Process development for a Cuban cholera vaccine based on the attenuated strain Vibrio cholerae 638

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Available online 22 July 2005

Abstract

Genetically modified Vibrio cholerae strain 638 (biotype El Tor, serotype Ogawa) has previously been shown to be immunogenic in animal models and in human trials. Our objective in the work reported herein was to describe the process development methods for the production of the 638 attenuated cholera vaccine. Cell seed bank, culture of biomass, lyophilization and final formulation were processes were developed. The results show kinetics of culture that fulfills a logistical model. The microbiological properties, colonizing capability, immunogenicity and non-toxigenicity of the final product were indistinguishable from the properties of the working seed lot. We conclude that the non-reactogenic, immunogenic and protective strain 638 is robust and can withstand the fermentation processes required for large-scale production of a vaccine.

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Keywords: Vaccine; Cholera; Process

1. Introduction

Vibrio cholerae is the infectious agent responsible for cholera. Only Vibrio cholerae O1 and O139 serogroups are known to cause epidemics of cholera. Isolates of Vibrio cholerae serogroup O1 are classified into two biotypes, El Tor and classical, on the basis of several phenotypic characteristics. Currently, the El Tor biotype is responsible for virtually all of the cholera cases throughout the world and classical biotype isolates have virtually disappeared worldwide. In addition, Vibrio cholerae O1 is classified into two main serotypes, Inaba and Ogawa, based on agglutination test using polyvalent antiserum against lipopolysaccharide (LPS) antigens. A third serotype, Hikojima, has been described, but it is very rare. The immunity due to previous infection of Vibrio cholerae is serogroup specific. There are other serogroups of Vibrio cholerae (for example, serogroups O5 and O37) that can cause isolated cases of watery diarrhea but they do not cause epidemics. The current seventh pandemic of cholera due to Vibrio cholerae O1 biotype El Tor has been reported from all regions of the world [1].

Some strains of Vibrio cholerae O1 show resistance to multiple antibiotics including to ampicillin, furazolidone, tetracycline and trimethoprim/sulphamethoxazole, drugs that are recommended by the WHO for treatment of clinical cholera. Massive prophylaxis has been shown to be unsuccessful and is discouraged. Although the organism remains susceptible to nalidixic acid (not used for the treatment of cholera but used as a proxy for sensitivity to all fluoroquinolones), minimum inhibitory concentrations are increasing [2].

Two oral cholera vaccines that are already licensed have recently been evaluated for their effectiveness and practicality as public health tools in controlling cholera outbreaks in developing country populations at high risk of cholera [1,3,4]. These experiences suggest that mass vaccination with oral cholera vaccines can be a useful adjunct tool for controlling outbreaks [3,4] particularly if they are implemented early, in association with other standard control measures.
Genetically modified *V. cholerae* strain 638 (biotype El Tor, serotype Ogawa) has previously been shown to be a good colonizer of the intestine and is strongly immunogenic and protective, as well as being non-virulent and non-toxigenic “in vitro” and in animal models and small scale human clinical trials [5]. Recently, it was shown to confer a high protection in an experimental challenge study involving human volunteers [6]. In a definitive vaccine formulation strain 638 also showed good viability “in vitro” and retained the capacity to colonize neonatal mice [6].

Herein we describe the process development methods for the consistent, potentially large-scale production of attenuated cholera vaccine strain 638. Methods for the working seed lot, culture of biomass, lyophilization and preparation of a final formulation were developed. The yield and process consistency, identity, potency, safety and stability of the product were documented.

2. Materials and methods

2.1. Working seed lot

A lyophilized 638 strain from the reference seed lot was cultivated in Tryptone Peptone Broth at 37°C, with shaking at 200 rpm for 4 h. Each 0.3 mL of this culture was mixed (v/v) with skim milk (10%) and glycerol (20%) and distributed in cryopreservation tubes (1.5 mL) and finally frozen at –70°C, until use.

Viability was determined in three tubes every 6 months by enumeration of colony forming units (CFU). Morphology studies were done by optic microscopy with Gram stain and by electron microscopy using negative staining [8].

Biochemical and serological tests for identity were done according to OPS/OMS/CDC/NCID [9]. These included the string test, kligler iron agar, lysine iron agar, Voges proskauer, oxidase, catalase, arginine dehydrolase and ornithine and lysine decarboxylase tests.

2.2. Colonization test

The colonization studies were done in neonatal mice (Balb/c, 2–4 days) [10]. Each animal was orally inoculated with 50 µL of bacterial suspension (10^8 CFU/mL) containing Evan’s blue dye (0.01%). After 24 h, the animals were sacrificed and the CFU/g of intestine was determined. Adherence of vaccine strain to intestinal mucosa of mice was observed by direct immunoperoxidase method [11]; mice were inoculated with 50 µL/cm of accumulated fluid, i.e., lack of enterotoxic activity. The assessment of colonization capability in neonatal mice demonstrated 2.4 × 10^7 ± 1.73 × 10^7 CFU/g of intestine. Moreover, by immunoperoxidase method it
was shown that the vaccine bacteria adhered to the mucosal layer of the mouse intestine. The serum antibodies showed a vibriocidal titer of $2.9 \pm 0.4$ (log T$_{-1}$) and an ELISA titer of IgG against LPS of $3.2 \pm 0.8$ (log T$_{-1}$). All these results are very similar to the results obtained with the original 638 strain, as previously described; accumulated fluid $0.28 \text{ mL/cm}^{14}$, intestinal colonization in neonatal mice $1 \times 10^6 \text{ UFC/g}$ [7], vibriocidal titer $2.75$ (log T$_{-1}$) [14] and ELISA titer (IgG against LPS) $2.64$ (log T$_{-1}$) [14].

3.2. Process

The fermentation showed a culture kinetics that fulfills the logistical model, with a growth rate of $1.6 h^{-1}$ (Fig. 2) and a consistent yield of $2.6 \times 10^{10} \pm 6.5 \times 10^9 \text{ CFU/mL}$ of culture. The final product kept the same morphological, biochemical, serological and genetic markers as the cultures of the working seed lot. The final product also maintained the colonizing capability, $1.1 \times 10^6$ ($2 \times 10^5$ to $1.6 \times 10^6$) CFU/g, and its immunological capacity tested in rabbits, vibriocidal titer of log T$_{-1}$ 3.3 (2.5–3.7) and ELISA titers of log T$_{-1}$ 3.1 (1.7–3.4). In general, after the production process the strain in the final product kept the relevant characteristics of the original strain 638 conserved in the working seed lot, without any divergence. Finally, the stability test of the final products was more than 80% of viability, for at least one year.

4. Conclusion

The non-reactogenic, immunogenic and protective live oral cholera vaccine strain 638 is robust and strain able to withstand the various production processes necessary to reliably and consistently manufacture an industrial formulation of this cholera vaccine.

References


