The Influence of Vancomycin and Linezolid on the Phagocytic Activity of Blood Neutrophils

in vitro Experiments in Preterm Newborns with “Ventilator-Associated” Pneumonia

ABSTRACT

The article is devoted to the study of the influence of vancomycin and linezolid antibiotics on the phagocytic activity of blood neutrophils in vitro studies among 15 premature newborns with ventilator-associated pneumonia.

The stimulating effect of linezolid in 12 infants on neutrophil phagocytosis was established with an increase of phagocytic number, index of phagocytosis and completion index of phagocytosis by 40% or more of the initial values. Vancomycin inhibited phagocytosis of neutrophils in 1 or 2 indicators by 32% or more in six infants. The phagocytic activity of neutrophils in these infants was normalized in the recovery period.

Therefore, the majority of newborns with ventilator-associated pneumonia, linezolid and vancomycin was not affected negatively on the phagocytic activity of blood neutrophils.

KEYWORDS newborns, pneumonia, phagocytosis, neutrophils, vancomycin, linezolid

INTRODUCTION

This study is focused on to determine the features of the effect of vancomycin and linezolid on the phagocytic activity of blood neutrophils in premature newborns with ventilator-associated pneumonia. Currently, the problem of effective treatment of severe infectious and inflammatory diseases, including ventilator-associated pneumonia (VAP) in preterm newborns is still paid much attention. The reason is the fact that the infant mortality rate remains high: 11.2 per 1000 infants born alive and the share of severe infection in the structure of causes of neonatal mortality is about 36%.

Treatment does not always allow to achieve rapid and lasting effect that may be associated with the spread of hospital strains, which have multidrug resistance to antibiotics. In addition, several authors note the suppression of certain immune system functions under the influence of antibiotics, which also reduces the effect of antibacterial treatment.

Over the last years, there has been an increase in the incidence of VAP caused by gram-positive cocci. In view of this, such antibiotics as vancomycin and linezolid are widely used in the complex treatment of newborns. The effect of these drugs on the immune system of newborns particularly on the phagocytosis of blood neutrophils is unknown. At the same time, the study of the effect of antibiotics on the activity of the most important element of anti-infective protection such as neutrophil phagocytosis would allow to determine possible ways to eliminate the negative consequences from their use and increase the effectiveness of treatment.

MATERIAL AND METHODS

The study was included 15 premature newborns with body weight at birth from up 1120 to 2170 g (M = 1784.4 ± 101.7 g), body length from up 39 to 44 cm (M = 31.2 ± 0.53 cm) and gestational age from 28 to 34 weeks.
(M = 41.5 ± 0.97 weeks). All infants from the first day of life were on the hardware artificial ventilation of the lungs about the respiratory disorder syndrome, complicated by the development of “ventilator-associated” pneumonia on the 4–8th day of life.

The study of phagocytic activity of blood neutrophils was carried out in the acute period of pneumonia on day 2–3 of the disease in all infants and in the recovery period on the 3–4 weeks of the disease in 6 infants according to the classical method Kost EA10. To evaluate the phagocytic activity of neutrophils, the phagocytic number (PhN), the phagocytic index (PhI) and the index of completion of phagocytosis (ICPh) were determined after 30 and 90 minutes incubation of the whole blood (37°C) with a suspension of the standard strain Staphylococcus aureus P209. The FH index reflects the percentage of neutrophils involved in phagocytosis. The PhI is a functional indicator that reflects the total number of captured microbes divided into the number of phagocytic cells. The ICPh is a functional indicator that reflects the digestive capacity of phagocytes and it calculates as the sum of the ratios of PhN 30 min/PhN 90 min and PhI 30 min/PhI 90 min, divided by 2. The index ICPh > 1 corresponds to the complete phagocytosis, ICPh ≤ 1-incomplete phagocytosis.

To determine the effect of vancomycin and linezolid on the phagocytic function of neutrophils, one of the antibiotics with a final concentration was additionally added to the reaction complex, which corresponded to the average concentration in the blood: 50 mcg/ml for vancomycin and 30 mcg/ml for linezolid was additionally added to the reaction complex.

All the indices of phagocytosis were calculated and determined by how many percent each index changed when the antibiotic was applied in comparison with the original (i.e., without the antibiotic). The normative indices of the phagocytic activity of the blood neutrophils were obtained in healthy newborns for comparison in the study of Kushnareva et al.11. These indicators are within the following limits: 40 ≤ PhN 30 ≤ 60%; 15 ≤ PhN 90 ≤ 30%, 2 ≤ PhI 30 ≤ 4; 1,5 ≤ PhI 90 ≤ 2,5.

RESULTS

The results of the study of the phagocytic activity of the blood neutrophils are presented in Tables 1–3.

The indices of phagocytosis of blood neutrophils were reduced (in 8 infants - 53%) or were not different from the norm (in 7 newborns) in the acute period of VAP (hereinafter the percentage of small numbers is given for comparison). The reduction of phagocytic activity of neutrophils could be on 1–4 indicators by 25–50% of the norm. As can be seen from Table 1, there was a significant decrease in the mean values of PhN 90 and ICPh in the studied group in comparison with the norm.

The phagocytosis was complete in 11 infants (73%), and it was incomplete in 4 infants (27%). In the latter, this indicated a weak digestive capacity of neutrophils. It is indicated the poor digestive capacity of neutrophils among those with the complete phagocytosis.

As can be seen from Table 1, there was a significant increase in the mean value of ICPh, which pointed at the increase in the digestive capacity of neutrophils in the in vitro studies with linezolid. This indicator increased by 39.8 ± 3.88% compared with the baseline.

As can be seen from Table 2, with an individual analysis of the results, this increase was observed among the majority of infants - 12 (80%). The reduction of phagocytosis after 90 minutes (PhI 90 and PhN 90) also indicated the strengthening of the digestive function. In none of the cases the ICPh was not decreased. It should be noted that this function of neutrophils became complete in the presence of linezolid in 4 newborns with initial incomplete phagocytosis.

The phagocytosis indicators did not change after 30 minutes in 10 infants (67%), but the PhN 30 was increased by 129 ± 78.3% in 5 infants (33%), and the PhI 30 was increased by 40% and 50% compared to the baseline values. This indicated an increase in the absorption capacity of neutrophils by linezolid.

In general, the stimulation of phagocytosis by 1 or more indicators is noted in vitro studies with linezolid in

### Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Index</th>
<th>Initial index</th>
<th>Test with vancomycin</th>
<th>Test with linezolid</th>
<th>Control group**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PhN 30</td>
<td>46.4 ± 3.33</td>
<td>38.9 ± 3.46</td>
<td>47.53 ± 3.53</td>
<td>45.2 ± 2.2</td>
</tr>
<tr>
<td>2</td>
<td>PhI 30</td>
<td>3.38 ± 0.41</td>
<td>3.40 ± 0.44</td>
<td>4.13 ± 0.71</td>
<td>3.1 ± 0.27</td>
</tr>
<tr>
<td>3</td>
<td>PhN 90</td>
<td>30.3 ± 2.88*</td>
<td>23.3 ± 9.50</td>
<td>26.27 ± 2.74</td>
<td>23.3 ± 1.74</td>
</tr>
<tr>
<td>4</td>
<td>PhI 90</td>
<td>3.01 ± 0.66</td>
<td>2.60 ± 0.33</td>
<td>2.31 ± 0.30</td>
<td>1.95 ± 0.13</td>
</tr>
<tr>
<td>5</td>
<td>ICPh</td>
<td>1.46 ± 0.125*</td>
<td>1.88 ± 0.19*</td>
<td>2.14 ± 0.24*</td>
<td>1.83 ± 0.13</td>
</tr>
</tbody>
</table>

*Significant difference from baseline (P < 0.05), †: The tendency to decrease of the index in comparison with the initial value (t = 1.85), *significant difference from the control group (P < 0.05). **For reference, the normative indices of phagocytic activity of neutrophils of blood were obtained in healthy newborns in the study of Kushnareva et al.11
In the clinical studies, it was observed that the phagocytic function was in 6 infants (40%). It is noteworthy that the phagocytic function in the presence of vancomycin indicated a decrease in the absorption capacity of neutrophils (PhI 30) and/or the decrease in the number of active neutrophils (PI 30) in 3 infants among them. Simultaneously, there was an increase in the digestive function of the cells with an increase in ICPh among these infants. However, the total number of microbial cells, which were captured by active neutrophils in these infants, was lower than normal. Therefore, in general, phagocytosis and its sanitizing effect were considered to be reduced, despite the activation of the digestive ability of phagocytes. The decrease of the PhN 30 was by a mean of 39.8 ± 3.88%, while the PhI 30 was 32% and 42% lower than the baseline. The increase of ICPH was by 70.4 ± 9.55% in 8 infants compared to baseline, the decrease was by 24% in only 1 infant. Thus, generally vancomycin contributed to an increase in the digestive capacity of neutrophils. However, the stimulation of this process was not always sufficient. Therefore, with initial incomplete phagocytosis, the phagocytosis became complete in the presence of vancomycin in only 1 infant out of 4 infants. Phagocytosis indices were not changed only among 4 (27%) infants in the presence of vancomycin.

**DISCUSSION**

It is known that the activity of phagocytes, including neutrophils, increases at the beginning and in the acute period of infectious and inflammatory diseases. Stimulation of immune cells by an infectious agent is a normal reaction of the human body to invasion of the pathogen\(^{6,7,12}\). However, an inadequate weak response to the introduction of the pathogen into the infant’s body or even the absence of such a reaction is observed in preterm infants, especially those with a very low birth weight. It may be linked to the morphofunctional immaturity of the infant’s immune system. In addition, low activity of phagocytes in infection may be a consequence of a deficiency in the power capacity of the phagocytic cell in conditions of hypoxia, hypotrophy, and other factors that disrupt the process of normal early adaptation of the newborn\(^{14,15}\).

There is a rapid depletion of the reserve capacity of the phagocyte in cases of massive bacterial contamination and colonization in infants with VAP\(^{15}\).

The effect of drugs, including antibiotics, on neutrophils depends on the functional state of the biological cell such as from the stock and activity of the enzymes involved in the phagocytic process and the cell’s function in general\(^{6,7,12}\). It is impossible to exclude the chemical structure and chemical properties of antibiotics on neutrophil functions during their interaction, as well as the effect on the organelles and the energy system of the cell.\(^{6,12,13}\)

The important question is the following: “How to eliminate or prevent the negative influence of drugs (antibiotics) on the phagocytic activity of neutrophils?” It is known that the increase of the neutrophils’ phagocytic activity occurs when the opsonizing properties of blood plasma are enhanced. The main opsonins are immunoglobulins\(^{10,12,16}\). In the clinical studies, it was shown that the treatment of newborns with immunoglobulins significantly increased the phagocytic activity of blood neutrophils\(^{11,15}\). It can be assumed that the combination of substitution immunotherapy with antibiotics will allow to eliminate or reduce the negative effect of the last one on the phagocytic function of neutrophils.

**Table 2** Changes of the indices of blood neutrophil phagocytosis in the test system with linezolid in premature newborns with ventilator-associated pneumonia.

<table>
<thead>
<tr>
<th>No.</th>
<th>Index</th>
<th>N change in index</th>
<th>Index increase</th>
<th>Index decrease</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PhN 30</td>
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<td>87</td>
<td>2</td>
<td>13</td>
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<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PhI 30</td>
<td>10</td>
<td>67</td>
<td>5</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>PhN 90</td>
<td>10</td>
<td>67</td>
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<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PhI 90</td>
<td>6</td>
<td>40</td>
<td>2</td>
<td>13</td>
<td>7</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ICPH</td>
<td>3</td>
<td>20</td>
<td>12</td>
<td>80</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(n\): number of infants. The percentage of small numbers given for comparison.

**Table 3** Changes in phagocytosis of blood neutrophils in the test system with vancomycin in premature newborns with ventilator-associated pneumonia.

<table>
<thead>
<tr>
<th>No.</th>
<th>Index</th>
<th>N change in index</th>
<th>Index increase</th>
<th>Index decrease</th>
<th>n</th>
<th>%</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PhN 30</td>
<td>10</td>
<td>67</td>
<td>0</td>
<td>0</td>
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<td>33</td>
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<td>PhI 30</td>
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<td>53</td>
<td>5</td>
<td>33</td>
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<td>13</td>
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</tr>
<tr>
<td>3</td>
<td>PhN 90</td>
<td>10</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>33</td>
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</tr>
<tr>
<td>4</td>
<td>PhI 90</td>
<td>9</td>
<td>60</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>27</td>
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</tr>
<tr>
<td>5</td>
<td>ICPH</td>
<td>4</td>
<td>27</td>
<td>10</td>
<td>67</td>
<td>1</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

\(n\): number of infants. The percentage of small numbers given for comparison.
CONCLUSION

Phagocytic activity of the blood neutrophils was reduced in half of the examined newborn infants and was not different from the norm of the other half in the acute period of VAP. Phagocytosis was incomplete in 27% of infants.

The average therapeutic concentration of linezolid stimulated the phagocytosis of blood neutrophils in vitro studies among 80% of premature newborns, mainly it was due to the increased digestive capacity of phagocytes, in the acute period of VAP. Phagocytosis was not changed in 20% of infants. Phagocytosis was completed in all infants in the presence of linezolid.

Changes in the phagocytic activity of blood neutrophils were multidirectional in the presence of the average therapeutic concentration of vancomycin in vitro studies among premature newborns in the acute VAP period. The intensification of phagocytic activity was in 33% of newborns, and the suppression of phagocytic function was in 40% of newborns. Phagocytosis was not changed in 27% of infants and it was incomplete in 20% of infants in the presence of vancomycin.

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CONFLICT OF INTERESTS

None.

REFERENCES