VACCINES FOR PREVENTING CHOLERA

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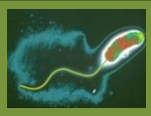


1. Antecedents

Cholera is an acute secretory diarrhoea disease caused by intestinal infection with *Vibrio cholerae*. Its estimated cholera affects 3–5 million people with **100,000 dead each year**, around the world.[1] Infection is usually asymptomatic or mild, but sometimes can be severe. Approximately **5% of infected people develop severe cholera** and without treatment, over 50% of people can die in a few hours. Cholera has probably existed on the Indian subcontinent for thousands of years. From 1817 cholera has caused seven important pandemics around the world, with thousands deads.[2] The transmission happens when food and water are contaminated through the faeces of infect people. Complications of cholera are causatives by the loss of fluids and electrolytes. Cholera's treatment consists only of quickly oral fluid replacement. But as it happened in the past, cholera remains largely a disease of **impoverishment**, where **sanitation water** is inappropriate or absent, social unrest and displacement, and continues to be a disease of major public health concern.

2. Objective

The principal objective is develop a new oral cholera vaccine, which would gives more protection to prevent the disease of cholera, inducing strong immune response, than prequalification oral vaccines.

3. Methodology and work plan

V. cholerae is Gram-negative proteobacteria, comma-shaped, facultative anaerobic, with a single polar flagellum. *V. cholerae* have two types or serogroups where only two, are **O1** and **O139**, can be epidemics, because they have the ability to produce **choleric toxins**.

Genome of *V. cholerae* has two pathogenic islands, where the genome has been lysogenized with a filamentous phage. It contains regulatory and virulence factors, such as **ctxA** and **ctxB** (cholera toxin genes), that help the pathogen colonization and toxin expression (figure 1).[3]

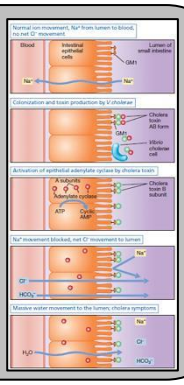


Figure 1: The action of cholera enterotoxin. Cholera toxin is a heat-stable AB enterotoxin that activates a second messenger pathway, disrupting normal ion flow in the intestine.[2]

Lipopolysaccharides and **cholera toxin** are virulence factors, which are important for the immune response, concretely **humoral immunity** (figure 2). Cholera toxin induces sIgA, which neutralizes pathogens of intraluminal mucus. LPS induce IgA and IgM, which plays an important role in affinity maturation IgG, important for the development of memory B cells.

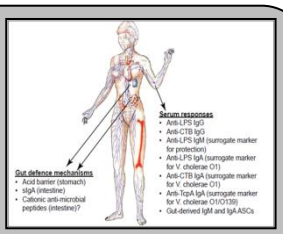


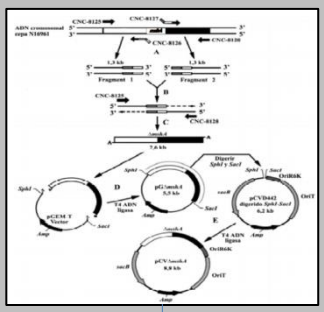
Figure 2: Schematic diagram showing gut defences and serum immune responses against *V. cholerae* infection.[4]

Currently, there are three prequalification oral vaccines for the World Health Organization (WHO), described in table 1. **Duration of protection** is the first problem we can see, it's less than **two years** long and less than **90%** of protection. In addition there are other troubles like storage, price, shelf life and presentation where is required an intervention.

Characteristic	WC-rCTB	CVD103-HgR	Reformulated WC-only
Commercial name	Dukoral®	Orochol® or Mutachol®	Vietnam: mORCVAX® India: Shanchol®.
Vaccine type	Killed whole cells of *V. cholerae* O1 plus CTB recombinant	Live attenuated *V. cholerae*.	Killed whole cells of *V. cholerae* O1.
Vaccine composition	Inaba Cairo 48 Classical and Ogawa Cairo 50 Classical (heat-killed), Inaba Phil 6973 El Tor and Ogawa Cairo 50 Classical (formalin killed)	Inaba Classical strain 5948	Ogawa Cairo 48/50 Classical (heater-killed), Inaba Phil6973 ET Tor, Ogawa Cairo 50 Classical and 42806 O139 (formalin killed).
Administration Route	Oral	Oral	Oral
Formulation	Liquid	Lyophilized	Liquid
Regimen	2 doses	1 dose	2 doses
Age range for vaccination	≥ 2 years	≥ 2 years	Vietnam: ≥ 2 years India: ≥ 1 year
Duration of protection	2 years	≥ 6 months	At least 3 years
Buffer requirement	Yes	Yes	No
Storage temperature	2° to 8° C	2° to 8° C	2° to 8° C
Shelf life	3 years	2 years	2 years
International acceptance	WHO-prequalified	Was WHO-prequalified until 2004	WHO-prequalified
Price to the public sector per dose	~$1.25/dose for small quantity of 250.000 doses	Vaccine not currently available	Vietnam: ~$0.75/dose India: $1.85 per dose
Protection 1st/2nd year	80%/60%	91% only six months	68%/50%

Table 1: Comparison of killed WC-rCTB vaccine, live CVD-HgR vaccine and reformulated WC-only vaccine.[2,5,6]

A new oral recombinant vaccine was synthesized by **outer membrane vesicles** derived from *V. Cholerae*. The idea is to use a method that can present LPS-modified with CTB-modified and it gives protection against both clinically relevant serogroups. These vesicles consist of outer membrane lipids, a subset of outer membrane proteins and soluble periplasmic components (figure 4).[7]



1. To construct the thermosensitive suicide plasmids pCTB/pLPS/pCAP, upstream and downstream fragments of the gene were PCR-amplified using the oligonucleotide pairs digested with the appropriate restriction enzyme, and finally ligated into the digested pCTB/pLPS/pCAP (figure3). Deletion mutants were generated using derivatives of the suicide vector pCTB/pLPS7pCAP.[7] Plasmids are carrying Amp^R and recombinase.

Abbreviations:
pCTB: Modified ctxB gene fused to the type 2 secretion signal sequence (derived from the β-lactamase gene).
pLPS: Truncated msbB (lacks a typical lipid A structure).
pCAP: Mutant otnA (alteration transporter of capsule precursors).

Figure 3: Schematic figure of one study that have shown the deletion of mshA by PCR and construction of suicide vector pCVDmshA.[8]

2. Obtaining our plasmids and confirmation by PCR.

- pCTB → ctb-modified + β-lactomase gene + Km^R
- pLPS → msbB-modified + Tet^R

- pCTB → ctb-Modified + β-lactomase gene derived + Km^R
- pLPS → msbB-Modified + Tet^R
- pCAP → otnA-Modified + Cm^R

E. coli Serogroup 01 Biotype El Torn

E. coli Serogroup 01 Biotype Classical

E. coli Serogroup 0139

3. Conjugation and homologous recombination.

4.High Tº selection was applied to obtain Amp^1 colonies and correct resistant deletions, by recombinase, were confirmed by PCR.

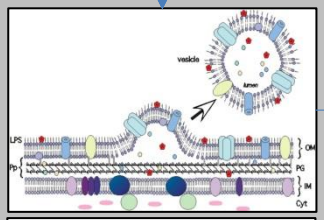


Figure 4: Model of vesicle biogenesis. OM vesicles are proteoliposomes consisting of OM phospholipids and LPS, a subset of OM proteins, and periplasmic proteins.[8]

Control, checking live-cell contamination

Vesicles were resuspended in buffer, filtered and mixed

5. Centrifugation and filtration of the supernatant.

Final vaccine

4. Expected results

- Vesicles, CTB-modified and LPS-modified, were produced correctly and no problems showed up.
- Results showed high levels of antibodies Anti-LPS O1 (Inaba and Ogawa), anti-LPS O139, anti-capsular O139 and anti-CTB.
- All immunization groups induced a robust and long immune response with similar levels of Ig titers against both serogroups.
- The protective efficacy of anti-LPS antibodies is mediated either through vibriocidal activity or inhibition of adhesion to the epithelial surface.
- Only one dose was required and the protection was always 97% in mice.
- The vaccine shelf life was 5 years and storage temperature should be between 2-8°C. But the antigenicity of OMVs is not significantly compromised after prolonged heating, the viability was 60 days.[7]
- Price was unclear, but when large scale production takes place, prices will be cheaper than the other prequalified vaccines.

5. Benefits of the project

We need cholera vaccine because cholera is endemic in developing countries. Although the provision and establishment of safe water, food, and sanitation systems are the principal way to prevent cholera, these measures cannot be implemented in the near future in most areas where there is cholera. It's known that *V. cholerae* shows increased resistance to antibiotics, complicating clinical management. These concerns and the improved protection of new-generation oral cholera vaccines have an importance in order to reduce cholera cases, alongside traditional cholera prevention measures.

6. Work diffusion

Regardless of whether the applicant group intends to publish the results in more impact journals in endemic areas, it's expected that the above results could be useful for professional sectors companies or users.

7. Bibliography

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