Vaccine 27 (2009) 6862–6868

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Safety and immunogenicity of GMZ2 — a MSP3–GLURP fusion protein malaria vaccine candidate

Meral Esen[a], Peter G. Kremsner[a], Regina Schleucher[a], Michael Gässler[a], Egeruan Babatunde Imoukhuede[c], Nathalie Imbault[c], Odile Leroy[c], Søren Jepsen[e], Birgitte Walther Knudsen[e], Michael Schumm[b], Jürgen Knobloch[a], Michael Theisen[d], Benjamin Mordmüller[a,*]

[a] University of Tübingen, Institute of Tropical Medicine, Wilhelmstraße 27, D-72074 Tübingen, Germany
[b] University of Tübingen, Children's University Hospital, Hoppe-Seyler-Straße 1, D-72076 Tübingen, Germany
[c] European Malaria Vaccine Initiative (EMVI), Statens Serum Institut, 5, Artillerivej, 20/317, DK-2300 Copenhagen S, Denmark
[d] Department of Clinical Biochemistry and Immunology, Statens Serum Institut and Centre for Medical Parasitology at the Department of International Health, Immunology and Microbiology, University of Copenhagen, 5, Artillerivej, 20/317, DK-2300 Copenhagen S, Denmark
[e] Statens Serum Institut, 5, Artillerivej, 20/317, DK-2300 Copenhagen S, Denmark

ARTICLE INFO

Article history:
Received 23 June 2009
Received in revised form 15 August 2009
Accepted 1 September 2009
Available online 13 September 2009

Keywords:
Malaria vaccine candidate
Phase I clinical trial
B-cell ELISPOT

ABSTRACT

Malaria is a major public health problem in Sub-Saharan Africa. In highly endemic regions infants, children and pregnant women are mostly affected. An effective malaria vaccine would complement existing malaria control strategies because it can be integrated in existing immunization programs easily. Here we present the results of the first phase Ia clinical trial of GMZ2 adjuvanted in aluminium hydroxide. GMZ2 is a malaria vaccine candidate, designed upon the rationale to induce immune responses against asexual blood stages of *Plasmodium falciparum* similar to those encountered in semi-immune individuals. Ten, 30 and 100 μg of GMZ2 were well tolerated in 30 healthy malaria-naïve German volunteers when given three times in monthly intervals. Antigen-specific antibodies as well as memory B-cells were induced and detectable throughout the one year follow-up of the study. We conclude that GMZ2 is a safe and immunogenic malaria vaccine candidate suitable for further clinical development.

1. Introduction

Malaria as resulting from *Plasmodium falciparum* infection is one of the most important causes of death and morbidity in children aged one to five years and pregnant women in Sub-Saharan Africa. Resistance against chemotherapeutics and insecticides and the poor economic situation of most affected populations requires the amelioration of existing, and the invention of new strategies to control malaria through international efforts. Over the last 90 years at the least, development of an effective, safe and protective vaccine against *P. falciparum* has been one of the major goals of malaria research [1]. Besides the scientific challenge, a main motivation is the possible integration of a potential malaria vaccine into present health interventions such as the expanded program on immunization. This approach would be an extremely cost-effective tool for the control of malaria.

Presently, two main lines of clinical malaria vaccine research dominate: (i) induction of immunity against pre-erythrocytic antigens, a strategy rooted in first experiments with UV-inactivated *P. gallinaceum* sporozoites [2], and (ii) identification of antigens that induce antibodies with specificities similar to immunoglobulin preparations of semi-immune adults with a therapeutic effect in malaria patients [3,4]. Both strategies rely on the identification of plasmodial proteins that induce a protective immune response. GMZ2 belongs to the second class of malaria vaccine candidates. It is a fusion protein of parts of *P. falciparum* glutamate-rich protein (GLURP) and merozoite surface protein 3 (MSP3), two antigens that induce antibodies with activities similar to immunoglobulin preparations from semi-immune individuals. $GLURP_{27–500}$ represents the R0 non-repeat region which is a major B-cell epitope [5] and is expressed in pre-erythrocytic and erythrocytic stages of parasite development. $MSP3_{212–380}$ is a conserved part of the otherwise highly polymorphic MSP3 [6,7]. It was one of the first vaccine candidates identified by analysis of "therapeutic" immunoglobulin preparations, when sera of semi-immune and first-time *P. falciparum* infected individuals were compared in their reactivity in Western blots of crude parasite lysates and antibody (Ab) dependent cellular inhibition (ADCI) of parasite growth in the presence of monocytes [8]. The dominant ADCI effect exerted by cytophilic anti-MSP3 Abs and the association of ADCI with

* Corresponding author. Tel.: +49 7071 2982187; fax: +49 7071 295189.
E-mail address: benjamin.mordmueller@uni-tuebingen.de (B. Mordmüller).

0264-410X/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2009.09.011

clinical protection in semi-immunes was the rational to select MSP3 as a vaccine-candidate. Besides their activity in semi-immune sera, immuno-epidemiological studies have shown an inverse association of high Ab titres against both antigens (Ag) with clinical malaria [9,10]. To date, it is not clear how Abs against GLURP and MSP3 might control parasite growth. Currently, the main hypothesis centres on the interaction between Ab, parasites and monocytes, since these three partners show an *in vitro* growth-inhibitory effect on *P. falciparum* which has been used as a surrogate marker for *in vivo* parasite growth [11–15]. Recombinant GMZ2 is produced as a secreted protein in *Lactococcus lactis*, a gram positive organism with rather low immune-stimulatory potential [16]. First studies of GMZ2 in splenectomized *Saimiri sciureus* showed good immunogenicity, safety, and partial protection against *P. falciparum* blood stage challenge [17]. These results led us to pursue the development of GMZ2 as a malaria vaccine candidate and progress towards clinical development.

Here we report the results of the first-in-man phase I clinical trial (EudraCT Number: 2005-004568-22, and ClinicalTrials.gov Identifier: NCT00397449) of GMZ2 adjuvanted in aluminium hydroxide administered in ascending doses, in thirty healthy, malaria-naïve adults from Germany.

2. Material and methods

2.1. Study subjects and study design

The study was performed from October 2006 until December 2007 at the Institute of Tropical Medicine of the University of Tübingen, Germany and received approval from the institutional Ethics committee (ethics committee of the medical faculty and university clinic). The study was a randomised, open-labelled, dose-escalating phase Ia clinical trial. Participants were healthy, non-pregnant, malaria-naïve, adult Europeans, negative for anti-GMZ2, -GLURP and -MSP3 antibodies. Enrolled participants were randomly assigned to one of three doses of GMZ2 (group I: 10 μg, group II: 30 μg, or group III: 100 μg), adjuvanted with aluminium hydroxide (alum). Vaccine preparation was extemporaneous and all doses were given subcutaneously. Vaccination of groups I, II, and III was done dose-staggered, with a 15 days lag until the next group's injection to ensure participant's safety and to be able to stop the trial within 14 days if unforeseen and severe reactions at one dose-level occur. Participants were observed for 30 min after each vaccination. Follow-up visits were done after 24 h and two weeks after each vaccination. In between face-to-face visits diaries were kept and regular telephone calls were made to the participants. In addition, four weeks after the last vaccination and one year after the first vaccination participants were re-examined and blood samples were taken to evaluate safety and immune responses at its peak as well as the decay phase, respectively. The clinical trial was conducted in accordance with the latest version of the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines.

2.2. GMZ2 vaccine

Recombinant GMZ2 was expressed in *L. lactis* and purified from culture supernatants following good manufacturing practice (GMP) to obtain one batch (G026/LCI/040301) for use in human subjects within this trial. Lyophilized GMZ2 (Henogen S.A., Belgium) was reconstituted into water and mixed with aluminium hydroxide (Batch 528601; Statens Serum Institut, Denmark) at a GMP facility in Tübingen before subcutaneous injection.

2.3. Objectives of the clinical trial

The primary objective was the evaluation of the safety of three doses of three different dosage of GMZ2 adjuvanted with alum and was done on intention to treat (ITT) population. The secondary objectives were (i) the assessment of humoral immune response to the vaccine before and one month after each vaccination as well as one year after the first vaccination, (ii) the assessment of the ability of antibodies to recognize the native antigen by indirect fluorescence assay (IFA), (iii) the assessment of antigen-specific memory B-cells. All analyses up to Day 84 were done on the ITT population. Exploratory analyses comprised further immunological tests and will be presented in a separate publication.

2.4. Evaluation of clinical and biological safety

Adverse events (AEs) were recorded one month after each vaccination and the serious adverse events (SAEs) throughout the study period. Participants were physically examined by the study physician on each day of vaccination and 24 h as well as 7 days thereafter. On Day 7 post-vaccination a telephone call was made to all participants. Between scheduled visits and calls a 24 hourly operated telephone line was maintained to ease reporting of adverse events. Solicited local AEs including pain, erythema, swelling, induration, pruritus, oedema and local heat occurrence were evaluated immediately (30 min) and within 14 days after each vaccination. Solicited systemic AEs such as fatigue, fever, headache, malaise, myalgia, joint pain, gastrointestinal symptoms (nausea, diarrhoea, vomiting, abdominal pain), generalized skin reactions, and contra-lateral local reaction were listed immediately (30 min) and within 14 days after each vaccination. Laboratory parameters included a full blood cell count and biochemical parameters (potassium, sodium, ASAT, ALAT, bilirubin, alkaline phosphatise, gamma GT, creatinine, glucose). Laboratory parameters were assessed before, one month after each vaccination and one year after the first vaccination. Physical examinations were performed at each visit to the study site.

2.5. Assessment of anti-GMZ2, -GLURP and -MSP3 antibodies

Determination of specific anti-GMZ2, -GLURP and -MSP3 immunoglobulin G (IgG) concentrations was done on Days 0, 28, 56, 84, and 365 by enzyme-linked immunosorbent assay (ELISA) as previously described [18]. In brief, ELISA microtitre plates (NUNC Maxisorp™, Germany) were coated with either 0.5 μg GMZ2, 0.5 μg GLURP, or 1 μg MSP3 per ml, diluted in phosphate buffered saline (PBS) overnight at +4 °C. Following four washes in PBS, 0.1% Tween 20, 0.5 M NaCl, plates were blocked in PBS, 3% non-fat milk powder, 0.1% Tween 20 for 1 h. Serum samples were diluted 1:100 in PBS, 1% non-fat milk, 0.1% Tween 20, 0.02% NaN_3. Further serial dilutions were done in the same dilution buffer without NaN_3. Ag-specific Abs were detected by a peroxidase conjugated goat anti-human IgG Ab (Caltag, USA) diluted 1:3000 in dilution buffer. As peroxidase substrate we used TMB ONE (KEM EN TEC, Belgium). Absorbance was read after addition of 0.2 M H_2SO_4 (32%, Merck, Germany) at 450 nm (reference 620 nm) on a plate reader (Asys Expert 96, Type G018065). Pooled sera from semi-immune individuals were used as positive control and sera from malaria-naïve Europeans with no cross-reaction to all three antigens served as negative controls. A reference standard curve was measured with a serially diluted, purified human polyclonal IgG (The Binding Site, UK) in PBS. Specific Ab concentrations were calculated from the standard curve and are given in μg per ml.