STUDY OF INHIBITORY EFFECT OF GLUTATHIONE-REDUCED, 
L-CYSTEINE AND ZINC IONS ON NEURAMINIDASE N2 ACTIVITY 
IN INFLUENZA VIRUS TYPE A

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The increased interest in studying the inhibitors of orthomyxoviruses and some paramyxoviruses is due to the fact that these inhibitors can be applied as chemotherapeutic drugs by means of suppression of the enzyme neuraminidase, too.

Sulfhydryl preparations inhibit neuraminidase activity of influenza viruses A(Japan) 305/72 (H2N2) and PR8 (HON1) at concentrations of 10^-3M (9).

There are literature data that some structurally different compounds such as phenyl-glioxane, phenyl-oxaminic acid, β-phenyl-L-mercapto-acrylic acid, etc. exert also an inhibitory action (2). Edmond et al. (1966) proved that derivatives of both phenyl-glioxane and of oxaminic acid inhibited neuraminidase activity of influenza virus A(England) 1/61 (H2N2). N-phenyloxaminic acid is proved to be the most effective inhibitor of the whole group of compounds. It inhibits the enzyme activity by 50 per cent at concentration between 2 and 5.10^-4M. In order to create specific neuraminidase inhibitors some investigators synthetize compounds which are derivatives of N-acetylneuraminic and of desoxyneuraminic acids specifically suppressing enzyme activity (2).

2-desoxy-2,3-dihydro-N-trifluoroacetyl neuraminic acid (FANA) possesses the strongest activity. It inhibits competitively enzyme activity of strains A/Me 1/35 (H0N1) with $K_m = 7.9$ at concentration of inhibitor of 10^-7M (2).

The aim of the present work is to study the influence of some inhibitors on neuraminidase N2 activity.

Material and Methods

Investigations were carried out of standard influenza virus type A strains with neuraminidase, serotype 2: A(Singapore 1/57 (H2N2); A(Hong Kong) 1/68(H3N2); A(Victoria) 35/72 (H3N2); A(Texas) 1/77 (H3N2); as well as of some influenza virus strains isolated in our country: A(Sofia) 1/57 (H2N2); A(Sofia) 142/69 (H3N2) (both provided kindly by Dr. Kotzeva from the Research Institute of Infectious and Parasitic Diseases, Sofia); A(Varna) 123/76 (H3N2), and A(Varna) 31/7/84 (H3N2) isolated in the Department of Microbiology and Virology of the Higher Institute of Medicine, Varna. The strains studied were selected with a view to neuraminidase incorporated in them.

Virus strains having passed through 2 passages on developing chick embryos with infectious titre 6.5 — 7.6 lg EID 50/0.2 cm^3 were used in our study.

The working inhibitor concentration was 0.1M t.c. Preliminarily, the following concentrations were selected: from 0.110^-3M till 0.1M. At the last concentration enzyme inhibition was maximal.
Table 1
Changes of N2 activity influenced by glutathione, L-cysteine and zinc ions

<table>
<thead>
<tr>
<th>No</th>
<th>Influenza viral strains</th>
<th>Inhibitor conc.</th>
<th>Controls x ± %</th>
<th>Glutathione x ± %</th>
<th>L-cysteine x ± %</th>
<th>Zinc ions x ± %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A(Singapore 1/57) (H2N2)</td>
<td>0.1M</td>
<td>23.19 ± 0.05</td>
<td>39.85</td>
<td>4.54 ± 0.04</td>
<td>13.45 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>A(Sofia 1/57) (H2N2)</td>
<td>0.1M</td>
<td>24.41 ± 0.02</td>
<td>40.02</td>
<td>6.22 ± 0.11</td>
<td>16.60 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>A(Hong Kong 1/68) (H3N2)</td>
<td>0.1M</td>
<td>23.04 ± 0.08</td>
<td>43.66</td>
<td>3.02 ± 0.08</td>
<td>10.47 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>A(Victoria 35/72) (H3N2)</td>
<td>0.1M</td>
<td>25.69 ± 0.05</td>
<td>41.33</td>
<td>6.10 ± 0.14</td>
<td>15.87 ± 0.20</td>
</tr>
<tr>
<td>2.</td>
<td>A(Sofia 142/69) (H3N2)</td>
<td>0.1M</td>
<td>25.76 ± 0.02</td>
<td>42.05</td>
<td>3.98 ± 0.05</td>
<td>19.40 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>A(Varna 123/76) (H3N2)</td>
<td>0.1M</td>
<td>24.40 ± 0.09</td>
<td>41.33</td>
<td>6.10 ± 0.14</td>
<td>15.87 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>A(Texas 1/77) (H3N2)</td>
<td>0.1M</td>
<td>25.40 ± 0.04</td>
<td>42.05</td>
<td>3.98 ± 0.05</td>
<td>19.40 ± 0.01</td>
</tr>
<tr>
<td>3.</td>
<td>A(Varna 31/784) (H3N2)</td>
<td>0.1M</td>
<td>25.76 ± 0.02</td>
<td>42.05</td>
<td>3.98 ± 0.05</td>
<td>19.40 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>A(Varna 31/784) (H3N2)</td>
<td>0.1M</td>
<td>31.46 ± 1.53</td>
<td>39.99</td>
<td>6.50 ± 0.23</td>
<td>20.49 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>A(Texas 1/77) (H3N2)</td>
<td>0.1M</td>
<td>23.52 ± 0.07</td>
<td>65.39</td>
<td>4.77 ± 0.04</td>
<td>60.24</td>
</tr>
<tr>
<td></td>
<td>A(Varna 31/784) (H3N2)</td>
<td>0.1M</td>
<td>24.52 ± 0.05</td>
<td>65.39</td>
<td>4.77 ± 0.04</td>
<td>60.24</td>
</tr>
</tbody>
</table>
The rest enzyme activity after inhibitory action was determined after Aminoff's method (1961). The results were calculated in mkg protein and then statistically processed by the methods of variation analysis for \( p < 0.05 \) (1, 3).

Results and Discussion

Our data are summarized on table 1 and fig. 1. One can see that glutathione inhibits relatively less standard strains as compared with local ones isolated by us excepting the strains A(Singapore) 1/57 (H2N2) and A(Sofia) 1/57 (H2N2) where inhibition is equally manifested (60.15 per cent and 59.98 per cent, respectively).

![Fig. 1. Changes of neuraminidase N2 activity influenced by glutathione-reduced, L-cysteine and zinc ions](image)

Legend:  
- □ standard strains  
- □ local strains

There are analogous results when L-cysteine action is concerned. Local strains isolated by us are inhibited to a greater extent as compared with the standard ones: A(Singapore) 1/57 (H2N2) - 40.43 per cent; A(Sofia) 1/57 (H2N2) - 50.05 per cent; A(Hong Kong) 1/68 (H3N2) - 42.62 per cent; A(Sofia) 142/69 (H3N2) - 50.85 per cent; A(Victoria) 35/72 (H3N2) - 44.96 per cent; A(Varna) 123/76 (H3N2) - 47.56 per cent; A(Texas) 1/77 (H3N2) - 39.96 per cent, and A(Varna) 31/84 (H3N2) - 45.64 per cent.

Zinc ions exert the same effect on strains A(Victoria) 35/72 (H3N2) (25.51 per cent inhibition) and A(Varna) 123/76 (H3N2) (34.87 per cent). Standard strains A(Singapore) 1/57 (H2N2), A(Hong Kong) 1/68 (H3N2) and A(Texas) 1/77 (H3N2) are inhibited to a greater extent in comparison with local ones isolated by us under zinc ion influence by 1.5 per cent, 7.62 per cent, and 7.21 per cent, respectively.

It is evident that glutathione is the best inhibitor among the three ones at these concentrations selected by us. Most probably, it is due to its structure inhibiting competitively this en-
zyme. Our investigations argue for an approximately equivalent inhibitory action of L-cysteine which is possibly due to its active sulfhydryl group – SH-group.

There are rather great variations when zinc ions are concerned. This probably confirms the statement that neuraminidase type 2 (N2) is heterogenous and divided into subtypes (4, 5).

We can conclude that at concentration of 0.1M glutathione, L-cysteine, and zinc ions inhibit neuraminidase N2. Glutathione and L-cysteine do it to a greater extent when local strains are concerned in comparison with standard ones. Zinc ions inhibit neuraminidase N2 of standard strains to a greater extent than that of local ones excepting A(Varna) 123/76 (H3N2).

REFERENCES