Vaccine 28 (2010) 171–178

Contents lists available at ScienceDirect

Vaccine

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

Safety and immunogenicity of whole-virus, alum-adjuvanted whole-virus, virosomal, and whole-virus intradermal influenza A/H9N2 vaccine formulations

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ARTICLE INFO

Article history:
Received 13 July 2009
Received in revised form
15 September 2009
Accepted 22 September 2009
Available online 30 September 2009

Keywords:
Pandemic vaccine
Serology
Immunogenicity

ABSTRACT

Avian influenza H9N2 viruses are considered as a pandemic threat. We assessed the safety and immunogenicity of fourteen H9N2 vaccine formulations. A randomized, phase I trial was done in 353 adults, aged 18–82 years. Subjects received two doses of A/Hong Kong/1073/99 (H9N2) whole-virus, alum-adjuvanted whole-virus, virosomal, or intradermal whole-virus vaccine at four doses (1.7, 5, 15 or 45 μg haemagglutinin). Sera were obtained before and three weeks after each vaccination (days 0, 21, and 42) for haemagglutination–inhibition (HAI) and neutralization assays. All formulations were well tolerated. Pre-vaccination sera from subjects aged below or above 40 years had baseline antibody to H9N2 in 1% and 16% of samples. Compared to intramuscular whole-virus vaccine, alum-adjuvanted vaccine was more immunogenic, intradermal vaccine was comparable, and virosomal vaccine less immunogenic. Among subjects under 40 years, two doses (45, 15, and 5 μg) of alum-adjuvanted vaccine achieved seroprotective HAI titres in 50%, 41%, and 39% respectively, and neutralization seroconversions in 83%, 82%, and 78% of recipients. Among subjects over 40 years, one dose (45, 15, and 5 μg) of alum-adjuvanted vaccine achieved seroprotective HAI titres in 50%, 25% and 0% respectively, and neutralization seroconversions in 88%, 63% and 63% of recipients. Among immunologically naive subjects under 40 years, two doses of vaccine are required and alum-adjuvanted vaccines were most immunogenic. Among immunologically primed subjects over 40 years, one dose of whole-virus or alum-adjuvanted vaccine induced immune responses; the second dose provided less additional benefit. However, no vaccine formulation satisfied all European regulatory criteria for pandemic vaccines.

1. Introduction

Pandemics of influenza A are unpredictable in their frequency and origin. At least three phylogenetic lineages of avian influenza A/H9N2 viruses are widely dispersed in poultry [1–3]. G1-like H9N2 viruses not only have genetic similarities with A/Hong Kong/97 (H5N1) viruses, but also have features in their haemagglutinin and neuraminidase that may facilitate transmission to humans [3–5]. Cross-species transmission of H9N2 viruses into humans, most recently in China in 2008, and swine has occurred, indicating that they pose a pandemic threat [5–10].

Current licensed vaccines to confront a pandemic include whole-virus, split-virion or subunit preparations, with or without alum or oil-in-water emulsion adjuvants, and a virosomal delivery system. Traditional non-adjuvanted H9N2 and H5N1 split-virion and subunit vaccines are poorly immunogenic [11–16]. Whilst the addition of oil-in-water emulsion or whole-virus formulation enhances immunogenicity providing dose-sparing for H5 and H9 antigens [11–14,17,18], it is uncertain whether alum facilitates consistent dose-sparing in adults [16–18]. Compared to trivalent seasonal split-virion vaccine, virosomal vaccine improves seroconversion rates in immunologically naïve children [19], but virosomal candidate pandemic vaccines have not yet been tested clinically. Intradermal vaccination offers the possibility of dose sparing, but trials during past pandemics and during the re-emergence of A/H1N1 during 1976/1977 gave contradictory results [20–22].

We conducted a dose-comparison study to evaluate the safety and immunogenicity of whole-virus, alum-adjuvanted whole-virus, virosomal, and intradermal whole-virus vaccines prepared

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doi:10.1016/j.vaccine.2009.09.103

from A/Hong Kong/1073/99 (H9N2) virus in healthy adults. The objectives were to measure short-term local and systemic reactions and serum immune responses to assess the amount of antigen needed to raise protective levels of antibody.

2. Materials and methods

2.1. Study subjects, timing and setting

Study subjects were healthy adults aged over 18 years. Exclusion criteria included: immunosuppression due to illness or treatment; pregnancy or women unwilling to use reliable contraception; immunomodulator therapy or receipt of blood products in the preceding three months; vaccination or use of experimental drugs in the preceding four weeks; anaphylaxis or allergy to eggs, vaccine, or mercury-containing products; acute respiratory illness in the previous 7 days; febrile illness in the preceding 3 days; or could not give informed consent. Eligible subjects were screened and enrolled during August 2006–January 2007 at the Leicester Royal Infirmary, UK after giving written consent in accordance with protocols approved by the Medicines and Healthcare products Regulatory Agency, Leicestershire Ethics Committee and University Hospitals of Leicester (Clinicaltrials.gov NCT00814229).

2.2. Vaccines

The vaccine virus, A/Hong Kong/1073/99 (H9N2), was provided by the UK National Institute for Biological Standards and Control (NIBSC). Crucell-Berna (Berne, Switzerland) prepared vaccines using virus propagated in hens' eggs. Virosomal formulations were produced by standard processes used for the seasonal trivalent vaccine Inflexal® V (Berna Biotech AG). Alum-adjuvanted whole-virus vaccine contained aluminium phosphate at a final concentration of 0.5 mg/mL. Whole-virus, alum-adjuvanted whole-virus, and virosomal-adjuvanted subunit vaccines were formulated in 0.5 mL liquid in coded, pre-filled monodose syringes at four antigen concentrations (1.7, 5, 15, and 45 μg haemagglutinin) for intramuscular delivery. Whole-virus vaccine was formulated as liquid in vials at two concentrations (5 and 15 μg) in 0.1 mL for intradermal delivery. Haemagglutinin content was determined by single-radial-immunodiffusion.

2.3. Study procedures

Subjects were screened with a medical history, examination, and for females, a urine pregnancy test before each vaccination. Participants were stratified by age (above and below 40 years) on the basis of previous findings suggesting that those born before 1969 are primed to H9 antigen [11]. Subjects were randomly assigned to receive two doses of vaccine, 21 days apart, either by intramuscular injection into the deltoid muscle of the non-dominant arm, or by intradermal injection into skin overlying the deltoid. The second vaccination was identical to the first and was given by the same route. A vaccine randomization list with a randomization block size of 14 was used to maintain group balance. Subjects remained under observation for 30 min after each injection for safety assessment. Subjects maintained a daily self-completed diary card for 7 days after each vaccination to record oral temperature, presence of local or systemic reactions and use of any medication.

Solicited and unsolicited adverse events were graded as: none; mild if symptom occurred but did not cause inconvenience; moderate if interfered with daily activities but did not need medical intervention; and severe if required medical intervention. Diary cards were reviewed with subjects at scheduled study visits. Vaccine injections were given by a nurse who was not involved in

Table 1
Demographic characteristics of study population by vaccine type, route of administration and dose.

Subject characteristic	Intramuscular route										Intradermal	
	Whole-virus				Whole-virus+alum			Virosomal subunit			Whole-virus	
	1.7 μg $N=26$	5 μg $N=25$	15 μg $N=25$	45 μg $N=25$	1.7 μg $N=24$	5 μg $N=26$	15 μg $N=25$	45 μg $N=26$	1.7 μg $N=24$	5 μg $N=25$	15 μg $N=25$	45 μg $N=27$	5 μg $N=25$	15 μg $N=25$
Age, years														
Mean, years	33.6	34.8	33.7	34.6	33.0	33.7	34.0	33.2	32.9	37.0	34.1	34.4	32.8	34.6
SD	11.9	12.9	11.1	11.4	12.5	14.2	12.8	13.4	11.7	16.6	12.1	12.0	10.3	13.1
Median, years	30	30	33	32	33	27	27	29	27	32	30	33	30	31
Range	19–54	20–60	18–57	20–62	18–57	19–66	18–67	18–66	19–59	19–82	19–58	18–57	18–57	19–58
Age <40 years	18	17	17	17	16	18	17	18	17	17	17	18	17	17
Sex														
Female	18	13	17	18	16	12	15	13	12	14	15	15	13	16
Male	8	12	8	7	8	14	10	13	12	11	10	12	12	9
Ethnic origin														
White	22	20	20	23	23	18	23	24	22	23	24	22	23	24
African	0	0	1	0	0	0	0	0	0	0	0	1	1	1
Asian	4	2	3	1	1	2	1	2	2	1	1	3	1	0
Oriental	0	3	1	1	0	2	1	0	0	1	0	1	0	0

Data are number of subjects, unless otherwise stated.