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Original Article

Primary Humoral Immune Responses to Formalin Inactivated Hemorrhagic Fever with Renal Syndrome Vaccine (Hantavax®): Consideration of Active Immunization in South Korea

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The efficacy of a formalin-inactivated hemorrhagic fever with renal syndrome (HFRS) vaccine and the effectiveness of a related vaccination program have not been previously evaluated. We measured the primary immune responses to Hantavax® by plaque reduction neutralizing antibody test (PRNT), hemagglutination inhibition test (HAI), ELISA and high density particle agglutination test (HDPA) in order to confirm a possible biological efficacy through independent substantiation of experimental results and to compare the results with previous studies. Following two doses of primary vaccination, the seroconversion rate of PRNT and HAI antibody was 33.3% (10/30)[95% C.I. 17.3-52.5%] and 26.7% (8/30) [95% C.I. 12.3-45.9%], respectively. The correlation between PRNT and HAI antibody showed a statistical significance ($r=0.58$, $p<0.01$). The seroconversion rate of HDPA and ELISA were both 76.7% (23/30) [95% C.I. 57.7-90.1%], which correlated well with each other($r=0.58$, $p<0.01$). In our study, Hantavax® elicited low neutralizing antibody responses, at least in the volunteers samples that we tested. The vaccination program, including the vaccine itself, that has been adopted by the national immunization program to protect against HFRS in Korea should be re-evaluated and re-formulated to produce a higher protective immune response rate.

Key Words: Hemorrhagic fever with renal syndrome, vaccine, immunogenicity, neutralizing antibody, vaccination policy

INTRODUCTION

In 1988, the first Hantaan virus vaccine (Hantavax®, Korea Green Cross, Seoul, Korea) capable of preventing Hemorrhagic Fever with Renal Syndrome (HFRS) was developed from suckling mouse brain infected with the ROK 84-105 strain, and inactivated with 0.05% formalin by Lee and colleagues.[1] With the acceptance of data showing a high level of seroconversion rate with immunofluorescent (IF) and enzyme-linked immunosorbent assay (ELISA)antibody response as a surrogate for vaccine efficacy, Hantavax® was approved in 1990 on condition that data indicating protective efficacy shown in a placebo controlled clinical trial and a demonstration of protective antibody titers and their long-term persistence be submitted within a few years.[2] The overwhelming opinion of the Health Authority at the time was that the early distribution of the vaccine would have beneficial impact on public anxiety over the risks of HFRS that was an important health problem in soldiers and farmers in Korea.[3]

Since Hantavax® has been available commercially from 1990, and the vaccine has been widely distributed to public health centers since its adoption into a national immunization program in 1992.[4] The recommended immunization schedule with Hantavax® is two doses one month apart as a primary vaccination, and one booster 12 months later. Not having established the persistency of long-term protective immunity and in spite of several post-marketing clinical studies,

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outcome assessments have been limited to measurements of IF antibody without measuring protective neutralizing antibody response.[5,6] In addition, a neutralizing antibody response following primary vaccination has not yet been well established, although there was one report showing a neutralizing antibody response measured with a 75% seroconversion rate 4 weeks after two doses of Hantavax®, which was decreased to 14.2% in the twelve months following vaccination.[7] We measured the primary immune responses to Hantavax® by plaque reduction neutralizing antibody test (PRNT), hemagglutination inhibition test (HAI), ELISA and high density particle agglutination test (HDPA) in order to confirm a possible biological efficacy through independent substantiation of experimental results and to compare with previous studies. Additionally, we reviewed recent reports of long-term immune response and evaluated the efficacy of Hantavax®.

MATERIALS AND METHODS

Two doses of commercially available Hantavax®, Korea Green Cross, (Lot No. 6001, 2360002) were adminstered intramuscularly to thirty healthy adults with informed consent. Sera was collected before vaccination and 4 weeks after each dose for a total of 90 serum samples from 30 vaccine recipients. PRNT, HAI and ELISA were performed [Peter L. Summers]as described previously[8-10] at the United States Army Medical Research Institute for Infectious Diseases (USAMRIID), Ft. Detrick, MD using a laboratory blinding method. PRNT antibody titers were expressed as the highest serum dilution that reduced the standard viral dose (45 - 58 plaque forming units of Hantaan virus strain 76 - 118) by 50 percent. All sera were heat-inactivated at 56℃ for 30 min. Sera were initially diluted 1:10 and subsequently in serial twofold dilutions. PRNT antibody titers were measured either with 5% normal human serum or without normal human serum and compared. For IgG ELISA, cobalt-irradiated HTN antigen at a 1:50 dilution and test sera at a 1:100 dilution were used. Also, horseradish peroxidase-labeled anti-human IgG conjugate was used. For HAI test, a serum sample and 4 units of histochoice fixative HTN antigen were incubated overnight at 4℃. A standard microtiter procedure was used, in which two-fold serum dilutions were tested beginning at 1:10. Serum binding antibodies also were measured using a HDPA commercial kit (Hantadia®, Korea Green Cross, Seoul, Korea) at Yonsei University.[11] Sera samples were tested in two fold dilutions beginning at 1:20 and reactions at dilutions of 1:40 or greater were regarded as positive, as specified by the manufacturers instructions.

RESULTS

HAI, ELISA IgG and PRNT antibodies were not detected in any of the 30 pre-vaccination samples, however HDPA antibody was detected by the commercial kit in 2 pre-vaccination samples [case 16, 18]. At four weeks only one of the 30 vaccine recipients developed a measurable neutralizing antibody response [PRNT antibody titer of 1:10] as tested without normal human serum. The PRNT antibody titer did not change in this recipient after two doses. Four others developed PRNT antibodies after two doses, for a total seroconversion rate of 16.7% (5/30) [95% C.I. 5.6 - 43.7%](Table 1). The titers of five reactive sera after two doses were 1:10, 1:10, 1:10, 1:40, and 1:320, respectively. However, when tested with 5% normal human serum in the virus diluent to consider complement-dependent neutralization, PRNT antibodies were detected in two cases 1:10, respectively after one dose and the titer was increased to 1:40, respectively after two doses. The final seroconversion rate of PRNT antibody was 33.3% (10/30) [95% C.I. 17.3 - 52.5%]. The titers of the ten reactive sera after two doses were 1:640, 1:40, 1:20, 1:40, 1:20, 1:20, 1:20, 1:10, 1:10 and 1:40, respectively. The seroconversion rate of HAI antibody showed 10.0% (3/30) after one dose and 26.7% (8/30) [95% C.I. 12.3 - 45.9%] after two doses. The correlation between PRNT and HAI antibody showed statistical significance ($r=0.58$, $p<0.01$). The seroconversion rate of HDPA and ELISA showed 33.3% (10/30) and 46.7% (14/30) after one dose respectively and after two doses, was equally 76.7% (23/30) [95% C.I. 57.7 - 90.1%], which correlated

Table 1. Immune Response in Vaccine Recipients after Two Doses of Formalin-inactivated Mouse Brain Derived HFRS Vaccine from Three Different Independent Laboratories

	HDPA[a]	ELISA IgG[b]	HAI[b]	PRNT[b]	
				Without NHS	With 5% NHS
Pre-Vaccination	6.60% (2/30)	0.00% (0/30)	0.00% (0/30)	0.00% (0/30)	0.00% (0/30)
After one dose	33.30% (10/30)	46.70% (14/30)	10.00% (3/30)	3.30% (1/30)	6.70% (2/30)
After two Doses	76.70% (23/30)	76.70% (23/30)	26.70% (8/30)	16.70% (5/30)	33.30% (10/30)

[a]High Density particle agglutination test (HDPA) tested by commercial kit (Hantadia®, Korea Green Cross) at Yongdong Severance Hospital, Yonsei University Medical Center.
[b]Enzyme-linked immunosorbent assay (ELISA) IgG, Hemagglutination inhibition test (HAI) and Plaque Reduction Neutralizing Test (PRNT) were performed at U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID).

well with each other ($r=0.58$, $p<0.01$) (Table 1).

DISCUSSION

HFRS has been an important public health problem in Korea and occurs mainly in rural areas, primarily in 20 - 50 year-old adults, and sporadic cases are occasionally recognized in urban residents.[3] Available surveillance data during the last decade indicated that approximately 1,000 HFRS cases were hospitalized annually and the case-fatality ratio was 2 to 3% (Table 2). However, childhood cases were much less common than adults, with 63 reported cases over the last 15 years.[12] The special risk groups-farmers, field workers, and soldiers stationed in the field in selected geographical areas were indicated for recommended vaccination. Korea adopted Hantavax® into its national immunization program in 1992, and vaccination has been mainly conducted at public health centers and schools (3,745,000 doses, 65.8%) through a mass immunization program due to overwhelming concern and the recommendation of health care providers rather than through the military (1,162,000 doses, 20.4%) and hospitals (783,000 doses, 13.8%)(Table 3).

In 1998, public health concerns of the vaccination policy and its practice arose regarding several unclear questions on the primary neutralizing immune response, the booster schedule for long-term immunogenicity, identification of risk groups, and the evaluation of cost-effectiveness. Providing evidence of a protective immune response, Cho et al.[7] and Chu et al.,[13] reported a similar observation of a neutralizing antibody response. The seroconversion rate was 66.7% (14/21) at 1 - 4 months after two doses of primary vaccination, and was reduced to 22.5% (9/40) at 12 months after primary vaccination.[13] With a booster dose at 12 months after primary vaccination, the positive rate was 100% (8/8) at one month after the first booster, and 75% (9/12) at two months. Additionally, with a second booster dose at 2 years following primary vaccination, the positive rate was 86% (6/7) at three months after the second booster. They asserted that the booster vaccination is necessary at one year following the primary two-dose vaccination in order to maintain high levels of neutralizing antibodies, and that antibody levels after the one dose booster vaccination persisted for at least two years. However, considering the inadequate sample size and the statistical significance of the results of this study, with wide 95% confidence intervals - 49.8 - 92.2% at 1 - 4 months after two doses of primary vaccination, 59.8 - 98.8%, 42.8 - 93.3% at one month and two months after the first booster, 42 - 99.2% at three month after the second booster it is difficult to accept such suggestions. In addition, according to our observation, only 33.3% (10/30) [95% C.I. 17.3 - 52.8%] developed a neutralizing antibody response. The WHO manual reports methods including PRNT developed at USAMRIID that are commonly used in laboratories.

Table 2. Reported Cases of HFRS in Korea from 1977 to 1998 in South Korea

Year	Civilian	Korean military personnel	U.S. Soldiers stationed in Korea	Total
1977	288	241	7	536
1978	207	168	10	385
1979	241	122	1	364
1980	185	72	1	258
1981	377	164	2	543
1982	378	123	3	504
1983	402	98	3	503
1984	568	156	6	730
1985	531	159	7	697
1986	530	166	14	710
1987	533	163	5	701
1988	264	97	6	367
1989	306	104	6	416
1990	964	73	6	1,043
1991	1190	44	0	1,234
1992	1116	49	2	1,167
1993	1230	62	1	1,293
1994	1006	34	1	1,041
1995	752	29	1	782
1996	662	23	1	687
1997	390	23	2	415
1998	709	37	4	750

Cases were tested serologically by IF antibody method since 1977.

The assay that Peter L Summers ran for our study in USAMRIID is the same one that USAMRIID used to measure neutralizing antibody response to their vaccinia vectored HFRS vaccine several years ago. This vaccine showed a poor neutral.

We have several serious concerns regarding Hantavax® being used publicly because of the low primary protective immune response, and an uncertainty as to whether the administration of a third dose in the primary immunization series would significantly improve the response rate above the observed proportion or whether neutralizing antibody could persist or might eventually yield an acceptable response, as asserted by Chu et al.[13] Using a binding assay for the diagnosis of HFRS based upon the agglutination of viral antigen-coated particles, a higher antibody response was seen in the vaccine recipients (76.7%). However, it cannot be said that a high level of seroconversion rate with IF and ELISA antibody response could be accepted as proof of adequate protective efficacy of Hantavax® as a surrogate in human.

The current limited observation of the decrease in the incidence of HFRS by passive HFRS surveillance in South Korea probably cannot be attributed solely to the effectiveness of the vaccination program of the last several years.[4] The total number of reported HFRS cases diagnosed by IF has decreased from 1,234 in 1991 when the

Table 3. Amount of Vaccine Doses and Number of HFRSP Patients in South Korea from 1990-1998

Year	Doses of vaccine used at			Total amount of vaccine used	No. of HFRS cases
	Hospital & Clinics	Public Health Centers	Military		
1990	0	0	0	0	1.043
1991	35,000	115,000	33,000	183,999	1,234
1992	122,000	493,000	145,000	760,000	1,167
1993	118,000	707,000	133,000	958,000	1,293
1994	107,000	660,000	142,000	909,000	1,041
1995	118,000	640,000	232,000	990,000	782
1996	105,000	460,000	155,000	720,000	687
1997	108,000	360,000	162,000	630,000	415
1998	70,000	310	160,000	540,000	750
Total	783,000	3,745,000	1,162,000	5,690,000	8,412

National immunization program started in 1992.

vaccination program started to 750 in 1998 (Table 3). It can not simply be said that the vaccination is effective, reducing the total number of reported HFRS cases following vaccination. Perhaps the HFRS incidence rate has changed over the years with fluctuating rodent populations and other natural factors. Incidence may have declined with improvements in the standard of living and housing in rural areas. In actuality, the vaccinations were conducted largely in children (65% of total vacciantion) who are not identified as a risk group. A seroepidemiological study in Chorwon, an area with one of the highest endemic transmission rates in Korea, found a seroprevalence rate of 2.1% in children 5-19 years old.[14] However, Chu et al.[15] reported that an efficacy field trial of Hantavax® conducted in areas of Yugoslavia showed a very high protective efficacy no cases among 1900 vaccinees and 20 cases among 2000 non-vaccinees. Unfortunately this report did not describe the method of randomization for the vaccine group and the placebo (saline injection) group and the authors mentioned they never obtained informed consent for this study in Yugoslavia.

The poor protective immune response reported in our observation and the unknown clinical efficacy beg the question whether the recommendation for the use of Hantavax® is appropriate, particularly in children. Although occupation-associated risks in rural areas, principally affecting adults, have been established, it is unclear whether the potential benefits of inactivated HFRS vaccine may be enough to support the immunization of school children even in rural areas, not to mention those in urban locations, considering the lower risk of HFRS in children and the possible adverse events associated with inactivated mouse brainderived vaccines, as seen with Japanese encephalitis vaccine produced by similar methods.[16,17] In our report, Hantavax® elicits low neutralizing antibody responses, at least in the samples that we tested. Although this does not necessarily mean that Hantavax® will not protect people from disease at all, generally speaking, highly effective vaccines induce the long-lasting presence of neutralizing antibodies. In terms of the low neutralizing antibody seroconversion rate (26%) in vaccinia preimmune, the people to vaccinia vectored Hantavirus vaccines that have been developed in USAMRIID, this vaccine would be effective only in a target population of a vaccinia virus-naive population that showed 72-98% neutralizing antibody response after vaccination.[18] Hantaviral nucleocapsid protein alone may be capable of inducing protection in animal infection

regardless of the absence of neutralizing antibodies in animal experiments.[19,20] However, Hantavax® has never been proven for the possible protective immune response in humans in terms of its low neutralizing antibody response with nucleocapsid protein.

In conclusion, the active immunization program with formalin inactivated mouse brain derived HFRS vaccine should be re-evaluated thorough a well designed case-control study and the primary protective immune response and long-term protective immunogenicity should be established. In addition, in regards to conducting a national immunization program, a cost-effectiveness study is required in Korea.

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