Study of Toxin Transmission Technique of Micrococcus Derived from the Pneumocytes Culture of Rat

Lotfollah Mahdavi

Ph.D. in Biology (Animal physiology), Shahid beheshti Faculty, Farhangian University

**ABSTRACT**

**Background:** Staphylococcus is from the Micrococaceae family. These bacteria are considered Hospital-acquired infection as *Bacillus coli*. Staphylococcus is consistently associated with the body surfaces of animals. These bacteria can cause more than 75 to 80 percent of purulent infection. Approximately 5 percent of people die from the toxin produced by the bacteria. Staphylococci have a good compatibility by parasitic life and are regarded as a successful pathogenic microorganism in the host. Only when the mucosal barrier is damaged, these organisms have invaded the submucosa tissue. The capacity of a bacterium to cause disease reflects its relative pathogenicity. By the invasion of bacteria into the blood, provides the possibility of acute endocarditis.

**Objective:** In this study have been prepared SEB toxin and ovalbumin and this material transmitted through the alveolar wall (Type II pneumocytes) to the culture medium. **Results:** The results are as follows: - These molecules (SEB and ovalbumin) pass through alveolar–capillary barrier in the culture medium. - Rate of passage of SEB decreases over time at 37 °C. SEB passes through Facilitated diffusion -SEB can have an effect on cell growth and mitogenesis and avoids passing by increasing cell volume. It seems that SEB can be effective on the disabled migration paths (the space between cells). **Conclusion:** This material passing has an inverse relation to with their molecular size and mass.

**INTRODUCTION**

*Staphylococcus* (from the Greek: σταφυλή, *staphylē*, "grape" and κόκκος, *coccus*, "granule") is a genus of Gram-positive bacteria. They appear round (cocci), and form in grape-like clusters. *Staphylococcus* species can be differentiated from other aerobic and facultative anaerobic and grow on the nutrient medium and make different pigments such as gold, yellow and white.

The word of Aureus means the golden color that the pigment of these bacteria is golden yellow in the culture medium [14]. The species of Staphylococcus include 19 types. *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Enterotoxin B is produced from strain S-6 and it is a Single-strand binding protein [5]. The molecular weight is about 30 KD.

The number of amino acids is 239 and the Type and sequence has known [18]. Therefore, this study will respond to the following questions: could be SEB toxins enter the body via inhalation? Is there defense mechanism against this substance (SEB) in the alveoli of the lungs? In this study have been prepared SEB toxin and ovalbumin and we used an in *vitro tissue culture* methodology. In this study has been examined the transition of SEB toxin and ovalbumin through the alveolar wall (Type II pneumocytes) and the results were analyzed [1].

2. Methodology:
   A) *Separation* technique of pneumocytes II
   B) Method of culture of pneumocystis II
   C) Study of substances passing across the cell monolayer
A) Separation technique of pneumocytes II:

The Lung tissue contains many cells that each has different tasks. To Separation of pneumocytes II was performed the following steps: First the Rat was anesthetized by chloroform and then intracardiac perfusion was done by 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.4 for partial fixation of the lungs. The combined lavages were centrifuged at 4°C.

Tissues were diced into proper sized pieces (1 mm 3) and fixed by immersion in 3% buffered glutaraldehyde (cacodylate buffer, pH 7.2) for 4 h at 4°C. Trypsin enzyme was prepared as two percent HBSS buffer solution and added to the tissue components to ratio about thirty times in 37°C and was stirred by a magnetic stirrer for 20 minutes to the trypsin enzyme could break the cell adhesion to the proper temperature.

The final solution contains different cells and lung tissue has passed through the following filters respectively:

- Plastic filter with a pore diameter of 120 microns (to isolate from tissue blocks of cells)
- Filter whatman, with a pore diameter of 20 to 25 microns (to isolate large cell and multi-cell components)
- Filter whatman, with a pore diameter of 165 microns (to isolate large cell from the pneumocytes II)

Finally, the filtered liquid, Suspensions are and particles smaller than it. Then, the centrifuge was performed for 5 minutes at 2700 rpm and precipitated the pneumocytes II at the bottom of sediment solution that contain other compounds as fetal calf serum (FCS) to 10 percent and antibiotics (Penicillin-Streptomycin) to one percent.

B) Method of culture of pneumocytes II:

Initially, the culture medium DMEM (Dulbecco’s Modified Eagle Medium) was prepared then added pneumocystis II to the culture medium and it has composed of a cell suspension. Cell suspension is poured into the filter with a diameter of 4.7 cm and a pore diameter of 0.45 microns and attach to the cells and permeable membrane (filter).

After 48 hours, pneumocytes II cells Begin to flatten and reduced the thickness. This process continues until the sixth day and increased cell surface area and Come together and formed a cellular alveolar [1]. substances passing across the cell monolayer was studied. Initially, were taken amounted to 0.5 mL from the top and bottom of the culture medium to as a sample in the beginning (time zero). Therefore, the sampling was performed at intervals of an hour.

The samples were analyzed by HPLC and has been studied parameters such as concentration of absorbing material (transferred) FA, passage rate constant (K) and flow velocity (flow) Flux.

3. Result:

The results of constant flow rate (K) with concentration of substances passing across the cell monolayer versus time (FA (cum/t)):

Figure 1 shows the comparison of SEB passing rate constant in the temperatures of 4 and 37 ° C and this result was obtained: In the primary majority of time, increasing the amount of SEB is consistent in both the temperature But transmission of SEB decreased in the 37 ° C gradually(Table1)

Figure2 shows the ovalbumin passing rate constant in the temperatures of 4 and 37 ° C and It indicates this fact:

- The ovalbumin passing across the cell monolayer as uniformly and linear.
- In the 4 ° C, The transmission of ovalbumin is less than transmission of this substance at 37 ° C.

Accountancy:

Calculation of Cumulative Fraction Absorbed=FA

\[ FA = \sum \frac{C}{C} \]

The Cumulative Fraction Absorbed for different samples has offered in two different temperatures, 37 and 4 ° C in the following tables

Table 1: The Rate of FA of SEB in 4 and 37°C.

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
<th>360</th>
<th>420</th>
<th>480</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA 37</td>
<td>1.8</td>
<td>4.2</td>
<td>6.1</td>
<td>7.6</td>
<td>9.0</td>
<td>9.8</td>
<td>10.5</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>FA 4</td>
<td>1.6</td>
<td>3.4</td>
<td>5.0</td>
<td>6.4</td>
<td>8.2</td>
<td>9.9</td>
<td>11.4</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Table 2: The Rate of Ovalbumin in 4 and 37°C.

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
<th>360</th>
<th>420</th>
<th>480</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA 37</td>
<td>1.3</td>
<td>2.9</td>
<td>4.3</td>
<td>5.5</td>
<td>7.3</td>
<td>8.6</td>
<td>10.1</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>FA 4</td>
<td>1.0</td>
<td>2.3</td>
<td>3.5</td>
<td>4.5</td>
<td>5.7</td>
<td>6.8</td>
<td>8.1</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Calculation of passing rate constant (K)

Table 3: The Rate of FA /t (k) of SEB and Ovalbumin in 4 and 37°C.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>SEB</th>
<th>Ovalbumin (Ou)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K rat in 1 37°C</td>
<td>3.72 .10</td>
<td>2.4 .10</td>
</tr>
<tr>
<td>K rat in 1 4°C</td>
<td>2.6 .10</td>
<td>1.8 .10</td>
</tr>
</tbody>
</table>

Calculation of Flux rate constant (K) (mol/ min. cm) by Concentration versus time

Table 4: The Rate of Flux t J mol/ mim.cm SEB and Ovalbumin in 4 and 37°C.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>SEB</th>
<th>Ovalbumin (Ou)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J (37°C)</td>
<td>2.19 .10</td>
<td>2.41 .10</td>
</tr>
<tr>
<td>J (4°C)</td>
<td>2.69 .10</td>
<td>1.89 .10</td>
</tr>
</tbody>
</table>

4. Discussion:

According to the diagrams of constant flow rate of SEB (Figure 1), this result has concluded that the metabolic activity of cells monolayer stops at a temperature of 4°C. Therefore, SEB passes through Facilitated diffusion (Figure 1, transmission path 1 and 2) Because in these temperatures are stopped any transaction is done with the consumption of energy ATP such as active transport and endocytosis; Also, it would not be a simple diffusion in the plasmatic membrane because SEB such as protein compound can not dissolved in lipids o membranes and transferred; hence , SEB passes through Facilitated diffusion in 4°C (figure3).

Transmission of SEB decreased in the 37°C gradually (figure2) and Diagrams are the similar process of facilitated diffusion and active transport [3,6]; On the other hand, the different proteins (in the tight junctions) that are connected the two adjacent membranes, with the presence of SEB, reacts and are contracted and prevent SEB passing. Also, Ions like Ca are causing contraction of the protein strands; apparently with the presence of SEB showed positive effects and are closer to adjacent cell membranes together and decreased transmission of substrate [12]. The SEB is known today as a cell growth factor and the effect it has metaplasia.

It seems that SEB causes growth and increased volume of epithelial cells (pneumocytes II) in the 37°C. Gradually, The cell monolayer is prevented gradually passing of this substance and show the passive mode and its transfer is done as uniformly.

The only passage way for the ovalbumin will be Intracellular path (figure1, transition path 2) because the passing of the material at a temperature of 4°C is almost the same as the uniform passing at the temperature of 37°C. Because in temperatures 4°C are stopped any transaction is done with the consumption of energy ATP such as active transport and endocytosis and it would not be a simple diffusion in the plasmatic membrane because ovalbumin such as protein compound can not dissolved in lipids of membranes and transferred hence , ovalbumin passes through Facilitated diffusion in 4°C [6]; In addition, the amount of absorbed ovalbumin at 4°C (transferred) is less than SEB; Because the mass and molecular size of ovalbumin was more than SEB and passes from the cell monolayer slowly (Table1,2). These molecules (SEB and ovalbumin) pass through alveolar–capillary barrier in Vitro.

It appears that at low temperature (4°C) with decreasing molecular movement, the transfer is slow and requires much more time to cross the alveolar barrier [2,14]. These proteins (SEB and ovalbumin) have not destructive effect on culture. In this case, the transition process began uniformly and rapidly increasing concentration; whereas, such a situation has not been observed in the results and graphs. The mass and molecules size are effective in the transition from monolayer; if molecular Mass and size is higher, the transition will be slower (Tables1, 2, 3).
Fig. 1: Transport routes in epithelial FA cum / time (SEB) Albert. B (1994).

Fig. 2: The Rate of Flux J mol/ mim.cm in 4 and 37° C.

Fig. 3: The Rate of Flux J mol/ mim.cm Ovalbumin in 4 and 37° C.

REFERENCE


