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BRIEF REPORT

A phase III, open-label, single-arm, study to evaluate the safety and immunogenicity of a trivalent, surface antigen inactivated subunit influenza virus vaccine produced in mammalian cell culture (Optaflu®) in healthy adults

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Abstract

Vaccination is an essential tool in reducing the impact of seasonal influenza infections. The viral strains responsible for seasonal outbreaks vary annually, and preventive vaccines have to be adapted accordingly. The aim of this study was to evaluate the safety, clinical tolerability and the antibody response to each of the three influenza vaccine antigens after vaccination with a cell-derived, trivalent, surface antigen, inactivated influenza vaccine (TIVc), as measured by single radial haemolysis (SRH) or haemagglutination inhibition (HI) assay in accordance with European Union licensing guidelines in place for years 2013/2014. This phase 3, open-label, single-arm study enrolled 126 healthy adults divided into two age groups (63 subjects aged 18 to ≤ 60 years and 63 subjects aged ≥ 61 years). Antibody titres were measured before and 21 days after vaccination. Adverse events were determined using diary cards, interviews and reviews of the available medical records. One subject was lost to follow-up and three subjects had protocol deviations. Following vaccination, protective HI antibody titres (≥ 1:40) were detected in 100%, 97%, and 94% of the younger adults (18–≤ 60 years) and in 97%, 95%, and 80% of the older adults (≥ 61 years) against the A (H1N1), A (H3N2), and B influenza strains respectively. The antibody response licensing criteria were met in both age groups. Solicited adverse events were reported by 57% subjects 18 to ≤ 60 years and 35% subjects ≥ 61 years. Among the younger adults 51% had local and 27% had systemic adverse events, whereas of the older subjects 29% had local and 13% had systemic adverse events (mainly injection site pain or headache in both age groups). Unsolicited adverse events at least possibly related to the vaccine were mild and detected in 3% of the younger adults and none of the older adults. Overall, the trivalent, surface antigen, inactivated subunit influenza virus vaccine produced in mammalian cell culture proved to be safe and immunogenic in younger and older healthy adults.

Keywords Seasonal influenza vaccine · Cell derived · Immunogenicity · Safety

Introduction

The impact of seasonal influenza can vary from year to year depending on virus activity and vaccine protection rates [1]. Due to changes in evolving influenza strains, the host immune response has to be adapted annually by means of revaccination. The WHO influenza committee decides on the antigenic composition of seasonal influenza vaccines approximately 6 months ahead of the following influenza season on the basis of surveillance data [2]. For the Northern hemisphere 2013/2014 influenza season, the recommendations for the two influenza A strains [A/California/7/2009 (H1N1)pdm09-like strain and A/Victoria/361/2011 (H3N2)-like strain] were unchanged from the previous (2012/2013)

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season. The recommendation for the influenza B strain changed from the B/Wisconsin/1/2010-like strain to the B/Massachusetts/2/2012-like strain [3].

Production of influenza vaccines is mainly based on embryonated chickens' eggs to cultivate prevalent influenza virus strains; alternative virus cultivation methods are based on mammalian cell lines [4]. The safety of Madin-Darby canine kidney (MDCK) cell culture-based influenza vaccines has been established in in clinical trials, where levels of immunogenicity and safety were found to be comparable to those of vaccines derived from embryonated chicken eggs [5–7].

The European Medicines Agency through the Committee for Medicinal Products for Human Use (CHMP) guidelines, in place for 2013/14, demanded a clinical trial to evaluate safety, clinical tolerability and immunogenicity before the licensing of the annual influenza vaccine [8]. The aim of this study was to evaluate the safety and immunogenicity of the cell-derived seasonal trivalent, surface antigen, inactivated influenza vaccine in healthy younger and older adults.

Methods

Ethical approval

The study was approved by the ethics committees at the participating study sites (FK-2013-0002) and by the local and national medicines agencies. Prior to participation, all participants provided written informed consent. The study was conducted according to current principles of Good Clinical Practice. The trial was registered at Eudra CT (2013-000621-30) and ClinicalTrials.gov (NCT01880697).

Participants and study procedures

This phase III study was originally planned as a multicentre study; however, it was actually possible to recruit all the subjects at a single study centre. Individuals were enrolled after informed consent had been obtained, a review of their medical history and a general physical examination, and their eligibility determined on the basis of study entry criteria (Supplement 1). Participants were required to visit the study site twice, on day 0 and day 22 (− 1/+ 3) and received two reminder calls within this period. The main study procedures were unchanged from those described in an earlier seasonal influenza vaccine study [9].

The primary objectives of this study were (a) to evaluate the antibody response to each influenza vaccine antigen 21 days after vaccination with the TIVc vaccine, as measured by single radial haemolysis (SRH) or haemagglutination inhibition (HI) assay and (b) to evaluate the safety of TIVc in adult subjects in compliance with the requirements of the current EU recommendations for clinical trials related to yearly licensing of influenza vaccines separately in two age cohorts (18 to ≤ 60 years and ≥ 61 years).

After blood sampling on day 1, all subjects received the study vaccine as a single intramuscular (IM) dose in the deltoid muscle of the non-dominant arm in an open-label manner.

The vaccine consisted of inactivated influenza virus hemagglutinin and neuraminidase surface antigens propagated in MDCK cells. To comply with WHO recommendations and EU decisions for the 2013/2014 season, ≥ 15 µg of hemagglutinin antigen of each of the following was included in 0.5 ml of study vaccine:

- A/California/7/2009 (H1N1)pdm09-like strain used: A/Brisbane/10/2010 wild type
- A/Victoria/361/2011 (H3N2)-like strain used: (NYMC X-223A) derived from A/Texas/50/2012
- B/Massachusetts/2/2012-like strain used: B/Massachusetts/2/2012 wild type.

The excipients of the prefilled syringe were: sodium chloride, potassium chloride, potassium dihydrogen phosphate, disodium phosphate dihydrate, magnesium chloride hexahydrate and water for injection.

Subjects documented all local and systemic adverse events, body temperature and concomitant medication using a diary card. Antibody responses were measured by single radial haemolysis assay (SRH) and haemagglutination inhibition assay (HI) before vaccination on day 1, and again at the end of the study. Antibody responses were evaluated according to the Committee for Medicinal Products for Human Use (CHMP) guidelines [8].

Statistical analysis

The safety and immunogenicity were analysed in two age groups, 18 to ≤ 60 years and ≥ 61 years. CHMP guidelines on the harmonization of requirements for influenza vaccines [8] recommend that groups be comprised of at least 50 individuals. On the assumption that 20% of subjects would be non-evaluable, a total of 63 subjects were included in each age group. The study populations were analysed in different groups: (a) all enrolled subjects; (b) exposed subjects (subjects who received a study vaccination); (c) an immunogenicity full analysis set (FAS), i.e. subjects who received a study vaccination and provided immunogenicity data both, before and after vaccination; (d) a per protocol set (PPS), i.e. all the subjects in the immunogenicity FAS who correctly received the vaccine, provided evaluable serum samples before and after vaccination and committed no major protocol violation; (e) a safety set, i.e. all exposed subjects who provided post-vaccination safety data.

All statistical analyses were performed using SAS® version 9.1 or higher (SAS Institute, Cary, NC). Where appropriate, the two-sided Fisher's exact test was used at a 95% confidence interval (CI) [this was based on a type 1 error probability (a) of 5%] to compare the number of subjects with local or systemic reactions after vaccination and to compare subject characteristics between the two age groups. All reported *p* values are two-sided; values of 0.05 or less were considered to indicate statistical significance.

Results

A total of 126 subjects were included in this study, 63 subjects aged 18 to ≤60 years and 63 aged ≥61 years. All but one subject completed the study according to protocol; one subject was lost to follow-up due to scheduled surgery on a pre-existing abdominal hernia. One subject ≤60 years of age and two subjects ≥61 years of age had protocol deviations and were, therefore, excluded from the PPS. The baseline characteristics are shown in Table 1.

In the SRH assay, 66% of the younger subjects had sera that generated an SRH area ≥25 mm^2, against A (H1N1), 44% against A (H3N2) and 68% against the B strain prior to vaccination in the per protocol set (PPS), whereas 41%, 39% and 77% of the older subjects had such a level against the three strains. On day 22 after vaccination, the percentage of subjects who either seroconverted (subjects with increase from SRH area ≤4 mm^2 prevaccination to SRH area ≥25 mm^2 postvaccination) or had a significant increase in antibody titre (at least a 50% increase in area from positive prevaccination serum) was 68%, 65% and 58% in the younger age group and 56%, 46% and 39% in the older age group for the three antigens in the per protocol analysis.

The HI assay detected GMT titres ≥1:40 in 68%, 89% and 58% of the younger subjects against the three strains prior to vaccination. In subjects ≥61 years, 66%, 87% and 46% had GMT titres ≥1:40 against the three strains prior to vaccination (Table 2 and Supplement 2). On day 22 after vaccination, the percentage of subjects who either seroconverted (from baseline titres <1:10 to ≥1:40 post-vaccination) or had a significant increase in HI antibody titre (≥1:10 at baseline and at least a fourfold increase post-vaccination) was 63%, 47% and 48% in the younger age group and 43%, 26% and 28% in the older age group for the three antigens in the per protocol analysis. However, 100%, 97% and 94% of the younger subjects had GMT titres ≥1:40 against A (H1N1), A (H3N2) and the B strain in the HI assay on day 22, as did 97%, 95% and 80% of older subjects (PPS).

Overall, in the full analysis set the CHMP requirements [8] were met in both age groups using both antibody assays (Supplement 3). However, in the SRH assay the geometric mean ratio requirement was not met in both the younger and the older age groups for the B strain, and in the HI assay the proportion of subjects achieving seroconversion or a significant increase in HI antibody titre was below 30% in the older age group for both, the A (H3N2) and the B strain.

Among the subjects included in this study, 45/63 (71%) of the younger age group and 59/63 (94%) of the older age group reported a previous seasonal influenza vaccine, and 30/63 (48%) of the younger adults and 50/63 (79%) of the older age group had received their last vaccination for the previous influenza season, meaning that it included the same A (H1N1) and A (H3N2) strains.

Table 1 Characteristics of the enrolled subjects

Group	18 to ≤60 years, *N*=63	≥61 years, *N*=63	Total, *N*=126
Age (years) ± SD	39.3 ± 10.7	68.3 ± 4.8	53.8 ± 16.7
Sex			
Male	25 (40%)	30 (48%)	55 (44%)
Female	38 (60%)	33 (52%)	71 (56%)
Child-bearing potential	26/38 (68%)	0/33	
Ethnic origin			
Caucasian	63 (100%)	63 (100%)	126 (100%)
Weight (kg) ± SD	78.13 ± 15.13	78.68 ± 11.77	78.41 ± 13.50
Height (cm) ± SD	171.6 ± 10	170.4 ± 9.2	171.0 ± 9.6
Body mass index ± SD	26.5 ± 4.2	27.1 ± 3.1	26.8 ± 3.7
Previous seasonal influenza vaccination[a]			
No	18 (29%)	4 (6%)	22 (17%)
Yes	45 (71%)	59 (94%)	104 (83%)
Lost to follow-up	0	1 (2%)	1 (1%)
Protocol deviation	1 (2%)	2 (3%)	3 (2%)

SD standard deviation

[a]Any documented previous seasonal influenza vaccine

Table 2 Vaccine immunogenicity assessed by the HI assay (per protocol analysis)

Strains	18 to ≤60 years			≥61 years		
	A (H1N1)	A (H3N2)	B	A (H1N1)	A (H3N2)	B
Prevaccination (day 1)						
GMT (95% CI)	58 (41–82)	154 (109–217)	41 (30–55)	46 (35–60)	135 (96–191)	23 (18–29)
Postvaccination (day 22)						
GMT (95% CI)	509 (388–688)	541 (369–793)	135 (104–173)	165 (121–223)	299 (204–438)	55 (43–70)
GMR (95% CI)	8.8 (5.66–14)	3.52 (2.4–5.15)	3.31 (2.45–4.47)	3.61 (2.61–5.01)	2.22 (1.63–3.01)	2.4 (1.81–3.17)

Bold: CHMP criteria met

GMT geometric mean titer, *95% CI* 95% confidence interval, *GMR* geometric mean ratio (values > 2.5 for 18 to ≤60 years and > 2.0 for subjects ≥61 years should be met according to CHMP criteria)

In total, 46% of subjects (57% subjects 18 to ≤60 years and 35% subjects ≥61 years) reported solicited adverse events(AEs) from six hours after vaccination through day 4 (Table 3). In the younger age group pain at the injection site was the most common solicited local AE (49%) and headache was the most common solicited systemic AE (17%). In the older age group, pain at the injection site was also the most common local AE (29%), and headache was the most common systemic AE (10%). Unsolicited AEs judged to be possibly or probably related to the study vaccine were detected in 2 (3%) of the younger subjects and none of the older subjects (Table 3). One severe adverse event occurred, but this was a dental infection not related to the study vaccine.

Discussion

A total of 123 vaccinated subjects (62 subjects aged 18 years to ≤60 years and 61 subjects aged ≥61 years) were analysed with respect to SRH and HI response to the influenza vaccine in the per protocol set (PPS). The trivalent, surface antigen inactivated subunit influenza vaccine produced in mammalian cell culture (Optaflu®) fulfilled the CHMP requirement of meeting at least one of the CHMP criteria in

Table 3 Overview of subjects experiencing solicited and unsolicited adverse events following vaccination (6 h post-vaccination through day 4)

	Number (%) of subjects experiencing adverse events		
	18 to ≤60 years, *N* = 63	≥61 years, *N* = 63	Total, *N* = 126
Solicited adverse events			
Any	36 (57%)	22 (35%)	58 (46%)
Local[a]	32 (51%)	18 (29%)	50 (40%)
Pain	31 (49%)	18 (29%)	49 (39%)
Induration	5 (8%)	1 (<2%)	6 (5%)
Systemic[b]	17 (27%)	8 (13%)	25 (20%)
Headache	11 (17%)	6 (10%)	17 (14%)
Fatigue	10 (16%)		
Malaise	3 (5%)		
Arthralgia	3 (5%)	3 (5%)	6 (5%)
Unsolicited adverse events			
Any AEs	6 (10%)	4 (6%)	10 (8%)
At least possibly related AEs	2 (3%)	0	2 (2%)
Serious AEs	1 (2%)	0	1 (1%)
At least possibly related SAEs	0	0	0
Medically attended AEs	4 (6%)	3 (5%)	7 (6%)
AEs leading to discontinuation	0	0	0
Death	0	0	0

[a]Threshold for erythema, ecchymosis and induration: grade 0 (< 10 mm), any (≥10 mm)

[b]Includes subjects with body temperature ≥38 °C irrespective of route of measurement

both the SRH assay and the HI assay for each strain in both age groups.

The results were comparable to those of a previous study in which protective HI titres of ≥ 40 were detected against the three strains (A/California/7/2009(H1N1)-like, A/Victoria/361/2011(H3N2)-like and B/Wisconsin/1/2010-like) in 98%, 100%, and 98% of subjects aged 18 years to ≤ 60 years and in 100%, 100%, and 85% of subjects ≥ 61 years of age [7]. Another study comparing cell-culture derived and egg-based trivalent influenza vaccines showed an overall vaccine efficacy (preventing influenza infection in comparison to placebo) of 69.5% and a vaccine efficacy against the vaccine-like stains of 88.2% (A/H1N1) and 100% (B strain) [5]. In that study, which involved adults aged 18 to 49, protective HI antibodies were detected against the three strains (A/H1N1, A/H3N2 and B) in 99%, 99% and 78% of subjects after vaccination [5]. Interestingly, subjects also showed some level of protection against influenza viruses antigenically different to those used in the vaccine: the study revealed a vaccine efficacy in comparison to placebo of 83.3%, 100% and 50% against non-vaccine-like A/H1N1, A/H3N2 and B strains, respectively. However, the efficacy of seasonal influenza vaccines before the beginning of an influenza season may be difficult to establish and antibody response to the influenza vaccine may serve as a maker for vaccine efficacy.

Among the subjects vaccinated the previous year, protective antibody titres ranged from 52 to 100%, rates comparable to another study in which 63% of healthy controls and 47% of organ transplant recipients exhibited protective a/H1N1 antibodies even 1 year after vaccination [10].

The adverse events reported fell within the expected range for seasonal influenza vaccines [6]. One serious adverse event of tooth infection at day 12 was judged not to be related to the vaccine. Generally, solicited adverse events were reported at a higher rate by younger subjects than older subjects. Among the unsolicited adverse events that were at least possibly related to the vaccine, one episode of diarrhoea and one of hyperhidrosis occurred.

Overall, the seasonal influenza vaccine tested here proved to be safe and immunogenic in both younger and older adults. In future, additional means of assessing vaccine performance are expected to be established for the licensing of influenza vaccines in Europe.

Compliance with ethical standards

Conflict of interest The authors declare the following conflicts of interest: EH and DK are employees of Seqirus, the company that acquired the sponsors' influenza vaccine division; ML and ECR have served as principal investigator or coordinating investigator for studies sponsored by GSK, Novartis Vaccines, Seqirus, and Valneva; all other authors declare that no competing interests exist.

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