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Local tolerance and systemic toxicity of single and repeated intramuscular administrations of two different formulations of the RTS,S malaria candidate vaccine in rabbits

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ABSTRACT

RTS,S malaria antigen is weakly immunogenic as such and needs to be formulated with an adjuvant to improve the magnitude and duration of the immune responses to RTS,S. Two Adjuvant Systems, AS01 and AS02 were evaluated during the development of the RTS,S vaccine. The evaluation included non-clinical studies in rabbits to evaluate the local intramuscular tolerance following administration on a single occasion, and the local and systemic effects following repeated administrations of RTS,S/AS01 or RTS,S/AS02 formulations. In the first study, rabbits were injected on one occasion with RTS,S/AS01, RTS,S/AS02 or controls, and the local intramuscular tolerance was evaluated up to 3 days after injection. In the second study, the different formulations were injected on Days 0, 14, 28 and 42. General health status, haematology and blood chemistry parameters were monitored on a regular basis. Macroscopic and microscopic evaluations were made after termination of the study.

No sign of toxicity was detected following single or repeated administrations of the adjuvanted RTS,S formulations. Changes in haematology or clinical chemistry parameters were indicative of a developing immune response in the groups receiving either RTS,S formulation. All examined parameters returned to normal within 28 days after the last injection.

The absence of toxicological effects following the injection of RTS,S/AS01 or RTS,S/AS02 in rabbits was supportive of further clinical evaluation of these two formulations.

1. Introduction

Vaccination against *Plasmodium falciparum* represents an important component in the multipronged approach to malaria disease control, particularly for <5-year-old children in endemic regions of Sub-Saharan Africa (Roll Back Malaria, 2008). The RTS,S malaria candidate vaccine is currently the most advanced in development globally, being evaluated in a large phase III clinical trial program (Leach et al., 2011; The RTS,S Clinical Trials Partnership, 2012). This vaccine targets the circumsporozoite (CS) protein of *P. falciparum* and is based on a recombinant fusion protein (RTS) comprising the CS central tandem repeats and the C-terminal regions of CS protein fused to the N-terminal region of the hepatitis B virus surface antigen (HBs). Co-expression of this fusion protein with native HBs results in the spontaneous formation of RTS,S virus-like particles (for review, see (Cohen et al., 2010)). This vaccine is expected to trigger the immune system to combat *P. falciparum* during its pre-erythrocytic phase.

RTS,S antigen alone is weakly immunogenic and needs to be formulated with an adjuvant to improve the magnitude and duration of vaccine-induced specific immunity. The first clinical trials identified AS02 as the most suitable Adjuvant System to be used with RTS,S (Garçon et al., 2003). AS02 contains two immunostimulants, 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL) and QS-21, in an oil-in-water emulsion. MPL is a detoxified bacterial lipopolysaccharide known to act as a Toll-like receptor-4 agonist, and QS-21 is a natural saponin purified from the bark of the South-American tree *Quillaja saponaria*. The RTS,S/AS02 formulation has proven to be immunogenic and provided protection against experimental challenge in malaria-naïve adults (Stoute et al., 1997; Lalvani et al., 1999; Kester et al., 2007, 2008), as well as partial protection in

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adults (Doherty et al., 1999; Stoute et al., 2006; Bojang et al., 2001, 2009), children (Aide et al., 2011; Alonso et al., 2004, 2005; Bojang et al., 2005; Guinovart et al., 2009; Macete et al., 2007a,b; Sacarlal et al., 2009) and infants (Aide et al., 2011; Abdulla et al., 2013; Aponte et al., 2007) in malaria endemic areas, associated with an acceptable safety profile. In parallel, another formulation was developed, RTS,S/AS01, with the aim to improve the cellular arm of the specific immunity, hence vaccine efficacy. AS01 contains the same immunostimulants as AS02, MPL and QS-21, but is a liposome-based formulation. Non-human primates and mice studies suggested that RTS,S/AS01 is more immunogenic than RTS,S/AS02 (Mettens et al., 2008; Stewart et al., 2006a,b), which was confirmed in healthy malaria-naïve adults (Kester et al., 2009). Since then, several clinical trials (Lell et al., 2009; Owusu-Agyei et al., 2009; Polhemus et al., 2009; Ansong et al., 2011) have confirmed these findings, and AS01 was selected for use with the RTS,S antigen in the final RTS,S/AS01 formulation currently in late phase development (Leach et al., 2011; The RTS,S Clinical Trials Partnership, 2012).

Nonclinical safety evaluation to detect toxic or adverse events that may possibly occur following injection of vaccines and adjuvants is an essential aspect of vaccine development. Inflammatory reactions at the injection site and systemic effects are some of the potential safety concerns investigated in toxicity studies (European Medicines Agency, 1997, 2005; World Health Organisation, 2005). The rabbit is often used for vaccine or adjuvant toxicity studies, as a full human dose volume can be injected intramuscularly in this animal at a single site (Diehl et al., 2001), giving a representative view of the vaccine reactogenicity.

In this work, we have investigated the local tolerance following two intramuscular 0.5 ml injections on a single occasion, as well as the local and systemic effects following four repeated intramuscular injections of 0.125 or 0.5 ml/occasion of RTS,S/AS01 or RTS,S/AS02 formulations in rabbits.

2. Materials & methods

2.1. Animals

Specific pathogen-free New Zealand white rabbits of both genders were obtained from Harlan (Bicester, UK). They were between 11 and 19 weeks of age and in the weight range of 2130–3315 g at Day 0. All rabbits were acclimatised to the experimental environment for a period of 12 days (study 1) or 32–33 days (study 2) prior to the start of the study and housed singly in stainless steel cages with perforated floors. A pelleted rabbit diet (STANRAB (P) SQC; Special Diet Services, Witham, UK) and drinking water were available without restriction, and small white untreated wood blocks were given for environmental enrichment. The animals also received autoclaved hay three times (study 1) or once (study 2) a week. Target values within the study rooms were 15–23 °C for temperature, 30–70% (study 1) or 49–81% (study 2) for relative humidity and at least 15 air changes per hour. Lighting was set on a 12-h light-12 h dark cycle.

The studies were conducted in accordance with the UK good laboratory practice regulations 1999 (Statutory Instrument 3106), the OECD principles of good laboratory practice (as revised in 1997), ENV/MC/CHEM (98)17, the CPMP guidelines for vaccines (CPMP/SWP/465/95 preclinical and toxicological testing of vaccines, December 1997), and the EC commission directive 1999/11/EC (Official Journal No L 77/8). The welfare of the animals was maintained in accordance with the general principles governing the use of animals in experiments of the European Communities (Directive 86/609/EEC). The studies were carried out at the testing facilities of Huntingdon Life Sciences Ltd. (Huntingdon, UK).

2.2. Test substances

AS02 is an Adjuvant System containing MPL, QS-21 (*Q. saponaria* Molina, fraction 21; Antigenics Inc., a wholly owned subsidiary of Agenus Inc., Lexington, MA, USA) in an oil-in-water emulsion (50 μg MPL and 50 μg QS-21 per 500 μl). AS01 is an Adjuvant System containing MPL, QS-21 and liposome (50 μg MPL and 50 μg QS-21 per 500 μl). AS01 was available in monodose vials, whereas AS02 was provided as monodose syringes. RTS,S was provided in monodose vials containing 50 μg of the antigen as a freeze-dried pellet. Adjuvants and antigen were GMP clinical batches.

Just prior to dosing, the whole content of a monodose of AS01 or AS02 was added to one vial of RTS,S antigen followed by gentle shaking until complete dissolution of the pellet. The reconstituted vaccine was then collected in a new syringe to perform the injection.

Control saline (0.9% sodium chloride solution) was supplied either by the Huntingdon Life Sciences Formulation department, Fresenius Kabi Ltd. (Warrington, UK) or Baxter Healthcare Ltd. (Thetford, UK).

2.3. Study design

2.3.1. Study 1. Local tolerance following intramuscular injections on a single occasion

This study was intended to assess the local tolerance following intramuscular injections of RTS,S/AS01, AS01, RTS,S/AS02, AS02 or saline on a single occasion on Day 0. Three groups of rabbits received either the test substances or physiological saline into the paravertebral muscle, as described in Table 1.

All animals were observed twice daily for signs of ill health, behavioural changes or toxicity. Careful examination was made of the injection sites and surrounding tissues approximately 1, 3, 24, 48 and 72 h after injection, evaluating erythema and oedema.

All animals were sacrificed on Day 3 by means of an overdose of pentobarbitone sodium (200 mg/ml) into the lateral ear vein and an autopsy was undertaken. The macroscopic appearance of the tissues was recorded and the injection sites and surrounding tissues were preserved in 10% buffered formalin. They were further processed and embedded in paraffin wax before sections were cut and stained in haematoxylin-eosin for microscopic examination.

2.3.2. Study 2. Local and systemic reactions following repeated intramuscular injections

This study aimed to evaluate potential local and/or systemic reactions following four consecutive intramuscular injections of RTS,S/AS01 or RTS,S/AS02 given at two-week intervals, and the reversibility of toxic effects if seen after the fourth injection. To this end, four groups of 10 males and 10 females received clinical doses (0.5 ml) or sub-clinical doses (0.125 ml) of either RTS,S/AS01 or RTS,S/AS02 at each dosing occasion. A control group of 10 males and 10 females received saline (0.5 ml).

Table 1
Description of the groups to assess the local tolerance to RTS,S/AS01 or RTS,S/AS02 malaria candidate vaccines following several intramuscular injections on a single occasion (Day 0).

Group	Paravertebral injections/animal	
	Two sites on left flank (0.5 ml/site)	Two sites on right flank (0.5 ml/site)
1 (3 males + 3 females)	RTS,S/AS01	AS01
2 (3 males + 3 females)	RTS,S/AS02	AS02
3 (2 males + 2 females)	Saline	Saline