aeruginosa* skin infection in dogs and to determine their ability to provide information for evaluating such type of infection. Suspension of *P.aeruginosa* (1×10^8 cfu/mL) was inoculated at a dose 0.3 mL/kg body weight, in five clinically healthy, dogs, 2–5 years old, weighing 24.3 ± 1.8 kg. The control group (n=5) was injected with the same dose of normal saline. The blood samples were taken in the following dynamics: before infection (0 h) and on 4th, 24th, 48th, 72nd hour and on 7th, 10th and 14th day after infection. The data presented clearly suggest that ESR was the most sensitive haematological parameter increasing significantly 4 hours after infection ($P<0.01$), with values remaining high ($P<0.001$) till the end of experimental period (day 14). WBC increased significantly on hour 72 ($P<0.001$). The changes in the leukogram demonstrated increase in band neutrophils on hour 48 and 72 ($P<0.01$), which is indicative for left shift. Eosinopenia was found on hour 24 and 72 ($P<0.01$). Analysis of biochemical parameters demonstrated that the period within 24th to 72nd hour was crucial in progress of *P. aeruginosa* skin infection. This statement is supported by the significant decrease of albumin concentration ($P<0.001$), decrease in A/G ratio and hyperglobulinaemia ($P<0.001$) within this period. These alterations in protein profile did not affect total protein concentration, which remained unchanged during the whole experimental period. The matched analysis of both haematological and biochemical parameters is more accurate and indicative for the progression of bacterial skin infections in dogs. Albumin and globulins concentrations and A/G ratio are sensitive, consistent and reliable parameters, which can be useful for evaluating skin *P. aeruginosa* infection in dogs.

Key words: albumin, dog, globulin, haematological parameters, *P. aeruginosa*, skin infection

Research Article

Evaluation of Nuclear Factor Kappa Beta, Nitric Oxide and Blood Neutrophil/Lymphocyte Ratio as Biomarkers of Inflammatory Response and Complementary Therapy in Dogs with Experimental Skin *Pseudomonas aeruginosa* Infection

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Abstract | The optimisation of strategies for control of *Pseudomonas aeruginosa* skin infections in dogs includes minimization of induced hyper-inflammation. We aimed at monitoring changes in serum nuclear factor kappa B (NF-κB) and nitric oxide (NO) concentrations, and blood neutrophil/lymphocyte ratio (N/L ratio), to evaluate their potential as inflammatory markers throughout the course of therapy in canine skin infection experimentally induced by *P. aeruginosa*. A complementary therapy including antibiotic against the pathogen and NF-κB targeted host modulatory therapy by parthenolide from the phytopreparation Feverfew was applied. NF-κB was determined by ELISA kit. The NO assay was based on the Griess reaction. The N/L ratio was calculated on the basis of CBC counts. The infection was induced by *P. aeruginosa* (1×10^8 CFU/mL) injected s.c. to 20 male dogs. Four experimental groups were formed (0 – untreated dogs and three treated groups: I – with antibiotic; II – with Feverfew and III – with antibiotic and Feverfew) and one control group. The results showed that NF-κB in group 0 was significantly higher than those in controls on hour 24 (p=0.0103), hour 48 (p=0.0001), hour 72 (p=0.0505), day 7 (p=0.0114), and day 10 (p=0.0094). The treatment with Feverfew (group II and III) achieved an efficient control on NF-κB. In groups I and II, biphasic increase in NO was observed, associated with the early – hour 4 (p=0.00007 for group I; p=0.0001 for group II vs. baseline) and late – day 14 (p=0.0092 for group II vs. controls) stage of infection development. By hour 24, N/L ratio in these groups increased (p=0.005 and p=0.0104 for groups I and II respectively vs. baseline). The established changes were generalised signs of inflammatory response accompanied by fever on hour 4 in infected dogs (p=0.0005 for group 0; p=0.0008 - I; p=0.0002–II; p=0.0047-III vs. controls).

Keywords | *Pseudomonas aeruginosa*, Canine skin infection, Inflammatory biomarkers, Therapy, Feverfew

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Short communication

ACUTE-PHASE RESPONSE AND THE EFFECT OF PHYTOPREPARATION FEVERFEW *(TANACETUM PARTHENIUM)* IN DOGS WITH EXPERIMENTAL *PSEUDOMONAS AERUGINOSA* SKIN INFECTION

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Summary

Andonova, M., V. Urumova, D. Dimitrova, E. Slavov, P. Dzhelebov, Ts. Chaprazov & T. Georgieva, 2016. Acute-phase response and the effect of phytopreparation Feverfew *(Tanacetum parthenium)* in dogs with experimental *Pseudomonas aeruginosa* skin infection. *Bulg. J. Vet. Med.,* **19**, No 1, 72–77.

Acute-phase response is most critical for the attenuation of the strong inflammatory response induced by bacterial opportunist *Pseudomonas aeruginosa*. The aim of the present study was to evaluate the acute-phase response in experimental *Pseudomonas aeruginosa* skin infection, by measuring changes in fibrinogen concentration (a positive acute-phase protein, APP) and activity of arylesterase (ARE – a negative APP). We also aimed to evaluate the effect of therapy with phytopreparation Feverfew, containing the active component parthenolide, which has anti-inflammatory properties. Fifteen male mongrel dogs at 2–5 years of age were divided into three groups: group 0 (n=5) with infection induced by subcutaneous injection of bacterial *Pseudomonas aeruginosa* culture 1×10^8 CFU/mL; group I (n=5) – infected and treated with Feverfew (standardised extract, active principle parthenolide 0.7% – Nature's Way, USA), by application of 1 capsule at 12-hour intervals. The per os treatment began on post infection hour 4 and continued for 6 days. Dogs from group C (n=5) were controls. Plasma fibrinogen and serum ARE activity were assayed before infection and on 4th, 24th, 48th and 72nd hour and on 7th, 10th and 14th day after infection. The results suggested that fibrinogen levels in dogs from group 0 increased on p.i. hour 24 vs baseline, attained a peak on hour 48 ($P<0.001$), and persisted high on hour 72. Infected dogs treated with Feverfew (group I) exhibited a similar time course of changes in fibrinogen levels, but the numeric values were lower compared to those of group 0 ($P<0.05$). ARE activity in experimental animals did not change significantly and was similar to control values.

Key words: acute-phase response, arylesterase, Feverfew, fibrinogen, skin *Pseudomonas aeruginosa* infection

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EFFECTS OF FEVERFEW (*TANACETUM PARTHENIUM*) AND ANTIBIOTIC CO-ADMINISTRATION ON BLOOD NEUTROPHIL FUNCTION AND SERUM PROCALCITONIN IN DOGS WITH EXPERIMENTAL SUBCUTANEOUS *PSEUDOMONAS AERUGINOSA* INFECTION

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ABSTRACT

The control of experimental canine *Pseudomonas aeruginosa* infection was optimised via a combined therapy including enrofloxacin against the bacteria and parthenolide (active component of the plant *Tanacetum parthenium*) for correction of inflammatory reaction. The aim of the present study was to evaluate the effect of this therapy on absolute neutrophil counts (ANC), blood neutrophilic function (phagocytosis, oxidative burst activity), serum naturally occurring antibodies (NAbs), procalcitonin (PCT) in infected animals. Blood samples were collected prior to the infection – hour 0 and post infection hours 4, 24, 48, 72 and day 7 from 25 male dogs, divided into five equal groups. Group C included healthy dogs (negative control). Dogs from Group 0 were injected subcutaneously with *P. aeruginosa* bacterial culture (1×10^8 CFU/mL) and untreated (positive control). The infected dogs from Group I were treated s.c. with enrofloxacin on post infection hour 48 at 5 mg/kg. Dogs from Group II were treated beginning from the 4th hour post infection with standardized feverfew extract – 90 mg, 0.7% parthenolide given orally at 2 capsules daily for 6 days. Group III included dogs with combined therapy - enrofloxacin and feverfew (at above mentioned doses and intervals). This therapy regulated ANC, NAbs, increased the phagocytic index, decreased hydrogen peroxide (H_2O_2) production of neutrophils and serum PCT. The observed correlations between PCT – marker of bacterial infection and blood markers – ANC, phagocytosis, H_2O_2 indicated that they could be used in combination to detect the progression of bacterial infection and can help to select the proper approach for treatment of infection.

Key words: Dogs, *Pseudomonas aeruginosa* infection, Neutrophil functions, Therapy.

INTRODUCTION

During the last years, the emergence of microbial pathogens resistant to numerous antimicrobial drugs is a serious problem. *Pseudomonas aeruginosa* is an element of the skin commensal flora of the dogs (Hoffmann *et al.,* 2014). This Gram-negative bacterium exhibits rapidly evolving antimicrobial resistance, biofilm formation (Breidenstein *et al.,* 2011). The choice of antimicrobial therapy in this infection is intricate (Kanj and Kanafani, 2011; Kmeid *et al.,* 2013).

The focus of therapeutic approaches used for treatment of *P. aeruginosa* infections are generally the antibiotics (Hillier *et al.,* 2014). The primary consideration in the selection of an antibiotic is clearly the sensitivity on the target microbial pathogen. Enrofloxacin, which is a third generation fluoroquinolone has good diffusion through bacterial membrane. After the entrance in the bacteria targets mechanism of its action has two major bacterial topoisomerase II (Gyrase) and the DNA topoisomerase IV (Topo IV) (Trouchon and Lefebvre, 2016). However, the potential of the antibiotic to interaction with the innate immune system of the infected host, may be of significance in some cases to affect the outcome of antibacterial therapy (Anderson *et al.,* 2010). Recent studies suggested that fluoroquinolones could enhance production of reactive oxygen species (ROS) in phagocytic cells, disturbed systemic oxidative balance and biofilm formation (Morita *et al.,* 2014). The most common side effect of enrofloxacin is an inflammatory reaction at the site of infection for injectable forms (Fauchier, 2013). *P. aeruginosa* also provokes a strong inflammatory response (Lavoie *et al.,* 2011). This bacterium inhibits the chemotaxis of neutrophils, suppresses the opsonins (both complement and immunoglobulin) and phagocytes, could rapidly react to abrupt changes in ROS (Lavoie *et al.,* 2011; Laarman *et al.,* 2012). The optimisation of strategies for control of *P. aeruginosa* infections requires combined therapy that includes an antibiotic against the pathogen and therapy that corrects the strong inflammatory response. Some researchers have used natural products with anti-inflammatory properties. The medicinal plant feverfew (*Tanacetum parthenium*), whose active principle is parthenolide, inhibits key signalling pathways of the inflammation – nuclear factor kappa beta (NF-kB),

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Биометодология за хуманното използване на лабораторни животни в медикобиологични и научни изследвания

2017

Биометодология за хуманното използване на лабораторни животни в медикобиологични и научни изследвания

Ръководство за упражнения
по Организация на експеримента

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"Биометодология за хуманното използване на лабораторни животни в медикобиологични и научни изследвания"

Второ преработено издание

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Ръководството за практически упражнения по Организация на експеримента е написано в съответствие с учебната програма по тази дисциплина, включена в новия учебен план на Ветеринарномедицински факултет при Тракийски университет – Ст. Загора, утвърден от Академичния съвет с Протокол № 10 от 21 януари 2017 год.

Въз основа на литературни данни и многогодишния опит на авторите, в него са включени нови знания при организирането и използването на животни в експерименти. Ръководството ще помогне в обучението на студентите по ветеринарна медицина и ще обогати с информация научни работници, практикуващи ветеринарни лекари, специалисти по медицина, биология, микробиология и животновъдство при работата им с лабораторните животни.

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ПРЕДГОВОР

Едни от най-използваните лабораторни животни за биомедицински изследвания са мишки и плъхове, но заедно с тях и редица други животни като хамстери, морски свинчета, зайци, кучета, птици и жаби продължават да служат като модели за изучаване на различни аспекти във физиологията и патологията на домашните животни и човека. Лабораторните животни са в помощ и при тестването на нови лекарствени продукти и изпитването на нови хирургически техники.

Още през далечната 1959 год. двама изследователи – Russell и Burch публикуват принципите за хуманните експериментални техники, заложени в концепцията за 3-те "R" – Reduction, Refinement и Replacement при използване на животни в опити. Днес тази концепция под формата на изисквания за защита и хуманно отношение към животните е въведена и в нашето законодателство. Тя се отнася до намаляването на броя на животните, нужни за получаване на валидни резултати във всеки експеримент, усъвършенстване на прилаганите техники за редуциране дискомфорта на опитните животни и заменяне на опитите с живи животни с алтернативни методи. Има и изисквания по отношение компетентността на специалистите и изследователите, работещи с лабораторни животни, което налага тяхното обучение.

Настоящето ръководство за практически упражнения по Организация на експеримента е второ преработено издание, изцяло съобразено с най-новите постановки за 3-те "R". Допълнени с актуална информация са разделите за различните видове лабораторни животни и биологичните им особености, техниките за фиксиране и обездвижване, както и процедурите по въвеждане на лекарства. Нови детайли са включени при прилагането на наркотични вещества за свеждане до минимум на стреса, болката и страданието по време и след въздействия върху опитните животни. Добавени са нови данни за използването на алтернативните методи.

Ръководството комбинира текстовия материал с прилагането на подходящи таблици, в които ясно и синтезирано е представена необходимата информация в достъпна форма за всеки разгледан биологичен вид. Всеки раздел е визуализиран с богат снимков материал.

Надяваме се промените в това второ издание да съдействат за подобряване качественото експериментиране с животни в различните биомедицински изследвания и за формиране на етични принципи у студентите и изследователите, свързани с защитата и хуманното отношение към опитните животни.

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PREFACE

The most common lab animal species used in biomedical research are mice and rats. Other common lab animal species like hamsters, guinea pigs, rabbits, dogs, birds and frogs are still being used as research models in different aspects of animal and human physiology and pathology. Lab animals are of great help in the process of development of new medications and surgical techniques.

As far back as 1959 the two researchers Russell and Burch published the principles of humane experimental techniques, which were implicated in the concept of the three "Rs" – Reduction, Refinement and Replacement. Today this concept is part of the legislation concerning protection and humane treatment of experimental animals. This concept implies reduction of the number of animals needed to obtain valid experimental results, refinement of experimental techniques aiming to reduce discomforted experienced by experimental animals and replacement of animal testing with alternative methods. Legislation has set regulations for research personnel competence, which requires their additional education and training.

This manual for practical training in the subject "Organization of experiments" is a second updated edition, which is in compliance with the latest concepts of the three "Rs". The chapters on the different lab animal species have been revised and modified and we have added the most current information on the biological features of lab animals, techniques of animal handling and restraint and methods for application of medications. The manual provides new details on the use of anesthetic drugs aiming to reduce to minimal levels stress, pain and suffering of animals during and after the experimental procedures. New information concerning the use of current alternative methods has been added.

Along with the text, the manual contains relevant tables providing concise information on each lab animal species. Each chapter is illustrated with appropriate figures.

We hope that the changes in the second updated edition will improve the quality of animal experimentation in various biomedical studies and will help to develop in all students and researchers ethical principles and understanding of humane treatment and protection of experimental animals.

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