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Safety and Immunogenicity of a Candidate Parvovirus B19 Vaccine

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Abstract

Parvovirus B19 is an important human pathogen causing erythema infectiosum, transient aplastic crisis in individuals with underlying hemolytic disorders and hydrops fetalis. We therefore evaluated a parvovirus B19 virus like particle (VLP) vaccine. The safety and immunogenicity of a 25 µg dose of parvovirus B19 recombinant capsid; 2.5 and 25 µg doses of the recombinant capsid given with MF59; and saline placebo were assessed in healthy adults. Because of 3 unexplained cutaneous events the study was halted after enrollment of 43 subjects and before any subject received their third scheduled dose. The rashes developed 5-9 days after the first or second injection and were seen in one placebo recipient (without an injection site lesion) and two vaccine recipients (with injection site reactions). No clear cause was established. Other safety evaluations revealed mostly injection site reactions that were mild to moderate with an increase in pain in subjects receiving vaccine and MF59. After dose 2 the majority of vaccine recipients developed ELISA and neutralizing antibody to parvovirus B19. Given the possible severe consequences of parvovirus B19 infection, further development of a safe and effective vaccine continues to be important.

Introduction

Parvoviruses are small DNA viruses that infect rapidly dividing cells and cause a diversity of diseases in animal species [1]. Parvovirus B19 and the newly discovered Bocavirus are the only members of the Parvoviridae family known to cause disease in humans. In healthy individuals, parvovirus B19 causes erythema infectiosum, more commonly known as fifth disease, a rash illness in children that can manifest as an arthralgia syndrome in adults. Its role in myocarditis may also have been underestimated [2, 3]. In individuals with underlying

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hemolytic disorders, parvovirus B19 infection results in transient aplastic crisis (TAC), a temporary cessation of red blood cell production with severe worsening of anemia, which is occasionally fatal [1, 4]. Similarly, parvovirus B19 infections play a significant role in the etiology of severe anemia in areas where malarial is endemic and contributes to the morbidity and mortality associated with severe anemia in such regions [5, 6]. In immunosuppressed individuals, parvovirus B19 can persist and cause severe anemia and pure red cell aplasia. In patients with organ transplantation, parvovirus B19 may account for considerable mortality [7].

Importantly, infection of a pregnant woman in mid-trimester can result in hydrops fetalis and fetal loss. Up to 40% of all pregnant women are seronegative and susceptible to parvovirus B19. Transplacental transmission rates have been reported to vary from 24% to 33%. In addition to hydrops fetalis, fetal parvovirus B19 infection is associated with fetal anemia, spontaneous abortion, and stillbirth. Fetal infection rates are estimated to range from 1%-9% [8]. Detection and treatment is complicated due to the fact that in many cases, pregnant women may be asymptomatic, requiring detection of IgM in maternal serum or via visualization of hydrops by ultrasound. In a seroepidemiologic maternal risk study in five European countries Mossong et. al. showed that the annual fetal loss was close to 900 and when considering Europe as a whole, the number could be several thousand [9]. There are no known effective antiviral drugs for the treatment of parvovirus B19 infections.

The major capsid protein of parvovirus B19, VP2, constitutes about 95% of the capsid structure. The minor capsid protein, VP1, is identical except for an additional 227 amino acids at the amino terminus. Studies in the Hematology Branch of the NHLBI have demonstrated that the individual capsid proteins can be expressed in a baculovirus system and, when recombinant vectors are cotransfected, VP1 and VP2 spontaneously assemble into empty parvovirus capsids [10]. The capsids were shown to be immunogenic in a variety of animals and in humans, eliciting neutralizing antibody responses [11, 12]. Enrichment of empty capsids by manipulation of the multiplicity of infection of the recombinant insect virus can produce capsids containing approximately 20% to 40% VP1. These capsids are particularly effective in promoting a neutralizing antibody response.

A previous evaluation of a capsid preparation that was approximately 25% VP1 and about 75% VP2 (Medi-491) was conducted as an open-label, dose-escalation study in healthy adults using doses of 1, 3, 10, 30, and 100 μ g. The vaccine was poorly immunogenic when given with the adjuvant alum (Balsley unpublished results). In a subsequent trial of this vaccine, all parvovirus B19 seronegative subjects (n=24) immunized with 2.5 μ g or 25 μ g of vaccine formulated with the adjuvant MF-59 (an oil in water emulsion) seroconverted after receiving at least 2 doses of vaccine [11]. Antibody titers were significantly higher in the 25 μ g dose group.

In the study presented here, we compared the safety and immunogenicity of a baculovirus expressed parvovirus B19 recombinant capsid vaccine at a dose of 25 μ g to 2.5 and 25 μ g doses of the vaccine given with MF59. Baculovirus derived vaccines present an alternative to cell culture based vaccines especially in the development of new influenza vaccines [13]. MF 59 is currently licensed as an adjuvant for influenza vaccines in several countries and is licensed in the USA for seasonal influenza vaccines in the elderly. Its mechanism of action is not fully understood, but enhancement of the interaction between the antigen and the dendritic cell appears to be involved [14].

Materials and Methods

Vaccine and adjuvant: The vaccine is a recombinant parvovirus B19 vaccine composed of the VP1 and VP2 proteins of the virus [11]. VP1 and VP2 were expressed in a baculovirus system in which the 2 capsid proteins self-assemble into virus like particles (VLP), as previously described [10]. The capsid preparation was approximately 22.6% VP1 and about 77.4% VP2, meeting pre-specified specifications. Vaccine was supplied by Meridian Life Science, Inc. (fdba Viral Antigens, Inc., Memphis, Tennessee) and has a designation of VAI-VP705 (referred to as parvovirus B19 vaccine in the remainder of the text). Tests for the presence of contaminating Sf9 host cell DNA was evaluated by quantitative PCR at the unprocessed bulk stage (the stage most likely to contain such contaminants) and was found to be below the detectable limit of the assay. The concentration of VLP proteins was determined by bicinchoninic acid assay (BCA). Purity of the preparation was evaluated by SDS-PAGE and silver staining and the ratio of VP1 to VP2 proteins comprising the VLPs was determined by SDS-PAGE and silver staining.

MF-59 (Novartis) is an oil (squalene)-in-water emulsion that was used at a concentration of 10 mg of squalene/dose. The vaccine and the adjuvant were available in separate vials, and were mixed prior to administration because the combined product was not stable for long periods of time. A diluent (20 mM tris buffer with 5% sucrose and 0.005% tween-80, pH 7.5) was used instead of the adjuvant for subjects randomized to the non-adjuvanted arm of the study. Placebo recipients received saline.

Subjects

Subjects were healthy 18 to 45 year old adults recruited at Cincinnati Children's Hospital Medical Center and Baylor College of Medicine. Subjects were screened for health status by history, vital signs and laboratory studies including negative tests for active HIV, hepatitis B and C infection, and normal hemoglobin, white blood counts and alanine transaminase (ALT). Only subjects, who were seronegative for antibodies to parvovirus B19, as assessed by a commercial ELISA assay, were enrolled into the study.

The study was approved by the Institutional Review Board at each site. Written informed consent was obtained before performing any study procedures. A Safety Monitoring committee (SMC) comprised of 3 Independent Safety monitors periodically reviewed and evaluated the accumulated study safety data in a blinded fashion.

Study Design

This study was a Phase I/II randomized, placebo-controlled, double-blind clinical trial evaluating the safety and immunogenicity of 2 dose levels of parvovirus B19 vaccine. Eighty-nine parvovirus B19 seronegative healthy adults were to be randomized to 1 of 4 groups: 26 to each vaccine group (parvovirus B19 2.5 µg with the adjuvant MF59, 25 µg with the adjuvant MF59, 25 µg without the adjuvant MF59) and 11 subjects to a placebo group (saline control). Each participant was to receive 3 separate vaccinations (at 0, 1, and 6 months).

Study participants were followed to evaluate safety and immune response. Safety was assessed by clinical evaluation and laboratory parameters on days 7 and 28 after each injection. Specific solicited injection site and generalized adverse events (AEs) were assessed for 7 days following vaccination beginning on the day of vaccination using a memory aid completed daily by the subject. The severity of solicited events was graded on a scale of 1-3, corresponding to mild (symptom present but does not interfere with activity), moderate (interferes somewhat with activity) and severe (incapacitating). Antibody

responses were to be measured at 28 days after the administration of each vaccine and at the 6-month follow-up visit.

Antibody Assays

A commercial parvovirus B19 IgG ELISA (Biotrin International, Dublin, Ireland) was performed according to the manufacturer's instructions to screen all subjects for antibody and to assess for seroconversion after the first and second dose. Results are reported as positive or negative. The absorbance (optical density) is also provided as a semi quantitative measure of activity. Two subjects who had a negative result on screening were found to have detectable antibody when retested just prior to vaccination. These subjects were not included in the analysis of immunogenicity.

The neutralization assay was performed in triplicate using methods similar to those previously described [15]. Briefly, 10 μ L of virus (2×10^7 genome equivalents/ml) and 10 μ L of serum dilutions of 10, 100, 1000) were pre-incubated in 96-well plates for 1 hour at room temperature. Then, $\sim 2 \times 10^4$ UT7/Epo-S1 cells in 10 μ L were added and allowed to incubate at 4°C for 2 h. After the incubation, 70 μ L of culture media was added to the well, bringing the final volume to 100 μ L. The cells were incubated at 37°C with 5% CO₂ and harvested for analysis on day 3.

RNA was then extracted from 25 μ L of cells using the TurboCapture 96 mRNA Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocols. mRNA was converted to cDNA using MMLV-RT (Invitrogen, Carlsbad, CA, USA) and 10 ng/ μ L Random Primers (Invitrogen) following manufacturer's protocol scaled up to a 50 μ L reaction volume. Parvovirus B19 RNA transcripts were quantitated by real-time RT-PCR designed to amplify products in the NS regions using the PerfeCTa™ Multiplex qPCR SuperMix (Quanta Biosciences Inc., purchased through VWR Scientifics, West Chester, PA, USA). In a final reaction volume of 25 μ L, the PerfeCTa™ Multiplex qPCR SuperMix was combined with 5 μ L of the RT reaction, 0.4 μ M of the NS amplification primers (5'-GGGCAGCATGTGTTAAAGTGGA - 3' and 5'-TGGCCATTGCCAAGTTTGT - 3', and 0.2 μ M of the NS probe TYE665-TTATGGGCCGCCAAGTACAGGAAA-IB RQ. After an initial activation step of 3 min at 95°C, 45 cycles of 15s at 94°C and 60s at 60°C were performed, the number of transcripts was determined by comparison against a standard curve created using serial dilutions of pYT103, a plasmid containing the parvovirus B19 coding region. To confirm extraction of RNA and to normalize the number of transcripts per cell, quantitative RT-PCR (qRT-PCR) was performed using the same amplification conditions, but with primers β -actin F (5'-GGCACCCAGCACAATGAAG - 3'), β -actin R (5'-GCCGATCCACACGGAGTACT - 3') and β -actin probe (MAX550-TCAAGATCATTGCTCCTCCTGAGCGC-IB FQ). An actin standard curve was obtained from serial dilutions of a plasmid containing an extended region of the actin coding sequence. All probes and primers were purchased from Integrated DNA Technologies (Coralville, IA, USA). Inhibition of at least 50% virus transcript copies compared to the negative control serum was defined as a positive response. The neutralization titer was defined as the highest serum dilution that met the definition of a positive response. For the calculation of GMT a value of 5 was used for samples that were not positive at the lowest dilution tested (1:10).

Statistics

The sample size for this study of this newly manufactured vaccine was selected to provide preliminary information on safety and immunogenicity. The sample size chosen (26 subjects per vaccine group and 11 placebo recipients) had at least 72% power to observe 1 or more SAEs of a particular type in a single active vaccine group when the underlying probability

was 5% or greater. Combining the vaccine groups, this power is increased to 92%. Due to the adverse events discussed below, full enrollment was not achieved. Forty-three subjects received the first dose and were available for safety analyses and twenty-eight were available for the main immunogenicity analyses distributed across four groups; substantially reducing the power to detect statistically significant differences. Student's T-test was used to compare ELISAs and log transformed neutralization results. Chi-square tests and McNemar's test were performed to compare response rate and response concordance. However, descriptive statistics should be viewed as the principal summary of the data.

Results

Because of the unexplained reactions described below the study was halted after enrollment of 43 subjects. A first dose of vaccine was received by 12-14 subjects in each vaccine group, while 5 received placebo. A second dose of vaccine was given to 8-10 subjects in each of the vaccine groups while 4 of the 5 subjects in the placebo group received the second dose. None of the subjects received the third dose of vaccine. Demographic characteristics of the enrolled subjects are shown in Table 1. The subjects were predominantly female, white and non-Hispanic.

Safety

Injection site reactions were common but were mostly mild or occasionally moderate (Figure 1). Severe injection site reactions were only reported by one subject who received placebo. The addition of MF59 appeared to increase the frequency of injection site pain and tenderness. For example, pain was reported by 17% and 13%, in the group receiving 25 µg of parvovirus B19 vaccine without MF-59 after the first and second dose respectively, but was reported by 43% and 51% in the group receiving 25 µg of parvovirus B19 vaccine with MF59. Injection site reactions did not increase in frequency or severity after the second dose. Systemic reactions were less common and fever was not detected in any subject. One subject, who received placebo injection, reported severe systemic adverse events (myalgia) following injection. No consistent abnormality in any laboratory parameter was noted.

Of special interest are unusual skin manifestations that were seen in three subjects, all at Baylor College of Medicine. The first subject, a 33 y/o male, developed injection site lesions 5 days after the first immunization with 25 µg of parvovirus B19 vaccine with MF59. He developed a 6×7 cm erythematous, indurated lesion that was 3.5 cm distal to his injection site as well as a 2×3 cm erythematous lesion around the injection site, with a few faint scattered lesions between these two larger lesions (Figure2). There was some itchiness but no systemic complaints and no abnormalities in his laboratory values. The lesions resolved over the next 3 days but the area became hyper pigmented. An immunology specialist thought that the rash was most consistent with an Arthus like reaction. The second immunization was not administered. He seroconverted as measured by ELISA and neutralizing antibody. The second subject was a 23 y/o female who received placebo injections. She developed a systemic rash 7 days after the second immunization but in contrast to the other 2 subjects she had no injection site reaction. The rash consisted of fine sparse papular lesions over the arms and legs that were mildly pruritic (Figure 2). Over the next few days the lesions became more defined as an erythematous macular papular rash on the upper arms thighs, and lower abdomen. The rash resolved within 9 days with no persistence. She did not seroconvert as measured by ELISA or neutralizing antibody. The last subject, a 28 y/o male developed a rash 9 days after receiving the second immunization with 2.5 µg of parvovirus B19 vaccine with MF59. The lesion was an 8×6 cm warm, erythematous, indurated plaque located slightly distally to the injection site (Figure2). This was accompanied by a maculopapular erythematous rash on all extremities and the trunk, back and abdomen. The rash consisted of 0.5-1 cm lesions along with 3-5 mm papular and

2-3 mm pinpoint papular, erythematous lesions. He had a biopsy 3 days after the onset of the rash which showed superficial and deep perivascular interstitial infiltrate of lymphocytes and eosinophils. A dermatologist thought the lesions were urticarial in nature. The area at the injection site remained hyper pigmented. He seroconverted as measured by ELISA but neutralizing antibody was not detected. None of the subjects had other systemic complaints, lymphadenopathy or abnormalities in their laboratory values. It is interesting to note that all three subjects worked in laboratories and the first subject worked with baculovirus.

Because of these unexplained reactions the study was halted after enrollment of 43 subjects and prior to the administration of the third dose to any subject. The trial was not restarted because the subjects had missed their third immunization window and the vaccine would have expired before completion of the study due to the lengthy period evaluating these reactions.

Immunogenicity

Serum was available from 30 subjects after the first dose and from 29 after the second dose of vaccine. Antibody was detected by ELISA approximately 28 days after the first dose of vaccine in 2 of 9 subjects who received the 2.5 µg of parvovirus B19 vaccine with MF59; 2 of 8 subjects who received 25 µg of parvovirus B19 vaccine; 6 of 8 subjects who received 25 µg of parvovirus B19 vaccine with MF59 and 0 of 3 subjects who received placebo. Approximately 28 days after the second dose of vaccine antibody was detected by ELISA in the majority of subjects regardless of dose or the addition of adjuvant (Table 2). Thus, there was no significant difference among the vaccine groups comparing the percent of subjects who developed detectable ELISA antibody or in the absorbance (O.D.) value. No placebo subject developed parvovirus B19 antibody.

Neutralizing activity was only evaluated on sera obtained before immunization and approximately 28 days after the second dose. When evaluated for neutralizing activity the majority of vaccine recipients developed antibody but there were fewer subjects with detectable neutralizing antibody compared to the ELISA assay (Table 2). The GMT of neutralizing antibody was highest in the group receiving 25 µg of vaccine with MF59.

Discussion

This is the third evaluation of VLP vaccines for prevention of parvovirus B19 infections. In the first trial the vaccine was poorly immunogenic when given with the adjuvant alum, (Balsley unpublished results). However, in a subsequent trial, the addition of the adjuvant MF-59 improved immunogenicity, so that all vaccines developed neutralizing antibody after the second dose with the highest titers in the group receiving 25 µg compared to the 2.5 µg dose. In the trial presented here, there was surprisingly little difference in the immune response after two doses of vaccine between the groups receiving 25 µg of vaccine with or without MF59 or between the groups receiving 2.5 vs. 25 µg with MF59. However, in general, responses were higher with MF59 and at the higher dose and ELISA antibody was more likely to be detected after the first dose in the group receiving 25 µg with MF59. Antibody responses also appeared to be diminished compared to the previous trial, as only 11-44% of vaccine recipients developed neutralizing antibody after 2 doses. Whether a boost would have been seen after a third dose is unknown. It should also be pointed out that the level of antibody required for protection has not been defined.

Previous evaluations of the vaccine reported the vaccine was safe and well tolerated except for local injection site reactions, predominantly pain [11]. In the study presented here pain was also common and increased with the administration of MF-59. Of more concern was the occurrence of rash in three subjects that lead to halting of the study. All rashes developed

5-9 days after receipt of vaccine at one of the two centers. A rash developed in one subject after the first immunization and in two subjects after the second immunization. A rash was seen in one placebo recipient, one recipient of vaccine containing 25 µg VLP with MF-59 and in one who received 2.5 µg VLP with MF-59. However, the skin reaction in the placebo (saline) recipient was generalized and no injection site reaction was noted. During a previous study using MF-59, one volunteer in the 25 µg group developed a rash and pruritus on his forearms after engaging in vigorous daily exercise. The rash was evaluated by a dermatologist and diagnosed as mild folliculitis. The rash was judged by the study investigator to be unrelated to the study vaccine. The investigation of the rashes in the study presented here was unable to determine the cause.

The presence of a rash illness in a placebo recipient raises the possibility of an unrelated event producing what appeared to be an allergic type reaction or the circulation of an unknown infectious agent in the Houston community but this seems unlikely given the marked injection site component of the rash in the two vaccines. It should also be noted that the two male subjects who received VLPs and adjuvant and subsequently developed the unusual cutaneous manifestations were laboratory technicians at the Baylor College of Medicine. Both were also born in Thailand and lived there until their early teens. It is not clear, what if any, genetic or previous exposures unique to Thailand would have increased their propensity to these reactions. Both subjects were also involved in multiple previous vaccine clinical trials. Further, the subject who received 25 µg VLP with MF-59 worked in a laboratory that routinely handled baculovirus and insect cells. This suggests that the rash illness that followed vaccination could be unique to people with unusual but yet unidentified previous exposures. However, it must be pointed out that many recruits at Baylor worked in laboratories at the center

If the two events reported here were related to the parvovirus B19 vaccine then one intriguing, possibility is that the Phospholipase A2 (PLA₂) activity of the VP1-unique region of the VP1 protein of parvovirus B19 [16] is responsible. PLA₂ can modulate the release of arachidonic acid and the precursor of eicosanoids of potent inflammatory mediators [17]. Insect and reptile venoms also contain PLA₂ which is a major allergen [18, 19] although they act to induce immediate hypersensitivity unlike what was seen in this trial. It may be possible to remove this enzyme activity from the VP1 protein of the vaccine. It is also possible that trace amounts of baculovirus or insect cell proteins that were below the limit of detection of the assays used may have been the significant immunogen triggering the reactions noted.

It should also be note that upon conclusion of the voluntary hold and review by the Safety Monitoring Committee, the final recommendation by the SMC, was that the study should be restarted as the benefits of the vaccine for the patient groups affected by Parvovirus B19 infection outweighed the risks. Upon resubmission to the FDA, they concurred that the investigators could restart the study. However, by the time FDA authorization for restart was provided, all of the subjects had missed their third vaccination window requiring re-enrollment of a new group of volunteers. Further, both the vaccine VLPs and the adjuvant would have reached the expiration date before new subjects could complete the study.

Conclusion

Parvovirus B19 is a global infection that can cause serious and life threatening complications in susceptible patient groups. Given the possible severe consequences, fatal anemia and the loss of an unborn child, further development of a safe and effective vaccine continues to be important.

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A. Local reactions

B. Systemic Reactions

Figure 1.

Evaluation of local and systemic adverse events within seven days of vaccination

A. Local Reactions

B. Systemic reactions

First bar is first immunization and second bar is second immunization. white = mild (score =1) black = moderate/severe (score =2 or 3 respectively)

Figure 2.

Photos of skin reactions that developed in subjects after receiving immunization with parvovirus B19 vaccine or placebo.

1a) Subject one: injection site/local reaction 5 days after the first immunization with 25 μ g of parvovirus B19 vaccine with MF59 1b) hyper pigmented area that developed. 2a) Subject two: rash on thigh 7 days after second placebo immunization 2b) rash on arm at same time 3a) Subject three: injection site/local reaction 9 days after the second immunization with 2.5 μ g of parvovirus B19 vaccine with MF59 3b) systemic component of rash.

Table 1

Demographics of study participants

	AIIN=43	2.5 µg+MF59 N=12	25 µg N=12	25 µg+MF59 N=14	Placebo N=5
Age					
Mean (STD)	31.8 (7.9)	32.4 (9.2)	32.5 (8.5)	29.8 (7.0)	34.3 (6.3)
Median	32.3	31.4	31.4	28.5	36.9
Range	19-45	20-45	19-43	19-42	23-38
Gender					
Male	18	6	4	8	
Female	25	6	8	6	5
Race					
White	24	7	4	9	4
African American	8	2	4	2	0
Other	11	3	4	3	1
Ethnicity					
Hispanic	7	2	2	2	1
Non-Hispanic	36	10	10	12	4

Table 2

Immune response following two doses of vaccine or placebo

ELISA									
Vaccine Group	N	N Positive	% Positive	% Positive (95% CI)		Mean Absorbance (O.D.) [†]	Mean (95% CI)		
				Lower	Upper		Lower	Upper	
2.5 µg dose group with MF59	9	9	100.0%	66.4%	100.0%	0.79	0.59	0.99	
25 µg dose group without MF59	7	6	85.7%	42.1%	99.6%	0.66	0.30	1.02	
25 µg dose group with MF59	8	7	87.5%	47.4%	99.7%	0.75	0.52	0.98	
Placebo (Saline)	3	0	0.0%	0.0%	70.8%	0.11	0.00	0.23	

Neutralization									
Vaccine Group	N	N Positive	% Positive	% Positive (95% CI)		GMT	GMT (95% CI)		
				Lower	Upper		Lower	Upper	
2.5 µg dose group with MF59	9	1	11.1%	0.3%	48.3%	5.40	4.52	6.45	
25 µg dose group without MF59	7	3	42.9%	9.9%	81.6%	6.73	4.78	9.48	
25 µg dose group with MF59	8	3	37.5%	8.5%	75.5%	8.65	3.63	20.59	
Placebo (Saline)	3	0	0.0%	0.0%	70.8%	5.00	--	--	