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Lapin Challenge Model for Laboratory Development of an Oral Carbohydrate Based Vi Vaccine for Typhoid

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Abstract

A vaccine seed strainVSS of Salmonella enterica serovar typhoid was elected from local isolates of human enteric fever.A cell free culture fitrate antigen was prepared from VSS for in-vitro use.Vi antigen was separated,characterized from propagation of VSS in a suitable growth media.Incomplete Freund adjuvant IFA was as immune preconditionar and as immunostimlant.In three groups of rabbits each of five.First,saline control,second IFA preconditioned and the third was the Vi-IFA combination in which,the vaccine specific immune priming protocol was IFA through SC route to one week only.Followed by and oral 3mg/5ml dosages in a week a part for five weeks.Two to three days post to the sixth week dose,live S.typhi challenge dose of1.5/10 to five was applied per Os for the three groups.Saline control group got clinical infection, IFA group has shown 80% immune efficacy and the Vi-IFA group has shown 100% immune efficacy.Vi-IFA prototype experimental vaccine formulation proved to be;pure,safe, immunogenic,non-allergenic and immune effective against experimental live challenge.It induces humoral agglutinins , hem agglutinin and inhibits migration of leukocyte in capillary test.Confirmation of these findings in other nonhuman primate model is suggestive.

Keywords: Adjuvant; challenge; enteric fever; model; lapin; prototype; seed strain; vaccine.

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1. Introduction

Typhoid circulating agglutinins had been used as an infection probe for human typhoid fever at Babylon province 1996[1].Typhoid seroprevelance had been reported at babylon province area[2].Mucosal and systemic S.typhi antibodies in typhoid patient have been determined by Ferial Abd[3].AlSarhan 2014[4] reported secondary cryoglobulinemia associated with typhoid fever.ALMOosawi and his colleagues [5],have been documented the development of Vi typhoid vaccine in a guina pig model.The aim of the present investigation was aimed at developing Vi typhoid vaccine in a lapin challenge model.

2. Material And Methods

2.1 Prototype Vi typhoid Vaccine Development

From the VaccineSeed Strain VSS [6, 7],an 18 hr growth onto DCA plate,five colonies of anallogus morophotypes were transfered to sterile Brain heart infusion broth BHIB tube and incubated for 2 hrs at 37C.From this growth, 0.1 ml of the growth transfered to a series of flasks containing 100cc BHIB and incubated at 37C in a shaking incubator with 60 cycle/minute for 24 hrs to obtain high density growth.The growth in the series of flasks,inocula were transfered and quadrate streaked on to DCA medium for purity check.The propagated growth were centrifuged in cooling centerfuge at 5000 rpm for 20 minutes.The pellets which represent the vaccine bacteria cells were deried for 48hrs at 37C incubator.The deried pellet cell populations were mixed with ethanol and centrifuged at 5000 rpm for 20 minutes.The precipitate was mixed with aceton and centrifuged again.Pellets were washed twice with ether and precipitate deried in an incubator at 40C.This pellet represented the vaccine bacterial dry weight.Ten grams of dry weight was suspended in a sterile 0.9 sodium chlorid solution and shaked for 30 minutes.The suspesion was centerfuged at 3000rpm for 30 minutes.Supernatents were withdrawn and dialysed against runing tap water for 12 hrs.NaCl was added to the dialyzing solution up to 0.9%.This preparation was precipitated with ethanol slowly to form ;0.1,0.2 and 0.3 M.The formed precipitate at each added concentration were collected by centrifugation.Pellets were dissolved in distilled water.Acetic acid were then added up to 0.1 M.The obtained purified precipitate was redissolved in DW and treated with acetic acid to 1M concentration.The solution was reflexed in a reflex condenser for 24 hrs then dialyzed and ppt in ethanol.PPTs were dispensed in 3 mg/ml amounts in ampoules and stored at 4 C[8].

2.2 Vi Prototype Vaccine Developmental Features

The Vi chemical identity was determined by Molish's test[8]. The purity was checked by quadri streack method on DCA[8]. Safety check through SC injection of the Vi prototype vaccine in five normal rabbits left for five days then eviscerated for checking any gross pathological and histological visceral changes[9].Immunization protocol was made as in[10] ,Table - 1 .Delayed skin hypersensitivity test DTH[11], agglutination and hem-agglutination [12,13], and leukocyte inhibitory factor was performed as in [14].Rabbit's live challenge was done and briefed as;in the third day after the 6th week,5 ml of containig 1.5x10 to 5 CFU of live S.typhi.Infected immune primed and control rabbits were watched for five days for survivors,morbed ity and mortality rates[5]

Table 1: Rabbits Immunisation protocols.

Immune Priming	Rout	Dose	Dosage frequency in time term	Number of test rabbits
Vi-IFA	IFA/SC Vi-IFA/oral	1 ml. 3mg/5ml	1 week Five weeks,one week a part	5 rabbits
IFA	SC	1 ml.	1 week	5 rabbits
Saline	Oral	5ml.		5 rabbits

3. Results

3-1 Purity

The quadri streaked Vi prototype vaccine preparation onto DCA plates have shown neither growth of contaminants nor growth of salmonella typhi colony morphotpes.

3.2 Safety

The prepared prototype Vi vaccine preparation SC injected and eviscerated rabbits have shown neither gross pathological nor histo-pathological changes.

3.3 Immune Identity

The sera of Vi-IFA and IFA primed rabbits have shown seropositiviity with Vi and Cell free culture filtrate antigens via agglutination and hem-agglutination tests

3.4 Allergenicity

Both of IFA non-specific and Vi-IFA specific immune primed rabbits have shown erythem in first few hour post to ID injection of CFCF antigen in their skins without any evidence of the following induration and necrosis event.Vi rat model injected with 1 ml IM and SC routes for one week the ID injected with 0.1 ml.CFCF antigen have shown erythema, induration and necrosis 48 hrs post ID priming.

3-5 Immunogenicity

The IFA primed rabbit have shown nill titres before live challenge with S.typhi.While postchallenge with life S typhi have shown titre means of anti O 160 anti H 120 agglutinins.While before challenge hemagglutinin titres were nill.The after challenge hemagglutinin titre means were 505.The Vi-IFA primed rabbits before challenge gave agglutinin titre means of 256 for both anti-O and anti-H.The postchallenge agglutinin titre means were 256 for anti-O and 320 for anti-H.The prechallenge and postchallenge hemagglutini titre means were 1024.Leukocyte inhibitory factor studies reveals that there were significant inhibition of leukocyte in pre and postchallenge Vi-IFA primed rabbits with dense splenic hyperplasia as compared to non-significant inhibition of leukocyte migration in IFA primed in prechallenge and significant in postchallenge state accompanyed by moderate splenic hyper plasia,Table 2.Vi-IFA mediate humoral(agglutinin and hemagglutinin responses) and

cellular immune responses(LIF responses).

Table 2: Leukocyte Inhibitory factor LIF in immune primed rabbits.

Vaccine priming	LIF inPre-experimental	LIF in prechallenge	LIF in postchallenge	Splenic hyperplasia
Vi-IFA	0.93-0.95	0.35-0.45	0.35-.45	Dense splenic hyperplasia
IFA	0.9-096	0.9-0.95	0.3-0.35	Moderate hyperplasia
Saline control	0.95-0.97	0.95-0.97	0.95-0.97	Normal splenic tissue archecture

3-6 Immune Efficacy

The survivore percentages of postchallenged rabbits was 5:5 100% in Vi-IFA primed group and 4:5, 80% in IFA group and 0:5 0% in saline control group.

3-7 Vi-IFA prototype Vaccine Developmental Criteria

The VI-IFA combination in separate application sites proved to be;pure, safe, immunogenic and efficieous with no evident adverse effects,Table-3.

Table 3: The Vi- IFA prototype vaccine developmental features.

Features	Vi-IFA	IFA	Vi local [5]	Vi commercial[5]
Understanding disease UD	UD	UD	UD	UD
Understanding Causal UC	UC	UC	UC	UC
Purity P	P	P	P	P
Safety S	S	S	S	S
Immunogenicity Imm	Imm	Imm	Imm	Imm
Efficacy E	E,100%	E 80 %	E90 %	E70%

4 Dissucssion

The search for typhoid vaccine in multiple versions hold the position of every present issue in past,present and future[15, 19].To develope a vaccine ; all what we need is vaccinal strain, series of developmental features , siutable lab animal models in preclinical phase of development and numbers of volunteers for the clinical phases of development [20].The typhoid versions of vaccine that have been tried were as; whole cell,attenuated, conjuagate and molecular makes [15, 19].The theme of the present work was to develope a prototype Vi-IFA typhoid vaccine version in a lapin model.

Understanding the pathogenesis and the causal of typhoid is a pre-requist for undestanding vaccine developmental phases[20, 21].The pathogenic mechanisms of enteric fever infection and disease is started by the translocation of infectious events from the intestinal mucosa to blood stream followed by the sytemic dissemination of the invading salmonellas to the distal organs facilitating the emergance of the enteric fever disease.Enteric infectious disease cause more than billion disease episodes per year worldwide and claim

nearly two million lives each year mostely in lesser developed countries[20, 21].

Several systemic vaccines have been developed for human typhoid fever[15].Though mucosal vaccines are preferable due to the fact that the infection are mostely encountered at mucosal sufaces[15, 16].This necesstate the development of mucosal vaccines which may be hampered by the; limited knowledge of childhood gut mucosal immune system,lack of suitable mucosal adjuvant and rather unclear correlates to immune protection and limited knowledge of the factors affecting oral vaccines in children of developing countries.The application of oral vaccines through vaccination protocol may initiate mucosal antibodies in small intestine ,colon,rectum and blood [15] .Among the known typhoid mucosal vaccines is Vi vaccine versions[17].

Mice,rat, gunia pig, and chimpanzees were the common experimental animals for investigation of pathogenesis and vaccine development for salmonellas[22] .Immuno-competant mouse found valid both for pathogenesis and vaccine production[23].Rabbit have been proved to be the model of use in matching pathogenesis of S.typhi[24, 25] .Chimpanzee a non-human primate laboratory animal have been proved to be valid for both studying pathogenesis and vaccine development of typhoid[26, 27].Huq and his colleagues [28] have been tempting Vi,Vi-OMP congujates in a mice model and reported high antibody responses in Vi-OMP conjugates than in Vi alone.Vi, and Vi conjugates have shown memory B cell activation in mice model[29].,Table – 4 .Vi conjugate vaccine has shown to sustain the efficacy of immune responses[30].

Table 4: Developmental immune features of various vaccines of Salmonella typhi in laboratory animal model.

Vaccine version	Laboratory animal model	Findindgs	References
Vi	Gunia pig	Effcacy 90 %	ALMousawi and his colleagues [5]
Vi,Vi-protein combinations	Mice	Combination vaccine showed interference,significant infleuence on B cell afinity maturation	Zhang and his colleagues [29]
Vi-IFA	Rabbit	Rabbit Challenge model showed 100% efficacy	This study

Repeated oral dosing protocol together with IFA SC priming rabbits higthen the immunity of Vi-IFA in primed rabbits and records 100% immune efficacy as compared to 80% protection in IFA primed rabbits,Table -3 and 90% in VI primed gunia pigs[5].The Vi-IFA vaccine development lapin challenge model,Tables- 1-4,Oral multiple doses of Vi make vaccine units available in contact with mucosal immune cells together with the action of separatly SC injected IFA induces continious cell-cell cooperation events during the immune response inducing high humoral and cellular responses affecting the production of specific antibodies and acivation of T lymphocytes specific to S.typhi vaccine units[19].

To this end, we laboratory develope Vi-IFA prototype experimental vaccine for typhoid disease with an evident and characteritic immune developmental featres,Tabl e– 3 as;Pure,safe,immunogenic,non-allergenic in rabbit and allergenic in rat and immune efficieous.It induces specific S.typhi agglutinin and hemagglutinin antibodies

and significantly inhibits leukocyte migration in pre and post challenge immune states.Rabbits were proved to be valid immune model for laboratory development of Vi vaccine of typhoid.The authors holds the idea that the vaccine design presented in Tables – 1-4 is being novel acheivment since it holds valid lapin model for typhoid vaccines in contrast to the in common issue that lapin are only valid to pathogenesis study of Salmonellas[22].

5 Suggestion

The authors are of the opinion that running this prototype vaccineVi-IFA in non-human primates like chipanzees model is advisible since the use of more than one mammalin immune system strengthen the drawn conclusion concerning the efficacy of prototype vaccine under laboratory development stages.

6 Conclusion

Vi –IFA prototype typhoid vaccine was developed via lapin live challenge model.Rabbits were proved to be valid models for development of Vi vaccine development.

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