Specific Inactivated Vaccines And Immunomodulators As Means Of Urgent Control Of Acute Viral Infection And Recurrent Chronic Viral Diseases

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Abstract. In order to enhance the immunogenicity of a vaccine, it should be applied in combination with immunomodulators. In our experimental and clinical studies of tick-borne encephalitis (TBE) and herpes simplex virus types 1 and -2 (HSV-1, -2) infection, the following immunomodulators manufactured in Russia have been used: ridostin, hyaferon, polyribonate and thymostine. The results of an experimental study on TBE in mice showed a reliable protection of the mice inoculated with ridostin. In the breedless mice infected with TBE virus (10 LD50), a reliable increase of protection and lifespan were observed. Combined use of ridostin with the inactivated anti-TBE vaccine has been recommended for prompt control in loci of infection.

At chronic herpetic infection, the Vitaherpavac inactivated vaccine was administered in combination with the hyaferon immunomodulator to 28 patients in order to prevent recurrences of the disease. The suggested combined therapy schedule led to an anti-recurrence effect in 96% cases of frequently recurrent genital herpases. Administering of the vaccine alone gave a similar effect in 84% cases. This difference is statistically highly reliable. The Vitaherpavac vaccine was recommended by Russia’s Ministry of Health for prophylactics of HSV-1 and -2 infection recurrences in 2010.

Thus, our study of TBE and chronic HSV-1 and -2 infections indicates to the prospect of combined application of vaccines and immunomodulators for prompt control of acute infections as well as prophylactics of chronic diseases recurrences.

Keywords: herpes simplex virus, tick -borne encephalitis virus, recurrence, viremia, vaccine, immunomodulator, cytokine, cell-mediated immunity, antibodies

1. Introduction

Over the last years, researches have become more interested in the use of immunomodulators for strengthening of overall resistance of the organism to viral infections. Immunomodulators have one thing in common, they can be applied to certain targets on immunocompetent cells. Clinically selected immunomodulators can be used for a range of viral diseases as they activate the immune system thereby increasing the resistance to those infections.

Besides, immunomodulators can be applied in combination with inactivated vaccines in therapy and control of acute and persistent viral infections by increasing the vaccine’s immunogenicity and preventing secondary immunodeficiencies at the same time [1]

A low immunogenicity of inactivated vaccines is likely to be due to insufficient amounts of specific antigens. Thus, the use of prophylactic and especially therapeutic vaccines is based on multiple injections in order to create a lasting antiviral immunity [1]. The other approach for enhancing the vaccine’s immunogenicity is combining the vaccination with administering adjuvants and / or immunomodulators [2]

The urgent specific prophylactics of viral infections suggests the application of either etiotropic antiviral preparations or vaccinal preparations combined with immunomodulators. In this regard, a study on the efficacy of a number of home-made immunomodulators applied in combination with the vaccines represents an interest for urgent control of experimental arboviral infections and tro control of recurrences of chronic herpetic infection.

2. Replace this text with your section Heading
Materials and methods

Viruses. Tick borne encephalitis virus (TBEV), the Sophin strain, the infecting dose 10 LD50/02 ml for subcutaneous injection, provided by the viruses museum of D. Ivanovsky Institute.

Animals. BALB/c, CBA (10 to 12 g) and breedless (6 to 7 g) mice were obtained from the academy of medical science nursery. All the manipulations with the animals were conducted strictly according to ‘The rules and regulations of work with experimental animals’.

Vaccines. The Encevir anti-TBE vaccine is a cultured purified concentrated inactivated adsorbed liquid vaccine. Injection dose: 0.2 ml, intraperitoneally, 3 days prior to infecting. The humoral immune response was assessed by the conventional neutralization test.

The inactivated vaccine against herpes simplex viruses types 1 and 2 were manufactured in Moscow, Russia.

Immunostimulators. Ridostin (Vector Medica enterprise), hyaferon (VitaFarma company), polyribonate and peptidoglu-cane-100 (PG-100, Vector enterprise, Berdsk, Russia), thymo-sine (Farmadone enterprise). All the listed preparations were manufactured in Russia. All the immunostimulators were administered to the animals according to the manufacturers’ recommendations, the doses calculated per 1 kg of body weight.

Activity of preparations. The immunostimulating activity of the above drugs was studied on the experimental TBE model. The activity was measured (i) by the level of protection of the animals vaccinated in combination with an immuno-
modulator versus the animals vaccinated with a vaccine alone; (ii) by the average lifespan (ALS) of the animals calculated conventionally [3] (iii) by the level of virus neutralizing antibodies (VNA) [1] (iv) by stimulation of the cell-mediated immune response measured by the activity of T-lymphocytes in vivo [4].

Statistics processing. For the statistic processing of the experimental results, Fischer criterion and the X2 criterion were applied. The difference between values was taken as sensible at p < 0.05.

Results and discussion

Table 1. The effect of the immunomodulators in various schedules of their administering on the surviving capacity and average lifespan of the mice infected with TBE

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Single dose of preparation and administration route</th>
<th>Protection, %</th>
<th>Average lifespan, days</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ridostine</td>
<td>50 mg/mouse, subcutaneous</td>
<td>-24</td>
<td>60</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-4</td>
<td>60</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+4</td>
<td>30</td>
<td>11.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+48</td>
<td>0</td>
<td>10.6</td>
</tr>
<tr>
<td>Peptidoglycane (PG-160)</td>
<td>0.5 mg/mouse, subcutaneous</td>
<td>-24</td>
<td>10</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-4</td>
<td>40</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+4+24</td>
<td>35</td>
<td>11.5</td>
</tr>
<tr>
<td>Polyribonate</td>
<td>0.5 mg/mouse, subcutaneous</td>
<td>-24</td>
<td>10</td>
<td>10.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3</td>
<td>20</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+3+24+48+72</td>
<td>20</td>
<td>11.4</td>
</tr>
<tr>
<td>Thymosine</td>
<td>4 ug/mouse, subcutaneous</td>
<td>-3</td>
<td>-</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+3</td>
<td>15</td>
<td>11.3</td>
</tr>
<tr>
<td>TBE virus control: subcutaneous</td>
<td>10 LD50/0.2 ml</td>
<td>Survival</td>
<td>5-10%</td>
<td>Average lifespan</td>
</tr>
</tbody>
</table>

The TBE model. The antiviral activity of the tested drugs was studied on breedless mice challenged with 10 LD50 of TBEV. The results presented in Table 1 show the reliability of protection as a result of vaccination with various administration schedules of these drugs: ridostin, polyribonate and peptidoglycane. It is remarkable that the statistically reliable percentage of protection was obtained after applying ridostin by the prophylactic administering schedule (-24 hrs, -4 hrs), and after applying polyribonate and PG-160 by the therapeutic schedule. Thymosine proved ineffective in this experiment.

Table 2. The protection percentage and average lifespan of the naïve and immune CBA and BALB/c mice at a single inoculation of the immunomodulators

<table>
<thead>
<tr>
<th>Preparation, dose, administration route</th>
<th>Protection %</th>
<th>Average lifespan, days</th>
<th>Preparation, dose, administration route</th>
<th>Protection %</th>
<th>Average lifespan, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymosine 4 ug/mouse</td>
<td>-</td>
<td>96</td>
<td>Vaccine+ Thymosine</td>
<td>40</td>
<td>10.3</td>
</tr>
<tr>
<td>Thymosine 50 mg/mouse</td>
<td>50</td>
<td>11.0</td>
<td>Vaccine+ Thymosine</td>
<td>40</td>
<td>10.3</td>
</tr>
<tr>
<td>Polyrribonate 0.5 mg/mouse</td>
<td>20</td>
<td>9.7</td>
<td>Vaccine+ Polyrribonate</td>
<td>55</td>
<td>12.0</td>
</tr>
<tr>
<td>PG-160</td>
<td>20</td>
<td>9.8</td>
<td>Vaccine+ PG-160</td>
<td>40</td>
<td>13.8</td>
</tr>
<tr>
<td>TBEV control, 10 LD50</td>
<td>0</td>
<td>92</td>
<td>Vaccine (undiluted)</td>
<td>30</td>
<td>10.4</td>
</tr>
</tbody>
</table>

Thus, the conducted study has detected a number of immunomodulators protective for experimental TBEV infection. The obtained results confirm the importance of a further research on antiviral drugs that possess immunomodulating properties, on arboviral models.

A comparative study of the immunomodulating drugs in the intact and vaccinated animals showed an enhanced anti-
viral activity when administered in combination with the vaccines, the fact that confirms the immunostimulating properties of the drugs studied. The stimulating activity of ridostin on the humoral immune response together with the previous data on its activity in regard to cytotoxic lymphocytes against the alphavirus infection [6] allow to recommend this drug for further clinical trials in TBE, given that this infection is endemic to Russia.

Chronic herpetic infection. A combined application of the Vitaherpavac vaccine without immunomodulators showed that six months 19 patients with chronic genital herpes out of 61 (31.1%) developed a marked improvement (a 3-time prolongation of the inter-recurrence period).

35 patients (57.3%) had a 1.5 to 2 times increased remission and only in 7 patients (11.6%) the therapeutic effect was low. None of the patients developed a post-vaccination reaction over the whole observation period (immediately, 10 days and 6 months post vaccination).

Considering our previous data on a decrease of a number of immunity markers in patients with recurrent genital herpes, we carried out a comparative clinic-immunological surveillance of two groups of patients. One group (28 patients) received the vaccine combined with hyaferon, the other group (25 patients) received the vaccine alone.

In order not to provoke a recurrence, we recommend the following therapy schedule for a remission period:
- hyaferon, 1 suppository twice a day, for 10 days;
- the Vitaherpavac vaccine is administered on day 8 of the hyaferon therapy and further with a 7- to 10-days interval

The efficacy of the therapy was assessed by the decrease of the intensity and duration of the herpes simplex clinical features at recurrences, by the shortening of the prodromal period and pain syndrome, by the prolongation of the inter-recurrence period to 6 months after the onset of therapy.

As Table 3 shows, the combined therapy has been more efficient compared to the monotherapy. In addition, the suggested schedule of combined therapy against the backdrop of vaccination allows to increase the efficacy of the treatment and to get an apparent positive effect in over 95% of cases.

Table 3. The results of a combined treatment of recurrent genital herpes patients with the vitaherpavac vaccine and the hyaferon immunomodulator

<table>
<thead>
<tr>
<th>Therapy schedule</th>
<th>Marked improvement</th>
<th>Improvement</th>
<th>No effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abs.</td>
<td>%</td>
<td>Abs.</td>
</tr>
<tr>
<td>Vitaherpavac + hyaferon (n=28)</td>
<td>21</td>
<td>39.3*</td>
<td>6</td>
</tr>
<tr>
<td>Vitaherpavac (n=25)</td>
<td>7</td>
<td>28.0</td>
<td>14</td>
</tr>
</tbody>
</table>

* The reliable difference of the results of the vaccine and hyaferon combined application compared with application of the vaccine alone

Therefore, the conducted study has shown the advantage of a combined application of vitaherpavac with hyaferon over a convenient vaccinotherapy. The results of this study together with our previously published results [1-6] allow to detect a number of prospective immunomodulators: ridostin, polyribonate, peptidoglycane PG-100, which can be used at exceptional arboviral infections for control and even therapy. At the same time, other well known immunomodulators (e.g. thymosine) lack this quality. The applicability of the selected immunostimulators combined with the specific vaccines increased the protection against the experimental arboviral infections by 10 to 15% (ridostin, polyribonate, PG-160). It should be also marked that a combined application of the vaccine with thymosine at the experimental TBE infection in mice allowed to increase the protection to 40-50%.

A preliminary examination in experiments will allow to obtain an information on a possibility of using immunostimulators in combination with vaccines in the clinical and immunological practice. A special attention should be paid to the fact that commercial vaccines have been in use for arboviral infections (TBE, YF) for a few decades. Recently, a new generation of immunomodulators came to light, immunophane, polyvidonium, roneoleukin and others [7] which are subject to similar experimental trials. Out of the studied homemade immunostimulators, ridostin has been shown to possess the highest activity and the widest range. It therefore can be recommended for clinical trials in TBE-infected persons in loci of infection.

Conclusion

At the chronic herpetic infections, we have shown the advantage of a combined application of the vitaherpavac vaccine and the hyaferon immunomodulator by comparison with a monotherapy. Thus, our studies on TBE and chronic herpetic infection testify for the prospect of combined application of vaccines and immunomodulators in prompt control of acute infections and in prophylactics of chronic diseases recurrences.

Acknowledgments

We are grateful to the Ministry of Health of Russia and the VitaFarma medical company for funding our research.

Conflict of Interest

We have had no conflict of interests in conducting experiments or writing the text for publication.

References:


