

Preclinical Development of a Prototype Protoplasmic Sonicate Protein Mucosal Vaccine Pspm of Salmonella Typhi in A Lapin Model

Ibrahim M S Shnawa

Department of Medical Biotechnology, College of Biotechnology,
Green University of Qasim, And Hilla University College Babylon /IRAQ

Mahdi H M Almmar

Department of Biology, College of Science, University of Kufa/IRAQ

ABSTRACT

Protein based mucosal vaccines of human communicable bacterial diseases are being within the current research mode. The present work was aimed at the preclinical development of a prototype protoplasmic sonicate protein PSP mucosal vaccine PSPMV for human typhoid via lapin live challenge model. From a local confirmed S typhi vaccine seed strain, a dense cell biomass was prepared and sonicated by cell sonicator. The resultant sonicate protein was considered as prototype vaccine. An amount of 3mg/ml. Of the PSPMV was injected through SC route to five rabbits followed by one week leave then eviscerated for gross and histologic changes. It was found to be safe. Then fifteen rabbits were divided into three groups each of five. Group I was primed per Os with five doses of 3 mg PSP each in 5ml. saline, in a week a part followed by sixth week leave. Group II received IFA through SC route for one-week followed by one week leave. Group III received 5ml saline per OS using same group I schedule. Three days after the leave week. Live S.typhi suspension of 1.5×10^5 to 5×10^5 CFU/ml., completed to 5ml in sterile saline were applied per Os to the three groups. Humoral agglutinin and heagglutinin responses were raised up to clinical titre limits were noted. In addition to significant leukocyte inhibitory factor response in challenged PSPMV group. Erythema, induration, necrosis was noted in skin DTH test of PSPMV group in a wistar rat model. Survival record after challenge were 5/5 100% in PSPMV group, 4/5,80% in FIA group and 0/5 0% in saline control group. The novelty in this achievement that PSPMV prototype S typhi showed 100% protection in rabbits, while OMP28 KDA vaccine was immunogenic but non-immune protective in a work done by other workers. These results are being promising but still need to be supported by developing a non-human primate challenge model.

Keywords; Agglutinins, heagglutinins, humoral, LIF, DTH, Protein Based Vaccine, Salmonella typhi.

INTRODUCTION

Protein is a polymer of amino acids with a characteristic profound stereochemical structure. This protein quaternary structure is considered to be a pre-requisite for the protein biological and immunological functions [1]. Eukaryotic derived proteins EP may function as antibody,

cytokine, enzyme and or venome beside it could served as structural entities of cell, tissues and organs forming the organisms. While, Prokaryotic proteins PP may function as; toxins, virulence associated enzymes and as short amino acid sequence signaling molecules. PP may form some structural entities as subcellular organelle [2].

In vaccine technology, bacterial proteins may hold a distinct group of protein based preventive vaccine versions [3]. Outer membrane proteins OMP of *S. typhi* has been tempted as a prototype experimental vaccine and found to be effective [4]. Flagellin and polysomes prepared from *S. typhimurium* designated as *S. typhi* complex prototype vaccine [5]. The aim of the present communication was to develop as an oral PSPMV prototype *S. typhi* vaccine through live challenge lapin model.

MATERIALS AND METHODS

Vaccine Seed Strain

A fecal culture of 38 clinically proven typhoid patients was done [1]. Five isolates were confirmed to be *Salmonella enterica* serovar *typhi* using classical biochemical tests, API 20 and serology. These five isolates were confirmed as *S. typhi* by the national Central laboratory, Ministry of Health, Baghdad. Among which one whose characteristics typify *S. typhi* was elected as vaccine seed strain following the below mentioned criteria [6].

1. Recovered from clinical typhoid case.
2. Produce serum and mucosal *S. typhi* specific antibodies and in patient
3. Produce significant mucosal and systemic leukocyte inhibitory factor in typhoid patients.

Purity

The purity check was performed both by direct wet slide preparation and quadrat streak culture onto DCA medium [7].

Cell Biomass

From 18 hrs fresh BHIB broth culture of the seed strain, 0.1 ml inocula were transferred into a series of 100 ml. flasks containing 60 ml. sterile BHIB broth. The inoculated flasks were incubated at 37°C in a shaker water bath with 60 shake per minute for 24 hrs. The growth in each flask was distributed into 5 ml amounts in centrifuge tubes, each of which was centrifuged at 5000 rpm for ten minutes. Pellets were washed twice with sterile saline at 3500 rpm. The resultant biomass was checked for purity. These constitute the cell biomass [8].

Protoplasmic Sonicate Protein PSP

Pellets from paragraph 2 were reconstituted with 5 ml. sterile saline solution and checked for purity. Pure suspensions were fractioned by cell sonicator at 18-20 oscillation for 15 minutes under cooling conditions. for five minutes. The sonicated preparations were centrifuged at 3000 rpm. Pellets were discarded while supernats were kept forming the protoplasmic mass PM. PM preparations were checked for purity [9]. Pure PM solutions were mixed with equal volumes of 6% 6000 PEG [10]. The mixtures were left for 1 hr at 4°C. Pellets were saved, dispensed in 1 ml. amounts in ampoules and tested for protein identity by Biuret test [11]. Biuret positive preparations were being PSP preparations.

Wister White Rat

A group of ten Wister white rats were grouped into two each of five one intraperitoneally injected 0.1 ml PSP solution and left for one week and the other injected by same route with saline. Then 0.1 ml in one ml. disposable syring was injected through intradermal route into each rat in both of the groups to detect skin DTH test [12]

Rabbits

Twenty rabbits were elected over a clutch of rabbits as proven free of ecto and endo parasites and free of natural bacterial infections, kept at libidum for two weeks at animal house. Then they were grouped into four groups as

Safety group..... five rabbits

PSP group..... five rabbits.

IFA group..... five rabbits

Saline Control group..... five rabbits.

Safety

From the dispensed PSP ampoules, one was elected and 0.1 ml amounts of PSP were injected via intraperitoneal route to each of the five assigned rabbits. The injected rabbits were left for one week in every now and then pattern of inspection for any noted whole-body abnormality. Then eviscerated to trace gross and histologic changes [13].

Specific immune Priming Protocol

The assigned rabbits PSP, IFA and saline control groups were primed as in Table – 1.

Table 1: The Specific Immune Priming Protocol [14].

Group	Route	Dose	Dosage frequency
PSP	Oral	3mg/ml completed to 5 ml sterile saline	Five weeks apart, the sixth week was leave week
IFA	Subcutaneous	1 ml	One week and other leave week
Saline	Oral	5ml	Five week followed by one week leave

Live Challenge Protocol

Three days post to the sixth week for the test and Saline groups. As well as, five mls dose containing 1.5×10^5 to five CFU were feed per Os to rabbits of the three groups. Survival, morbidity and mortality rates were recorded within one week period post to challenge [4].

PSPMV Developmental Features

Skin DTH test were performed to the test and control rabbit groups as 0.1 ml. amounts of PSP were injected to each primed rabbits in each group. Erythem, induration, and necrosis were noted within 48 hr post injection [15].

Serum agglutinin and hemagglutinin responses were done as in [16]. Leukocyte inhibitory responses were tested by capillar method as in [17]. Lymphocyte density in splenic sections were done as in [13]. The preclinical developmental features of PSPMV were made as in [7].

RESLUTS

Purity

The prepared; biomass, protoplasmic mass, crude protoplasmic sonicate, protoplasmic sonicate protein preparations were found pure on direct wet slide preparation and quadrate DCA media.

Safety

PSP was found to be of neither gross nor tissue pathology in a rabbit model.

Antigen Identity and Immunogenicity

Sera from PSP pre and post challenge and IFA post challenge were showing frank and specific positive agglutinins and heagglutinins. The humoral agglutinin and hemagglutinin responses were higher in PSP and IFA test groups as compared to nill titres in saline control group.

PSPMV Immunity

Cellular:

Dense and moderate hyperplasia were noted in splenic stained tissue sections of PSP and IFA groups in postchallenge state and normal lymphocyte distribution insplenic tissue sections of saline control rabbits. The leukocyte inhibitory factor LIF in PSP primed, IFA preconditioned and control rabbits were 0.3-0.45, 0.3-0.43 and 0.93-0.95 respectively, Table-2.

Table 2: Leukocyte Inhibitory factor LIF % for the rabbits and control test groups.

Vaccine	LIF% control	LIF % prechallenge	LIF% postchallenge
PSP	0.92-0.95	0.31-0.45	0.31 – 0.44
IFA	0.93 – 0.95	0.90 -0.95	0.3- 0.43

Delayed Type Hypersensitivity:

Variable degrees of erythema were noted in PSP and IFA groups but not in saline control group. Induration of 12 mm size around skin injected site for PSP group but not in IFA and saline groups with 48 hr post to skin sensitisation with PSP in a rat model.

Agglutinin and Hemagglutinin Responses:

The mean prechallenge and postchallenge agglutinin titres for S typhi anti-O antibody titre was; 224,224 and prechallenge and post challenge agglutinin titre means for S. typhi anti-H were 320,320 in PSP rabbit group.

The postchallenge mean anti-0 agglutinin titre in IFA group was 160 and anti-H mean agglutinin titres were 120. The mean prechallenge and postchallenge hemagglutinin titres were 696 and 696 respectively in PSP group while the postchallenge mean hemagglutinin titres was 505 in IFA group.

Efficacy

The PSP Lapin challenge model with live S. typhi have shown neither morbidity nor mortality with 5:5, 100% survival rate. As compared to IFA group have shown 4:5, 80% survival rate and 1:5, 20% morbidity rate. Saline control group have shown 0:5 100% morbidity rate.

Preclinical Development

The PSPMV was found; pure, safe, antigenic, immunogenic and immune protective in lapin challenge model and were matching to that of Ty 21a and classical vaccine, Table-3.

Table 3: Preclinical developmental features of PSPMV of Typhoid fever in lapin challenge mode.

Features [18]	PSPMV	Ty21a [19]	Classical [20]
Understanding Disease	U	U	U
Undestanding causal	U	U	U
Vaccine Candidate	Prepared	Prepared	Prepared
Purity	Pure	Pure	Pure
Safety	Safe	Safe	Safe
Antigenic Immunogenic	Antigenic Immunogenic	Antigenic immunogenic	Antigenic Immunogenic
Efficacy in lab animals	Rabbit immune effective 100%	mice Immune effective 90%	Mice Immune effective Up to 60%

DISCUSSION

Current information concerning oral mucosal vaccination with protein vaccines are indicating that there were no avialable approved mucosal protein-based vaccine candidate against infectious diseases such as influenza [21], tetanus [22], plague [23] and diphtheria [24]. Oral vaccination are the most effective routes among other mucosal routes and the availability of an oral derlivary devise bearing the advantage of being superior for patents complaiance, easy for administration and mass immunization capacity [25]. Since the mucosal surface is protected by major and specialised innate and adaptive mucosal immune system. Innat immune system exhibits an important interplay in fighting aganist initial infectious events and orchestrate the generation of the adaptive immune responses. While the adaptive responses are essential for providing immune protection against the previously encountered bacterial pathogens [26]. Mucosal vsaccination can be done through three steps;

1. effieicent vaccine antigen sampling and uptake,
2. antigen processing and presentation and
3. B & T lymphocyte activation, production of effector cells and genertion of their memory lymphocytes counterparts [27].

Protein antigens are widely tackedled in in vaccine development to protect human infectious diseases but they sufer from an inherent limitations like low stability and variable immunogenicity these two limitations may pose some difficulties in practice which may shed influence on their ability to induce granted humoral and cellular responses. Though some microbial proteins were proved to be efficient immunogens [6]. Salmonella outer membrane protein [4], flagellin [5] were proved as prototype candidate vaccine for typhoid. The present developed PSPMV was proved to be as; pure, safe, antigenic, immunogenic and immune protective, Table – 3. It did not need adjuvant may be due to an intrinsic adjuvanicity inborn in its native structure [28,29]. Protein based S.typhi vaccine candidates were traced from 2005 till date and dipicted as a timeline in Table- 4.

Table 4: Protein based S. typhi vaccine candidates Timeline.

S. typhi Protein based vaccine candidates	Model	Effective Immune protection	References
Fim-C recombinant	Ydd mice	Effective	[30]
OMP- 49KDA		Effective	[31]
Recombinan OMP-28 KDA	Rabbit	Immunogenic non- protective	[32]
Fim-C recombinant	Mice	Immunogenic induce antibody and protective	[33]
Outer membrane protein viscile	Mice	TH1, TH2, Th17 cytokines	[4]
Fim-C recombinant	Balb /c mice	Effective potential vaccine candidate	[34]
OMP-ChitosanMulti-epitopic	Mice	Protective in live challenge model. induce cytokine responses	[35]

S. typhi OMP-28KAD has been found immunogenic, but non-effective for live challenge in lapin model [32]. While, PSPMV was found immunogenic and immune protective, Tables-2-3. This situation may hold the novelty point of this contribution.

CONCLUSION

The prototype experimental PSPMV typhoid vaccine was prepared in atepwise manner as;

1. Preparation of Cell biomas and purity check,
2. Preparation of protoplasmic mass and purity check,
3. sonication by cell sonicator and purity check,
4. protein precipitation and purity check,
5. chemical identity determination,
6. safety check,
7. antigen identity check,
8. immunogenicity and ix-immune protectivity.

It was found as; pure safe, antigenic, immunogenic and immune protective in a rabbit model. The authors are of the opinion holding a suggestion to tempting this prototype vaccine in a non-human primate model.

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