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A Phase I trial to evaluate the safety and immunogenicity of WRSs2 and WRSs3; two live oral candidate vaccines against *Shigella sonnei*

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ABSTRACT

Effective vaccines are needed to combat diarrheal diseases due to *Shigella*. Two live oral *S. sonnei* vaccine candidates, WRSs2 and WRSs3, attenuated principally by the lack of spreading ability, as well as the loss of enterotoxin and acyl transferase genes, were tested for safety and immunogenicity.

Healthy adults 18–45 years of age, assigned to 5 cohorts of 18 subjects each (WRSs2 (n = 8), WRSs3 (n = 8) or placebo (n = 2)) were housed in an inpatient facility and administered a single oral dose of study agent 5 min after ingestion of oral bicarbonate. Ascending dosages of vaccine (from 10^3 CFU to 10^7 CFU) were evaluated. On day 8, treatment with ciprofloxacin (500 mg BID for 3 days) was initiated and subjects were discharged home 2 days after completing antibiotics. Subjects returned for outpatient visits on day 14, 28 and 56 post-vaccination for monitoring and collection of stool and blood samples.

Both WRSs2 and WRSs3 were generally well tolerated and safe over the entire dose range. Among the 80 vaccinees, 11 subjects developed diarrhea, 8 of which were mild and did not affect daily activities. At the 10^7 CFU dose, moderate diarrhea occurred in one WRSs2 subject while at the same dose of WRSs3, 2 subjects had moderate or severe diarrhea. Vaccinees mounted dose-dependent mucosal and systemic immune responses that appeared to correlate with fecal shedding.

S. sonnei vaccine candidates WRSs2 and WRSs3 are safe and immunogenic over a wide dose range. Future steps will be to select the most promising candidate and move to human challenge models for efficacy of the vaccine.

1. Introduction

Shigella species, comprised of 4 major serogroups, *S. dysenteriae*, *S. flexneri, S. boydii*, and *S. sonnei,* are estimated to cause over 164,000 deaths annually with 55,000 among children <5 years of age, which represents 11% of all global diarrhea deaths [1]. While *Shigella* typically is a self-limiting infection, due to low infectious dose, efficiency of transmission and infection associated sequalae, antibiotics often are prescribed to treat the infection. However, multidrug resistance among *Shigella* is common and increasing in frequency [2]. Therefore, efforts have been focused on development of vaccines to prevent the infection.

A first generation oral, live, attenuated *S. sonnei* vaccine candidate, WRSS1, was constructed by deleting virulence-plasmid encoded VirG(IcsA) [3]. Phase 1 trial of WRSS1 showed that dose of 10^4 CFU was safe and immunogenic, but higher doses were associated with diarrhea and fever in over 12% of the recipients [4,5]. To widen the window of safety, while retaining immunogenicity, two second-generation *VirG(IcsA)*-based oral live vaccine candidates, WRSs2 and WRSs3 were constructed. Animal studies demonstrated that WRSs2 and WRSs3 compared favorably with the safety, immunogenicity and efficacy of WRSS1 with less reactogenicity [6–8]. The current study was conducted to determine whether one or both vaccine candidates strikes the right balance between reactogenicity and immunogenicity and provides a wider window of safety for VirG(IcsA)-deletion-based live vaccines.

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2. Materials and methods

2.1. Vaccine candidates

WRSs2 and WRSs3 vaccine candidates were derived from the *S. sonnei* strain, Moseley, [3,4,7]. Both candidates were manufactured under cGMP at the Walter Reed Army Institute of Research (WRAIR) Pilot Bioproduction Facility. Each vial of WRSs2 (lot #1501) and WRSs3 (lot #1486) contained 9.6×10^8 CFU and 1.6×10^9 CFU, respectively, formulated in 2 mL of phosphate buffer saline containing 7.5% dextran T10, 2% sucrose and 1.5% glycerol and then lyophilized. Since manufacture, the stability of the vaccine candidates remain unchanged with >90% *Form I phenotype for both WRSs2 and WRSs3* candidates. The vaccines were shipped on dry ice with a temperature monitor to Cincinnati Children's Hospital Medical Center (CCHMC) and stored at −80 °C ± 10 °C until used.

2.2. Study subjects and study design

Following extensive screening, healthy adults (18–45 years) were enrolled after informed consent. The eligibility criterion included no abnormal lab tests, negative for HLA-B27 and an anti-*S. sonnei* LPS serum IgG ELISA titer of <1:2500 (for detailed eligibility criteria see https://clinicaltrials.gov/show/NCT01336699). The study was conducted as a double-blind dose-escalation (10^3–10^7 CFU, sequential 10-fold increases) trial with five cohorts of 18 subjects randomly assigned to receive a single oral dose of WRSs2 (n = 8), WRSs3 (n = 8) or placebo (n = 2). Subjects were admitted to the inpatient unit the day prior to receipt of the study agent and remained in the inpatient unit for 10 days or after having 2 stool cultures negative for *Shigella*, whichever was longer. Subjects were administered ciprofloxacin (500 mg twice daily for 3 days) beginning on day 8 Outpatient visits occurred on days 14 ± 1, 28 ± 2 and 56 ± 4 and a final phone call on day 180 ± 14 after vaccination.

The primary objective of the study was to evaluate the safety and tolerability of each candidate with secondary objectives to assess the fecal shedding of the vaccine strain and immune responses in blood and stool.

2.3. Vaccine administration

On the day of vaccination, two vials of each of the vaccine candidates were thawed on ice and immediately reconstituted in 2 mL of ice cold sterile water. After combining the contents of the vials, additional dilutions were made in ice cold saline to the desired concentrations. The vaccine was administered within 2 h of reconstitution. At the time of vaccine administration, an aliquot was removed to determine by culture the actual dose administered.

Subjects fasted for 90 min prior to and after receiving the study product. Subjects ingested 150 mL of sodium bicarbonate (2 gm in 150 mL of sterile water) followed within 5 min by the 30 mL of saline containing 1 mL vaccine suspension.

2.4. Clinical assessment

During the inpatient period, subjects were evaluated at least twice daily to capture reactogenicity (Table 1). All signs and symptoms were graded as mild, moderate or severe based on the impact of the symptoms on the daily activities of the subject. Clinical signs and abnormal laboratory values were graded based on predefined criteria. Serious adverse events (SAEs) were recorded through day 180 post-vaccination.

Diarrhea was defined as per 24 h period; **mild** if the subject had 2–3 loose or watery stools with <400 gm/loose stools, **moderate** if the subject had 4–5 loose or watery stools or 400–800 g/loose stools and **severe** if ≥6 loose or watery stools or >800 gm/loose stools in 24 h. **Dysentery** was defined as two or more diarrheal stools with gross blood and mucous and reportable constitutional symptoms. Shigellosis was defined as shedding of *S. sonnei* in the stool accompanied by moderate-severe diarrhea and/or dysentery along with moderate fever or one or more severe intestinal symptoms. **Dehydration** was defined as a negative fluid balance associated with *any* of the following symptoms: syncope or near syncope, orthostatic hypotension, urine specific gravity >1.030, or no urine output within an 8-hour period.

A Safety Monitoring Committee reviewed all safety data collected through Day 28 and provided recommendation for the subsequent cohort. Halting rules included 2 or more subjects in a single dosage cohort experiencing the same ≥3 grade severe event, or 3 subjects developing the same severe event across dosage groups for WRSs2 or WRSs3, in the 15 days after vaccination.

2.5. Sample collection

2.5.1. Blood

Blood was collected before vaccination and on days 7, 14, 28 and 56 post-vaccination for whole blood count, renal and liver function tests as well as measurement of *Shigella* antigen-specific serum IgA and IgG. Blood for enumerating antibody secreting cells (ASCs) was collected before vaccination and on days 5, 7 and 9 post-vaccination. Sera for immunological testing were stored at −20 °C until assayed.

Table 1
Maximum severity of solicited adverse events.

Vaccine and vaccine dose[a]		Diarrhea		Fever	Headache	Cramps	Vomiting	Myalgia	Arthralgia
		Mild	Mod-Severe	Any	Any	Any	Any	Any	Any
10^3	WRSs2	1	0	0	1	1	0	1	0
	WRSs3	1	0	0	0	1	0	0	0
10^4	WRSs2	1	0	0	3	2	1	0	1
	WRSs3	0	0	0	2	1	0	0	0
10^5	WRSs2	0	0	0	4	2	0	0	0
	WRSs3	1	0	0	4	1	0	1	0
10^6	WRSs2	1	0	0	1	1	0	0	0
	WRSs3	1	0	0	0	2	0	0	0
10^7	WRSs2	2	1[b]	1 (38.4 °C max)	4	2	0	1	0
	WRSs3	0	2	0	4	2	0	1	0
Placebo		0	0	0	2	1	0	1	1

[a] n = 8 for each vaccine and dose, n = 9 for placebo.
[b] Subject had 4 loose stools over 24 h with a total weight of 178 gm with one of the stools being 0.2 gm in weight.