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Review

The state-of-the-art of approved and under-development cholera vaccines

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ABSTRACT

Cholera remains a huge public health problem. Although in 1894, the first cholera vaccination was reported, an ideal vaccine that meets all the requirements of the WHO has not yet been produced. Among the different approaches used for cholera vaccination, attenuated vaccines represent a major category; these vaccines are beneficial in being able to induce a strong protective response after a single administration. However, they have possible negative effects on immunocompromised patient populations. Both the licensed CVD103-HgR and other vaccine approaches under development are detailed in this article, such as the *Vibrio cholerae* 638 vaccine candidate, Peru-15 or CholeraGarde® and the VA1.3, VA1.4, IEM 108 and VCUSM2 vaccine candidates. In another strategy, killed *V. cholerae* vaccines have been developed, including Dukoral®, mORCAX® and Sanchol™. The killed vaccines are already sold, and they have successfully demonstrated their potential to protect populations in endemic areas or after natural disasters. However, these vaccines do not fulfill all the requirements of the WHO because they fail to confer long-term protection, are not suitable for children under two years, require more than a single dose and require a distribution chain with cold storage. Lastly, other vaccine strategies under development are summarized in this review. Among these strategies, vaccine candidates based on alternative drug delivery systems that have been reported lately in the literature are discussed, such as microparticles, proteoliposomes, LPS subunits, DNA vaccines and rice seeds containing toxin subunits. Preliminary results reported by many groups working on alternative delivery systems for cholera vaccines demonstrate the importance of new technologies in addressing old problems such as cholera. Although a fully ideal vaccine has not yet been designed, promising steps have been reported in the literature resulting in hope for the fight against cholera.

Contents

1. Introduction

In 1854, Filippo Pacini described a curved bacterium from the stools of a cholera victim and called it *Vibrio cholerae* (VC) [1]. Subsequently, in 1884, Robert Koch isolated *V. cholerae* (VC) in pure culture for the first time. Since then, this organism's biology has

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been studied in depth. However, VC remains a public health issue associated with a high disease burden [1–3].

2. Physiopathology of *V. cholerae*

V. cholerae is a gram-negative, curved-rod bacterium with a single flagellum. It is classified into different serogroups according to differences in its O side chain of the lipopolysaccharide (LPS). Although the O antigen presents a vast diversity of variants, only the O1 and O139 variants are known to cause epidemic or pandemic outbreaks [4–6]. The O1 serogroup can be further divided into two biotypes, Classical and El Tor. Furthermore, both biotypes can be classified into Ogawa and Inaba serotypes. The Ogawa serotype is able to produce the A and B antigens as well as the C antigen, but the C antigen is produced to a relatively low extent. In contrast, the Inaba biotype produces the A and C antigens. A third serotype, Hikojima, is known to produce the three antigens, A, B and C, but it is infrequent [3,7].

V. cholerae has two main virulence factors, the toxin co-regulated pilus (TCP) [8] and cholera toxin (CT) [9]. However, *V. cholerae* is capable of producing other enterotoxins, such as the zona occludens toxin (ZOT) [10] and the accessory cholera enterotoxin (ACE) [11], Shiga-like toxins [12], the heat stable toxin (ST) [13] and the new cholera toxin [14,15].

3. Disease burden: transmission, treatment and facts

Cholera is transmitted by the fecal–oral route; however, it is not strictly a waterborne malady, and the role that contaminated food plays in the disease's spread should also be considered. A high inoculum, 10^5–10^8 bacteria, is necessary for infecting a healthy individual. Nevertheless, in hypochlorhydrial situations, lower doses (as low as 10^3 CFU) are capable of causing cholera. In fact, 10^6 *V. cholerae* O1 organisms given with either buffer or food can cause an attack rate of ~90% in volunteers. [3,16,17]. However, it should be taken into account that a single infected individual discharges as many as 10^{13} *V. cholerae* bacteria per day in their stools [18]. In addition, *Vibrios* can persist in environmental water associated with other microorganisms, constituting a reservoir of infective organisms [19–21].

Without treatment, the cholera case fatality rate can be as high as 70% [1]. Fluid replacement must be carried out rapidly as the first measure. First, intravenous rehydration should be conducted. When vomiting allows it, oral hydration should be undergone, and antibiotic therapy should be administered to reduce the stool volume and decrease the duration of the illness [5,22]. Unfortunately, since 1977, antibiotic-resistant *V. cholerae* strains have been reported. *V. cholerae* has developed many types of resistance over recent decades, such as resistances to quinolone, fluoroquinolone and ciprofloxacin, as summarized in Table 1 [1,23].

Epidemic outbreaks occur frequently, especially after natural disasters or wars. These explosive outbreaks are more likely to appear in locations with poor sanitation and inadequate hygiene conditions and where the supply of drinking water is restricted [22,24]. Since the 19th century, seven cholera pandemics have been described, and the currently ongoing one began in 1961 [25]. This pandemic is caused by the *V. cholerae* O1 biotype El Tor, which was originally found in Indonesia and has replaced the Classical strain around the world. The strain spread to Africa during the 1970s and reached South and Central America in 1991 [7,16,21]. In 1992, a new serogroup, denominated O139 Bengal, was found to provoke cholera outbreaks [6].

The lack of laboratory case confirmation and the potential for tourism or economic impacts leads to an under-reporting of cholera cases. During 2011, only 589,854 cases were reported, of which 7816 were fatal, leading to a case fatality rate of 1.3% [26]. It is estimated that approximately 3–5 million cholera cases per year might actually arise around the world, giving rise to 100,000–120,000 deaths [2,21]. During the last three years, a sharp increase in cholera cases has been described, with an almost 3-fold increased number of cases reported to the WHO, mainly as a consequence of the Haiti earthquake [1,26,27]. Due to this scenario, the re-emergence of cholera as a public health issue was highlighted at the 64th World Health Assembly during 2011, mainly because of the outbreaks in Zimbabwe and Haiti [28]. Similarly, the need to implement an integrated and comprehensive approach to controlling cholera was also emphasized [26]. The use of oral cholera vaccines (OCV) seems to be a promising strategy; nevertheless, safe water, food safety and sanitation system implementations are also imperative. At a previous WHO meeting held during 2005 in Cairo, the WHO described a method for deciding whether a mass vaccination is feasible in a complex emergency (Fig. 1). OCV played a role in disease prevention, and two main approaches were proposed, (i) OCV introduction into national immunization programs in endemic areas and (ii) OCV stockpile strategy initiation as an alternative preventative measure against cholera outbreaks [29]. In this regard, this review will focus on the currently available cholera vaccines and on the vaccines under development.

4. Cholera vaccines

The lesson learned from the Spaniard Jaume Ferran is noteworthy because he demonstrated that mass vaccination with parenteral cholera vaccines could control an epidemic outbreak during 1884 [30,31]. In the late 90s of the 19th century, Haffkine and Kolle developed a whole-cell parenteral cholera vaccine; however, it provided only a short protective effect, from 3 to 6 months, and a low rate of effectiveness, ≈48% [32,33]. In addition, the vaccine also produced a painful local inflammatory reaction. This vaccine might have been useful for those who could afford it during the early 20th century, when treatment was not effective and sanitation was inappropriate; however, it is not currently recommended by the WHO [3,5,21,34].

Since then, several cholera vaccines have been introduced; still, most of the current efforts are orally administered cholera vaccines. Herein, attenuated, killed and new approaches to oral cholera vaccines are described in more detail.

5. Attenuated live oral cholera vaccines

Attenuated vaccines display some advantages over killed or inactivated vaccines, such as being potentially more reactogenic or effective after only single-dose inoculations. It has been postulated that attenuated vaccines are able to mimic natural infections, and therefore they should provide long-lasting and specific responses [35]. However, they may possess some drawbacks, such as presenting overly high reactogenicity, as was the case with the Texas Star™ strain whose development was finally abandoned [36]. On the other hand, killed cholera vaccines have shown to be safe and effective. However, they require a two-dose administrations schedule, which in certain disaster situations is difficult to carry out [37].

A summary of the attenuated vaccines developed using O1 and O139 strains is presented in Table 2.

CVD103-HgR, Orochol™ (Berna, Crucell, Switzerland), is the only licensed attenuated *V. cholerae* vaccine. It is provided in two sachets, one containing the vaccine and the other the neutralizing buffer needed to protect the formulation from the gastric environment. This vaccine requires administration together with 100 ml of cold or warm water. Once reconstituted, it should be kept below

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