Peru-15 (CholeraGarde®), a live attenuated oral cholera vaccine, is safe and immunogenic in human immunodeficiency virus (HIV)-seropositive adults in Thailand

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Abstract

Background: Many areas with endemic and epidemic cholera report significant levels of HIV transmission. According to the World Health Organization (WHO), over 95% of reported cholera cases occur in Africa, which also accounts for nearly 70% of people living with HIV/AIDS globally. Peru-15, a promising single dose live attenuated oral cholera vaccine (LA-OCV), was previously found to be safe and immunogenic in cholera endemic areas. However, no data on the vaccine's safety among HIV-seropositive adults had been collected.

Methods: This study was a double-blinded, individually randomized, placebo-controlled trial enrolling HIV-seropositive adults, 18-45 years of age, conducted in Bangkok, Thailand, to assess the safety of Peru-15 in a HIV-seropositive cohort. **Results:** 32 HIV infected subjects were randomized to receive either a single oral dose of the Peru-15 vaccine with a buffer or a placebo (buffer only). No serious adverse events were reported during the follow-up period in either group. The geometric mean fold (GMF) rise in *V. cholerae* O1 El Tor specific antibody titers between baseline and 7 days after dosing was 32.0 ($p < 0.001$) in the vaccine group compared to 1.6 ($p < 0.14$) in the placebo group. Among the 16 vaccinees, 14 vaccinees (87.5%) had seroconversion compared to 1 of 16 placebo recipients (6.3%). *V. cholerae* was isolated from the stool of one vaccinee, and found to be

genetically identical to the Peru-15 vaccine strain. There were no significant changes in HIV viral load or CD4 T-cell counts between vaccine and placebo groups. **Conclusion:** Peru-15 was shown to be safe and immunogenic in HIV-seropositive Thai adults.

1. Introduction

Cholera is an acute watery diarrheal illness with an estimated 2.8 million cases and 91,000 deaths occurring annually in endemic countries [1]. Many areas with cholera transmission also report significant levels of HIV transmission, possibly due to similar predisposing socioeconomic factors including poverty and lack of education. Excluding the recent cholera outbreak in Haiti, over 95% of officially reported cholera cases to the World Health Organization (WHO) come from Africa, which also accounts for nearly 70% of total people living with HIV/AIDS globally [2-5]. Nearly two-thirds of cholera outbreak reports to ProMed came from Sub-Saharan Africa from 1995 to 2005 [6]. Additionally, a study in a cholera endemic area in Mozambique suggests that HIV infection is associated with an increased risk of cholera [7].

The WHO recommends that available oral cholera vaccines (OCVs) should be used in conjunction with other preventive and control strategies in cholera prone countries [8]. Currently available OCVs (Dukoral and Shanchol) are both composed of killed whole-cell, require at least two dose regimens, and are not licensed for use in infants. While Dukoral has been shown to be safe and immunogenic in HIV infected individuals [9], there is no similar data for Shanchol [10]. Live OCVs, which may have the potential of higher and longer duration of protection and of possible single dose regimens, must also be safe and immunogenic in HIV infected persons in order to be implemented in cholera-stricken communities.

Peru-15 (CholeraGarde®), a live attenuated OCV, is derived from a Peruvian strain of *Vibrio cholerae* O1, biotype El Tor by removing cholera toxin genetic elements and by reinserting the cholera toxin B subunit (*ctxB*) [11]. It has been evaluated in phase I and II clinical trials for its safety and immunogenicity in different age-groups in America and Bangladesh where Peru-15 was well tolerated and showed satisfactory immunogenicity at doses ranging from 1×10^7 to 1×10^9 Colony Forming Units; however, there is no such data in HIV-infected individuals (CFU) [11-16].

In Thailand, there have been sporadic reports of cholera outbreaks for several decades. During 2007-2010, an unusually high number of cholera cases were reported from northeastern regions and the Thai-Myanmar border [17, 18]. The HIV prevalence rate in Thailand was 1.0-1.4% in adults aged 15 to 49 years during 2007 to 2010 (<http://www.unaids.org/en/regionscountries/countries/thailand/>). Given the comorbidity of cholera and HIV infection, the aim of this study was to further evaluate the safety and immunogenicity of Peru-15 in HIV-seropositive individuals in Thailand.

2. Materials and methods

2.1. Study site, participants and ethics

The study was conducted at the Siriraj Hospital in Bangkok, Thailand, which has an ongoing HIV program and follows HIV-seropositive individuals for regular healthcare. HIV-seropositive, non-pregnant adults, aged 18-45 years old who had been on standard Highly Active Anti-Retroviral Therapy (HAART) for at least 6 months prior to enrolment or who had never started HAART regimen were recruited for the study. We

used such recruitment criteria primarily because OCVs are often used in mass vaccination campaigns where some individuals who will participate would be those who may not know they are HIV positive and relatively healthy. To simulate this state, we included only those who had never been on treatment or had been on standard HAART for at least 6 months. The study was approved by the Thailand Food and Drug Administration, the Ethical Review Committee of the Siriraj Hospital, and the Institutional Review Board of IVI. This study was registered in the clinical trials registration (<http://clinicaltrials.gov/> NCT00741637).

2.2. Eligibility

HIV-seropositive, non-pregnant adults, aged 18-45 years old subjects who gave the written informed consent, were available for follow-up visits and who had CD4 T-cell count > 500 cells/mm³ for at least 6 months prior to enrolment were eligible for the study.

Subjects who had never started HAART regimen needed to meet the following criteria at the start of the study:

- (1) Asymptomatic HIV infection as determined by medical history, physical examination, laboratory tests and clinical judgment of the investigator

Subjects who had been on HAART regimen for at least 6 months prior to enrolment needed to meet the following criteria at the start of study:

- (1) History of CD4 nadir > 150 cells/mm³
- (2) Viral load (HIV -1 RNA levels) < 200 copies/mL for at least 6 months prior to enrolment

Exclusion criteria included any overt signs of immunodeficiency and ongoing serious chronic illness. Subjects reporting abdominal pain or cramps, loss of appetite, nausea, general ill-feeling or vomiting in the past 24 hours before dosing were also excluded, as were subjects whose stool samples were found to be positive for *V.cholerae* O1 or O139, or *Shigella* spp. or *Cryptosporidium* spp. at baseline. Additional exclusion criteria included intake of anti-diarrheal medicine in past week, presence of any acute illness in one week prior to enrollment, receipt of antibiotics in past 2 weeks, receipt of live or killed enteric vaccine in last 4 weeks, receipt of killed OCV in the past, history of diarrhea (3 or more loose stools within a 24-hour period) in the past 6 weeks, one or more episodes of diarrhea or abdominal pain lasting for more than 2 weeks in past 6 months, receipt of blood, blood products, parenteral immunoglobulin preparation, or any immunosuppressive therapy in the previous 6 months, prior to enrolment, woman planning to be pregnant during her participation in the study, and any condition which in the opinion of the investigator, might interfere with the evaluation of study objectives.

2.3. Study design

The study was conducted as an individually randomized, double-blinded and placebo-controlled trial. The sample size calculation was carried out at a power of 80% and significance level of one tailed 2.5%, on the assumption of a 5 % diarrhea rate among placebo and vaccine groups to exclude 35% as the upper boundary of one-tailed 97.5% CI for the vaccine minus placebo difference in the rate of diarrhea. With a 20% dropout rate, 32 subjects were required divided into two equal groups (16 vaccine and 16

placebo groups) [19]. The randomization list was sequential numbers unique to each individual. A randomization blocking scheme (1:1) was used to ensure balance between the two groups. We randomly selected pairs with block size of 2 and 16 times and then added random number from uniform distribution and finally, sorted by random number and assigned the subject ID. A single treatment number uniquely identified the dose to be administered to the subject. Using SAS version 9.1 software, eligible subjects were assigned to receive either vaccine or placebo in a 1:1 ratio according to a randomization list generated by IVI staff, who were not involved in the study. The randomization envelopes were sent to the un-blinded study personnel responsible for preparing study vaccine for administration at the Siriraj Hospital in Bangkok, Thailand. To protect blinding of subjects and assessors of outcomes, study vaccine and placebo doses were prepared by the designated individual, third party un-blinded dispenser, and it was instructed not to reveal the identity of the agent to the subject nor to personnel who were involved in the conduct or monitoring of the study.

2.4. Formulation of study agents

Each single dose vial of CholeraGarde® contained approximately 5×10^7 to 1×10^9 CFU of Peru-15, 6.75 mg KH_2PO_4 and 300mg trehalose as a lyophilized cake in a 5mL single-dose vial. The vaccine was mixed in 5 mL of chlorine-free bottled water and then mixed with 95 mL of a buffer containing 2.5g sodium bicarbonate and 1.65g ascorbic acid. The placebo contained only 100 mL of prepared buffer solution. Since the vaccine and placebo were not similar in their appearance, we had a third party unblinded

vaccine preparer and after reconstitution, they were administered with dark colored cups in order to further ensure blinding.

2.5. Follow up for adverse events

All subjects were required to stay at the vaccination area for observation of any adverse events for at least 30 minutes after dosing. Follow up for adverse events was continued passively for 90 days after dosing using a daily diary card to collect information about possible symptoms.

Systematically solicited adverse events included abdominal cramps, gas, nausea, vomiting, myalgia, fever (otic or oral temperature ≥ 38 °C or axillary temperature ≥ 37.5 °C), diarrhea, and headache. Serious adverse events were defined as any event that resulted in death, life-threatening, persistent or significant disability/incapacity, requiring inpatient hospitalization or prolonged hospitalization, a congenital anomaly/birth defect and other important medical events. The relationship to study agent and intensity of an adverse event were assessed and determined by the principal investigator.

2.6. Immune response analysis

Approximately 5-10 ml of venous blood was obtained from each participant in each visit prior to enrolment, and at 7, 30 and 90 days after vaccination for blood tests including those for immunogenicity test and CD4-T cell counts. Blood samples were obtained just prior to administration of vaccine or placebo and 7 days after dosing for immune response analysis. The serum vibriocidal antibody assay using the microtiter

technique was performed at the International Center for Diarrheal Disease Research, Bangladesh (icddr,b) laboratory. Vibriocidal antibody assays were carried out with *V. cholera* O1 (strain 19479 El Tor Inaba) using sera (initial dilution 1:10) tested in duplicate. The vibriocidal antibody titer was defined as the reciprocal of the highest serum dilutions resulting in greater than 50% optical density (OD) reduction when compared to control wells without serum. Vibriocidal antibody titers less than 10 for O1 Inaba were considered as 5 for statistical analyses. An increase of titer by 4-fold or greater between baseline and on day 7 after dosing was regarded as a seroconversion.

2.7. Bacteriologic analysis

In order to confirm the presence of the vaccine strain, stool samples or rectal swabs were collected on screening (pre-dosing), and 1, 3, 7, 14 and 30 days after dosing. If there was any colony found positive by one or more of specific DNA probes (*ctxA*, *RS* and *attRS*), further analysis was carried out to test whether these were derived from the vaccine strain as previously described [11, 20]. Bacteriological examination of the stool was also carried out to exclude the presence of other enteric pathogens including *V. cholerae* O139.

2.8. CD4-T lymphocyte and HIV viral load

Blood specimens were obtained for CD4-T cell count at screening and 90 days after dosing. The CD4 T-cell count was measured using flow cytometry within 12 hours of venipuncture. Quantitative Plasma HIV RNA was assessed to measure viral load, using

the real time PCR assay, at screening and 7, 30 and 90 days after dosing. Both tests were performed at the Siriraj Hospital laboratory.

2.9. Data management and statistical analyses

Data was entered into a web based data capture system designed for this study. After data verification and validation, and monitoring of data by the independent clinical monitor, data were locked. Cleaned data sets were reviewed by the Principal Investigator and the Data Safety Monitoring Board (DSMB) prior to analysis. Data analyses were performed after the study was un-blinded. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary NC).

Comparisons of dichotomous outcome such as adverse events and seroconversion between vaccine and placebo groups were performed with the chi-square test or Fisher's exact test when cell counts were sparse. For comparisons of continuous outcome such as serum vibriocidal titers and fold rises between the two groups, normality of serum vibriocidal titers and fold rises using Shapiro-Wilk and Kolmogorov-Smirnov test was investigated. When the data was normally distributed, Student's t-test or Satterthwait's t-test were performed depending on equality of the variances. The F test was used for testing equality of variances. When the data was not normally distributed, nonparametric approaches - Wilcoxon rank-sum test and Kolmogorov-Smirnov test-were used depending on the identical spreads or not using Ansari-Bradley test. Serum vibriocidal titers and fold rises were logarithmically transformed prior to analysis. Baseline and post dosing CD4 T-cell counts were compared using Wilcoxon signed-rank test. The primary outcome, which was the

proportion of diarrhea adverse events and as well as the proportion of subjects with solicited adverse events within 7 days following dosing, was evaluated with one-tailed 97.5% CI. Statistical significance in all comparison was set at $p < 0.05$ and all reported p-values were 2-tailed.

3. Results

3.1. Study population

From July 2010 to March 2011, 37 subjects provided informed consent and were screened for their eligibility, of which 32 were enrolled and randomized to each group (vaccine recipients and buffer recipients). Of the 5 ineligible cases, 1 was more than 45 years old at time of screening, 1 received antibiotics 2 days prior to dosing, and 3 had a CD4 T-cell count less than 500 cells/mm^3 for the past 6 months prior to enrollment (Figure 1). Of the 32 subjects, 10 were male and 22 were female; with a median age of 37.4 years (Table 1).

FIGURE 1. CONSORT FLOW DIAGRAM

TABLE 1. BASELINE CHARACTERISTICS

3.2. Adverse events (AEs)

No subjects experienced a serious adverse event during the study period (90-day follow up period). The most frequently reported solicited adverse events in vaccine group were: gas (62.5%), headache (18.8%), and abdominal cramps (18.8%), which were mild in intensity. Nausea (12.5%), myalgia (12.5%) and diarrhea (12.5%) which were all mild. All of solicited AEs occurred between day 0 to day 7 after dosing.

Among the vaccine group, upper respiratory tract infection, depressed mood and back pain were also reported as unsolicited AEs, which were all mild in intensity, except for depression, and were assessed as unlikely or unrelated to the vaccine. There were no significant differences in symptoms between the two groups.

TABLE 2. ADVERSE EVENTS FOLLOWING DOSING

3.3. Fecal excretion of Peru-15

Shedding of vaccine in stool was observed in one vaccinee at 3 and 7 days after dosing. This patient whose HIV viral load was 6356 copies/ml and whose CD4 counts was 725 cells/mm³ prior to receipt of vaccine, had not been placed on HAART. It was shown to be negative for *attRS*, *RS* and *ctxA*, and positive for *ctxB* gene and nonmotile, which confirmed the strain to be Peru-15.

3.4. Serum vibriocidal antibody responses

The baseline serum vibriocidal antibody reciprocal titers in vaccine and placebo groups ranged from a minimum of 5 to a maximum 320, and there was no significant difference between the two groups. The geometric mean fold (GMF) rises between baseline and 7 days after dosing were 32 ($p < 0.001$) in vaccine group and 1.6 ($p = 0.14$) in the placebo group. Overall, 87.5% (14/16) of vaccinees and 6.3% (1/16) in placebo recipients exhibited seroconversion between baseline and post dosing . Two of the 16 vaccinees who did not show seroconversion had high baseline titers of 320. Among placebo recipients, one subject had increased vibriocidal antibody titers at day 7 (5120).

TABLE 3. SERUM VIBRIOCIDAL ANTIBODY TITERS (AT BASELINE, 7 DAYS AFTER DOSING)

3.5. The level of HIV viral load

No difference in HIV viral load increase was shown between vaccine and placebo groups at each time point (screening, day 7, 30, 90 after dosing). Most subjects in both groups (14 of 16 vaccinees and 14 of 16 placebo recipients) maintained < 40 copies/mL in viral load over time. Viral load increase (more than 10,000 copies/mL) was observed in 2 vaccinees (subject #1= 24,304 at day 90, subject #2= 13,889 at day 30 and 34,751 at day 90), who were identified without HAART and had relatively high viral load at screening (subject #1= 4,098, subject#2=6,356)

3.6. Changes in blood CD4 lymphocytes counts

Median CD4 T-cell counts (cells/mm³) were 730 (Min 506, Max 1638) and 665 (Min 516, Max 1074) at baseline and 826 (Min 448, Max 1901) and 770.5 (Min 529, Max 1142) at day 90 post dosing, among vaccine and placebo groups respectively without any significant difference between the two groups. However, among vaccinees, significant increases between baseline and day 90 were observed ($p < 0.01$). One subject in vaccine group who had been under HAART had a count of 448 at day 90 with a baseline value of 559 (Figure 2).

FIGURE 2. BOXPLOT OF CD4 LYMPHOCYTES COUNTS AT SCREENING DAY AND DAY 90

4. Discussion

This is the first clinical trial to evaluate the safety and immunogenicity of a single dose of Peru-15 when administered to HIV-seropositive adults.

Our results show that a single dose of Peru-15 is safe and well tolerated with only minor adverse events in HIV-seropositive adults. Most solicited adverse events in vaccine group observed during 7 days of follow up were gastrointestinal symptoms including gas, nausea, abdominal cramp and diarrhea. There were no additional solicited events reported during the day 7 to day 90 follow up period. However, the frequency of adverse events was low when compared with the earlier studies of Peru-15 from North America [11-14].

This study demonstrates comparably high vaccine immunogenicity (vibriocidal antibody response with ≥ 4 fold increases in titers of 88%), when compared with the 75% of titers in Bangladeshi adults ($\sim 2 \times 10^8$ CFU) but lower than that of 97% of titers in American volunteers respectively [14, 15]. This is likely because the baseline titers of participants in Bangladesh were higher compared to the participants in Bangkok, where cholera is not endemic [15]. Previous studies have shown that there is an inverse relationship between baseline serum vibriocidal antibody titers and vaccine response [21-23]. The geometric fold increase of titers (32-fold vs 8-fold) and individual maximal fold titers (1024-fold vs 256-fold) at day 7 compared to pre-dosing titers was higher compared with Bangladeshi adults [15].

One (6%) vaccine recipient excreted the vaccine strain and this is somewhat similar to the excretion in earlier studies in healthy Bangladeshi adults and children (5%) [15, 16]. Larger studies will be necessary to further understand the release of the vaccine strain in the environment [27].

A potential concern for vaccination of HIV infected subjects is that vaccination may stimulate HIV replication and decrease the CD4 T-cell count, which may accelerate the progression of HIV disease [25, 26, 28-30]. This study has demonstrated no significant changes in HIV viral load and CD4 T-cell count after immunization with Peru-15.

Alternatively, the study was small and may have failed to detect significant differences. Only 2 vaccinees showed elevation of HIV viral load after administration of Peru-15 and they were never treated with HAART making interpretation of this observation difficult.

This study had several limitations.

Previous studies indicate that immune responses after vaccination could vary depending on the progression of HIV infection, [9, 30-33] or vaccines, such as MMR and varicella. Indeed these vaccines are not recommended for HIV- positive adults with CD4 T-cell count $< 200 \text{ cells/mm}^3$. In a study with CVD HgR 103, a live attenuated OCV among HIV-seropositive Mali adults, lower vibriocidal GMT were identified in those with $< 500 \text{ cells/mm}^3$ compared to subjects with $\geq 500 \text{ cells/mm}^3$ [25]. Since this is the first study using Peru-15 among HIV infected individuals, vaccine safety had to be ensured first in this immunocompromised population. Future studies assessing the safety and immunogenicity of the vaccine in individuals with more progressive disease and perhaps longer duration for follow up will be necessary.

Though serum vibriocidal antibody titers are widely used for determination of immune responses to cholera vaccines, these are not true correlates of protection against cholera;

the findings cannot be interpreted as Peru-15 is protective against clinical cholera in HIV infected individuals [34].

5. Conclusions

Our findings suggest that Peru-15 is safe and immunogenic when given to HIV infected adults. Additional studies to confirm the safety and immunogenicity among HIV-seropositive individuals in alternative settings are warranted.

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Conflicts of interest statement: None.

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Figure legends

FIGURE 1. CONSORT FLOW DIAGRAM

FIGURE 2. BOXPLOT OF CD4 LYMPHOCYTES COUNTS AT SCREENING AND DAY 90

* p value was derived using Wilcoxon signed rank test of paired samples

TABLE 1. BASELINE CHARACTERISTICS

		Vaccine group (n=16)	Placebo group (n=16)	p- value
Sex	n (%) of male	3 (18.8%)	7 (43.8%)	0.25
	n (%) of female	13 (81.3%)	9 (56.3%)	
Age (years)	mean (sd)	37.3 (4.2)	38 (3.1)	0.61
	median (min, max)	37 (26.3, 43.1)	37.4 (32.7, 44.2)	
HAART	n (%) of HAART	14 (87.5%)	14 (87.5%)	1.00
	n (%) of no HAART	2 (12.5%)	2 (12.5%)	
HAART duration (years)	mean (sd)	8.6 (3.5)	7.4 (3.1)	0.25
	median (min, max)	10 (1, 12)	7 (2, 12)	
CD4 T-cell count (cells/mm³)	mean (sd)	787.5 (287.9)	716.3 (165.6)	0.78
	median (min, max)	730 (506, 1638)	665 (516, 1074)	
CD4 T-cell count (cells/mm³) (category)	500 - ≤1000	14 (87.5%)	15 (93.8%)	1.00
	>1000	2 (12.5%)	1 (6.3%)	
HIV-1 RNA levels (copies/ml)	mean (sd)	670.9 (1825.7)	465.1 (1687.1)	1.00
	median (min, max)	20 (20, 6356)	20 (20, 6783)	

HIV-1 RNA levels (copies/ml); category	<40	14 (87.5%)	14 (87.5%)	1.00
	40 - <10,000	2 (12.5%)	2 (12.5%)	

TABLE 2. ADVERSE EVENTS FOLLOWING DOSING

N (%)		Vaccine group (N=16)	Placebo group (N=16)	p- value
		All grade	All grade	All grade
Within 7 days following dosing	Gas	10 (62.5%)	9 (56.3%)	0.72
	Nausea	2 (12.5%)	1 (6.3%)	1.00
	Headache	3 (18.8%)	1 (6.3%)	0.60
	Vomiting	0	0	--
	Abdominal Cramps	3 (18.8%)	0	0.23
	Myalgias	2 (12.5%)	0	0.48
	Diarrhea	2 (12.5%) [32.7%] ^a	1 (6.3%)	1.00
	Fever	0	0	--
Number (%) of subjects with ≥ 1 solicited AEs within 7 days following dosing		13 (81.3%) [56.3%] ^a	9 (56.3%)	0.25
Number (%) of subjects with ≥ 1 solicited AEs OR unsolicited AEs within 90 days following dosing		14 (87.5%)	9 (56.3%)	0.11

^a Value in brackets represents the upper boundary of one-tailed 97.5% confidence interval for the proportion difference in the adverse events among vaccinees compared to placebo recipients. For the diarrhea adverse events, the vaccine group is non-inferior to the placebo group as the upper boundary of the proportion difference is lower than pre-defined cut-off (35%).

TABLE 3. SERUM VIBRIOCIDAL ANTIBODY TITERS (AT BASELINE, 7 DAYS AFTER DOSING)

		Vaccine group (n=16)	Placebo group (n=16)	p-value
Baseline	GMT ^a (95% CI)	30.8 (14.8, 64.5)	19.2 (8.4, 43.7)	0.70
7 days after dosing	GMT ^a (95% CI)	987 (503.7, 1934)	30.8 (11.0, 86.8)	<0.01
	GMF ^b rise (95% CI)	32 (11.8, 87)	1.6 (0.6, 4.1)	<0.01
	Number (%) of subjects who seroconverted ^c	14 (87.5%)	1 (6.3%)	<0.01

^a Geometric mean titer

^b Geometric mean-fold rise from baseline to 7 days after dosing

^c Number of subjects with ≥ 4 fold rise in titers from baseline to 7 days after dosing