

Phagocytosis and the Effect of Imupurin

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Abstract

The purpose of the study was to investigate a new drug of entomological origin isolated from the pupae of *Lepidoptera*. Experiments were performed on young mice 18-20 g, which were divided into 3 groups: a control group and two groups that received a suspension of the drug in the amount of 0.5 ml of saline at doses of 1000 and 100 micrograms per mouse, respectively. There was a strong stimulation of nonspecific resistance of the organism as a result of the use of Imupurin as demonstrated by increased phagocytosis capacity of peritoneal neutrophils and macrophages (marked increase in phagocytant neutrophils and macrophages, namely reducing the nonphagocytant). The results showed that in doses of 100 and 1000 micrograms Imupirin increased the number of phagocytant neutrophils by 3.16 and 3.49 times and increased the number of phagocytant macrophages by 3.66 and 3.55 times, respectively, compared to the control. The observed effects of the entomological drug did not depend on the dose, which indicates that the effects on phagocytosis depend mainly on the composition of Imupurin (proteins, lipids) and to a lesser extent on the quantity.

Key words: Imupurin, nymph, immunostimulation, nonspecific resistance, phagocytosis, *Lepidoptera*.

Фагоцитоз и его изменения под влиянием имупурина

Цель исследования – изучение действия нового препарата энтомологического происхождения, выделенный из куколок *Lepidoptera*. Опыты были произведены на молодых мышках 18-20 г, которые были подразделены на 3 группы с использованием суспензии препарата в объеме 0,5 мл физиологического раствора в дозах 1000 и 100 микрограмм на мышку. Наблюдается сильная стимуляция неспецифической резистентности организма вследствие употребления имупурина, в результате повышения способности к фагоцитозу нейтрофилов и макрофагов. Результаты опытов показали, что имупурин в дозах 100 и 1000 микрограмм увеличил количество фагоцитирующих нейтрофилов в 3,16 и 3,49 раз и количество фагоцитирующих макрофагов в 3,66 и 3,55 раз, соответственно по сравнению с контролем. Именно в этих пропорциях имупурин уменьшил количество нефагоцитирующих нейтрофилов и макрофагов, не участвующих в фагоцитозе. Для определения фагоцитарного индекса была выделена тенденция к повышению способности фагоцита поглощать большее количество стафилококков. Анализ фагоцитарного индекса позволил констатировать, что последний, при использовании имупурина в соответствующих дозах, вырос по отношению к контролю в 3 раза. Выявленные эффекты энтомологического препарата не зависят от дозы, что указывает на то, что стимулирующее действие на фагоцитоз зависит, в основном, от состава имупурина (протеины, липиды) и, в меньшей степени, от количества.

Ключевые слова: имупурин, куколка, иммуностимуляция, неспецифическая резистентность, фагоцитоз, *Lepidoptera*.

Introduction

The research done so far has shown that insects' tissues contain lipoprotein complex structures with immune modulatory effect, i.e. consumed products in various ways considerably increase the human immune system against unfavorable environmental actions and infections of various pathogenic agents, including viral infections. Thus, we can ascertain a possible method to stimulate the immune system, which surely would increase human life expectancy [2, 3].

The recent increase in researchers' interest in insects is partly due to their immune system. Although their immune system is different from that of humans, studying them might help us overcome many illnesses. For instance, insects are able to destroy microbe cells with the help of some receptor molecules in their body and to synthesize peptide as a protection system against microbes [1, 4].

Material and Methods

To identify the action of the entomologic drug Imupurin upon the process of phagocytosis, the recommendations approved by State Pharmacological Committee of the Russian Ministry of Health (Moscow 2005) have been followed [5]. To identify the action of phagocytosis *in vivo*, 30 mice were

used. The control number contained 10 mice which were given 0.5 ml of intraperitoneal physiological solution. The following two experimental groups (10 mice each) were given drug suspension in an amount of 0.5 ml physiological solution according to the methodical recommendations (1000 and 100 mkg for each mouse) [5].

Activity of phagocytosis of peritoneal neutrophils and macrophage were observed. The mice were given 3-4 ml of 2-3% of intraperitoneal bullion and after 24 hours they were sacrificed with chlorophorm. Subsequently, the intraperitoneal liquid was extracted according to the recommendations mentioned above by injecting the material with *Staphylococcus aureus*.

The results were determined microscopically. The number of peritoneal neutrophils and macrophages were counted from the sample. The index of phagocytosis and the percent of phagocytant cells of each group were counted. We also determined the phagocytosis activity of neutrophils and macrophage by analyzing the phagocytary index (PI), i.e. the percent of phagocytant cells out of their total amount, and the phagocytary number (PN), i.e. the number of phagocyted bacteria by a neutrophils (obtained out of the media of phagocyted bacteria and non phagocytant neutrophils).

Results and discussions

The results of the experiments proved that Imupurin contributes to the increased amount of phagocytant neutrophils, and respectively, the decrease of those non-phagocytant (tab. 1).

Thus, the drug of 100-1000 micrograms per mouse increased the number of phagocytant neutrophils from 3.16 to 3.43.

Respectively, Imupurin reduced the number of non phagocytant neutrophils. Concomitant, an essential increase was established in the number of phagocyted *Staphilococcus* (tab. 1). Thus, neutrophils phagocyted 226.6 ± 11.4 microbes in the control group, this number increased to 820.3 ± 16.8 and 801.6 ± 27.3 *Staphilococcus* or 3.6 and 3.5 times at the doses 100 mkg and 1000 mkg.

Table 1

The action of Imupurin on the neutrophyls activity and number of phagocyted *Staphilococcus*

Groups and doses of Imupurin	Nr. of animals	Phagocytant neutrophils	Non-phagocytant neutrophils	Phagocyted <i>Staphilococcus aureus</i>
Control	10	24.6±1.4	75.4±4.43	226.6±11.4
1000 mkg/ per animal	10	82.5±1.26*	17.5±4.0*	801.6±27.3*
100 mkg / per animal	10	77.7±0.87*	22.3±2.75 *	820.3±16.8*

* - veridical deviation, $p < 0,001$.

For detailed phagocytosis analysis, the activity of neutrophils was determined characterized by the phagocytary index and the phagocytary number.

The phagocytary number increased from 9.5 ± 0.85 in the control group to 10.6 ± 0.18 and 9.7 ± 0.36 in study groups with Imupurin in the doses 100 and 1000 mkg per animal (tab. 2).

The analysis of respective parameters allowed us to find that the phagocytary indexes have increased from 24.6 ± 1.4 (in the control group) to 74.7 ± 0.87 and 82.5 ± 1.27 in groups given Imupurin ($p < 0.001$).

The increasing phagocytary index, though non-veridical, shows the phagocytary capacity of a neutrophil. All this permit us to conclude, that Imupurin increases the number and phagocytary activity of neutrophils (fig. 1).

Table 2

The action of imupurin in the doses of 1000 and 100 mkg/ per animal on the functional neutrophils activity

Parameters	Control	II group Imupurin 1000mkg / per animal	III group Imupurin 100mkg/ per animal
Phagocytary index	24.6±1.4	82.5±1.27*	77.7±0.87*
Phagocytary number	9.5±0.85	9.7±0.36	10.6±0.18

* - Veridical deviation, $p < 0.001$.

The obtained data suggest a hypothesis that Imupurin stimulant action on phagocytosis doesn't depend on the administrated doses therefore it is not a dose dependent action, but it depends on lipoproteic complexes from Imupurin composition, which are inductors of neutrophils activity.

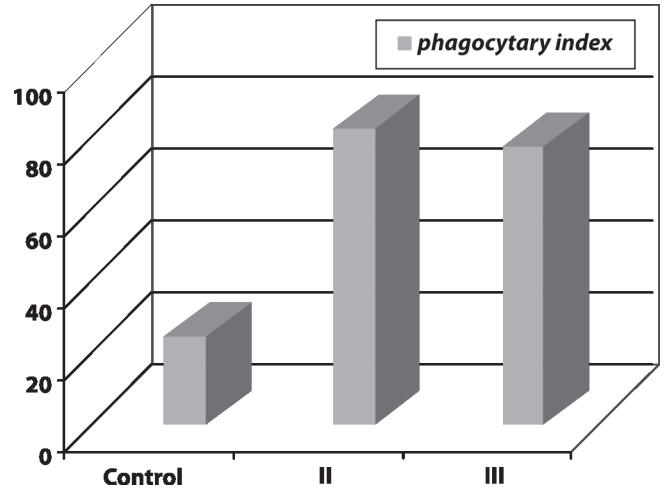


Fig. 1. The action of Imupurin on the functional neutrophils activity

Table 3

The effect of Imupurin on the activity of macrophages and the number of phagocyted *Staphilococcus*

Groups and doses of Imupurin	Nr. of animals	Phagocytant macrophages	Non-phagocytant macrophages	Phagocyted <i>Staphylococcus aureus</i>
Control	10	22.7±1.13	77.3±1.13	217.1±10.5
II group 1000mcg// per animal	10	81.0±1.49*	20.0±0.9*	828.2±22.1*
III group 100mcg // per animal	10	83.1±1.65*	16.9±1.65*	820.3±14.8*

* - Veridical deviation, $p < 0.001$.

Because phagocytes are one of the first defense barriers of the body against infection, the influence of Imupurin on the number and phagocytary activity of macrophages was studied (tab. 3).

The experimental study has demonstrated that Imupurin (100 and 1000 mkg per animal) increases the phagocytant macrophages number (by 3.66 or 3.55 times) in comparison with the control group (tab. 3). Concomitant, the same drug proportion reduced the number of non-phagocytant macrophages (tab.3). Simultaneously, Imupurin increases the number of phagocyted *Staphylococcus aureus* by macrophages. In this way, this parameter constituted 820.3 ± 14.8 and 828.2 ± 22.1 comparative with the control group (217.1 ± 10.5) ($p < 0.001$).

The phagocytary index is in correlation with the reported data, and its increase is similar with the number of phagocytant macrophages (tab. 4). The phagocytary number noted an intensification of one macrophage's activity to phagocyte more *Staphylococcus* (fig. 2). Accordingly, the contrary with a significant increase of phagocytant macrophages it is noted a light activity of the phagocytary number.

The constant effects of entomologic drugs are not dose dependent. Probably, the stimulatory action on phagocytosis depends more on the composition of Imupurin (proteins, lipids) than their quantity.

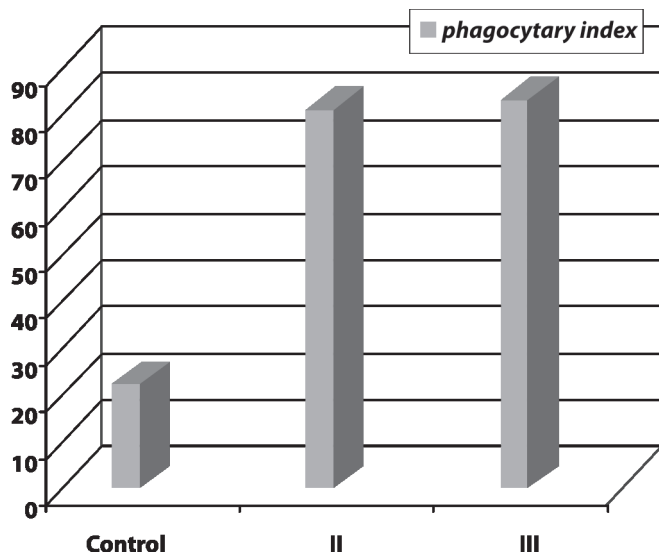


Fig. 2. The action of Imupurin on the functional macrophages activity

Tabel 4

The action of Imupurin in the doses of 1000 and 100 mkg/ per animal on the functional macrophages activity

Parameters	Control	II group Imupurin 1000mkg / per animal	III group Imupurin 100mkg/ per animal
Phagocytary index	22.7±1.13	81.0±1.49*	83.1±1.65*
Phagocytary number	9.73±0.64	10.24±0.29	9.90±0.26

* - veridical deviation, $p < 0.001$.

Conclusions

1. Imupurin is an entomologic drug with certain immunotropic properties.
2. Imupurin has a stimulant action on phagocytosis, a fact manifested through the increasing of the indexes characteristic for phagocytosis.
3. Lipoproteic complexes from Imupurin composition may be considered responsible for the stimulatory action on phagocytary macrophages and neutrophils action and *Staphylococcus* infection eradication.

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