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INFLUENCE OF INACTIVATED TOXINS OF E.COLI ON PHAGOCYTIC PROPERTIES OF NEUTROPHILIC GRANULOCYTES

Research article

Abstract

The mechanisms of activation of the immune defense of a macro-organism in response to the ingestion of pathogens of infectious genesis are of practical interest for medicine and veterinary science, in particular, for developing and improving biological drugs for treatment and prevention. Given the relevance of the problem of the efficiency of preventive vaccination of colibacillosis in animals, the most promising areas in biotechnology are singled out, and they include the creation of immune preparations based on toxoid components of *E. coli*. This way, the effectors of innate immunity — neutrophilic granulocytes — come to the forefront of the immune response, and the scenario of the immune response to antigenic irritation will largely depend on their regulatory function. In this regard, the aim of our research was to study functional parameters of neutrophils under antigenic load, in this case – inactivated toxins of *E. coli*. White rats were used for biological research, and their blood was taken on the 1st, 3rd, 5th, and 7th days after the immunization performed with the toxoid components of *E. coli* to study the phagocytic activity of neutrophils, their capturing and digesting ability, and in order to determine the index of completion of phagocytosis. In the course of the experiment, it was found that, generally, *E. coli* toxins activate phagocytic immunity in vaccinated rats. At the same time, a preparation containing LT, ST, STX-toxins of *E. coli* stimulates the phagocytic activity of neutrophilic granulocytes in the first days after administration in comparison with separate use of toxins. The digesting and capturing ability of neutrophils was most pronounced on the third day of the research. Afterwards, there was a decrease in the functional activity of cells.

Keywords: neutrophilic granulocytes, innate immunity, toxins of *E. coli*, phagocytosis, colibacillosis infection.

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ВЛИЯНИЕ ИНАКТИВИРОВАННЫХ ТОКСИНОВ КИШЕЧНОЙ ПАЛОЧКИ НА ФАГОЦИТАРНЫЕ СВОЙСТВА НЕЙТРОФИЛЬНЫХ ГРАНУЛОЦИТОВ

Научная статья

Аннотация

Механизмы активации иммунной защиты макроорганизма в ответ на попадание патогенов инфекционного генеза представляют практический интерес для медицины и ветеринарии при конструировании и совершенствовании биологических препаратов лечебно-профилактического направления. Учитывая актуальность проблемы эффективности вакцинопрофилактики эшерихиоза у животных, в настоящее время наиболее перспективными направлениями в биотехнологии является создание иммунных препаратов на основе токсоидных компонентов кишечной палочки. При этом на первый план иммунного реагирования будут выходить клетки-эффекторы врожденного иммунитета – нейтрофильные гранулоциты, от их регуляторной функции во многом будет зависеть сценарий иммунного ответа на антигенное раздражение. В связи с этим целью наших исследований являлось изучение функциональных показателей нейтрофилов под антигенной нагрузкой, роль которой выполняли инактивированные токсины кишечной палочки. В качестве биологической модели исследований использовали белых крыс, у которых на 1, 3, 5 и 7 сутки после иммунизации токсоидными компонентами *E. coli*, отбирали кровь для изучения фагоцитарной

активности нейтрофилов, их захватывающей и переваривающей способности и определения индекса завершенности фагоцитоза. В ходе опыта установлено, что токсины кишечной палочки в целом активируют фагоцитарное звено иммунитета у вакцинированных крыс. При этом препарат, содержащий в совокупности LT, ST, STX-токсины *E. coli*, в течение первых суток после введения в 2 раза и более стимулирует фагоцитарную активность нейтрофильных гранулоцитов по сравнению с применением токсинов по отдельности. Переваривающая и захватывающая способность нейтрофилов наиболее выражена на третьи сутки исследований, после чего происходит снижение функциональной активности клеток.

Ключевые слова: нейтрофильные гранулоциты, врожденный иммунитет, токсины кишечной палочки, фагоцитоз, эшерихиозная инфекция

1. Introduction

Effective modern cattle breeding is inextricably intertwined with the well-being of animals in relation to various diseases. A macro-organism may already be exposed to microorganisms in the prenatal period, including pathogenic genesis. Natural mechanisms of animal protection such as innate and adaptive immunity are crucial in relation to causative agents of infectious diseases [3], [7]. One of such innate response mechanisms is the phagocytosis system, in particular, neutrophilic granulocytes, which not only destroy foreign pathogenic agents but also participate in the regulation of the immune response and inflammation [1], [2]. Neutrophils are the main effectors of innate immunity and act on the first line of the immune response when pathogens enter a body. Next, neutrophils have a helper and suppressor effect on other mechanisms of both innate and adaptive immunity [5], [8], and [9]. As they are soluble antigenic structures, according to recent studies, inactivated *E. coli* toxins can have a stimulating effect on the body [6]. It is important to understand how the phagocytic system of immunity will respond to the introduction of *E. coli* toxin devoid of toxic properties.

The aim of the research was to study the phagocytic activity of neutrophilic granulocytes during antigen load with inactivated toxins of *E. coli*.

2. Material and research methods

To conduct an experiment, we selected 25 white rats and divided them into five groups, five animals in each one. Animals from groups 1 to 4 were experimental (they were given a culture suspension of toxins of *Escherichia coli* (*E. coli*)), group 5 was a control one (animals were not immunized). Rats from group 1 were injected with a suspension of thermostable *E. coli* toxin (ST); the 2nd group of animals – with a thermolabile toxin (LT); the 3rd group – with a suspension of Shiga like toxins (STX). Rats from group 4 were given a complex preparation containing LT, ST, and *E. coli* STX toxins. Inactivation of toxoid components of *E. coli* was carried out by adding formalin. The immunizing dose was established by previous studies and amounted to 0.15 cm^3 by the intramuscular route of administration.

After the administration of toxins, blood was taken from rats on days 1, 3, 5, and 7. As indicator values of the phagocytic immunity link, the phagocytic activity of neutrophils (PAN), the phagocytic number (PN), and the phagocytic index (PI) were determined. The completion of the phagocytic reaction and the digestion process was evaluated by the percentage of digestion (% digest.). The most common micro-organism, *Staphylococcus aureus* strain 209P, was used as a test object for phagocytosis [4].

Obtained data were drawn up in graphic and tabular material using *Microsoft Office Word and Excel 2010*.

3. Research results

In the course of the experiments, we determined the degree of stimulation of the phagocytic immunity unit under antigen load in time dynamics, the numerical values of which are shown in the table. The most significant were the PAN, PN, and digestion percentage.

Table 1 – Dynamics of changes in the phagocytosis of neutrophilic granulocytes under the influence of inactivated toxins of *E. coli*

Groups of animals	Values				
	PAN,%	PN 30'	PI	% digest	IZF
24 hours after administration					
1 (ST-toxin)	20,2±2,6	2,3±0,2	1,16±0,06	57,3±5,6	1,01±0,13
2 (LT- toxin)	32,5±4,9	2,3±0,2	1,17±0,09	52,0±1,6	1,65±0,21
3 (STX- toxins)	38,2±3,8	2,2±0,3	1,15±0,05	59,6±1,5	1,9±0,15
4 (LT+ST+STX- toxins)	64,3±4,2*	5,1±0,1	2,74±0,02	55,7±2,8	1,8±0,6
Control	8,4±5,5	2,0±0,2	0,56±0,04	42,7±3,9	1,3±0,17
72 hours after administration					
1 (ST- toxin)	34,0±3,8	2,7±0,4	2,92±1,4	48,2±3,7	1,22±0,33
2 (LT- toxin)	44,7±6,4	2,8±0,4	2,7±1,5	51,2±1,3	1,86±0,47
3 (STX- toxins)	40,6±6,7	2,7±0,2	2,6±1,97	56,2±9,1	2,37±1,54
4 (LT+ST+STX- toxins)	47,7±1,5*	2,4±0,1	3,7±0,74	57,7±14,1	1,82±0,4
Control	12,2±3,6	2,3±0,2	0,58±0,24	52,9±5,9	1,57±0,15

120 hours after administration					
1 (ST- toxin)	18,7±1,5	1,9±0,5	1,49±1,08	48,9±0,95	1,1±0,44
2 (LT- toxin)	21,3±5,0	2,5±0,5	1,72±1,14	46,9±14,4	1,5±1,1
3 (STX- toxins)	19,0±2,2*	2,3±0,8	1,25±0,14	52,5±5,99	1,69±0,5
4 (LT+ST+STX- toxins)	28,0±4,2*	2,1±0,7	1,36±0,07	55,66±6,3	1,85±0,09
Control	11,0±2,1	2,4±0,2	0,58±0,24	54,9±5,9	1,57±0,15
168 hours after administration					
1 (ST- toxin)	14,25±1,36	1,9±0,25	0,24±0,08	49,69±14,8	0,88±0,36
2 (LT- toxin)	13,59±5,47	1,5±0,25	0,23±0,01	50,3±12,6	0,9±0,24
3 (STX- toxins)	14,67±6,35	1,9±0,7	0,25±0,09	47,8±15,6	1,1±0,56
4 (LT+ST+STX- toxins)	15,3±5,37	1,7±0,56	0,25±0,2	45,3±11,6	1,1±0,29
Control	12,0±0,99	1,6±026	0,58±0,24	49,9±5,9	1,57±0,15

**P≤0,05 in relation to intact animals*

As can be seen from Figure 1, the phagocytic activity of neutrophilic phagocytes 24 hours after the administration of inactivated toxins of *E. coli* increased depending on the type of toxin. The highest indicator was observed in animals from group No. 4, which were immunized with a complex toxoid drug (64.3%). A less pronounced capturing ability of neutrophils was noted after the exposure to other toxins, which were 26.1–44.1% lower than in animals immunized with toxins in the complex. After 72 hours, the situation changed somewhat; in the fourth group, phagocytic activity of neutrophils in rats decreased but remained higher than in other tested animals (47.6%). In groups under No.1, 2, and 3, on the contrary, there was a slight change in the neutrophilic capturing ability, which amounted to 34, 44.7, and 40.6%, respectively.

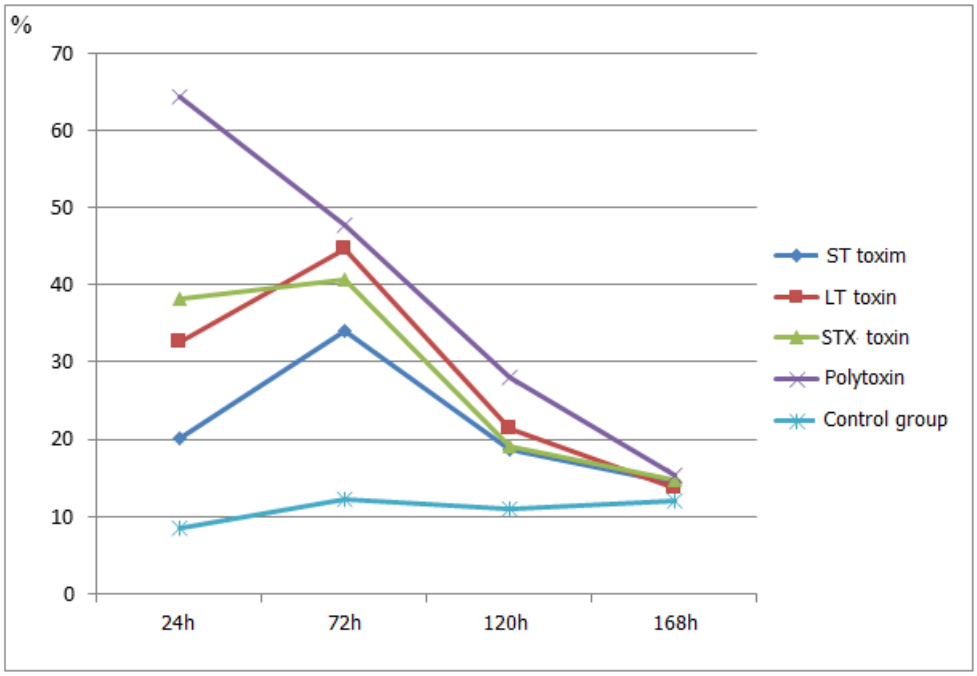

Figure 1 – Dynamics of changes in the phagocytic activity of neutrophils under the influence of inactivated toxins of *E. coli*

On the 5th day of studies, there was a decrease in the phagocytic activity of neutrophils in all experimental groups, by 19.7% in rats from the fourth group, by 21.6% in the third group, by 23.4%, in the second group and by 15.3% in the first group. Moreover, all values were higher than those in animals of the control group. The maximum rate was in rats of group No. 4 (28%). 168 hours after the administration of inactivated *E. Coli* toxins, the phagocytic activity in all experimental groups was the same as of the animals in the control group and did not differ significantly.

The dynamics of the number of cells captured by neutrophils are shown in Figure No. 2, and it can be seen that this indicator was maximum in animals of the 4th group on the first day of research and amounted to 5.1 cells. In groups under No. 1.2 and 3, the phagocytic number was 2.2–2.3 times lower than in group 4, but still higher than in animals of the control group.

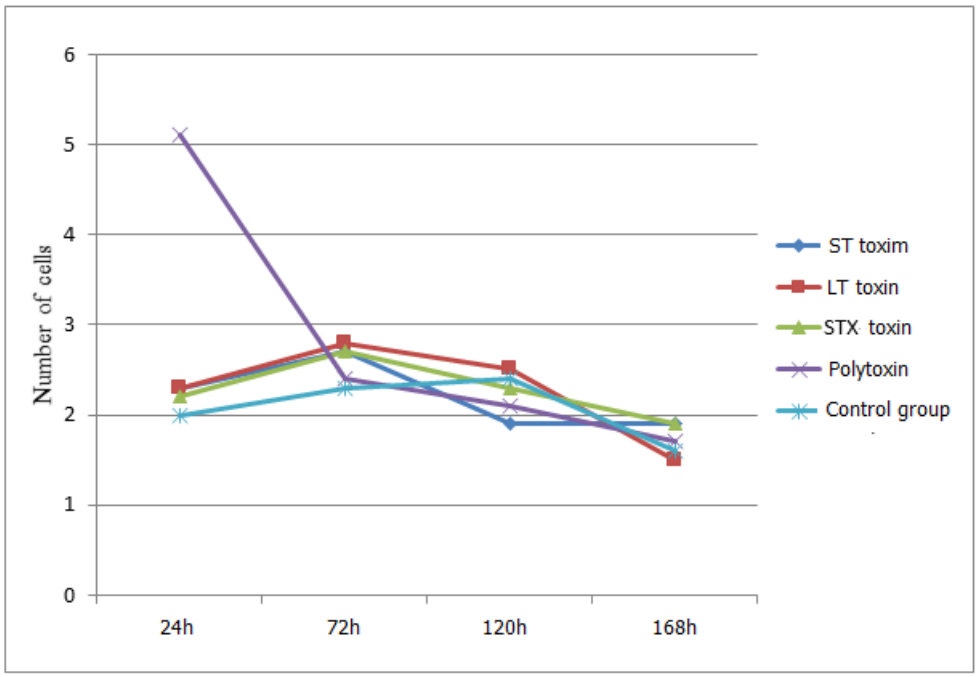

Figure 2 – Dynamics of changes in the phagocytic number of neutrophils under the influence of inactivated toxins of *E. coli*

After 72 hours, the dynamics changed – in animals that were injected with polytoxin of captured staphylococci, it decreased by 2.1 times in the remaining experimental groups; on the contrary, there was an increase in the phagocytic number compared to the previous indicators. On days 5 and 7, the capturing ability of neutrophils decreased to the indices of the control group of animals.

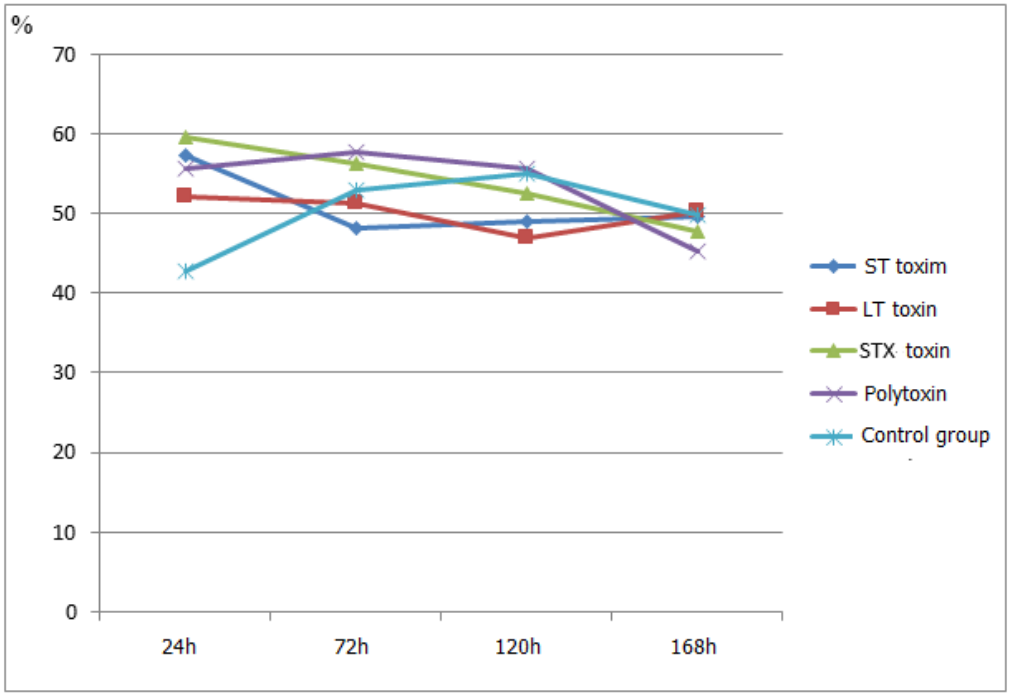

Figure 3 – Dynamics of changes in digestibility neutrophils under the influence of inactivated toxins of *E. coli*

The digestibility of neutrophils is shown in Figure 3, which indicates that the best percentage of digestion was in groups of animals where toxins were administered separately in the first 24 hours under the influence of antigenic load. In the fourth group, against the background of a significant value of a phagocytic activity, the percentage of digestion was relatively low – 55.7%, with an index of completion of phagocytosis of 1.8. After 72 hours, the rate of digestion in the experimental groups remained within the range of 48.2–57.7%, but whereas it was higher in animals of groups 3 and 4 than in the control group, in groups 1 and 2 the values did not significantly differ from those of intact animals. The index of completion of phagocytosis exceeded the control values in all groups except the first one. On days 5 and 7, the percentage of digestion remained within the range of 45.3–55.66%, which did not significantly differ from the intact animals. The completion index of phagocytosis in the first and second groups decreased on the 5th day, the same occurred in the 3rd and 4th groups on the 7th day, which indicates the end of antigenic irritation of the substrates introduced into the animal.

4. Conclusion

The administration of inactivated *E. coli* toxins stimulates the phagocytic immunity in white rats. The response of the body was accompanied by an increase in phagocytic activity and the digesting ability of neutrophils in the first 72 hours after the administration. Moreover, immunization with three toxins of *E. coli* had a potentiating effect on the phagocytic activity, and the number of captured cells, which were toxins administered separately, was two times higher in comparison with the same indicators of animals. After 72 hours, there was a gradual decrease in the phagocytic activity of neutrophils, which minimized on the 7th day of the research.

Conflict of Interest

None declared.

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Конфликт интересов

Не указан.

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