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RESEARCH ARTICLE

EFFECT OF INTRAVENOUS IMMUNOGLOBULINS ON PHAGOCYTIC ACTIVITY OF NEUTROPHILS IN PREMATURE INFANTS WITH RESPIRATORY DISTRESS SYNDROME AND PNEUMONIA

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ABSTRACT

Material and methods: We investigated the effect of intravenous immunoglobulins (IVIG) on the phagocytic activity of blood neutrophils and the relationship between phagocytosis and the type of pathogen in 16 preterm infants (the main group) with respiratory distress syndrome (RDS) complicated by "ventilator-associated" pneumonia (VAP). Phagocytosis was investigated by cytological method. Microbiological examination of tracheo-bronchial aspirate was performed using standard methods. Infants received IVIG for 3 consecutive days, starting from the second day of life. ***Results:*** It was found that phagocytosis rates in these infants were lower than in 20 healthy premature newborns. IVIG stimulated phagocytosis of blood neutrophils a day after the start of treatment with the achievement of normal values and maintained the immunocorrective effect for at least three days after the course of immunotherapy. In newborns with VAP from the comparison group (19 infants did not receive IVIG), phagocytosis was lower than normal at the beginning and in the acute period of the disease. Neutrophil phagocytosis was lower before IVIG administration and one day after IVIG administration in newborns of the main group when *Mycoplasma hominis*, *Ureaplasma urealiticum*, *Chlamydia trachomatis* were isolated from the respiratory tract than in infants with bacterial pneumonia, but phagocytosis was high in infants with intracellular pathogens after a course of immunotherapy. ***Conclusion*:** Early use of IVIG in premature infants with RDS at risk of developing pneumonia stimulates neutrophil phagocytosis. This can contribute to the rapid elimination of pathogens and a less severe course of pneumonia.

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INTRODUCTION

Currently, the problem of treating infectious and inflammatory diseases in premature newborns is given great attention. The use of hardware artificial ventilation (mechanical ventilation) in infants with respiratory distress syndrome (RDS) can lead to the development of severe complications such as ventilator-associated pneumonia (VAP) and sepsis (Cernada, *et al*, 2014; Kolls, 2017). Antibiotic treatment does not always provide a quick and lasting effect, as many pathogens are hospital strains with multiple antibiotic resistance (Kushnareva, *et al*, 2019). Often, the etiology of VAP in newborns is represented by a combination of bacteria and intracellular microorganisms (*Mycoplasma hominis*, *Ureaplasma urealiticum*, *Chlamydia trachomatis* - *M. hominis*, *U. urealiticum*, *Ch. trachomatis*). However, the intracellular localization of microorganisms significantly limits the effect of antibiotics, which create low concentrations inside phagocytes, and also violates the functional properties of the latter (Rakovskaya, 2008; Kohlhoff *et al*, 2015). Intravenous immunoglobulins (IVIG) are used to treat infection in newborns, but their effectiveness and usefulness in the neonatal period is controversial (Antonov, *et al*, 2007; Aronskind, *et al*, 2007; Zwiers, *et al*, 2018). Until now, the mechanism of action of IVIG has not been sufficiently studied, and it is mainly explained by the ability of immunoglobulins to bind pathogens to the formation of immune complexes. Do not take into account the opsonizing effect of IVIG. The effect of IVIG on neutrophil phagocytosis, which is one of the leading links in anti-infective protection in premature newborns, has not been sufficiently studied. These facts dictate the need for a more in-depth study of the mechanisms of immunocorrective action of IVIG for their reasonable use in the complex treatment of VAP and for the prevention of this disease in infants with RDS.

The Aim: To determine the effect of intravenous immunoglobulins on the phagocytic activity of blood neutrophils in premature newborns with RDS complicated by "ventilator-associated" pneumonia", and to study the

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characteristics of phagocytosis in infants, taking into account the type of pneumonia pathogen.

MATERIAL AND METHODS

Patients: The study of phagocytic activity of blood neutrophils was carried out in 49 preterm newborns with a gestational age at birth from 28 to 37 weeks and a body weight from 920 to 2700 g. The VAP developed on day 5-8 of life in 29 infants. These infants received basic treatment. It included oxygen therapy, antibiotic therapy, and syndromic and symptomatic treatment. The main group (I) consisted of 16 premature infants with RDS complicated by VAP. These infants, in addition to basic treatment, received IVIG starting from the second day of life due to the presence of several risk factors for the development of infectious and inflammatory diseases (prematurity, burdened infectious history of the mother; especially in the third trimester, complicated course of childbirth, severe asphyxia suffered during childbirth, mechanic ventilator; deterioration in the child's condition during the first two days of life in the presence of adequately conducted syndrome therapy). VAP developed on the 4th – 8th day of life. The comparison group (II) consisted of 13 infants with VAP who were not treated with immunoglobulins. These infants were in the basic treatment. The control group (III) consisted of 20 conditionally healthy premature newborns without infectious pathology. These infants were on physiological nursing. Indicators of gestational age, body weight and body length at birth did not have significant differences in all groups.

Immunotherapy: For the treatment of infants, we used immunoglobulin preparations for intravenous administration of different generations (6 infants received Octagam ®; 5 infants received Pentaglobin ® ; 5 infants received Intraglobin ®). The IVIG was administered from 2 days of life until the development of clinical symptoms of pneumonia. All IVIGs were administered by slow intravenous infusions, three consecutive days, once a day. We used the dose recommended for use in newborns (Formular on the use of drugs of intravenous immunoglobulins in neonatology, 2006/2007; Instructions for the use of intravenous immunoglobulins): the dose of Octagam ® ("OCTAPHARMA AG", Austria) was 500 mg/kg; the dose of Pentaglobin ® ("BIOTEST PHARMA GMBH", Germany") was 250 mg/kg (5 ml/kg); the dose of Intraglobin ® ("BIOTEST PHARMA GMBH", Germany) was 6 - 8 ml /kg per day.

***Survey periods*:** The study of phagocytic activity in group I was performed three times: before IVIG administration, one day after the first infusion, and three days after the end of a three-day course of immunoglobulin treatment in the acute period of pneumonia. In 13 premature newborns with pneumonia from group II, the study of neutrophil phagocytic activity was studied at the onset of pneumonia when the diagnosis was made (5-8 days of life) and in the acute period of the disease on 7-8 days (12-15 days of life). The control group included 20 conditionally healthy premature newborns. In these infants, neutrophil phagocytosis was studied once on day 4-7 of life.

***Determination of phagocytic activity of neutrophils*:** The neutrophil phagocytosis was studied by the method of E. A. Kost and M. I. Stenko (Khaitov, et al, 2009). We incubated 0.1 ml of capillary blood with 0.1 ml of suspension of the control strain Staphylococcus aureus P209 (concentration of Staphylococcus aureus was 10^9 microbial cells in 1 ml of suspension) at 37°C for 30 minutes and 90 minutes. Then we made the preparations on the glass slide, dried them in air, fixed them with 90° ethanol, stained them with azur-eosin dye, and examined them with a light microscope.

We determined the following indicators.

A) Percentage of neutrophils involved in phagocytosis: this is Phagocytic number (PhN). PhN 30 and PhN 90 were calculated after 30 and 90 min of incubation. accordingly.
B) The index of phagocytosis (IPh) is the total number of captured microbes in 50 neutrophils divided by 50, that is, the average number of microbes in one active neutrophil (IPh 30 and IPh 90, after 30 and 90 minutes, respectively).
C) We determined the Index of completion of phagocytosis (ICPh), which reflects the digesting capacity of neutrophils and is calculated by the formula:

$$ICPh = \frac{PhN30/\ PhN90 + IPh30/\ IPh90}{2}.$$

ICPh>1 corresponds to completed phagocytosis, ICPh≤1 corresponds to incomplete phagocytosis.

***Microbiological research*:** The study of the microflora of tracheobronchial aspirates (TBA) to identify the causative agent of infection was carried out using conventional microbiological methods (Labinskaya, 2015).

***Statistics*:** Statistical processing of the results was carried out using the Statistica 7 computer software package. The indicators M±m and percent (%) of the occurrence of the trait in the groups were calculated. A significant difference between the compared indicators was considered indicators $p \leq 0.05$. The percentage of small numbers is given for comparison in groups.

RESULTS AND DISCUSSION

The results of the study of phagocytosis of blood neutrophils in premature newborns are presented in the table. As can be seen from the table, in infants of group I before the introduction of IVIG, there was a significant decrease in the average PhN 30 ($p<0.05$), a tendency to decrease IPh30 and ICPh, which indicated a violation of neutrophil function. Individual analysis revealed that 13 infants of this group (81%) had a decrease in neutrophil phagocytosis by 1 or more indicators before IVIG administration (mainly by 2 indicators: in 9 infants, 56%). The phagocytosis was incomplete in 3 newborns. The phagocytosis rates were at the lower limit of normal in 3 infants. The use of IVIG in infants before the development of pneumonia stimulated the phagocytic activity of the blood after a day. There was a positive dynamics in the activity of phagocytosis: the pool of activated neutrophils involved in phagocytosis increased in 13 out of 16 infants (81%). The phagocytic activity of each activated neutrophil increased, namely, their ability to adhere and absorb microorganisms in 15 infants (94%). In all newborns, this indicator corresponded to the norm. An increase in ICPh was observed in 15 infants (94%), and phagocytosis was complete in 14 newborns of this group (88%). Three days after the IVIG course, phagocytic activity stabilized in 11 of the 16 infants

Table 1. The Phagocytosis of blood neutrophils in premature newborns with VAP and in the control group

Group and period of examination	The Phagocytic number, 30 minutes (%)	The index of phagocytosis, 30 minutes	The Phagocytic number, 90 minutes (%)	The index of phagocytosis, 90 minutes	The Index of completion of phagocytosis
The main group before the introduction of the IVIG	35.6±3.86#	2.56±0.23	23.4±2.71	2.3±0.39	1.6±0.16#
The main group a day after the introduction of the IVIG	47.3±1.88*	3.34±0.35*	21.6±2.38	1.9±0.17	2.06±0.14*
The main group 3 days after the introduction of the IVIG	41.3±3.55	3.37±0.36*	22.2±2.21	1.8±0.2	2.1±0.23
The Comparison group (onset of VAP)	39.1±1.68#	3.4±0.19	21.5±3.61	2.4±0.88	2.24±0.31#
The comparison group (acute period of VAP)	42.4±2.91	2.59±0.13*	23.8±2.43	2.35±0.13	1.34±0.12*#
Control group of conditionally healthy newborns	46.2±2.2	3.1±0.27	23.3±1.74	1.95±0.13	1.83±0.13

Notes: 1. "*" - the differences in indicators significantly differ from the initial value ($p<0.05$).
2. "#" – significant difference between the indicator in the groups of infants with VAP and the indicator in the control group

(69%), and all indicators were within normal limits. However, there was a decrease in phagocytosis by one of the three indicators in 5 newborns in the dynamics of observation at the height of the disease, but they remained within the normal range and were higher than their initial values. For this reason, we can note a positive dynamics of phagocytosis in comparison with the initial state for the same infants, but with insufficient pharmacological effect of immunoglobulin. The phagocytosis of blood neutrophils was completed in all infants after a course of IVIG. The average phagocytosis rates one day after IVIG administration and after the course of immunotherapy did not differ from the control group. There was a decrease in phagocytic activity of neutrophils by PhN 30 ($p<0.05$) in group II infants compared to the norm at the beginning of VAP. ICPh was significantly higher than in the control group ($p<0.05$), which characterized an increase in the digesting ability of cells in response to infection. However, there was a decrease in ICPh ($p<0.05$) and a downward trend in IPh30 in these infants at the height of the disease within a week of its onset. This appears to be due to a decrease in the opsonizing capacity of blood plasma due to the expenditure of IgG, which was accompanied by inhibition of the capturing and digesting ability of neutrophils. In this group of infants in 3 newborns, phagocytosis was incomplete both at the beginning and in the acute period of the disease. ICPh decreased in 7 infants of the comparison group in the acute period compared to the baseline, which could be associated with depletion of the body's energy reserves. At the same time, the average PhN30 increased a week after the onset of the disease, although it did not reach the average of the control group. This was associated with the activation of a larger pool of neutrophils due to an acute infectious process (Khaitov, et al, 2009).

When comparing the phagocytic activity of neutrophils with the condition of infants in this group, it was found that the course of the disease was very severe in 7 newborns with initially low indicators of the phagocytosis. Including 3 infants developed bronchopulmonary dysplasia, two infants later died. We have established a relationship between the features of phagocytic activity of blood neutrophils and the etiological structure of pneumonia in the treatment of infants with immunoglobulins. Thus, when isolating associations of bacteria and one or two types of intracellular microorganisms (M. hominis, U. urealiticum, Ch. trachomatis) from TBA in 7 infants, the PhN30 was significantly lower before IVIG administration and a day after the first dose of the drug and averaged 32.3±2.77% and 45.4±3.06%, respectively, compared to 6 infants with IVIG bacterial pathogens of pneumonia (41.0±2.5% and 55.3±2.61%, respectively; $p<0.05$). Particularly low phagocytosis rates were found in one infant with M. hominis+ U. urealiticum (28% and 38% before IVIG administration and one day after administration, respectively) and in one infant with M. hominis+U. urealiticum+Ch. trachomatis (27.5% and 37.0%, respectively). In these two infants phagocytosis was incomplete. It can be assumed that the intracellular localization of M. hominis, U. urealiticum, Ch. trachomatis in newborns was possible as a result of the initially impaired killer and digesting ability of neutrophils. However, on the contrary, the penetration of these microorganisms into the cell and the production of metabolites by them could disrupt the initially normal function of the phagocyte. The following option cannot be ruled out. The pathogen penetrated into the neutrophil with low phagocytic activity, became involved in cellular metabolism (the use of chemical components of the phagocyte as nutrient substrates, the incorporation of the mycoplasma genome into the genetic apparatus of the phagocyte) and led to the aggravation of the existing disorders (Rakovskaya, 2008). IVIG increased the phagocytic activity of neutrophils in infants with mixed bacterial, mycoplasma and / or Ch. trachomatis infections. This function of neutrophils returned to normal after a course of immunotherapy. Based on our studies, we identified risk factors for the development of VAP in premature newborns with RDS who are on mechanical ventilation. In these infants, in 1-2 days of life, there was a decrease in the phagocytic activity of blood neutrophils by one or two indicators: PhN 30 is less than 30% and / or IPh30 is less than 2.0. The listed risk factors were combined with unfavorable medical anamnesis data (pathological course of pregnancy and childbirth, severe asphyxia of the child at birth), severe condition of the newborn in the early neonatal period, critically low level of IgG in the blood (less than 3 g / l).

Conclusion

The use of the intravenous immunoglobulins in newborns with respiratory distress syndrome and a high risk of developing pneumonia is accompanied by the stimulation of phagocytosis of blood neutrophils against the standard Staphylococcus aureus P209 strain one day after the start of treatment. The immunocorrective effect continued for at least three days after the course of immunotherapy. The relationship between the characteristics of the phagocytic activity of blood neutrophils and the etiological structure of pneumonia in infants who were treated with intravenous immunoglobulins was established. Thus, when intracellular microorganisms (M. hominis, U. urealiticum, Chlamydia trachomatis) were isolated from the respiratory tract, the phagocytic activity of neutrophils was significantly lower before IVIG administration and one day after the first dose of the drug against the indicators in infants with bacterial pneumonia.

The bulleted key points

1. The phagocytic activity of neutrophils in the blood of premature newborns with RDS was lower than in conditionally healthy premature infants.
2. Phagocytic activity of blood neutrophils in premature infants with “ventilator-associated” pneumonia caused by intracellular pathogens Mycoplasma hominis, Ureaplasma urealiticum, Chlamydia trachomatis was lower than in infants with bacterial pneumonia and in conditionally healthy newborns.
3. Intravenous immunoglobulins stimulate the phagocytic activity of blood neutrophils in premature newborns with RDS and " ventilator-associated " pneumonia caused by both intracellular pathogens and bacteria.

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