RESEARCH PAPER

Immunogenicity and safety of a novel seasonal influenza preservative-free vaccine manufactured in Kazakhstan: Results of a randomized, comparative, phase II clinical trial in adults

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ABSTRACT

Background: The study was aimed at comparative evaluation of seasonal influenza vaccine RIBSP versus commercial vaccine VAXIGRIP® for immunogenicity and safety in the course of clinical trial phase II on healthy volunteers aged 18–60 years. **Methods:** The trial involved 150 subjects in randomized 2:1 groups that received either RIBSP vaccine or comparator vaccine VAXIGRIP®. One dose (0.5 ml) of either vaccine contained 15 µg of hemagglutinin of each influenza virus strain recommended by WHO for the Northern hemisphere in 2016–2017 flu season. The observation period lasted 21 day. The trial was registered at ClinicalTrials.gov identifier NCT 03016143. **Results:** Assessment of immunogenic activity of the vaccine under study showed that in 21 day the portion of participants with 4-fold seroconversions was 87.0% to A/H1N1; 63.0% to A/H3N2 and 59.0% to B virus. Antibody titer increase factor in the group of subjects that received RIBSP vaccine was 23.3 for A/H1N1; 4.4 for A/H3N2 and 4.5 for B virus. The volunteers that received RIBSP vaccine demonstrated 95% seroprotection level against A/H1N1; 84% against A/H3N2 and 80% against B virus. RIBSP vaccine met the CHMP criteria of the Committee for Medicinal Products for Human Use (CPMP/BWP/214/96). In the course of evaluating the vaccine safety no serious undesirable effects were recorded. All changes of laboratory data were slight and single in most cases. All recorded local reactions have been light in character and these have been predicted reactions observed at vaccination against influenza. **Conclusion:** Comparison of the allantoic inactivated split vaccine obtained in vaccines RIBSP and VAXIGRIP®, showed similar immunogenic activity. Both vaccines were safe for the study participants.

ARTICLE HISTORY

Received 7 August 2017
Revised 6 September 2017
Accepted 28 September 2017

KEYWORDS

influenza; immune responses; vaccine; phase II trial; seasonal; safety

Introduction

The influenza virus annually infects up to 20% of the world's population, and causes up to one million fatalities. Influenza pandemics, which occur on an average of three or four times per century and are characterized by very rapid spread, affecting entire continents or the whole world, present a serious threat to mankind.^{1,2} Preventive vaccination remains the principal weapon against influenza as the most effective, safe and economically justified way of prevention. The influenza virus variability leads to the necessity of constant renovation of strains in vaccines composition. The World Health Organization (WHO) implements worldwide influenza surveillance collecting information on circulating and potentially pandemic viruses and making annual recommendations on vaccine candidates to vaccine producers.^{3,4}

There are no manufacturers of influenza vaccines in Central Asian countries. The Republic of Kazakhstan will be the first among them to produce its own influenza vaccines including pandemic ones.^{5,6} An inactivated allantoic split vaccine against seasonal influenza (RIBSP vaccine) for seasonal influenza

prophylaxis has been developed in the Research Institute for Biological Safety Problems.^{7,8} This vaccine is easily manufactured and was shown to be safe and highly immunogenic.^{9–11} Application of RIBSP vaccine without adjuvants and preservatives makes it different from commercially available vaccines. We believe that the components of annually applied influenza vaccines (aluminium hydroxide, thiomersal etc.) accumulating in humans can be the health risk factor.^{12–14} The RIBSP was a WHO grantee and received funding from the global action plan to increase the supply of influenza vaccine. This was an incredibly important initiative that aimed at the establishment of influenza vaccine production capacities in low- and middle-income countries, so that those countries were independent of vaccine supply from high-income countries (especially in case of a pandemic).¹⁵

The previous preclinical and phase I clinical trials of RIBSP vaccine showed excellent results in comparison with the split vaccine VAXIGRIP®.^{10,11}

The phase I clinical study of the vaccine in healthy subjects below 50 years of age showed good safe profile. Humoral

immune response to the vaccine met three requirements of the European Committee to human influenza vaccines (CPMP/EWP/214/96).^{11,16} 21 days post vaccination (DPV) seroconversion rate was 100.0% to influenza virus A/H1N1pdm09, 95.5% to influenza virus A/H3N2 and 81.8% to influenza B virus. Seroprotection level against influenza viruses A/H1N1pdm09, A/H3N2 and type B was 95.5%, 86.3% and 72.7, respectively. Geometric mean titers (GMT) of antibodies on 21 DPV were 175.7 for influenza virus A/H1N1pdm09, 64.2 for influenza virus A/H3N2 and 37.6 for influenza B virus.¹¹

The results of phase II clinical trials of RIBSP vaccine are promising for the optimization of influenza vaccine production for middle- or low-income countries and can make a significant contribution to the global influenza prevention program.

Results

Subject disposition

Between October 24, 2016, and November 3, 2016, 441 subjects were screened; vaccination started on November 26, 2016.

Screening has shown that 150 subjects aged within 18–60 years meet inclusion criteria of the clinical trial of the RIBSP (100 subjects) and VAXIGRIP® (50 subjects) vaccines.

Upon signing a written informed consent form containing information about the trial as well as about all aspects and procedures 150 subjects were randomized into groups and vaccinated with RIBSP vaccine and VAXIGRIP® as a comparator vaccine. All 150 subjects were involved in vaccines' immunogenicity and safety analyses; none of them stopped his/her participation or excluded from the trial during the entire study period

are shown in Fig. 1. There were no significant differences in age, gender, weight and height of subjects in both groups (Table 1).

Immunogenicity

The allantoic inactivated split RIBSP vaccine and VAXIGRIP® vaccine showed similar immunogenic activity. Immunogenicity was determined by antibody titers for hemagglutinin in HAI.

On 21 DPV seroconversion to A/H1N1pdm09 influenza virus were registered in 87.0% of subjects in RIBSP vaccine group; to A/H3N2 influenza virus– in 63.0% and to influenza B virus– in 59.0%. In VAXIGRIP® group the percentage of subjects with seroconversion was 80.0% to A/H1N1pdm09 influenza virus; 60.0% to A/H3N2 influenza virus and 56.0% to influenza B virus.

Antibody titer growth among RIBSP-vaccinated volunteers was 23.3 for A/H1N1pdm09 influenza virus; 4.4 for A/H3N2 influenza virus and 4.5 for influenza B virus. In VAXIGRIP® group these parameters were respectively 13.4, 5.2 and 4.4 for each component of the vaccine.

Seroprotection level among volunteers of RIBSP vaccine group was 95% for A/H1N1pdm09 influenza virus; 84% for A/H3N2 influenza virus and 80% for influenza B virus. Among those who received VAXIGRIP® vaccine seroprotection level was respectively 96%, 88% и 80% for A/H1N1pdm09, A/H3N2 and influenza B viruses.

Statistically significant difference in seroprotection levels of the inactivated allantoic split RIBSP vaccine and VAXIGRIP® vaccine was not detected ($P > 0.01$, F-criterion). F-criterion values in comparison of reliability difference in groups of volunteers are shown in Table 2 for each component of vaccines.

Figure 1. Flow—diagram of the study.

Table 1. Summary of demographic data.

Variable		Vaccine VAXIGRIP® (N = 50) n (%)		Vaccine RIBSP (N = 100) n (%)	
		Female	Male	Female	Male
Gender (n (%))		30 (60.0)	20 (40.0)	47 (47.0)	53 (53.0)
Age	Mean (SD)	29.96 (13.03)	26.0 (9.19)	28.76 (11.38)	33.32 (12.34)
Height (cm)	Mean (SD)	162.53 (5.2)	174.21 (8.0)	161.8 (6.7)	174.98 (5.4)
Weight (kg)	Mean (SD)	57.85 (10.09)	75.26 (10.73)	60.60 (11.16)	75.32 (10.57)

Table 2. Seroconversion and seroprotection rates in two vaccinated groups.

Group	Day	A/H1N1 virus, %	A/H3N2 virus, %	B virus, %
RIBSP vaccine (n = 100)				
Seroconversion [95% CI]	Day 21	87.0 [79.0–92.2]	63.0 [53.2–71.8]	59.0 [49.2–68.1]
Mean GMT increase	Day 21	23.3	4.4	4.5
Seroprotection [95% CI]	Day 1	30.0 [21.9–39.6]	33.0 [24.6–42.7]	34.0 [25.5–43.7]
	Day 21	95.0 [88.8–97.8]	84.0 [75.6–89.9]	80.0 [71.1–86.7]
Mean GMT	Day 1	17.1	17.4	16.9
	Day 21	396.7	77.3	76.7
VAXIGRIP® (n = 50)				
Seroconversion [95% CI]	Day 21	80.0 [67.0–88.8]	60.0 [46.2–72.4]	56.0 [42.3–68.8]
Mean GMT increase	Day 21	13.4	5.2	4.4
Seroprotection [95% CI]	Day 1	36.0 [24.1–49.9]	32.0 [20.8–45.8]	34.0 [22.4–47.8]
	Day 21	96.0 [86.5–98.9]	88.0 [76.2–94.4]	80.0 [67.0–88.8]
Mean GMT	Day 1	18.9	17.7	19.7
	Day 21	256.3	72.6	65.9
Comparison of sample rate (%) on the 21 st day (F-criterion)		P = 1.00	P = 0.63	P = 1.00

Safety

The safety profile was assessed as the proportion of subjects experiencing adverse events (AE). Immediate local adverse events (ILAEs) within 2 hours post vaccination are shown in

Table 3. Adverse events within 21 days after vaccination.

Events	RIBSP Vaccine (N = 100)	VAXIGRIP® Vaccine (N = 50)
<i>AE within 2 hours</i>		
Local AE		
Induration at the injection site	2 (2%)	2 (4%)
Hyperimia at the injection site	2 (2%)	—
Oedema at the injection site	2 (2%)	—
Painfulness at the injection site	1 (1%)	—
Number of subjects experiencing AE	3 (3%)	2 (4%)
<i>AE within 7 days</i>		
Local AE		
Induration at the injection site	3 (3%)	2 (4%)
Hyperimia at the injection site	3 (3%)	1 (2%)
Oedema at the injection site	2 (2%)	—
Painfulness at the injection site	2 (2%)	1 (2%)
Number of subjects experiencing AE	5 (5%)	2 (4%)
<i>AE according to self-observation diaries within 8–20 days</i>		
Local AE		
	—	—
Systemic AE		
Headache	3 (3%)	2 (4%)
Sore throat	2 (2%)	3 (6%)
Runny nose	3 (3%)	2 (4%)
Cough	2 (2%)	3 (6%)
Dizziness	1 (1%)	—
Fever	1 (1%)	1 (2%)
Stomach upset	1 (1%)	—
Number of subjects experiencing AE	10 (10%)	6 (12%)
Changes of laboratory values		
Biochemical blood analysis	15 (15%)	7 (14%)
Clinical blood analysis	15 (15%)	10 (20%)
Number of subjects experiencing AE	29 (29%)	13 (26%)

Table 3. ILAEs were reported in 2 subjects from the VAXIGRIP® vaccine group and only 1 subject from the RIBSP vaccine group. The most frequent local symptom in both groups was induration at the injection site.

The recorded local AEs during the first 7 days post vaccination was similar to the immediate local AEs. Systemic reactions were demonstrated by 5 subjects in VAXIGRIP® group and 2 subjects in RIBSP group. Most frequent local symptoms were as follows: induration, painfulness and hyperaemia at the site of vaccine administration; there were no differences in these reactions between groups. All registered reactions were categorized as mild and self-limiting, and did not need treatment.

From the 8th to the 20th day of the study subjects from both groups recorded systemic AEs with equal frequency: 10.0% in RIBSP and 12.0% in VAXIGRIP® group. The symptoms typical for acute respiratory viral infections (ARVI) were most frequent. The revealed frequency of ARVI symptoms can be explained by higher morbidity rate in the autumn period. Majority of adverse events was mild. One of the participants vaccinated with VAXIGRIP® had fever (38.5°C) against the background of ARVI from 14th to 16th DPV. The febrility was considered as AE of moderate severity not associated with vaccination.

Changes in laboratory values post vaccination (overall clinical and biochemical blood analyses) were registered on days 3, 7 and 21 in both groups; frequency of laboratory abnormalities was comparable between 2 groups. The changes in laboratory values were revealed for 29% of subjects immunized with the RIBSP vaccine and 26% of the VAXIGRIP® recipients. All these adverse reactions were mild and single (one elevated indicator) in most cases. Increase in numbers of lymphocytes was registered on days 7 and 21 for 1 volunteer from VAXIGRIP®

group; another participant of the same group demonstrated increase in eosinophils number on days 3 and 7 and decrease in lymphocytes number on days 7 and 21. There were no pathological findings in urine test results.

The serious adverse events were not registered during the study.

Discussion

This study financed and conducted on the basis of orders of the Ministry of Education and Science and Ministry of Health of the Republic of Kazakhstan to provide the public their own seasonal influenza vaccine for annual vaccination. The obtained results showed high immunogenicity and good tolerability of the vaccine in adults aged within 18–60 years.

Immunogenicity was evaluated in comparison with VAXIGRIP® vaccine, serological assays were carried out by comparing antibody levels in the pairs of sera samples collected prior to vaccination and on the 21st DPV. Statistically reliable increase of antibody titers was recorded in vaccinated subjects from both experimental groups.^{17,18}

Percentage of participants with 4-fold and higher increase of antibody titers (seroconversion index) is an important characteristic of immune response to vaccination. The highest seroconversion index (87.0%) was demonstrated for the RIBSP vaccine against influenza virus A/H1N1 component. It was 7% higher than the same index demonstrated for VAXIGRIP® vaccine.^{19,20} The seroconversion indices of RIBSP vaccine to influenza A/H3N2 and B components also exceeded the indices demonstrated by VAXIGRIP®. These results allowed concluding that the observed antibody titer increase to all influenza virus vaccine components considerably exceeds CPMP/EWP/214/96 criteria which require seroconversion index to be over 40%.^{16,21,22}

Significant difference in antibody titer increase between RIBSP and VAXIGRIP® vaccines was not revealed.

In RIBSP group mean fold increase of the antibody titer for influenza virus A/H1N1 has been 23.3; for influenza virus A/H3N2- 4.4 and for influenza B virus- 4.5, thus satisfying the requirements of the European Committee to influenza vaccines (CPMP/BWP/214/96) according to which this index should be no less than 2.5.

The seroprotection rate was at least 80% for all vaccine components in both groups, the highest one being for influenza virus A/H1N1. The rates of VAXIGRIP® vaccine were insignificantly higher than the rates of RIBSP vaccine in this case.

In accordance to CPMP/BWP/214/96 guidance on immunogenicity of influenza vaccines the vaccine is considered to be immunogenic when it meets at least one of criteria. So, based on the results of the study, the RIBSP vaccine without any preservatives and adjuvants as immunity activators could be recommended for immune protection against seasonal influenza via single vaccination for subjects aged 18–60 years.^{23,24}

RIBSP vaccine with regard to its safety is well tolerated and does not induce unexpected adverse events and reactions. Reactogenicity and rates of adverse events in case of RIBSP vaccine had no significant difference versus VAXIGRIP® vaccine. Indurations, hyperemia, oedemas and painfulness at the injection sites were the most frequent reactions. All recorded local

reactions were mild, foreseen and typical for vaccination with influenza vaccines.

Following the above mentioned hypothesis that in case of annual immunization against influenza it is possible to avoid inclusion of adjuvants and preservatives in the vaccine composition first of all for the sake of its safety, the vaccine of this kind can be used in children after performing the appropriate clinical trials. The second advantage is the feasibility of its production due to less number of steps and appropriate analytical trials. Keeping all above mentioned in mind the researchers of the RIBSP developed the vaccine against seasonal influenza.^{25,26}

The influenza morbidity rate can be reduced via total annual vaccination. Registration of a domestic influenza vaccine in Kazakhstan will allow decreasing the economic losses due to influenza outbreaks and moreover will contribute considerably to the global program of influenza prophylaxis.

Material and methods

Study design

This was phase II randomized, blind trial (VSI-II-01/2016) of the seasonal influenza split vaccine conducted in the Clinical Pharmacology Institute of S.D.Asfendiyarov, Kazakh National Medical University, Almaty, Kazakhstan, within the period of October 24 – November 26, 2016. The trial was registered at ClinicalTrials.gov identifier NCT 03016143 and conducted in accordance with the Code of Ethics of the World Medical Association Declaration of Helsinki and Good Clinical Practice Guidelines of the International Council for Harmonization.

Clinical study according to the protocol “Randomized blind comparative clinical trial, phase II, of allantoic inactivated split vaccine against seasonal influenza in healthy adults” was conducted under permission of Ministry of Healthcare of the Republic of Kazakhstan (order No. 322 dated October 24, 2016), approved by the Central Ethics Committee, and the Local Committee of Kazakh National Medical University (minutes No. 2 dated September 21, Ethics 2016).

Participants

The trial involved 150 subjects aged 18–60 years that were randomly divided in ratio 2:1 to be vaccinated either with RIBSP or VAXIGRIP® (comparator) vaccine. The major criteria of exclusion were as follows: allergic reactions to chicken egg albumin, severe chronic or acute disease, significant immunodeficiency, Guillain-Barré syndrome (acute polyradiculitis) in anamnesis, vaccination against influenza during 2015/2016 season, positive pregnancy tests and reluctance to use contraceptives during the trial period (for women), autoimmune disorders, using antiviral preparations, participation in any other clinical study within the recent three months. Each volunteer signed a written informed consent and was involved in the study.

Vaccines and vaccination

Name of the test vaccine: Liquid allantoic inactivated split vaccine against seasonal influenza (RIBSP vaccine), produced on chicken embryos, a trivalent inactivated split vaccine.

A commercially available trivalent inactivated split vaccine against seasonal influenza VAXIGRIP® (Sanofi Pasteur SA, Lyon, France) was used as a comparator preparation. One dose (0.5 ml) of both vaccines contained 15 µg of hemagglutinin of each influenza virus strain recommended by WHO for influenza season in the Northern hemisphere for years 2016–2017: A/California/7/2009 (H1N1)pdm09, A/Hong Kong/4801/2014 (H3N2), B/Brisbane/60/2008 and auxiliary substances. Viruses for RIBSP vaccine production were obtained from the National Institute of Biological Standards and Control. The vaccine differs from commercially available vaccines by absence of thiomersal, preservatives and adjuvants.

All subjects were injected intramuscularly with 1 dose of vaccine into deltoid muscle. Duration of the observation period was 21 day.

Randomization and masking

Between October 26 – November 03, 2016, investigators randomly allocated eligible participants (2:1) to intramuscular injection of a 0.5 ml dose of the RIBSP vaccine group or VAXIGRIP® vaccine group. Randomization was done with sequentially numbered, opaque, sealed envelopes opened by the study clinician at enrolment. Variable blocks (block sizes two to eight) in the randomization sequence ensured allocation concealment. Participants and immunologists were masked to treatment allocation, which was achieved through the paired-placebo trial design.

Assessment of immunogenicity

To evaluate immunogenicity blood was sampled in volume of 3–5 ml from each subject prior to vaccination (Day 1) and upon the trial completion (21 Day). HAI assays were performed at the Influenza Research Institute, St. Petersburg, Russia, using 0.5% chicken erythrocytes with 4 hemagglutinin units per 25 µl of virus based on the WHO-recommended procedure.¹⁵

Humoral immune was evaluated in HAI assay with chicken erythrocytes according to European Guideline CPMP/BWP/214/96¹²:

- the proportion of subjects achieving a haemagglutination inhibition (HI) titre ≥ 40 or single radial haemolysis (SRH) titre > 25 mm should be 70% (seroprotected);
- number of seroconversions or significant increase in anti-haemagglutinin antibody titre $> 40\%$ (seroconverted)
- increase in geometric mean titre (GMT) > 2.5 .

Assessment of safety

The second outcome of this trial was safety, which we assessed by the frequency and severity of vaccine related local and systemic adverse events. Safety and reactogenicity of the vaccine was evaluated during the entire observation period after vaccination (21 days). Type and frequency of local and systemic adverse events (AE) were daily analyzed after vaccine administration. The participants were examined by the investigator before vaccination, in 20 minutes and 2 hours post vaccination and in the evening (by phone). Follow-up care was carried out

within 7 days by examination of the volunteers in the morning and by telephone inquiries in the evening. Reactogenicity of RIBSP and VAXIGRIP® vaccine preparations was evaluated via registration of local and systemic AE as well as on the basis of subjective assessment of tolerance to the preparation by volunteers. Safety of the preparations was evaluated by the laboratory test results (clinical and biochemical blood analyses, urine analysis). Possible side effects of the vaccine may become evident as local reactions at the injection site (pain, hyperemia, infiltration and so on) and as systemic reactions (fever, coughing, pharyngitis, undue fatiguability, arthralgia, myalgia, headache, giddiness, sickness, abdominal pains, diarrhea and so on).

Within days 7 to 21 each volunteer recorded the presence/absence of adverse events in his/her self-observation diary card. Subjects were requested to inform the investigator of any serious adverse event occurring up to 21 days after vaccination.

Statistical analysis

Exact Clopper-Pearson method was used to calculate 95% confidence intervals. Statistical analysis of the data was carried out with the help of free software R Studio.

Biochemical and hematological parameters of peripheral blood on the 3rd, 7th and 21st DPV have been compared to the same parameters on day 1 before the vaccination by Dannette test that is a modification of Student's t-test for multiple comparisons with one control.

Effect of the allantoic inactivated influenza split vaccine on biochemical and hematological parameters of peripheral blood was compared with the effect of VAXIGRIP® vaccine on the same parameters with the help of non-parametric Mann-Whitney U test.

Disclosure of potential conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

The authors would like to thank Inkarbekov D. and Kozhamkulov E. (Laboratory of Infectious Disease Prevention, RIBSP) for the vaccine preparation and its staff as well as Nurpeissova A., Sagymbay A and Abytay R. (Laboratory Control of technology and biopreparations, RIBSP) for their help in research.

Funding

The Ministry of Healthcare of the Republic of Kazakhstan provided funding.

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