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Research Article

COMPARATIVE IMMUNOGENICITY OF TYPHOID Vi CONJUGATE VACCINE AND TYPHOID POLYSACCHARIDE VACCINE IN MICE

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ABSTRACT

Typhoid fever is a generalized acute systemic infection caused by *Salmonella typhi*. The Vi-polysaccharide vaccine is said to have some limitations – the immune response is a T cell independent phenomenon and the antibody response is not boosted by additional doses. Comparative immunogenicity study in mice was done in Typhoid Vi Conjugate Vaccine (Bio-Med (P) Ltd.), Typhoid Polysaccharide Vaccine (Bio-Med (P) Ltd.) and Typhoid Vi conjugate vaccine (Bharat Biotech International Ltd.). A randomized controlled study was conducted on Swiss albino mice weighing 17-22 gram. Mice were injected with primary (1st) dose and booster dose after 14 days by subcutaneous route. Control group was inoculated with normal saline. Peda TyphTM induced significantly higher anti Vi IgG serum antibodies than Bio TyphTM and Typbar TCV[®] in all dose variations and number of vaccination schedules carried out in the study.

Keywords: Typhoid Vi conjugate vaccine, typhoid polysaccharide vaccine, Vi polysaccharide –tetanus toxoid vaccine, typhoid vaccine.

INTRODUCTION

In developing countries, typhoid fever is very common and increasingly difficult disease to treat because of the increasing level of antibiotic resistance against *Salmonella typhi* (Lin *et al.*, 2001). It remains a serious disease with a disease burden in major population living in South America, African continent, South-East Asian countries. Despite the availability of several antimicrobial agents for its treatment; the emergence of antibiotic resistant strains has posed a significant challenge in the treatment of typhoid and so it still continues to remain

an important cause of morbidity. Therefore strategies to prevent the disease would include effective sewage treatment, safe potable drinking water and vaccination against the disease (Acharya *et al.*, 1987; Klungman *et al.*, 1987).

The vaccines for typhoid currently available in India are injectable Vi polysaccharide vaccine and Vi-TT conjugate polysaccharide vaccine (Klungman *et al.*, 1996). The Vi-polysaccharide vaccine is said to have some limitations – the immune response is a T cell independent phenomenon and the antibody response is not boosted by

additional doses (Kossaczka *et al.*, 1999; Singh *et al.*, 1999). In order to improve the efficacy of the Vi-polysaccharide vaccine and to protect the susceptible infants and under five years old children, a new technology for conjugation with a suitable protein was developed (Szu, 2013; Szu *et al.*, 2013). Clinical studies of tetanus toxoid conjugated Vi polysaccharide typhoid vaccine in infants and young children shows that the efficacy of conjugated typhoid vaccine have high titre compared to typhoid polysaccharide vaccine (Garg *et al.*, 2014; Vadrevu *et al.*, 2015).

The objective of this study was to compare the immune response induced by different typhoid conjugate vaccines in mice. No comparative immunogenicity study between two different conjugate vaccines was done previously in human or animal test models. Mice models are indispensable tools

for accessing candidate vaccine with pre-clinical, safety and immunogenicity.

MATERIAL AND METHOD

Study Design

6 groups of 10 mice (Swiss albino) each of 17-22 gram were inoculated with different vaccines, in different concentration and control group with normal saline (0.9% w/v sodium chloride injection IP) as detailed in Table 1.

Vaccine

First dose of vaccine was given on day 0 and second dose on day 14 by subcutaneous route using 0.1 ml per dose. Blood was collected on day 14 and day 21, the individual serum samples of mice were separated and tested for anti Vi IgG antibodies by ELISA (Szu *et al.*, 2013).

Table 1: Details of vaccination and blood collection schedule of mice.

Details	Group A	Group B	Group C	Group D	Group E	Group F
No of Mice	10	10	10	10	10	10
Vaccine	Typbar-TCV™	Typbar-TCV™	Bio Typh™	Peda Typh™	Peda Typh™	Normal saline
Dose	0.1µg/0.1 ml	0.5µg/0.1ml	0.1µg/0.1ml	0.1µg/0.1 ml	0.5µg/0.1 ml	0.1ml
Route	SC	SC	SC	SC	SC	SC
Day of Vaccination	0 & 14	0 & 14	0 & 14	0 & 14	0 & 14	0 & 14
Day of Blood Collection	14 & 21	14 & 21	14 & 21	14 & 21	14 & 21	14 & 21

Safety

Mice were monitored on weekly basis to observe the side effects of vaccination. Mice in all groups remained healthy with gradual increase in body weight.

Immunogenicity Assay

To evaluate the level of vaccine induced IgG anti-Vi antibody level, blood from mice of each group was collected on 14th day and 21st day (i.e. after 7 days of

booster dose) to prepare serum samples. Serum IgG anti-Vi antibodies were assayed by Enzyme linked immunosorbent (ELISA) (Szu *et al.*, 2013) and expressed in ELISA units relative to a positive control with standard arbitrary assigned a value of 100 ELISA units. For the study on mice a clearance was taken from the IAEC vide approval no 022/BM/R&D/2018.

ELISA

Coating antigen was prepared by Vi polysaccharide covalently conjugated to bovine serum albumin via adipic acid dihydrazide linker (Vi-ADH-BSA) having concentration 1725 µg of Vi polysaccharide/ml. Lot No. Vi-ADH-BSA/01. Positive control serum was prepared by pooling sera of mice vaccinated with Typhoid Vi conjugate vaccine (Peda TyphTM), (3 doses of 1µg/0.1ml concentration, injected in group of 10 mice by subcutaneous route at interval of 14 days, blood collected 7 days after the last dose) Positive control serum is assigned an arbitrary value of 100 ELISA units.

Vi polysaccharide covalently conjugated to bovine serum albumin (coating antigen) was prepared in phosphate buffered saline (pH 7.4). The optimum coating concentration of antigen was determined using checkerboard titration method, concentration of 10µg/ml was found suitable. Coating antigen was dispensed in an ELISA plate (Nunc MaxisorpTM). Wells were blocked by blocking solution for overnight at 2-8°C. After blocking, dilutions of test serum were prepared (1:100), negative control (1:100) and standard reference for Vi

polysaccharide IgG antibodies (1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200) in PBS-T with 1% BSA. 200µl of each dilution was dispensed in ELISA plate and incubated for 2 hours at room temperature in humidified box. Anti mouse IgG-HRP conjugate (1:25000) was diluted in PBS-T with 1% BSA, dispensed and incubated at room temperature in humidified chamber box for 2 hours. Horse reddish peroxidase (substrate) was dispensed and incubated at room temperature. Reaction was stopped by the addition of 1N H₂SO₄. Optical density was measured in ELISA reader at 490 nm. Pooled serum of control group inoculated with normal saline was set as blank.

Statistical Analysis

ELISA units were calculated with the program of ELISA for windows version 2.00 of the Center for Disease Control, U.S. Department of Health and Human Services, National Center for Infectious Diseases, Division of Bacterial and Mycotic Diseases, Atlanta, USA. This module uses a four-parameter logistic-log function to describe standards data and form calibration curves.

RESULTS AND DISCUSSION

Results are shown in Table 2.

Table 2. ELISA antibody response.

S.No.	GROUPS	ELISA unit on 14 th day	ELISA unit on 21 st day after booster dose
1	Group A (0.1µg/0.1ml Typbar TCV)	3.917	17.440
2	Group B (0.5µg/0.1ml Typbar TCV)	15.976	20.282
3	Group C (0.1 µg/0.1ml Bio Typh)	3.343	5.360
4	Group D (0.1µg/0.1ml Peda Typh)	10.553	82.798
5	Group E (0.5µg/0.1ml Peda Typh)	31.190	51.362

CONCLUSION

Peda TyphTM induced significantly higher anti Vi IgG antibodies than Bio TyphTM and Typbar TCV[®] in all dose variations and number of vaccination schedules. It can be seen from the results that unconjugated Vi polysaccharide vaccine (Bio TyphTM) induced lower immune response and booster response as compared with conjugated Vi polysaccharide vaccines (both Peda TyphTM & Typbar-TCVTM). Peda Typh immune response with 0.1 µg dose was higher than immune response of Typbar-TCV at 0.1 µg

and 0.5 µg. This points to superior antigenic response of Peda Typh over Typbar in mice model. Further, the results significantly show that the immune response to Peda typhTM after one booster dose induced much higher immune response than Typbar-TCVTM.

Comparative immunogenicity study using Typbar –TCVTM and Peda TyphTM may be conducted in human volunteers to see if similar correlates to mice immunogenicity study are observed.

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