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The status of extracellular antimicrobial potential of phagocytes genitals of cats

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This article shows the results of the experimental researches in the cellular link of local immune protection of cats' genitals. Due to the urgency of the problem the aim of our study was to investigate the functional state of the phagocytic cells and to explore and interpret their role in the formation of the antimicrobial potential of homeostasis in the system of local immunity of the cats' reproductive organs. Cytochemical research determined the antimicrobial reactive Oxygen-dependent mechanism for the protection of neutrophil granulocytes in reaction with NBT

It was found out, that the antimicrobial potential of phagocyte cells actively realizes via Oxygen dependent protection mechanisms. The total number of NBT + phagocytic cells in the investigated micropreparations was $21.35 \pm 0.86\%$. Cytochemical reactivity of primary phagocytic cells was shown on the I and II level, which reflects the full manifestation of the phagocytic defense. The activated phagocytes have also shown the active adhesion and phagocytosis of epithelial cells and of apoptotic macrophages, which is also one of the signs of the maintenance of cellular homeostasis. The interpretation of the results was done according to the outcomes of the research and the conclusions and recommendations on the application of cytochemical studies in a comprehensive assessment of the antimicrobial potential of phagocytic cells and for predicting clinical evaluation of reproductive diseases in small animals were developed.

It is recommended to perform cytochemical studies during complex testing of the local immune protection of the reproductive organs of animals, which will allow to objectively assess the state of cellular immunity, diagnose subclinical manifestation of reproductive pathology and predict the risk of complications.

Key words: cats, reproductive system, local immunity, cellular homeostasis, neutrophilic granulocytes, cytochemical reactivity, NBT-test.

Стан екстрацелюлярного потенціалу фагоцитів статевих органів у кішок

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У статті наведені результати експериментальних досліджень вивчення стану клітинної ланки локальної імунного захисту репродуктивних органів у кішок. Метою нашої роботи було дослідити функціональний стан фагоцитарних клітин, а також вивчити їх роль у формуванні імунного гомеостазу в системі локальної захисту органів розмноження у кішок. Встановлено, що протимікробний потенціал фагоцитарних клітин активно реалізується через Оксигензалежні механізми захисту. Загальна кількість НСТ + фагоцитарних клітин в мікропрепаратах становило $21,35 \pm 0,86\%$. Цитохімічний потенціал активованих фагоцитарних клітин проявлялась на I та II рівні реактивності. Дослідженнями встановлено також кореляційний зв'язок між цитологічним індексом та мікрофагами з активованим цитохімічним (протимікробним) потенціалом, що вказує про часткову праймерізацію клітинної ланки неспецифічного імунного захисту. Активовані фагоцити проявляли активну адгезію епітеліоцитів і апоптотичних мікрофагів. За результатами досліджень проведено інтерпретацію та

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зроблені висновки про перспективи застосування цитохімічних досліджень в комплексній оцінці протимікробної потенціалу фагоцитарних клітин для клінічної оцінки і прогнозування розвитку репродуктивної патології у дрібних домашніх тварин.

Ключові слова: кішки, статевая система, локальний імунітет, клітинний гомеостаз, нейтрофіли, цитохімічна реактивність, НСТ-тест.

Состояние экстрацеллюлярного противомикробного потенциала фагоцитов половых органов у кошек

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В статье приведены результаты экспериментальных исследований изучения состояния клеточного звена локальной иммунной защиты репродуктивных органов у кошек. целью нашей работы было исследовать функциональное состояние фагоцитарных клеток, а также изучить их роль в формировании иммунного гомеостаза в системе локальной защиты органов размножения у кошек.

Определено, что противомикробный потенциал фагоцитарных клеток активно реализуется через кислородзависимые механизмы защиты. Цитохимическими исследованиями кислородзависимого механизма фагоцитарной защиты клеток слизистой оболочки в реакции нитросиним тетразолием определено, что фагоциты принимают активное участие в реализации противомикробной защиты. Общее количество НСТ+ фагоцитарных клеток в исследованных микропрепаратах составило $21,35 \pm 0,86\%$. Цитохимическая реактивность праймированных фагоцитарных клеток проявлялась на I и II уровне. Исследованиями определено корреляционную связь между цитологическим индексом и количеством микрофагов с активированным цитохимическим (противомикробным) потенциалом, что указывает о частичной праймеризации клеточного звена неспецифической иммунной защиты. Активированные фагоциты проявляли активную адгезию эпителиоцитов и апоптотных микрофагов. По результатам исследований проведено интерпретацию и сделаны выводы о перспективах применения цитохимических исследований в комплексной оценке противомикробного потенциала фагоцитарных клеток для клинической оценки и прогнозирования развития репродуктивной патологии у мелких домашних животных.

Ключевые слова: кошки, половая система, локальный иммунитет, клеточный гомеостаз, нейтрофильные гранулоциты, цитохимическая реактивность, НСТ-тест.

Introduction

Immune animal protection system was formed in the course of a long evolution. More than a century has already passed since the phenomenon of phagocytosis was discovered by Mechnikov I.I. Despite this, scientists around the world continue actively to carry out a comprehensive study of cellular factors protecting the immune defense and of the interaction of immunocompetent cells (Ignacio et al., 2005; Michael, 2012).

The system of local immune protection of animal breeding has complicated ontogenetic development, which is clearly subordinated to the genetic determination and neurohumoral mechanisms of regulation (Yablonsky et al., 2004; Kjelgaard-Hansen et al., 2007). The modern scientific publications show more data on the role of phagocytes in the induction of cytokines, peptides fusion, mediators and other biologically active substances, which take part in the formation of immune homeostasis as well as in triggering the inflammatory response cascade Jursza-Piotrowska et al., 2016). Today, the central object of research are mechanisms for the implementation of the antimicrobial protection of phagocytic cells (urinary antimicrobial compounds, the formation of the protective traps and so on). Scientists are increasingly interested in the study of regulatory factors of functional state of phagocytes (William et al., 2012, Zhelavskiy et al., 2016).

According to many researchers the origin and development of the reproductive pathology (vaginitis, endometritis, pyometra) often occur because of immunological

disorders (Batista-Arteaga et al., 2007; Payan-Carreira et al., 2015; Zhelavskiy et al., 2016).

Due to the urgency of the problem the aim of our study was to investigate the functional state of the phagocytic cells and to explore and interpret their role in the formation of the antimicrobial potential of homeostasis in the system of local immunity of the cats' reproductive organs.

Material and methods

Clinical and experimental studies were carried out on 2 to 8 years old cats of various breeds. Laboratory studies were done in a specialized laboratory of animal reproduction in Podillya State Agrarian and Engineering University. The laboratory was founded by the doctor of biological sciences, professor, corresponding member of NAAS of Ukraine Yablonsky V. Immunological study determined cellular structure and functional state of the phagocytic cells. Cytochemical research determined the antimicrobial reactive Oxygen-dependent mechanism for the protection of neutrophil granulocytes in reaction with *nitro blue* tetrazolium (NBT +) using our own patented method (Zhelavskiy et al., 2016). At the same time we have determined the total percentage of reactive phagocytes (NBT +) index activation of phagocytic reactivity of neutrophils (IAN), cytological index of reactivity of the antimicrobial potential of cells (CLI). During the processing of biometric data set we were using the statistical software Statistica v.10.

Results and discussion

Cytological studies have determined that the cellular composition of the vaginal mucosa in the metestrus stage was mainly represented by intermediaries (Pic. 1) and parabasal epithelial cells. In the microscope slide cyto-gramme the overall share of neutrophils was $14.70 \pm 0.68\%$. Neutrophilic granulocytes were localized either singly or in groups and had a clear-cut segmented nucleus and cytoplasm (Pic. 2).

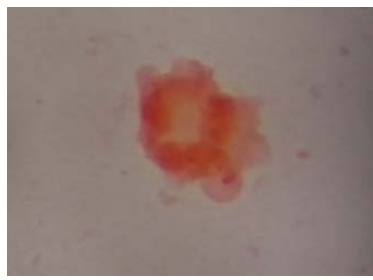
It is known that neutrophil granulocytes are able to activate the cellular part of the immune defense (Th1, Th2), as well as to coordinate the interaction of humoral and cellular immunity.

Neutrophils are located in the peripheral bloodstream only for 6 – 10 hours, and then get into the tissue where

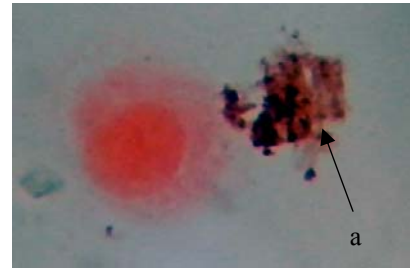
they perform their effector function (Kjelgaard-Hansen et al., 2012; William et al., 2012). Priming phagocytic cells are capable of destroying pathogenic agents both in the immediate attack (killing) as well as by absorption and digestion. Phagocytes are also able to realize its function by activating metabolic reactivity, followed by the extra-cellular release of antimicrobial compounds. This phenomenon has been called in the scientific literature as a respiratory burst. In the phagocytes occurs biochemical activation of the hexose monophosphate shunt and *NADPH oxidase of phagosome cell*. This metabolic reaction occurs against the backdrop of increasing (in ten times) consumption of cell glucose and Oxygen. *NADPH oxidase* converts O_2 – superoxide anion (O_2^-).



Pic. 1. Micro photo.
Intermediate epithelial cell
(x 2500)



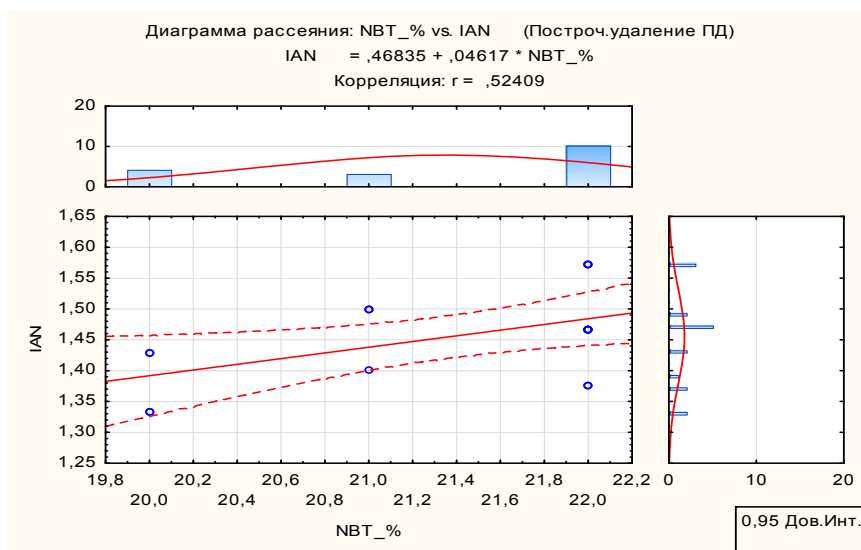
Pic. 2. Micro photo.
Neutrophil of vaginal mucosa
(x 2500)



Pic. 3. Micro photo.
Metabolic neutrophilic granulocyte reactivity in the reaction NBT (x 2500)

Furthermore, with the participation of superoxide dismutase other active forms of Oxygen are produced (H_2O_2 , $\bullet OH$, 1O_2 et al.) that are released by phagocyte into extracellular space. All this is accompanied by the launch of a cascade of other immune-biological reactions: there is an active synthesis of chemotactic peptides, the IL is being induced and others (Michael, J., 2012, Jursza-Piotrowska, E. et al., 2016). It was determined by

cytochemical studies of Oxygen-phagocytic mechanism of protection of mucosal cells in the reaction of NBT that phagocytes take an active part in the implementation of anti-microbial protection. The granules of diformasane were clearly visualized in the cytoplasm of reactive phagocytes. The total count of NBT + phagocytic cells in the investigated micropreparations was $21.35 \pm 0.86\%$ (Pic. 3).



Pic. 4. Correlational connection of IAN and NBT+

It was also determined that the intensity of antimicrobial potential of priming neutrophils appears mainly on the of I and II reactivity levels, which can clearly be seen in the graph (Pic. 4).

Statistical studies also identified a positive correlation ($r = 4.42$, $P < 0.001$) between the cytological index and the number of macrophages with activated cytochemical (antimicrobial) potential. All this indicates a partial activation of phagocytic cells and their incomplete primarization.

Recently, researchers are increasingly interested in the role of phagocytic cells in the formation of microbiocenosis and regulation of cellular homeostasis (Michael, 2012, Jursza-Piotrowska et al., 2016; Zhelavskiy et al., 2016). Reactive phagocytic cells have been often identified in our experiments, which carried out adhesion of epithelial cells and apoptotic neutrophils. We assume that phagocytes are involved not only in antimicrobial defense, but also involved in the formation of cellular homeostasis of mucosal tissues. This phenomenon leads us to the hypothesis that the functional state of neutrophils and epithelial cells, as well as the regulation of their apoptosis can be regulated by immunocompetent cells (under the influence of antimicrobial compounds, peptides, neurotransmitters and other biologically active compounds), which is going to be a subsequent stage of our research.

Conclusions

1. Thus, it can be concluded that in the cytogram of cats' vaginal mucosa during metestrus period intermediate and parabasal epithelial cells are mainly dominated. Cellular link of local security of the nonspecific immunity is presented by phagocytes.

2. The total number of neutrophils in cytogramme is $14.70 \pm 0.68\%$. The intensity of the antimicrobial potential of the Oxygen-dependent mechanism for the protection of neutrophils is found in the I and II levels of cytochemical reactivity.

3. It is recommended to perform cytochemical studies during complex testing of the local immune protection of the reproductive organs of animals, which will allow to objectively assess the state of cellular immunity, diagnose subclinical manifestation of reproductive pathology and predict the risk of complications.

Prospects for further research. Further research will be used to study the diagnostic criteria of reproductive pathology in cats.

References

Ignacio, G., Nordone, S., Howard, K. (2005). Toll-like receptor expression in feline lymphoid tissues. *Veterinary Immunology and Immunopathology*. 106, 229–237.

Michael, J. (2012). *Clinical Immunology of the Dog and Cat Revised And Updated*. J. Michael. Day Second Edition Copyright: Manson Publishing Ltd.

Yablonsky, V.A., Homin, S.P., Kalinovskiy, G.M. (2006). *Veterinarne akusherstvo, ginekologija ta biotehnologija vidtvorennya tvarin z osnovami andrologii*. Vinnicja : Nova kniga (in Ukrainian).

Kjelgaard-Hansen, M., Luntang-Jensen, M., Willesen, J. (2007). Measurement of serum interleukin-10 in the dog. *Veterinary Journal*. 173, 361–365.

Jursza-Piotrowska, E., Marta, J. (2016). Siemieniuch Identifying diagnostic endocrine markers and changes in endometrial gene expressions during pyometra in cats. *Reprod. Biol.* 8, 16(2), 174–180.

Jursza-Piotrowska, E., Socha, P., Dariusz, J. (2016). Prostaglandin release by cultured endometrial tissues after challenge with lipopolysaccharide and tumor necrosis factor α , in relation to the estrous cycle, treatment with medroxyprogesterone acetate, and pyometra. *Theriogenology*. 10, 85(6), 1177–1185.

Jursza-Piotrowska, E., Siemieniuch, E., Marta, J. (2016) Identifying diagnostic endocrine markers and changes in ndometrial gene expressions during pyometra in cats. *Reprod. Biol.* 8, 16(2), 174–180.

William, A., Rose, I.I., McGowin, C.L. (2012). Commensal Bacteria Modulate Innate Immune Responses of Vaginal Epithelial Cell Multilayer Cultures <http://dx.doi.org/10.1371/journal.pone.0032728>.

Zhelavskiy, M., Shunin, I. (2016). Cell factors' condition of local immunity of vaginas mucosa in cats. *Scientific Messenger LNUVMBT named after S.Z. Gzhytskyj*. 18, 32–36 (in Ukrainian).

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Zhelavs'kij, M.M. (2016). Osoblivosti sistemnogo ta lokal'nogo imunitetu u period vagitnosti : metodichni vkazivki. *Podil's'kij derzhavnij agrarno-tehnichnij universitet. Kam'janec'-Podil's'kij*, 22 (in Ukrainian).

Batista-Arteaga, M., Alamo, D., Herraes, P. (2007). Segmental atresia of the uterus associated with hydrometra in a ferret. *Vet. Rec.* 161, 759–760.

Payan-Carreira, R., Pina, J., Costa, M. (2015). Oestrogen receptors in a case of hydrometra in a bitch. *Vet. Rec.* 158, 487–489.

Zhelavskiy, M.M., Shunin, I.M. (2016). Perspektivi doslidzhennja klitinnih faktoriv lokal'nogo imunitetu slizovih obolonok statevih organiv kishok. *Zbirnik materialiv HV Mizhnarodnoi naukovo-praktichnoi konferencii profesors'ko-vikladač'kogo skladu ta aspirantiv «Problemy veterinarnoy medicini ta jakosti i bezpeki produkcii tvarinnictva» fakul'tetu veterinarnoy medicini*, 36–37 (in Ukrainian).

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