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Original Article

Evaluation of Seroconversion in Guinea Pigs Following Inoculation of New Formulations of Rubella Vaccine

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Abstract

Background and Aims: Rubella is a contagious viral disease mostly with mild clinical symptoms and often remains undiagnosed. Rubella infection may adversely affect pregnancy, especially in the first trimester and this mother to child transmission can cause congenital rubella and may be lead to permanent disability and mortality in children. Effective rubella vaccine should be prepared using suitable stabilizers. New stabilizers should be selected carefully in the manners that final product meet all requirements of stability, immunogenicity and safety. The aim of this study was to evaluate the immunogenicity of rubella vaccines that are formulated using two different new stabilizers in comparison to a commonly used vaccine that gelatin is used in it as a stabilizer.

Materials and Methods: 28 guinea pigs were prospectively divided into four groups (one control and three test groups) according to the materials that they were subjected to receive, including the vaccines that formulated with hydrolyzed gelatin (G), Sorbitol (S) and Trehalose dehydrate (T) stabilizers. Control group was received sterile water for injection (WFI). Anti-rubella antibodies in the sera were measured using haemagglutination inhibition (HI) technique. The results were analyzed by Generalized Estimating equations (GEE) statistical test.

Results: Our results showed that all three formulated vaccines (G, S and T) induced seroconversion in guinea pigs; however the vaccine that contained Trehalose dehydrate (T) induced a slightly higher level of antibody against rubella virus ($p<0.01$).

Conclusion: As an important part of final bulk of rubella vaccines, stabilizers are continuously studied to achieve safer, more stable and more effective vaccines. In this study, the immunogenicity of newly formulated rubella vaccines (T&S) was comparable to the vaccines which formulated with routine stabilizer (G).

Keywords: Rubella virus; haemagglutination inhibition (HI); Seroconversion

Introduction

Rubella, known as German measles, is a disease caused by the rubella virus (RV) (1), a togavirus that is enveloped and has a single-standard RNA Genome (2). The disease is often mild and attacks often pass unnoticed. The disease has an incubation period of 2-3 weeks (3). It is known that infection of the mothers by rubella virus during pregnancy can be serious. Within the first 20 weeks of pregnancy, the child may be born with congenital rubella syndrome (CRS) which entails a range of serious incurable illnesses inducing cardiac, cerebral, ophthalmic and auditory defects (4). The risk of major

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defects or organogenesis is high for infection in the first trimester. Miscarriage occurs in up to 20% of cases (5).

Rubella infections are prevented by active immunization programs using live attenuated vaccines. In 1969, a live attenuated virus against rubella was licensed. Among vaccine strains, RA27/3 and Takahashi are the most widely used vaccine strains. In the early 1970s, triple vaccine containing attenuated measles, mumps and rubella (MMR) viruses was introduced (6). Currently, live attenuated vaccine against rubella is used worldwide.

Specific IgM antibody against Rubella virus appears in people recently infected by RV, but these specific antibodies can persist for over a year (7). The presence of these antibodies along with, or a short time after, the characteristic rash confirms the diagnosis (8).

Serological methods and isolation is usually faster and easier and is used to diagnose and determine the society immune status (9). Haemagglutination Inhibition (HI) test is used in diagnostic laboratories, as a standard procedure.

Due to concerns about possible teratogenic effects, administration of MMR vaccine is not recommended during pregnancy. Instead, susceptible pregnant women should be vaccinated as soon as possible in the post-partum period (10).

This study was performed to compare the immunogenicity of two new formulations of rubella vaccine (containing two new stabilizers, briefly are named as S and T) with a routinely used formulation (containing a gelatin-based stabilizers, G in brief).

Methods

Takahashi strain of RV was propagated in human diploid cells (MRC-5) at human viral vaccine department (HVVD), Razi Vaccine & Serum Research Institute (RVSRI), Karaj, Iran. Vesicular stomatitis virus (VSV) for interference test was prepared by quality control unit, HVVD, RVSRI.

All cell monolayers for the virus propagation and quality control tests including MRC-5, RK-13 and Vero cells was prepared by cell culture unit, HVVD, RVSRI.

All media, stabilizers and buffers were prepared and tested by culture media preparation unit, RVSRI.

Propagation, quality control tests, formulation and lyophilization processes were done at HVVD, RVSRI.

Young male per bright short hair guinea pigs (weight, 250-350 g) were prepared by animal husbandry department, RVSRI.

As the first step, stabilizers (S and T) were prepared according to defined components and values (Tables 1 and 2). Sterility test were performed on the samples of prepared stabilizers and routine stabilizer (G).

Rubellaviruses were propagated in MRC-5 cell monolayers and harvested after a 7-days incubation period at 33°C. Samples were taken from harvested viruses and tested for sterility and viral titer. To make the final bulks, harvested viruses were mixed with equal volumes of prepared stabilizers, separately and final bulks were dispensed in vaccine vials. Using a 48 hours Lyophilization cycle, the vaccines were freeze-dried. Samples which were taken before and after lyophilization process were tested for sterility and potency. The vaccines were inoculated to animals. 28 young male guinea pigs were adapted to their

Table 1. Components of "T" stabilizer

Components	Amount (g/L)
Hydrolyzed gelatin	111.11
Trehalose	111.11
KH_2PO_4	1.258
Sodium glutamate	88.88
$Na_2HPO_4(12\ H_2O)$	5.6

Table 2. Components of "S" stabilizer

Components	Amount (g/L)
Hydrolyzed gelatin	40.2
Sorbitol	70.02
NaCl	5.07
Sucrose	60
Bicarbonate	0.88
Glucose	70.2
Human Serum Albumin (HSA)	0.69
Phenol Red	0.004
Sodium phosphate Na_2HPO_4 (12 H_2O)	4.236

Table 3. Antibody response following inoculation of "S' vaccine to guinea pigs.

Titers of antibodies against rubella virus, as measured by the HI method.			Number of samples	Vaccine
Thirtieth day	Fifteenth day	before inoculation		
1:8	1:2	0	1	S
1:8	1:4	0	2	S
1:8	1:4	0	3	S
1:4	1:4	0	4	S
1:4	1:2	0	5	S
1:4	1:2	0	6	S
1:8	1:4	0	7	S

Table 4. Antibody response following inoculation of "T' vaccine to guinea pigs.

Titers of antibodies against rubella virus, as measured by the HI method.			Number of Samples	Vaccine
Thirtieth day	Fifteenth day	before inoculation		
1:4	1:2	0	1	T
1:8	1:8	0	2	T
1:8	1:2	0	3	T
1:16	1:4	0	4	T
1:2	1:2	0	5	T
1:4	1:2	0	6	T
1:2	1:2	0	7	T

new cages for one week and were divided to four groups. Three groups of guinea pigs (G, S and T respectively) were inoculated by 0.5 ml of reconstituted vaccines subcutaneously (s.c). The 4th group (control) was only inoculated by diluent (WFI). Blood samples were taken from all guinea pigs before inoculation and at days 15 and 30 after inoculation. Blood samples were kept overnight at 4°C and then centrifuged to remove serum. Their sera were stored at -20°C until HI test.

Lyophilized Haemagglutination (HA) antigen was reconstituted. It was tittered using standard procedures and four HA antigen units were defined to use in the HI tests on guinea pig (GP) sera samples. A two-fold serial dilution of each treated serum sample was used in HI test. Fresh pigeon red blood cells were washed and a 0.34% preparation of compact red blood cells was used in HI test.

Results

All sera samples (G, S, T and control groups) were treated and tested by a four-units HA antigen of RV. The results of HI test on a total of 84 serum samples (28 animals, 3 bleeding each at days 0, 15 and 30) are mentioned on table 3 to 6. Seroconversion is seen in all three test groups (G, S and T). No detectable reaction was seen in control group. The data were analyzed using GEE statistical method which could be seen on table 7.

Discussion

Control and elimination of rubella infection is one of the world health organization`s goals. Vaccination as the most effective preventive action has dramatically reduced the morbidity and mortality of the disease worldwide. Live attenuated rubella virus strains are used to manufacturing of effective vaccines in different companies. One of the most important aspects of a live attenuated viral vaccine is it`s stability. Many companies have directed their efforts to develope more stable vaccines. According to physical and chemical properties of certain viruses, the components of their stabilizers may be different. In this study, two new stabilizers were used to formulate rubella vaccine. The produced vaccines then were compared with a vaccine that was formulated using a routinely used stabilizer at Razi Institute for the extent of specific antibody induction in guinea pigs. According to main component of each stabilizer, the vaccines were nominated as S and T beside the routine vaccine; G. Based on the results, all of produced vaccines have induced antibody against rubella virus. The extent of induction of specific antibody among three vaccines (T, S and G) were pretty close, but in the case of "T" vaccine was slightly more effective than the others. A recent study investigated the effect of the same stabilizers on the antibody induction against mumps virus (RS-12 strain) and the results suggest more

Table 5. Antibody response following inoculation of "S' vaccine to guinea pigs.

Titers of antibodies against rubella virus, as measured by the HI method.			Number of Samples	Vaccine
Thirtieth day	Fifteenth day	before inoculation		
1:4	1:2	0	1	G
1:8	1:8	0	2	G
1:8	1:4	0	3	G
1:8	1:4	0	4	G
1:4	1:2	0	5	G
1:8	1:2	0	6	G
1:4	1:2	0	7	G

Table 6. Antibody response following inoculation of WFI to control guinea pigs.

Titers of antibodies against rubella virus, as measured by the HI method.			Number of Samples	Vaccine
Thirtieth day	Fifteenth day	before inoculation		
0	0	0	1	Control
0	0	0	2	Control
0	0	0	3	Control
0	0	0	4	Control
0	0	0	5	Control
0	0	0	6	Control
0	0	0	7	Control

Table 7. Summarized GEE analysis on the results

Vaccine	Beta coefficient	SE	95% CI		Test		P Value
			Lower	Upper	Wald Test	Df	
S Vaccine	.178	.0236	.132	.224	57.061	1	<0.001
T Vaccine	.202	.0278	.148	.257	52.864	1	<0.001
G Vaccine	.187	.0284	.132	.243	43.518	1	<0.001
Control	0[a]	.	.	.	.	.	.
Time	.044	.0099	.025	.063	19.765	1	<0.001

SE: Standard Error, CI: Confidence Interval

effectiveness of the vaccine which has been formulated by "T" stabilizer, which is consistent with our results (11). Indeed, a rise in antibody titers could be seen between sera samples of day 15 and 30 post infection against rubella virus which reflects increasing immune reaction to the all inoculated vaccines. Because of the patent nature of such an important researches, no more data was available. Since the RV is mostly administrated as MMR vaccine, more studies are needed to prove the effectiveness of rubella vaccines whose stabilizers are "T" or "S". Satisfying results in similar studies may prove the reproducibility and lead to producing more stable MMR vaccine with no effects on its immunogenicity.

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