ABSTRACT

Cholera, the acute diarrheal disease caused by *Vibrio cholerae* serogroup O1 or O139 and evolution of new toxigenic strains remain an important global health challenge. It is transmitted primarily by contaminated water or food. Improved water and sanitation are the mainstays of cholera-prevention efforts supported with cholera vaccination. WHO licensed killed oral cholera vaccines: Dukoral®, Shanchol™ and Euvichol® which are cold-chain supply (2-8°C) dependent. As an alternative, a prototype cold chain free Live Attenuated Cholera Vaccine formulation (LACV) was developed with the patented *V. cholerae* (VCUSM14P) strain protective against toxigenic wild type (WT) O139 serogroup. The LACV formulated with 5 x 10^6 CFU/ml of vaccine strain is stable and retains its potency at room temperature: 25°C ± 2°C, and 60% ± 5% humidity for 140 days. In this study, the LACV was evaluated for its repeated dose toxicity, colonization, reactogenicity, immunogenicity and protective efficacy in animal models. In acute toxicity testing, the single dose of vaccine does not cause any adverse effects and mortality up to 10 x 10^6 CFU in Sprague Dawley (SD) rats. In sub chronic studies, 30 repeated doses of cholera vaccine at three different concentrations ie; group G-II (1.25 x 10^6 CFU), G-III (2.5 x 10^6 CFU) and G-IV (5 x 10^6 CFU) were evaluated in SD rats. No significant difference ($p>0.05$) was observed in the body weight, haematological and biochemical parameters, compared to the control group G-I after 15 and 30 repeated doses. However, compared to the control group a significant increase in the organ to body weight ratio of lungs, ureter, liver, kidney, heart and spleen in G-II, G-III and G-IV were recorded. These histopathology findings indicate a mild to moderate degeneration in the liver, kidney, heart and spleen in the treated rats. A mild to moderate lymphocytic infiltration in the lungs was observed in G-II and G-III and it was severe in the G-IV group rats. These histopathology findings might be attributed to 30 doses of vaccine given in succession daily for 30 days. In suckling mouse, the LACV recorded with highest recovery (7.2 x 10^7 CFU/ml) from the intestine compared to unformulated VCUSM14P (6.1 x 10^7 CFU/ml) and WT O139 strain (3.8 x 10^7 CFU/ml). LACV showed no reactogenicity even at an inoculation dosage of 10^4-10^6 CFU/mL in rabbit ileal loop model. The rabbits vaccinated with LACV or unformulated VCUSM14P survived the challenge with WT O139 and showed no signs of diarrhea in Reversible Intestinal Tie Adult Rabbit Diarrhea (RITARD) model. In the rabbits, a significant increase in anti-CT IgG/IgA antibodies titer was recorded in first-week post-vaccination and the booster dose on day 14 further enhanced the induction of anti-CT IgG/IgA.

After the second week of vaccination, there was (> 4 fold) increase in vibriocidal antibody titer. After the booster dose, there was a 24 fold increase in vibriocidal antibodies in the rabbits vaccinated with LACV as against 9 fold increase in the rabbits vaccinated with unformulated VCUSM14P. In summary, LACV is found to be a potential non-toxigenic cholera vaccine formulation against *V. cholerae* O139 capable of eliciting high antibody titers and protective immune responses in animal models.