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Evaluation of the intramuscular administration of *Cervarix*™ vaccine on fertility, pre- and post-natal development in rats

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ABSTRACT

Cervarix™ is a prophylactic human papillomavirus (HPV)-16/18 vaccine for the prevention of cervical cancer. It contains GSK Biologicals' proprietary Adjuvant System AS04. The objective of this study was to investigate the effects of *Cervarix*™ and of AS04 on female fertility and pre- and post-natal development in Sprague Dawley rats. Female rats were injected with vaccine, AS04, or saline 30 days before mating and on Gestation Days 6, 8, 11 and 15. Each dose of vaccine was one-fifth the human dose volume. Treatment of rats with vaccine or AS04 was not associated with any systemic toxicity and had no impact on female fertility. There were no adverse effects on pre- or post-natal development of litters from treated rats, as judged by fetal evaluation at Gestation Day 20, and growth and survival of pups to postnatal Day 25. These results support the use of the vaccine in the targeted human population.

1. Introduction

Cervical cancer is the second most common type of cancer in women worldwide. Globally, HPV-16 and HPV-18 are the predominant oncogenic HPV types that are responsible for 76% of cervical cancer cases in North America [1] while HPV-31, HPV-33 (HPV-16-related) and HPV-45 (HPV-18-related) account for a further 11% of cervical cancer cases [1].

To protect young girls and women against cervical cancer, GlaxoSmithKline (GSK) Biologicals has developed and marketed a prophylactic vaccine, *Cervarix*™. The efficacy, immunogenicity and safety of this vaccine has been evaluated in several pre-clinical (data not published) and clinical studies [2–4]. An analysis of a large database of approximately 30,000 girls and women aged 10 years and above, followed for up to 5.5 years post-vaccination, shows that *Cervarix*™ has a favourable safety profile similar to that of other vaccines licensed and in general use [2]. The vaccine demonstrated up to 100% protection for up to 6.4 years against cervical intraepithelial neoplasia grade 2 (CIN2) and higher abnormalities related to HPV-16 and HPV-18 [5–7]. Moreover, protection was associated with sustained levels of neutralizing antibodies in more than 98% of the subjects up to 6.4 years following the first vaccination [7]. *Cervarix*™ was also shown to provide protection against CIN2+ lesions that were associated with non-vaccine types HPV-31, HPV-33, and HPV-45 [3].

Cervarix™ contains HPV-16 L1 and HPV-18 L1 proteins, assembled into virus-like particles (VLPs), and adjuvanted with GSK Biologicals proprietary Adjuvant System AS04. The HPV-16 and HPV-18 L1 VLPs result from the self-assembly of purified recombinant HPV-16 and HPV-18 L1 major capsid proteins using a baculovirus expression system [8]. AS04 consists of aluminium hydroxide, one of the most widely used adjuvants in vaccines globally, with over 80 years of experience, and 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL), derivative of the lipopolysaccharide (LPS) isolated from the Gram negative bacterium *Salmonella minnesota* R595 strain through sequential acid and base hydrolyses [9–11]. MPL has been shown to retain the capacity of the natural LPS and lipid A compounds to act as an immunostimulant, but with a much reduced toxicity. MPL was shown to be approximately

Abbreviations: AS, Adjuvant System; CIN, cervical intraepithelial neoplasia; DPBS, Dulbecco's phosphate buffered saline; ELISA, enzyme-linked immunosorbent assay; HPV, human papillomavirus; LPS, lipopolysaccharide; MPL, 3-*O*-desacyl-4′-monophosphoryl lipid A; VLP, virus-like particle.

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0.1% as toxic as LPS when tested in pre-clinical rabbit pyrogenicity assays [12], which is in strikingly good agreement with Ribi's early estimates using lethal chick embryo assays [11].

Cervarix™ was designed to bring an effective vaccine to young girls and women worldwide by taking into consideration the current understanding of natural oncogenic HPV infection, including the ability of the virus to evade the immune system, repeated exposure throughout life, and the lack of reliable protection against re-infection by natural immunity. Also, other factors were taken into account in the development of *Cervarix*™: the prevalence of the most important oncogenic HPV types; the need for induction of high neutralizing antibodies at the site of infection (the cervix); and the need for long-term protection. The feasibility of a parenteral vaccine using L1 VLP to protect against papillomavirus challenge infection was demonstrated in animal models [13–17]. The selection of AS04 was based on its capacity to ensure the induction of a broad and sustained immune response as shown in clinical studies [18]. The induction of the robust adaptive response as observed after vaccination is believed to result from its capacity to lead to a rapid and spatially localized activation of the innate response as recently shown [19]. AS04 is currently a component in two licensed vaccines, *Cervarix*™ [5–7] and *FENDrix*™, a vaccine against hepatitis B virus [20].

Although the vaccine is not intended for administration to pregnant women, it is designed to protect young girls and women worldwide, and consequently the target population includes women of childbearing potential. Therefore, the potential effects of *Cervarix*™ and AS04 on female fertility and pre- and post-natal development was investigated in a pre-clinical model to support the clinical development of the vaccine.

2. Materials and methods

2.1. Animals, husbandry and study design

A total of 240 virgin female Crl:CD® BR Sprague Dawley rats were obtained from Charles River UK.

Animals were housed in a barrier-controlled rodent facility and each animal room had its own supply of 15 room changes per hour of filtered fresh air, which was passed to atmosphere and not re-circulated. The temperature and relative humidity (RH) in the animal room were controlled at 19–23 °C and 40–70% RH, respectively, and lighting was controlled to provide a 12-h light:12-h dark cycle.

Rats were housed up to 4 per cage before pairing and pair housed during mating in grid-bottomed cages. Females were singly housed in solid bottomed cages after mating. Grid-bottomed cages were suspended in batteries over trays covered with absorbent paper which was replaced at least twice weekly, or daily during pairing. Solid bottomed cages used during littering were provided with Lignocel 3/4 wood flakes as bedding which was changed at least twice weekly.

Tap water from the public supply was freely available and the rats were allowed free access to a commercially available pelleted laboratory animal diet (UAR VRF1 Certified), supplied by Charles River UK with a Certificate of Analysis for various nutritional components and chemical and microbiological contaminants.

The study design for non-clinical evaluation of the effects of *Cervarix*™ on pre- and post-natal development in the rat was based upon the European Medicines Agency (EMEA) Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines (CPMP/SWP/465/95) [21], the EMEA Guideline on Adjuvants in Vaccines for Human Use (EMEA/CHMP/VEG/134716/2004) [22], the US Food and Drug Administration (FDA) Considerations for Developmental Toxicity Studies for Preventive and Therapeutic Vaccines for Infectious Disease Indications [23] as well as on previous developmental toxicity studies with other vaccines conducted by GSK Biologicals. When this study was conducted in 2003, the FDA guidance document was still in draft form. Consequently, the study outline was submitted to the Center for Biologics Evaluation and Research (CBER)/FDA for review and discussion prior to study start. The rat was chosen for this study since it is suggested as a test species in EMA, FDA and WHO guidelines and is the most widely used species for pre- and post-natal developmental toxicity assessments of pharmaceuticals, chemicals and consumer products, with a large amount of historical control information available. A strong and sustained immunogenic response was observed following intramuscular injection of the *Cervarix*™ vaccine in the rat. Furthermore, the design of the current study included both pre- and post-natal assessments of fetuses and pups. Both the rat and rabbit have been frequently used for the teratology studies. However, the rabbit has been rarely used for post-natal evaluations, and presents some technical difficulties as compared with the rat [24].

Table 1
Description of the different study groups.

Group	Treatment 30 days before pairing (100 μl)	Treatment 6, 8, 11 and 15 days after mating (100 μl)	Number of females[a]
1	Saline	Saline	56
2	Saline	*Cervarix*™	56
3	*Cervarix*™	*Cervarix*™	56
4	AS04	AS04	56

[a] 56 animals were allocated to each group with a view to obtaining 44 females with positive evidence of mating in each group (and within a compact time frame), for treatment during gestation.

The study protocol was reviewed and approved by an ethical committee and was conducted in accordance with GSK policies regarding the care and ethical treatment of animals.

2.2. Allocation to treatment groups

During the 6-day acclimatisation period, the animals were weighed and ranked by bodyweight (5 g ranges). Grossly atypical animals were excluded and animals were selected from each bodyweight range in turn to each group to ensure that all groups contained populations of rats with similar initial mean and range of bodyweights. Each animal was assigned a number and identified by tail tattoo. Animals were allocated to the treatment groups described in Table 1.

2.3. Test material

A consignment of 160 vials, each containing 0.5 ml of *Cervarix*™, and 100 vials each containing 0.5 ml of AS04 was received for use in this study. The identity, strength, purity and composition, which defined the batch of test material for this study, were determined by GSK Biologicals.

Each vaccine vial contained 20 μg of HPV-16 L1 VLP, 20 μg of HPV-18 L1 VLP, 50 μg of MPL and 500 μg of aluminium hydroxide salt for a total volume of 500 μl. Each AS04 vial contained 50 μg of MPL and 500 μg of aluminium hydroxide salt in a total volume of 500 μl. Saline (sterile, non-pyrogenic, Baxter Health Care Ltd., UK) was also used in the study. The test materials were stored in a refrigerator (approximately 4 °C), protected from light.

2.4. Dose administration

Prior to starting this study, a preliminary investigation was undertaken to determine the optimal dose and to ensure the strong and sustained vaccine take in pregnant rats administered *Cervarix*™ in accordance with the dosing schedule planned for the reproductive toxicity investigation.

The vaccine and AS04 adjuvant were used as supplied. The animals received the vaccine, AS04 alone, or saline, by bolus intramuscular administration at a volume of 100 μl per rat (one-fifth the human dose volume). Animals were dosed into the anterior thigh (quadriceps muscle), with alternate hind limbs used for subsequent injections and a new sterile disposable needle for each injection. Injections were performed 30 days before pairing and on Gestation Days 6, 8, 11 and 15.

2.5. Procedures with F0 females

All animals were inspected at least twice daily for evidence of reaction to treatment or ill health and a more detailed physical examination was performed each week. Individual observations were recorded before and after each dose administration. Records of the appearance of the injection sites were made daily from Day −30 prior to pairing to Day 25 of lactation.

The animals were weighed on the first day of treatment (Day −30) before dosing and at weekly intervals until pairing with males. Thereafter they were weighed on Days 0, 3, 6, 8, 11, 15, 17 and Day 20 of gestation and on Days 1, 4, 7, 11, 14, 18, 21 and 25 of lactation. Food consumption for all animals was recorded weekly on a cage basis prior to pairing and then for the following periods: Days 0–2, 3–5, 6–7, 8–10, 11–14, 15–16 and 17–19 after mating. For animals allowed to litter, food consumption was also recorded for the following periods: Days 1–3, 4–6, 7–10, 11–13, 14–17, 18–20 and 21–24 of lactation.

Blood samples (0.8 ml) were taken from the retro-orbital sinus of each animal under isoflurane anaesthesia on Days −35 and −5 before pairing. All samples were collected without anticoagulant and the serum was separated after centrifugation at approximately 3000 rpm for 5 min. Serum samples were frozen (approximately −20 °C) until the antibody analysis was performed.

Thirty days after the first injection, females were paired on a one-to-one basis with stock males of the same strain. The trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for the presence of spermatozoa until mating