LONG-TERM PROTECTIVE RABIES ANTIBODIES IN THAI CHILDREN AFTER PRE-EXPOSURE RABIES VACCINATION

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Abstract. Rabies is a viral zoonosis affecting around 16,000-39,000 people annually. Pre-exposure vaccination should be offered to individuals at high risk of rabies exposure and is suggested for children living in rabies endemic areas.The incidence rate of dog bite in the test group was 14.1/1,000 person-years. Intradermal (ID) route of administration is considered an alternative to the standard intramuscular (IM) administration, and reduces vaccination cost. A 3-year clinical study of a rabies vaccine administered IM or ID to 12–18-month-old Thai children, simultaneously with a Japanese encephalitis (JE) vaccine (M49P2 study), revealed that all regimens used elicited an adequate immune response. In order to determine the long-term (4-8 years) rabies neutralizing antibody titers induced by the pre-exposure regimens, blood was collected annually from 68 from the M49P2 study and analyzed using a rapid fluorescence focus inhibition test. Full-IM(three doses of 1 ml/dose), half-IM(three doses of 0.5 ml/dose), and 3-ID(three doses of 0.1 ml/dose) regimens induced antibody titers above the seroprotective level throughout the study period. However, the 2-ID(two doses of 0.1 ml/dose) group had sub-seroprotective titer of 6.7%, 13.4%, 25.0%, and 36.4% in year 5, 6, 7, and 8, respectively. A secondary immune response was induced by rabies booster vaccination. This study demonstrates a reduced-dose rabies regimen may lower the cost of long-term protection against rabies for vulnerable populations, thus improving the cost-effectiveness of pre-exposure rabies vaccination in children.

Keywords: children, immunity, rabies vaccine, Thailand

INTRODUCTION

Rabies virus is a zoonotic RNA virus in the genus *Lyssavirus*, family Rhabdoviridae. Around 16,000-39,000 people are in contact with potentially rabid animals annually (Manning *et al*, 2008). Rabies vaccine made from killed Rabies virus may elicit rare systemic reaction after intramuscular (IM) or intradermal (ID) injection (WHO, 2014; Rupprecht *et al*, 2010).

Pre-exposure rabies vaccination is recommended for children living in rabies endemic areas. The standard recommendation for pre-exposure vaccination is three IM doses of a cell-, culture- or embryonated-egg-based vaccine given on days 0, 7, and 21 or 28 (Fishbein and Arcangeli, 1987; LeGuerrier *et al*, 1996). However, rabies vaccination can be given

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with a dose (1.0 ml) administered IM or with 0.1 ml via ID route, the latter can be used to reduce cost of the rabies vaccine (WHO, 2014).

A previous (M49P2) study of a pre-exposure rabies vaccine administered to Thai children simultaneously with a Japanese encephalitis (JE) vaccine in 2003-2006 demonstrated antibodies against rabies are induced in all the subjects, regardless of the pre-exposure rabies regimen (Pengsaa *et al*, 2009). A pre-exposure booster dose of rabies vaccine may be required in high-risk individuals if their antibody titer is less than the acceptable level or if complete neutralization is not achieved at a 1:5 serum dilution in a rapid fluorescence focus inhibition test (RFFIT) (Manning *et al*, 2008). In order to determine the long-term (4-8 years) induction of rabies neutralizing antibodies from pre-exposure vaccination in Thai children, the rabies neutralizing titers of 68 pre-school-age children who had previously participated in the M49P2 study were analyzed.

MATERIALS AND METHODS

Subjects

A total of 68 children aged 4 years old who had participated in the 3-year clinical study of the immunogenicity, safety, and booster response of a purified chick-embryo-cell-cultured rabies vaccine (PCECV) administered IM or ID together with a JE vaccine to 12-18 month old Thai children (M49P2 study) were enrolled in this study. Exclusion criteria were: (i) having received further rabies vaccination outside the M49P2 study, and (ii) having a history of chronic disease, immunodeficiency or autoimmune disease, which would interfere with their immune status.

The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, permission no. TM-IRB040/2004. Written prior consent of the parents or legal guardian was obtained.

Pre-exposure rabies vaccination regimens of M49P2 study

These consisted of (i) full-IM regimen: three doses of PCECV (1 ml/dose) given IM as the primary immunization on days 0, 7and 28, and a booster after 1 year; (ii) half-IM regimen: three doses of PCECV (0.5 ml/dose) given IM as the primary immunization on days 0, 7and 28, and a booster after 1 year; (iii) 3-ID regimen: three doses of PCECV (0.1 ml/dose) given ID as the primary immunization on days 0, 7and 28, and a booster after 1 year; and (iv) 2-ID regimen: two doses of PCECV (0.1 ml/dose) given ID as the primary immunization on days 0 and 28, and a booster after 1 year. All subjects received 0.25 ml of JE vaccine subcutaneously on days 0 and 7 and after 1 year, simultaneously with the rabies vaccine

Annual visits (in years 4-8)

Any previous medical illness was recorded and a physical examination was performed on all subjects, including weight, height, history of animal bites, and rabies vaccination. A 5-ml aliquot of each subject's blood was drawn and serum aliquots (2.5 ml) were stored at -70 °C before testing at the Department of Medical Science, Ministry of Public Health, Thailand. Each sample was tested using an in-house RFFIT with a cutoff value for seroprotection of rabies-virus-neutralizing antibodies (RVNA) > 0.5 IU/ml (WHO, 1992). In cases of suspected rabid animal bite, the subject received the appropriate treatment according to WHO recommendation, including rabies vaccine (WHO, 2014), and anti-rabies antibody titer was determined using RFFIT.

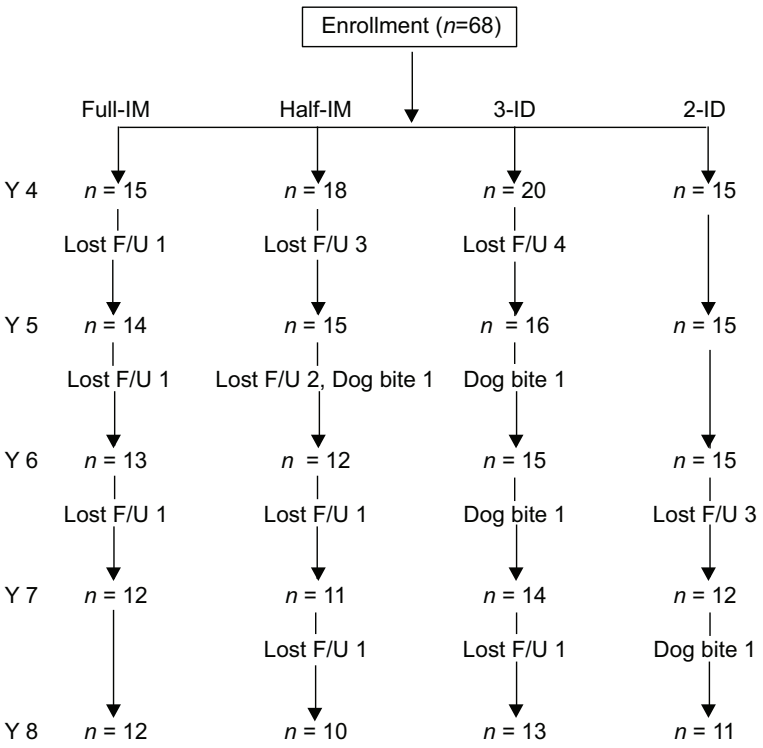

Fig 1–Flow diagram of the study. Full-IM:three doses of chick-embryo-cell-cultured rabies vaccine (1 ml/dose) given intramuscular (IM) on days 0, 7, and 28, and a booster after 1 year. Half-IM: same as full-IM except 0.5 ml/dose given. 3-ID: same as full-IM except 0.1 ml/dose) given intradermal (ID). 2-ID same as 3-ID except two doses of 0.1 m/dose given on days 0 and 28. All subjects received simultaneously with the rabies vaccine 0.25 ml of JE vaccine subcutaneously on days 0 and 7, and after 1 year. Y, year post-vaccination regimen; Lost F/U, lost to follow-up.

Data analysis

A descriptive analysis was used for demographic characteristics and RFFIT titers of the subjects. Data were analyzed using a statistical test with a preset 95% confidence interval (CI) and a p-value < 0.05). A χ^2 test was used to compare categorical variables and Mann–Whitney U test to compare continuous variables employing statistical software package SPSS 16.0 (SPSS, Chicago, IL).

RESULTS

The 68 subjects were divided into four groups according to the regimen administered (Fig 1). The proportion lost to follow-up, including dog bite victims, at the end of the study was 32.4%. Geometric mean titer (GMT) of RVNA declined from 7.5 IU/ml at year 4 to 2.2 IU/ml at year 8, and seroprotective rate gradually declined from 100% to 91.3% at the end of study (Fig 2). Antibody titers of children treated via IM route (full-IM and half-IM) were higher than those treated via ID route at every time point. The lowest titer was obtained with the 2-ID regimen. Seropositive rates after full-IM, half-IM and 3-ID regimen were 100%, while in the 2-ID group the rate declined from 100% to 63.6% at year 4 and 8, respectively.

The overall male:female ratio was 1:1.1. Interestingly, RVNA titers are significantly higher in females receiving IM than males throughout the study (Table 1). On the other hand, there is no significant difference between RVNA titer of males and females in the ID group between years 4 and 8.

The incidence rate of dog bite in each year ranged from 0 to 32.3/1000 person-years (overall incidence rate of 14.1/1000

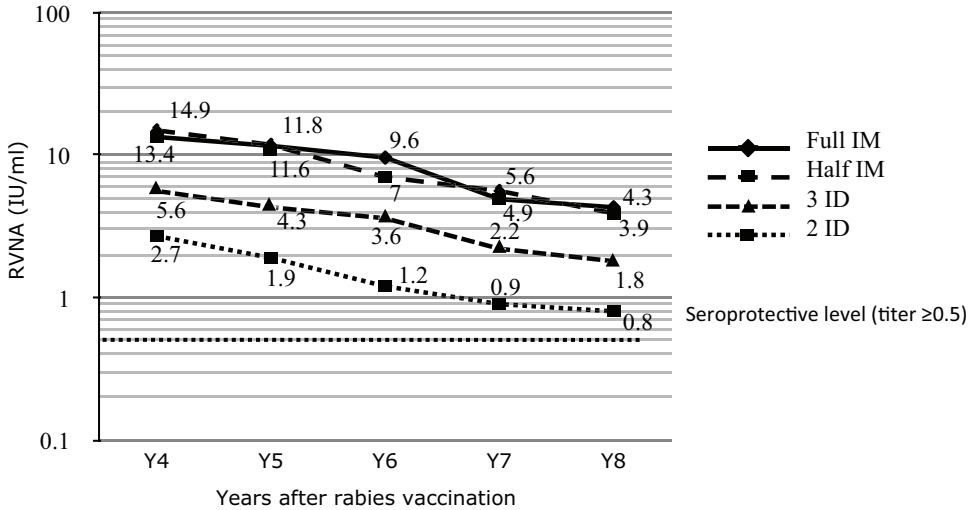

Fig 2–Geometric mean titer of rabies-virus-neutralizing antibodies (RVNA) of each vaccine regimen according to year following vaccination. RVNA was measured using rapid fluorescence focus inhibition test. Full IM:three doses of chick-embryo-cell-cultured rabies vaccine (1 ml/dose) given intramuscular (IM) on days 0, 7, and 28, and a booster after 1 year. Half IM: same as full IM except 0.5 ml/dose given. 3 ID: same as full IM except 0.1 ml/dose given intradermal (ID). 2 ID same as 3 ID except two doses of 0.1 m/dose given on days 0 and 28. All subjects received simultaneously with the rabies vaccine 0.25 ml of JE vaccine subcutaneously on days 0 and 7, and after 1 year.

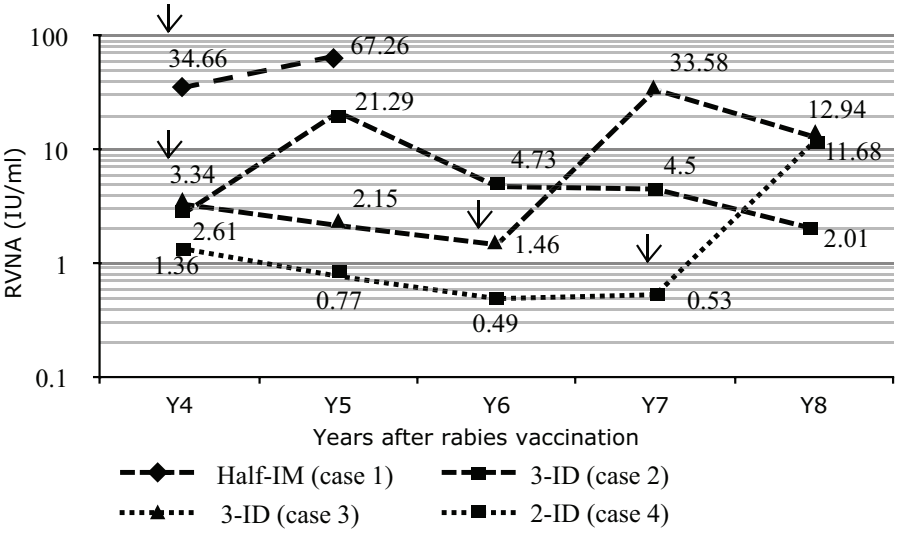

Fig 3–Geometric mean titer of rabies-virus-neutralizing antibodies (RVNA) of dog bite cases before and after chick-embryo-cell-cultured rabies vaccine (CECCRV) booster. RVNA was measured using rapid fluorescence focus inhibition test. Half-IM:three doses of CECCRV(0.5 ml/dose) given intramuscular on days 0, 7, and 28, and a booster after 1 year. 3-ID: same as half-IM except 0.1 ml/dose given intradermal (ID). 2-ID same as 3-ID except two doses of 0.1 m/dose given on days 0 and 28. All subjects received simultaneously with the rabies vaccine 0.25 ml of JE vaccine subcutaneously on days 0 and 7, and after 1 year. Arrow (↓) indicates dog bite event.

Table 1
Annual rabies-virus-neutralizing antibodies (RVNA) titer according to vaccination regimen and gender.

Year post-vaccination	RVNA titer (GMT, 95%CI)					
	IM primary series		*p*-value	ID primary series		*p*-value
	Male	Female		Male	Female	
4	10.1 (7.3-13.8)	20.4 (15.0-27.7)	0.006	4 (3.0-5.4)	4.1 (2.8-6.1)	0.474
5	7.8 (5.8-10.6)	17.9 (26.1-12.2)	0.001	2.9 (2.1-4.0)	2.9 (1.7-5.0)	0.361
6	6.4 (4.5-9.0)	12.1 (22.0-6.6)	0.013	2 (1.3-3.0)	2.2 (1.1-4.5)	0.286
7	3.7 (2.5-5.6)	8.1 (4.4-14.9)	0.018	1.3 (0.9-1.9)	1.6 (1.0-2.6)	0.329
8	3.1 (2.1-4.7)	6.1 (2.9-12.2)	0.066	1.1 (0.7-1.9)	1.4 (0.8-2.3)	0.387

GMT, geometric mean titer; ID, intradermal; IM, intramuscular.

person-years). In a case of dog bite, an IM rabies vaccine booster was administered (4 cases in all). There was an increase in neutralizing antibody titer in all dog bite cases during the study period, with an average increase of 13.78 folds, which included a rapid increase after the booster injection (Fig 3), implying a secondary immune response after the booster, even up to 5 years after the primary vaccination.

DISCUSSION

Pre-exposure rabies vaccination is recommended for all children in high-risk area (Manning *et al*, 2008). However, no routine pre-exposure rabies vaccine is currently available, which may be attributable to a lack of informationon on the long-term immunity induced by such vaccines. A 3-year study of a pre-exposure rabies vaccine administered simultaneously with a JE vaccine to Thai children found that all subjects expressed antibodies against rabies, regardless of the vaccine regimen used (Pengsaa *et al*, 2009).

The current study demonstrated antibody titer in children participating in the above mentioned study is significantly higher in those treated with IM regimens than in ID regimens, and the full-IM, half-IM and 3-ID regimens were 100% seroprotective, while the 2-ID regimen was about 40% lower. Thus, long-term immunity tended to persist longer using either IM or 3-ID regimens. Another study of two vaccination regimens administered to 18–24-year-old subjects in Thailand, the group given 2-ID PCECV followed by an ID booster after 1 year has a GMT titer of 0.35 IU/ml at year 1 (prior to booster) and 14.38 IU/ml two weeks after the booster compared to those given half-IM PCECV plus booster with GMT titer of 0.76 and 14.06 IU/ml, respectively (Wongsaroj *et al*, 2013). In our study, the GMT titers at year 4 after the 2-ID and half-IM regi-

mens (without any booster) were higher. These findings suggest that the immune responses of the toddlers were better than those of young adults (Shanbag *et al*, 2008).

A two-year study of school children in Thailand found that 12/703 children were exposed to suspected rabid dogs, equivalent to an incidence of dog bite of 1.7% (or incidence of 8.5/1000 person-years) (Kamoltham *et al*, 2011), a lower incidence rate than in our study. This difference-might be attributable to a more thorough follow-up or differences in the children's environment or living standards.

Surprisingly, our study demonstrated a higher antibody titer in male than female subjects given the IM regimen, both at years 4 and 8. This finding has never been demonstrated in other rabies vaccine studies. However, in a study conducted by an Australian travel medicine clinic over a period of 2 years, in which a human diploid cell rabies vaccine (HDCV) was administered to travelers in a 3-ID regimen, with a booster after 12 months, mean antibody titer for males and females is 7.83 and 8.69 IU/ml, respectively, but the values do not reach statistical significance (Lau and Sisson, 2002). According to the basic tenets of immunology, females have a stronger immune response after exposure to an antigen than males. A previous study have identified sex-specific differences in immune response, with women having, on average, 1.7 times the frequency of self-specific T cells than men (Su *et al*, 2013). According to a systematic analysis, androgens and genes involved in lipid metabolism may drive the differences in the immune responses of women and men.

The results of our study confirm the long-term efficacy of a pre-exposure regimen involving administration of IM and 3-ID doses of PCECV in eliciting a rapid immune response in children aged 4-4.5 years. The incidence of dog bite is sufficiently high in school-age children to justify the cost of the pre-exposure rabies vaccination program (Chulasugandha *et al*, 2006). However, the seroprotective rate of the 2-ID regimen after 8 years was only 63.3%, so another booster may be required to maintain adequate immunity.

In conclusion, this study demonstrates the long-term induction of protective antibodies by a pre-exposure vaccine against rabies and provides useful information that should further the proper control and management of rabies in humans.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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