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Safety and immunogenicity of an intranasal *Shigella flexneri* 2a Invaplex 50 vaccine☆

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ABSTRACT

Background: Shigella flexneri 2a lipopolysaccharide 50 is a nasally delivered subunit vaccine consisting of a macromolecular complex composed of LPS, IpaB, IpaC and IpaD. The current study examined vaccine safety and immunogenicity across a dose range and the clinical performance of a new intranasal delivery device.

Methods: Volunteers ($N = 36$) were randomized to receive vaccine via the Dolphin™ (Valois of America, Congers, New York) intranasal spray device at one of three doses (240, 480, and 690 μg) on days 0, 14, and 28. Another group ($N = 8$) received the 240 μg dose via pipette. Vaccine safety was actively monitored and antigen-specific humoral and mucosal immune responses were determined.

Results: There were no serious adverse events and the majority of adverse events (98%) were mild. Antibody secreting cells (ASC), plasma, and mucosal immune responses to *Shigella* antigens were detected at all three dose levels with the 690 μg dose inducing the highest magnitude and frequency of responses. Vaccination with comparable doses of Invaplex 50 via the Dolphin™ resulted in higher plasma and ASC immune responses as compared to pipette delivery.

Conclusion: In this trial the *S. flexneri* 2a Invaplex 50 vaccine was safe, well-tolerated and induced robust levels of antigen-specific intestinal IgA and ASC responses. The spray device performed well and offered an advantage over pipette intranasal delivery.

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1. Background

Shigellosis is a leading cause of diarrheal disease worldwide particularly in developing countries [1], as well as a continuing problem for civilian travelers and military visiting endemic regions [2–5]. Vaccine development remains a high priority given the disease burden, increasing antibiotic resistance, and growing appreciation of post-infectious sequelae associated with shigellosis [6,7]. *Shigella flexneri* accounts for 30–60% of shigellosis cases in developing regions necessitating coverage of prevalent *S. flexneri* serotypes in a multivalent *Shigella* vaccine [1].

Several vaccine approaches to prevent shigellosis are under active investigation including live-attenuated vaccines, inactivated whole cell vaccines, subcellular vaccines and purified subunit vaccines such as O-specific polysaccharide conjugate vaccines [8,9]. The lack of a clear correlate of protection for *Shigella* vaccines has hampered vaccine development over the past several decades [8,10,11]. Even so, the importance of the serotype specific LPS antigen is widely recognized and included as a component of all vaccine approaches actively being pursued. Protein antigens, such as the Ipa protein effectors of the type three-secretion system, remain attractive vaccine candidates due to their active role in pathogen-directed

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cellular invasion and the highly conserved sequences among these essential virulence factors [12,13]. An effective *Shigella* vaccine must deliver the appropriate immunogens and also stimulate the proper immune response phenotype. For shigellosis, this likely consists of local immunity within the intestinal tract. Oral immunization may achieve intestinal immunity but is often difficult to consistently accomplish due to delivery restrictions, particularly for inactivated or subunit vaccines. Mucosal immune responses can also be achieved with intranasal immunization, although it is not clear if this is an effective route for stimulating intestinal immune responses in humans. The *Shigella* Invaplex 50 vaccine, currently under clinical investigation, is a macromolecular complex isolated from wild-type *S. flexneri* 2a that included both serotype specific (LPS) and conserved antigens (IpaB, IpaC and IpaD) and upon intranasal immunization stimulates robust intestinal and pulmonary immune responses [14–16].

An initial phase 1 dose-escalation study evaluating the safety and immunogenicity of the *S. flexneri* Invaplex 50 vaccine produced under current Good Manufacturing Practices (cGMP) was recently conducted using a pipette to deliver the vaccine intranasally [16]. The Invaplex 50 vaccine was well tolerated and resulted in antigen-specific humoral and mucosal immune responses at doses $\geq$ 240 μg. In the current study, the *S. flexneri* 2a Invaplex 50 vaccine was evaluated using a new lot of cGMP Invaplex 50 to expand the dose range and safety monitoring. Additionally, a nasal delivery device was evaluated to facilitate vaccine delivery and potentially induce a more robust mucosal immune response due to distribution of the vaccine over a greater mucosal surface as compared to pipette delivery.

2. Materials and methods

2.1. Manufacture of Invaplex 50 vaccine

The cGMP Invaplex 50 vaccine was prepared from virulent *S. flexneri* 2a, strain 2457T as previously described [14,16] with the following modifications. The 300 l culture was incubated at 37 °C with an agitation speed of 200 rpm and air flow of 150 l/min, in animal product-free modified Antibiotic Medium 3 with 0.003% antifoam. The animal product-free modified Antibiotic Medium 3 contained the following components per liter: Bacto yeast extract (Becton Dickinson, Sparks, MD), 1.5 g; Vegetable Peptone No. 1 (Oxoid/Remel, Lenexa, KS), 5 g; Bacto Dextrose (Difco/BD, Sparks, MD) 1 g; sodium chloride (molecular-biology tested, Sigma–Aldrich, St. Louis, MO), 3.5 g; potassium phosphate, dibasic (Sigma–Aldrich), 3.68 g; and, potassium phosphate, monobasic (Sigma–Aldrich), 1.32 g. After 18 h of growth, the culture was harvested by centrifugation (Sharples AS-26 continuous feed centrifuge) and an aliquot of the final culture was used for determination of bacterial colony forming units (cfu), gram stain, purity, colony uniformity, percent Congo red positive colonies and organism identity. The collected cells were extracted with water and the resulting extract was applied to an anion-exchange column (Q Sepharose High Performance, GE Healthcare) for isolation of the Invaplex 50 product in an elution step consisting of 500 mM NaCl in 20 mM Tris, pH 9.0 as previously described [16]. The final Invaplex 50 product was adjusted to 250 mM NaCl in 20 mM Tris, pH 8.8 and a final protein concentration of 3.46 mg protein/ml, sterilized by filtration (0.22 μm Millipak-20 filter unit) and dispensed in 1.0 ml volumes into sterile, depyrogenated 2 ml glass vials without preservative. All vials were stored at −80 °C.

2.2. Analysis of cGMP S. flexneri 2a Invaplex 50 lot 1307

The total protein concentration was measured by the BCA (bicinchoninic acid) assay (BioRad). SDS-PAGE Coomassie blue-stained gels, silver-stained gels and western blots using anti-IpaB, IpaC and *S. flexneri* 2a LPS mAbs [17,18] were used to assess the protein and LPS composition in the Invaplex 50 product. The amount of IpaB and IpaC in Invaplex 50 was determined using a modified ELISA procedure [19]. The LAL (*Limulus* amebocyte lysate) gel clot method (Pryotell, Associates of Cape Cod, Inc.) was used to measure the quantity of lipopolysaccharide [16]. For the various analyses, the animal product free-derived Invaplex 50 lot 1307 was compared to previous cGMP lots 0994 and 0808 that were prepared from *S. flexneri* 2a grown in Antibiotic Medium 3 containing animal derived products [16].

2.3. Immunogenicity and efficacy in small animals

The immunogenicity and efficacy of the cGMP *S. flexneri* 2a Invaplex 50 lot 1307 vaccine was evaluated in mice and guinea pigs. These procedures have been previously described for other lots of cGMP Invaplex 50 [16].

2.4. Stability of Invaplex 50 vaccine

The Invaplex 50 product lot 1307, stored at −80 °C, was assessed annually for antigen content by quantitative and qualitative assays described above and immunogenicity in guinea pigs. At the time of the study described in this report, lot 1307 had undergone three immunogenicity evaluations at 4, 12 and 20 months and the serum antibody response to *S. flexneri* 2a LPS and Invaplex 50 was evaluated.

2.5. Stability of Invaplex 50 in the Dolphin™ device

To improve the distribution of antigen on the nasal mucosa and enhance antigen uptake, the Dolphin™ system (Valois of America, Congers, NY), a single use prefillable, dispensing device was used. The Dolphin™ ensures reproducible droplet distribution on the nasal mucosa with a consistent size of ~100 μm which is more effective for antigen uptake by M cells, or antigen delivery to antigen presenting cells (APCs) of the mucosal immune system [20]. The Dolphin™ intranasal spray device has not been used previously to deliver vaccine products. A study was conducted to evaluate the stability of the Invaplex 50 lot 1307 in the Dolphin™ device under conditions that simulated the product formulation and storage in the device on the day of immunization. Multiple devices were loaded with Invaplex 50 vaccine (230 μl, 2.4 mg protein/ml) and placed at 4 °C. At 0, 2, 4, 5 and 6 h a device was removed from the refrigerator and discharged twice (100 μl per spray) into a 50 ml conical tube to collect the 200 μl dose volume (spray). The dead volume of the device is approximately 30 μl. The sprayed vaccine was centrifuged (2000 rpm, 5 min, 4 °C), collected and stored at −80 °C. Prior to and following the discharge event the Dolphin device was weighed to estimate the total volume discharged. Similar studies to measure reproducibility of the spray volumes were also conducted with saline. Collected samples were evaluated for total protein concentration by the BCA protein assay and the stability of IpaB, IpaC and LPS by western blots (IpaB and IpaC) and silver-stained gels (LPS). The relative content of LPS, IpaB and IpaC at each time point (as compared to untreated sample) was determined by densitometry analysis of the bands in silver-stained gels or western blots.

2.6. Clinical trial design

The study was conducted as an outpatient, single center, randomized, double-blind study in 36 healthy adult volunteers to assess vaccine safety and immunogenicity. Volunteers (12 per group) received one of three intranasal dose amounts (240, 480 or 690 μg) by the Dolphin™ device. In addition, a fourth