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Antibody response to an eight-site intradermal rabies vaccination in patients infected with Human Immunodeficiency Virus

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ABSTRACT

Objective: To investigate the rabies virus neutralizing antibody response in HIV-1-infected patients with CD4+ cell count ≤200 cells/μL or >200 cells/μL after post-exposure prophylaxis using an eight-site intradermal rabies vaccination regimen.
Methods: In a prospective cohort study, 27 HIV-1 infected patients were recruited, none of which had a history of rabies vaccination. All patients provided informed consent and were separated into two groups according to their CD4+ cell count (patients with CD4+ counts of ≤200 cells/μL and patients with CD4+ counts of >200 cells/μL). All patients received Purified Chick Embryo Cell rabies Vaccine (PCECV) using a modified eight-site regimen in which 0.1 mL of vaccine was injected intradermally on each of days 0, 3, 7, 14, and 30 (8–8–8–8–8). CD4+ cell counts, HIV-1 viral load and rabies virus neutralizing antibody (RVNAb) concentrations as determined by the Rapid Fluorescent Focus Inhibition Test (RFFIT) were evaluated on blood samples taken on days 0, 3, 7, 14, 30, 90, 180 and 365 after vaccination.
Results: Of the 27 patients included in the study, 18 patients (67%) had CD4+ cell counts of >200 cells/μL and 9 patients (33%) had CD4+ counts of ≤200 cells/μL. No patients had detectable RVNAb concentrations on day 0. By day 14, all patients had adequate RVNAb concentrations (≥0.5 IU/mL). There was no statistically significant difference in RVNAb concentrations between the two groups on days 3, 7, 14, 30, 90, 180 and 365 after vaccination.
Conclusion: PCECV is immunogenic in HIV-1-infected patients with CD4+ cell counts below 200 cells/μL when administered in a modified eight-site intradermal PEP regimen.

1. Introduction

Rabies is an acute fatal viral encephalitic disease generally transmitted through bites of infected animals to man. Rabies is distributed worldwide, except Antarctica. Although effective modern rabies vaccines are available for post-exposure prophylaxis (PEP), the annual number of human death caused by rabies is still estimated to be 55,000 [1]. The majority of all global human rabies deaths occur in Asia and Africa [2]. In Thailand, rabies remains a public health problem with 300,000–400,000 persons seeking PEP annually [3,4]. In 1994, the Ministry of Public Health (MoPH) in Thailand officially recommended an intradermal (ID) vaccination regimen, that has since become known as the Thai Red Cross (TRC) regimen, as a means by which to reduce the cost of PEP. The TRC regimen has been recommended since 1992 by the World Health Organization (WHO) [5]. The WHO indicates that the TRC can be used with the cell culture rabies vaccines Purified Chick Embryo Cell Vaccine (PCECV) or Purified Vero cell Rabies Vaccine (PVRV) [6]. The TRC regimen has the benefit of allowing patients to receive PEP at moderate cost and has resulted in the dramatic decline of human rabies deaths since its inception [7]. The WHO also recommends another ID regimen, the Eight-site (Oxford) ID regimen, originally developed as an 8–0–4–0–1–1 schedule [8]. In addition to PEP, another cornerstone of rabies prevention is pre-exposure prophylaxis (PreP), which is not only administered to persons at occupational risk of rabies exposure but is recommended to be administered to travelers visiting canine rabies endemic countries for extended periods of time and increasingly is being administered to people living in canine rabies endemic countries. Modern tissue culture rabies vaccines like PCECV can be used for PreP and PEP by

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intramuscular (IM) or ID injection of rabies vaccine. The immunogenicity, as well as efficacy and tolerability of PCECV have been demonstrated in immuno-competent persons in numerous clinical trials [9–15]. In HIV-infected patients, both IM and ID vaccination regimens have been reported to be less immunogenic [16–18]. Only one published report has indicated that rabies vaccine is immunogenic (rabies virus neutralizing antibody (RNVAb) concentrations >0.5 IU/mL) in HIV-infected patients, when administered via a four-site ID regimen [19]. A previous report from our group reported adequate immunogenicity in two patients infected with HIV-1 that received PEP using a modified eight-site ID vaccination regimen (8–8–8–0–8–8) [20]. To our knowledge, the enclosed report is the first prospective, cohort study to evaluate the immunogenicity of rabies vaccination in HIV-infected patients, using a modified eight-site regimen.

2. Methods

This study was conducted at Bamrasnaradura Infectious Diseases Institute (BIDI), Nonthaburi, Thailand between August 2004 and September 2005. Subjects that were enrolled in this study were HIV-1 infected patients above 15 years of age without a history of exposure to animal bite or previous rabies vaccination.

The study was approved by the Ethics Committee for Research in Human Subjects, Ministry of Public Health, Thailand. Written informed consent was obtained from all subjects. (Although inclusion criteria allowed participation above 15 years of age including minors, all subjects enrolled in the study were adults above the age of 20.) Patients were excluded if they had a history of PreP or PEP, were participating in another study, had a history of drug allergy, or were taking antimalarial drug within two months prior to the initiation of the study, patients who were on long term corticosteroid therapy, and pregnant or lactating women. The subjects enrolled in the study were separated into two groups according to their CD4+ cell counts, as follows: Group 1 included patients with CD4+ counts $\leq$200 cells/μL and Group 2 included patients with CD4+ counts >200 cells/μL. Blood samples were taken from all subjects enrolled in the study on days 0, 3, 7, 14, 30 and 365. These blood samples were then evaluated for CD4+ cell counts, HIV-1 viral load and RVNAb concentration. A chi-square test was used to evaluate potential differences in the RVNAb concentrations between the two groups.

The vaccine used for the study was Purified Chick Embryo Cell rabies Vaccine (PCECV, Rabipur®, Novartis Vaccines, Ankleshwar, India, batch No. 957 with a potency of 9.05 IU/mL). Eight ID doses of 0.1 mL of PCECV were administered to every subject on each of days 0, 3, 7, 14 and 30 (8–8–8–8–8). The sites of the ID injections were one in each upper arm, one in each lateral thigh, one on each side of the suprascapular region, and one on each side of the lower quadrant region of the abdomen, as recommended by the WHO [6]. None of the subjects received rabies immunoglobulin (RIG). Blood was collected from all subjects on days 0, 3, 7, 14, 30, 90, 180 and 365 and evaluated for CD4+ cell counts, HIV-1 viral load and RVNAb concentrations. The CD4+ cell counts and HIV-1 viral load were determined at BIDI. CD4+ cell count was performed by Flow cytometry/TriTEST/FACScan (Becton Dickinson BioScience, USA). HIV-1 viral load was measured by RT-PCR/COBAS Amplicor HIV-1 Monitor test version 1.5 (Roche Molecular Systems, USA). RVNAb concentrations were determined using the Rapid Fluorescent Focus Inhibition Test (RFFIT) at the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand, as described earlier [21]. Briefly, 200 FFD_{50} of the challenge virus (CVS-11) was added to diluted serum samples in 96-well tissue culture plates and incubated at 37 °C for 90 min; MNA cells were added to each well and incubated at 37 °C overnight. Plates were fixed with 80% acetone and stained with FITC-labeled anti-rabies conjugate at 37 °C for 30 min. Cells were observed under fluorescent microscope and RVNAb concentration was determined using the Reed and Muench method, and expressed in IU/mL by comparison to a standard solution of RVNAb (1 IU/mL) obtained from NIBSC,

Table 1
Patient data (age, antiretroviral therapy) and course of CD4+ cell counts from Day 0 to Day 365 in HIV-1-infected patients with baseline CD4+ cell counts $\leq$200 cells/μL (patients 1–9) and >200 cells/μL (patients 10–27).

Patient no.	Age	ART[a]	CD4+							
			Day 0	Day 3	Day 7	Day 14	Day 30	Day 90	Day 180	Day 365
1	36	GPOVir	69	71	70	76	55	33	111	298
2	52	GPOVir	114	131	130	104	94	149	155	161
3	39	GPOVir	118	123	144	112	155	65	182	282
4	28	GPOVir	142	125	160	129	103	78	56	NA
5	35	GPOVir	169	154	173	147	162	157	177	229
6	32	ddI, d4T, EFV	177	170	203	148	176	185	262	327
7	33	GPOVir	183	147	195	165	140	183	188	249
8	33	GPOVir	188	181	153	174	133	244	217	426
9	30	GPOVir	190	180	156	170	209	225	281	412
10	33	GPOVir	205	162	171	188	179	166	140	157
11	33	GPOVir	222	213	216	225	223	237	334	498
12	45	GPOVir	235	281	222	136	253	225	225	295
13	40	GPOVir	273	259	235	345	327	318	321	523
14	36	GPOVir	275	244	303	267	373	355	303	304
15	32	d4T, 3TC, EFV	301	289	362	255	301	181	243	353
16	51	GPOVir	311	276	312	150	306	306	274	287
17	36	GPOVir	312	276	319	341	252	250	253	304
18	21	no ART	329	475	414	426	377	443	449	417
19	36	GPOVir	337	341	389	301	396	216	543	393
20	21	no ART	342	273	297	228	357	313	332	356
21	38	d4T, 3TC, EFV	354	390	405	329	387	506	362	506
22	31	GPOVir	404	399	333	483	426	429	399	504
23	55	GPOVir	422	364	313	394	329	331	484	461
24	26	no ART	429	446	360	399	516	299	442	439
25	35	GPOVir	432	405	368	361	384	417	456	648
26	30	no ART	455	474	406	437	453	NA	519	NA
27	22	no ART	397	381	388	319	351	317	321	241

[a] ART, antiretroviral therapy; GPOVir, fixed combination of d4T, 3TC (lamivudine) and nevirapine; d4T, stavudine; 3TC, lamivudine, ddI, didanosine; EFV, efavirenz.