Медицинская иммунология
2021, Т. 23, № 4,
стр. 915-920

Краткие сообщения
Short communications

Medical Immunology (Russia)/
Meditsinskaya Immunologiya
2021, Vol. 23, № 4, pp. 915-920

СОВЕРШЕНСТВОВАНИЕ СПЕЦИФИЧЕСКОЙ ПРОФИЛАКТИКИ ХОЛЕРЫ С ПОМОЩЬЮ ИММУНОМОДУЛЯТОРОВ

Филиппенко А.В., Омельченко Н.Д., Пасюкова Н.И., Труфанова А.А., Иванова И.А.

ФКУЗ «Ростовский-на-Дону противочумный институт» Роспотребнадзора, г. Ростов-на-Дону, Россия

Резюме. Известно, что совместное использование вакцин, цитокинов и различных иммуномодулирующих препаратов способствует развитию полноценного иммунного ответа. Такой подход позволяет усилить иммуногенность современных вакцин и направить развитие иммунных реакций по гуморальному или клеточному типу в зависимости от свойств возбудителя той или иной болезни. Совершенствование профилактических средств за счет их сочетанного применения с цитокинами и иммуномодуляторами увеличивает напряженность иммунитета, повышает уровень продукции специфических иммуноглобулинов, протективность антигенов, а также снижает проявление побочных реакций, приводящих к поствакцинальным осложнениям.

Иммуномодуляторы уже успешно используются в препаратах, предназначенных для терапии и профилактики хронической герпетической инфекции и противогриппозной вакцине. Многочисленные экспериментальные и клинические данные свидетельствуют о положительном эффекте применения иммуномодулирующих препаратов при вакцинопрофилактике различных вирусных и бактериальных инфекций, в том числе и особо опасных.

Совершенствование специфической профилактики холеры может быть достигнуто за счет иммуномодулирующих средств, способных стимулировать формирование местного и системного иммунного ответа.

Нами проведена сравнительная оценка целесообразности сочетанного применения вакцины холерной бивалентной химической (ФКУЗ РосНИПЧИ «Микроб» Роспотребнадзора) и иммуномодуляторов с целью повышения эффективности вакцинопрофилактики холеры.

Так как холерная вакцина вызывает активацию гуморального иммунного ответа, оценивали выработку специфических иммуноглобулинов в организме привитых экспериментальных животных и влияние иммуномодуляторов на этот процесс в разные сроки поствакцинального периода.

Способность иммуномодуляторов повышать протективную активность холерной вакцины изучали, заражая животных вирулентным штаммом холеры через месяц и семь месяцев после вакцинации.

Выявлено, что иммуномодуляторы повышают иммуногенность и протективность антигенов, входящих в состав противохолерной вакцины. Использование всех иммунопрепаратов увеличивает продукцию специфических иммуноглобулинов в сыворотке вакцинированных экспериментальных

Адрес для переписки:

Филиппенко Анна Владимировна
ФКУЗ «Ростовский-на-Дону противочумный институт» Роспотребнадзора
344002, Россия, г. Ростов-на-Дону,
ул. Максима Горького, 117/40.
Тел.: 8 (863) 240-91-22.
Факс: 8 (863) 267-02-23.
E-mail: plague@aaanet.ru

Address for correspondence:

Filippenko Anna V.
Rostov-on-Don Plague Control Research Institute
344002, Russian Federation, Rostov-on-Don,
Maxim Gorky str., 117/40.
Phone: 7 (863) 240-91-22.
Fax: 7 (863) 267-02-23.
E-mail: plague@aaanet.ru

Образец цитирования:

А.В. Филиппенко, Н.Д. Омельченко, Н.И. Пасюкова, А.А. Труфанова, И.А. Иванова «Совершенствование специфической профилактики холеры с помощью иммуномодуляторов» // Медицинская иммунология, 2021. Т. 23, № 4. С. 915-920.
doi: 10.15789/1563-0625-IOS-2248

For citation:

A.V. Filippenko, N.D. Omelchenko, N.I. Pasyukova, A.A. Trufanova, I.A. Ivanova "Improvement of specific cholera prevention using immunomodulators", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2021, Vol. 23, no. 4, pp. 915-920.
doi: 10.15789/1563-0625-IOS-2248

DOI: 10.15789/1563-0625-IOS-2248

животных. Показано, что в первый месяц после вакцинации наиболее эффективно стимулировал образование антител полиоксидоний, однако ликопид способствовал более длительному сохранению противохолерных иммуноглобулинов в сыворотке вакцинированных кроликов. Сочетанное применение вакцины и ликопида предотвращало развитие холеры у всех взятых в эксперимент животных, в том числе вакцинированных сниженной дозой. В отдаленные сроки поствакцинального периода этот иммуномодулятор повышал протективность противохолерной вакцины в три раза. Полиоксидоний и деринат также увеличивали защитный эффект противохолерной вакцины, но несколько уступали в этом ликопиду. Совместное применение противохолерной вакцины и иммуномодуляторов, особенно ликопида, может быть использовано для совершенствования специфической профилактики холеры.

Ключевые слова: профилактика, холера, иммуногенность, протективность, вакцина, иммуномодуляторы

IMPROVEMENT OF SPECIFIC CHOLERA PREVENTION USING IMMUNOMODULATORS

Filippenko A.V., Omelchenko N.D., Pasyukova N.I., Trufanova A.A., Ivanova I.A.

Rostov-on-Don Plague Control Research Institute, Rostov-on-Don, Russian Federation

Abstract. It is known that the combined use of vaccines, cytokines and various immunomodulatory drugs contributes to the development of a full-fledged immune response. This approach makes it possible to enhance the immunogenicity of modern vaccines and to direct the development of immune responses according to the humoral or cellular type, depending on the properties of the pathogen of a particular disease. The improvement of preventive drugs due to their combined use with cytokines and immunomodulators increases the intensity of immunity, increases the level of production of specific immunoglobulins, the protectivity of antigens, and also reduces the manifestation of adverse reactions leading to post-vaccination complications.

Immunomodulators are already successfully used in drugs intended for the treatment and prevention of chronic herpes infections and influenza vaccines. Numerous experimental and clinical data indicate a positive effect of the use of immunomodulatory drugs in the vaccination of various viral and bacterial infections, including particularly dangerous ones.

Improving the specific prevention of cholera can be achieved through immunomodulatory agents that can stimulate the formation of a local and systemic immune response.

We conducted a comparative assessment of the feasibility of the combined use of the cholera bivalent chemical vaccine (the Federal Government Health Institution Antiplague Research Institute "Microbe") and immunomodulators in order to increase the effectiveness of cholera vaccination.

Since the cholera vaccine causes the activation of the humoral immune response, the production of specific immunoglobulins in the body of vaccinated experimental animals and the effect of immunomodulators on this process at different times of the post-vaccination period were evaluated.

The ability of immunomodulators to increase the protective activity of the cholera vaccine was studied by infecting animals with a virulent strain of cholera one month and seven months after vaccination.

It was found that immunomodulators increase the immunogenicity and protectivity of antigens that are part of the anti-cholera vaccine. The use of all immunopreparations increases the production of specific immunoglobulins in the serum of vaccinated experimental animals. It was shown that in the first month after vaccination, polyoxidonium most effectively stimulated the formation of antibodies, but lycopide contributed to a longer retention of anti-cholera immunoglobulins in the serum of vaccinated rabbits. The combined use of the vaccine and lycopide prevented the development of cholera in all animals taken in the experiment, including those vaccinated with a reduced dose. In the long-term post-vaccination period, this immunomodulator increased the protectiveness of the anti-cholera vaccine by three times. Polyoxidonium and derinate also increased the protective effect of the cholera vaccine, but were slightly inferior to lycopide. The combined use of cholera vaccine and immunomodulators, especially lycopide, can be used to improve specific cholera prevention.

Keywords: cholera, cholera vaccine, immunomodulators, immunotherapy, protectivity, immunogenicity

Introduction

One of the approaches to improving the strategy for combating infectious diseases of any etiology is the development of schemes for the integrated use of vaccines and various groups of immunobiological drugs – immunomodulators, cytokines, etc., which can stimulate the formation of post-vaccination immunity [14]. An active search is underway for drugs that can, firstly, enhance the immunogenic effect of modern vaccines, especially in individuals with secondary immunodeficiency, and secondly, direct the development of an immune response via humoral or cellular type, depending on the properties of the pathogen [13]. Improvement of prophylactic agents due to their combined use with cytokine preparations and various immunomodulators can reduce the manifestation of systemic and local reactions, increase the intensity of immunity, the level of production of specific immunoglobulins, and increase the antigen immunogenicity and protectivity. In practical medicine, several vaccines that contain immunomodulators are already used. Polyoxidonium is included in the polyvaccine for the treatment and prevention of chronic herpetic infection caused by herpes simplex viruses of groups 1 and 2 (HSV-1 and HSV-2), and in subunit influenza vaccine "Grippol". Another multivaccine against HSV-1 and HSV-2 called Vitaherpavac contains sodium hyaluronate [6, 11].

In addition, it has been shown that the inclusion of cytokines and various immunomodulators in the vaccination regimen ensures the development of a full-fledged immune response to various antiviral vaccines and increases their immunological safety [1, 2, 3, 4, 5, 8]. Positive results of using immunomodulators to improve the specific prevention of particularly dangerous infections have been obtained [9, 10, 12].

The effectiveness of specific cholera prevention can be also enhanced by immunomodulatory agents that can stimulate the formation of both local and systemic immunity.

The aim of the study was to experimentally investigate the effect of immunomodulators on immunogenic and protective activity of the cholera bivalent chemical vaccine (the Federal Government Health Institution Antiplague Research Institute "Microbe") in order to assess the feasibility of their use in specific cholera prevention.

Materials and methods

Taking into account that drugs that can be effective for improving specific cholera prevention should have a complex immunomodulatory effect on both systemic and local immunity, we chose the following immunomodulators: Polyoxidonium (azoximer bromide) (NPO Petrovax Pharm, Russia), which stimulates antibody production, phagocytosis, restores immune responses in secondary immunodeficiency conditions, increases the body's resistance to local and generalized infections, has the ability to control the immune response of the immune system, anti–inflammatory effect, etc.; Derinat (sodium deoxyribonucleate) (CJSC "FP "Technomedservis", Russia) increases resistance to viral, fungal, bacterial infections, and also stimulates reparative processes on the mucous membranes: Lycopide (glucosaminylmuramyldipeptide) (CJSC "Peptek", Russia) – a synthetic analog of the structural fragment of the (peptidoglycan) of bacterial cell wall is an activator innate and acquired immunity, enhances the body defense against viral, bacterial and fungal infections, exerts an adjuvant effect in developing immunological reactions.

Experiments were conducted on 45 adult rabbits (weighing 1.8-2.0 kg) purchased from the nursery of the Rostov-on-Don Plague Control Research Institute.

Before oral immunization, the animals were orally inoculated with 5% solution of baking soda (2 ml each) to reduce damaging effect of gastric juice on the cholera vaccine. The vaccination dose of cholera bivalent chemical vaccine and immunopreparations was calculated according to the weight of experimental animals, based on the human doses recommended by the manufacturer. Immunomodulators were administered to rabbits once simultaneously with the vaccine: Polyoxidonium at 0.17 mg; Derinate at 2.0 mg; Lycopide at 0.285 mg.

The total pool of rabbit serum anti-cholera antibodies was determined by solid-phase enzyme-linked immunosorbent assay in three replicates.

The potential of immunomodulators to increase protective activity of the cholera vaccine was evaluated by challenging animals with the virulent strain Vibrio cholerae O1 569B one month and seven months after vaccination. We used a model of a bandaged loop of the adult rabbit small intestine, which allows us to obtain a pathogenetic picture of the disease in animals similar to that in humans [7]. The development of the disease in rabbits was assessed by collecting fluid in the experimental bandaged loops of the small intestine, determining the coefficient of the loop stretching (K) by the formula: K=volume of liquid/length the presence/severity of edema of the mucous and submucosal membranes, hemorrhages pointing at enteropathogenic effect (K > 1 indicates the development of a cholerogenic effect), as well as the presence / severity of edema of the mucous and submucosal membranes, hemorrhages indicating an enteropathogenic effect.

Statistical data analysis was performed by using the Microsoft Excel software. The magnitude of the confidence intervals (L) of the arithmetic mean (M) for the 95% confidence level (P) were determined.

Results and discussion

Given that the cholera vaccine causes the production of specific antibodies in vaccinated rabbits, we evaluated an effect of immunomodulators on this process after vaccination. It was found that in the first week of the post-vaccination period, all animals in experimental groups had similar serum level of produced anti-cholera immunoglobulins (titer value – 1: 625). By day 14, the number of specific Ig in rabbits vaccinated and treated with immunomodulators increased significantly (titer value – 1:3125), compared with that in monovaccinated animals (titre value – 1:625). On day 21, the group of vaccinated animals showed decreased serum antibody production (titer value – 1:125). A decrease in the number of anti-cholera immunoglobulins on day 21 (titer value – 1:625) was also observed in vaccinated rabbits treated with Derinat and Lycopide. Only animals primed with Polyoxidonium had titer of specific antibodies that remained unchanged (titer value – 1:3125).

While studying the duration of sustained serum specific immunoglobulin level in vaccinated rabbits, it was found that seven months after the onset of the experiment, the titer of anti-cholera antibodies in the group of immunized animals decreased to 1:64. Vaccinated rabbits treated with Derinate and Polyoxidonium had the titer at 1:128, whereas after treating with Lycopide during vaccination retained immunoglobulins at fairly high titer 1:256.

While assessing the pathoanatomical picture in the bandaged loops of the small intestine in all intact (control) infected rabbits, it was revealed that the experimental loops were strongly stretched and filled with cloudy contents ($K = 1.14 \pm 0.06$), hemorrhages and mucosal edema were recorded, which indicated the development of cholerogenic and enteropathogenic effects.

The results of challenging vaccinated animals one month after vaccination showed the presence of a rather durable post-vaccination immunity: 75% of rabbits lacked both cholerogenic and enteropathogenic effects. Only 25% of rabbits showed signs of developing disease in the form of a cholerogenic effect ($K = 1.1 \pm 0.08$).

In all groups of vaccinated animals treated with immunomodulators, no pathomorphological changes in the experimental loops of the small intestine were detected during infection one month after the onset, i.e., enteropathogenic and cholerogenic effects were absent in 100% of cases, which indicated a positive effect of immunopreparations on the protectivity of the cholera vaccine.

Seven months in the post-vaccination period, the intensity of cholera immunity in vaccinated rabbits decreased. The protective effect of the vaccine was recorded only in 25% of animals. Cholerogenic ($K = 1.12 \pm 0.08$) and mild enteropathogenic effects were observed in 75% of rabbits.

While assessing the ability of immunomodulators to increase the protective activity of the cholera vaccine in the long-term period after vaccination, it was found that the effect of Polyoxidonium, Lycopide, and Derinate was manifested to varying degrees. No signs of developing disease were registered in 75% of animals treated with Lycopide ($K = 0.12 \pm 0.06$), 25% of rabbits from this group developed only a cholerogenic effect ($K = 1.05 \pm 0.02$). The pathomorphological pattern characteristic of cholera was observed in 50% of animals from the groups with Polyoxidonium and Derinate ($K = 1.04 \pm 0.02$ and $K = 1.06 \pm 0.04$, respectively), whereas vaccinated animals rabbits treated with Derinat also had a weak enteropathogenic effect. Half of the animals from these groups, showed no cholerogenic ($K = 0.15 \pm 0.09$ and $K = 0.14 \pm 0.07$, respectively) and enteropathogenic effects.

The results of experiments assessing a potential to reduce the recommended dose of the cholera vaccine combined with immunomodulators showed that after infection of rabbits one month after vaccination, signs of disease development were not detected only in experimental animals treated with Lycopide ($K = 0.19 \pm 0.02$). Polyoxidonium and Derinate prevented the development of cholera in 50% of rabbits vaccinated with a reduced dose of the vaccine. It should be noted that in other animals from these groups, cholerogenic ($K = 1.08 \pm 0.02$ and $K = 1.06 \pm 0.04$, respectively) and mild enteropathogenic effects were recorded .

In animals vaccinated with half a dose of the vaccine, a pathogenetic picture characteristic of cholera was observed: the development of cholerogenic ($K = 1.12 \pm 0.02$) and enteropathogenic effects. This was evidenced by the presence of edema and hemorrhages in the experimental bandaged loops of the small intestine, which were stretched and filled with reddish, moderately turbid contents.

Thus, we found that the use of immunomodulators enhances the immunogenic and protective properties of the antigens included in the chemical cholera bivalent vaccine. The use of immunopreparations increases the production of serum specific immunoglobulins of vaccinated experimental animals. Moreover, in the first month of the post-vaccination period, Polyoxidonium most effectively abrogated this process. Seven months after vaccination, a sufficiently

high anti-cholera antibody titer was maintained in animals treated with Lycopide. The most effective method was to use this immunomodulator while vaccinated animals were infected with a virulent cholera strain. Lycopide prevented the development of infection in the small intestine in all adult rabbits, including those vaccinated with a reduced dose. Also, the combined use of Lycopide and the vaccine tripled its protectivity in the long-term post-vaccination period. Polyoxidonium and Derinate stimulated the protective ability of the cholera vaccine, but they were somewhat inferior to Lycopide. Considering the data noted above, it can be concluded that increased immunogenic activity of the cholera vaccine combined with immunomodulators, especially Lycopide, may be one of the approaches to improving specific cholera prevention.

References

1. Abramova N.N., Simbirtsev A.S., Dolgushin I.I. The effect of bestim and betaleukin on the immune status of patients with secondary immunodeficiency states during vaccination against viral hepatitis B. *Cytokines and Inflammation, 2004, Vol. 3, no. 4, pp. 29-35.* (In Russ.)
2. Avdeeva Z.I., Akolzina S.E., Alpatova N.A. Nikitina T.N., Medunitsyn N.V. Indicators of cellular immune response in mice immunized with anti-rabies vaccine against cytokines. *Cytokines and Inflammation, 2008, Vol. 7, no. 4, pp. 15-20.* (In Russ.)
3. Avdeeva Z.I., Akolzina S.E., Alpatova N.A. Nikitina T.N., Rassadina M.N., Medunitsyn N.V. The effect of cytokines on the immunogenic properties of the tick-borne encephalitis vaccine. *Cytokines and Inflammation, 2009, Vol. 8, no. 2, pp. 25-29.* (In Russ.)
4. Barinsky I.F., Lazarenko A.A., Alimbarova L.M. To study the effectiveness of the use of domestic immunomodulators, as well as their combined effect with specific vaccines in experimental arbovirus infections. *Immunologiya, 2012, no. 4, pp. 181-183.* (In Russ.)
5. Barinsky I.F., Alimbarova L.M., Lazarenko A.A., Davydova A.A. Immunomodulators and specific inactivated vaccines in the emergency prevention of experimental arbovirus infections. *Problems of Virology, 2013, Vol. 58, no. 4, pp. 35-39.* (In Russ.)
6. Barinsky I.F., Alimbarova L.M., Lazarenko A.A., Mahmudov F.R., Sergeev O.V. Vaccines as a means of specific immunocorrection in herpetic infections. *Problems of Virology, 2014, Vol. 59, no. 1, pp. 5-11.* (In Russ.)
7. Burrows W., Sack R. Animal models of cholera. Cholera. Ed. D. Barua, W. Burrows, Philadelphia, London, Toronto, 1974, Chapter 9, pp. 189-205.
8. Chebotareva T.A., Karyaeva S.K., Malinovskaya V.V., Mazankova L.N., Kaloeva Z.D. Modern possibilities of improving the effectiveness of influenza vaccination in children at high risk of morbidity. *Immunologiya, 2011, no. 3, pp. 146-150.* (In Russ.)
9. Duckett N.S., Olmos S., Durrant D.M, Metzger D.W. Intranasal interleukin-12 treatment for protection against respiratory infection with the *Francisella tularensis* live vaccine strain. *Infect. Immun., 2005, Vol. 73, no. 4, pp. 2306-2311.*
10. Karalnik B.V., Ponomareva T.S. Deryabin P.N. Effect of immunomodulation on the immunogenic and protective activity of live plague vaccine. *Journal of Microbiology, 2014, no. 6, pp. 108-112.* (In Russ.)
11. Pinegin B.V., Latysheva T.V. Immunodeficiency states: possibilities of using immunomodulators. Attending *Physician, 2001, no. 3, pp. 101-106.* (In Russ.)
12. Ponomareva T.S., Deryabin P.N., Karalnik B.V. The effect of betaleukin on the parameters of the antigen-specific immune response in model experiments of animal immunization with a live anti-plague vaccine. *Cytokines and Inflammation, 2014, Vol.13, no. 1, pp. 57-62.* (In Russ.)
13. Simbirtsev A.S., Petrov A.V., Pigareva N.V., Nikolaev A.T. New possibilities of using recombinant cytokines as adjuvants in vaccination. Biopreparations, 2011, Vol. 41, no.1, pp. 16-20. (In Russ.)
14. Vorobiev A.A. Medical Microbiology, Virology and immunology. Moscow: MIA, 2004. 690 p.

Авторы:

Филиппенко А.В. *— младший научный сотрудник лаборатории иммунологии ООИ ФКУЗ «Ростовский-на-Дону противочумный институт» Роспотребнадзора, г. Ростов-на-Дону, Россия*

Омельченко Н.Д. *— к.м.н., старший научный сотрудник лаборатории иммунологии ООИ ФКУЗ «Ростовский-на-Дону противочумный институт» Роспотребнадзора, г. Ростов-на-Дону, Россия*

Authors:

Filippenko A.V., *Junior Research Associate, Laboratory of Immunology of Particularly Dangerous Infections, Rostov-on-Don Plague Control Research Institute, Rostov-on-Don, Russian Federation*

Omelchenko N.D., *PhD (Medicine), Senior Research Associate, Laboratory of Immunology of Particularly Dangerous Infections, Rostov-on-Don Plague Control Research Institute, Rostov-on-Don, Russian Federation*

Пасюкова Н.И. *– научный сотрудник, врио заведующего лабораторией экспериментально-биологических моделей ФКУЗ «Ростовский-на-Дону противочумный институт» Роспотребнадзора, г. Ростов-на-Дону, Россия*

Трufанова А.А. *– младший научный сотрудник лаборатории иммунологии ООИ ФКУЗ «Ростовский-на-Дону противочумный институт» Роспотребнадзора, г. Ростов-на-Дону, Россия*

Иванова И.А. *– к.б.н., ведущий научный сотрудник, врио заведующего лабораторией иммунологии ООИ ФКУЗ «Ростовский-на-Дону противочумный институт» Роспотребнадзора, г. Ростов-на-Дону, Россия*

Pasyukova N.I., *Research Associate, Acting Head, Laboratory of Experimental Biological Models, Rostov-on-Don Plague Control Research Institute, Rostov-on-Don, Russian Federation*

Trufanova A.A., *Junior Research Associate, Laboratory of Immunology of Particularly Dangerous Infections, Rostov-on-Don Plague Control Research Institute, Rostov-on-Don, Russian Federation*

Ivanova I.A., *PhD (Biology), Leading Research Associate, Acting Head, Laboratory of Immunology of Particularly Dangerous Infections, Rostov-on-Don Plague Control Research Institute, Rostov-on-Don, Russian Federation*

Поступила 12.03.2021
Отправлена на доработку 31.05.2021
Принята к печати 09.06.2021

Received 12.03.2021
Revision received 31.05.2021
Accepted 09.06.2021