Immunogenicity of a reduced dose of A∕H3N2 in the 2005 southern hemisphere formulation of inactivated split influenza vaccine

Christopher Burrell,[a,b] Robert Booy,[c] Nicholas Wood,[c] Anne-Marie Egan,[c] David Taverner,[d] Ken Williams,[e] Winston Liauw,[e] Alan Moskwa,[f] Stephanie Pepin-Covatta,[g] Melanie Saville[g]

[a]School of Molecular and Biomedical Science, The University of Adelaide, Adelaide, Australia; [b]Infectious Diseases Laboratories, Institute of Medical and Veterinary Science, Adelaide, Australia; [c]National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases, The Children's Hospital at Westmead and the University of Sydney, NSW, Australia; [d]Department of Pharmacology, The University of Adelaide, Adelaide, Australia; [e]St Vincent's Clinical Trials Centre, St Vincent's Hospital, Darlinghurst, Australia; [f]CMAX Royal Adelaide Hospital, Adelaide, Australia; [g]Sanofi Pasteur, Marcy l'Etoile, France
Correspondence: Stephanie Pepin-Covatta, Sanofi Pasteur, 1541, Avenue Marcel Mérieux, F-69280 Marcy l'Etoile, France.
Email: stephanie.pepin@sanofipasteur.com

Background The 2005 southern hemisphere formulation of the inactivated split-virion influenza vaccine Vaxigrip® unintentionally contained a lower concentration of haemagglutinin (HA) than European Pharmacopoeia (EP) and WHO specifications for one of the three strains.

Objectives To evaluate the immunogenicity of the 2005 southern hemisphere formulation of an influenza vaccine containing 9 μg/dose of HA for A/Wellington/1/2004(H3N2) strain, and 15 μg/dose for each of the A/New Caledonia/20/99(H1N1) strain and B/Shanghai/361/2002-like strains.

Patients/methods In an open, non-controlled multicentre clinical trial, 75 healthy adults (18–59 years) and 65 healthy older adults (≥60 years) were vaccinated once. Serum samples were obtained on D0 and 21 for haemagglutination inhibition (HAI) antibody titration.

Results A high proportion of adults (64%) and elderly (68%) were already seroprotected (HAI titre of ≥40) against A/Wellington/1/2004(H3N2) before vaccination, probably due to high circulation of an antigenically similar H3N2 strain and a high 2004 vaccination rate. By D21, seroprotection rates against H3N2 attained 93·8% and 96·0% in adults and elderly respectively. The other two immunogenicity criteria for annual licensure of influenza vaccines in Europe were also met in both age groups for the H3N2 strain, and also for the H1N1 and B strains.

Conclusions These results enabled the 2005 southern hemisphere vaccine to be used in expectation that it would provide satisfactory protection against influenza, despite the reduced H3N2 antigen content.

Keywords Antigen content, immunogenicity, inactivated vaccine.

Introduction

Influenza is a highly contagious, vaccine-preventable acute respiratory disease. It affects all age groups and is associated with considerable morbidity, mortality and financial costs.[1–4]

Annual influenza vaccination is the single most important preventive measure against influenza and is generally recommended for the elderly (≥65 years) and other groups at increased risk of developing influenza-related complications. Studies have shown that immunization can significantly reduce morbidity and death in the elderly.[5–8]

Influenza vaccination also has substantial health and economic benefits for healthy working adults.[9] Despite the recent availability of antiviral agents, vaccination remains by far the most effective tool for the prevention of influenza.

Twice a year – i.e. once a year for the winter season in each of the northern and southern hemispheres – a new formulation of influenza vaccine is produced. Each season's formulation contains 15 μg of haemagglutinin (HA) from three influenza virus strains: two variants of sub-type A (H1N1 and H3N2) and a variant of sub-type B, in compliance with the World Health Organisation (WHO) recommendations.

The 2005 southern hemisphere formulation of the inactivated split-virion influenza vaccine Vaxigrip® (Sanofi Pasteur, Lyon, France) unintentionally contained a concentration of HA that was lower than the applicable European Pharmacopoeia (EP) and WHO specifications for one of the three strains. It contained 9·16 μg of HA per dose for the A/Wellington/1/2004(H3N2) strain.[10,11] The concentration for the A/H1N1 (A/New Caledonia/20/99-like strain) and B (B/Shanghai/361/2002-like strain) strains adhered to the specifications of 15 μg of HA from each strain per dose. We therefore conducted a clinical trial to evaluate whether this reduced dose of HA affected the immunogenicity of the vaccine. During the previous southern hemisphere influenza season (February to September 2004), outbreaks of illness caused by A/H3N2 viruses had been reported in Africa, the Americas, Asia, Europe and Oceania.[12] The Australian WHO Collaborating Centre analysed influenza isolates received from 1 January to 24 September 2004 from the 13 countries of the Asian Pacific WHO region: A/H3N2 was the predominant strain, accounting for 75% (464/621) of isolates.[13] Some 79% of Australians aged over 65 years had received the 2004 southern hemisphere influenza vaccine.[13] This formulation was composed of an A/Fujian/411/2002 like virus that is antigenically similar to the A/Wellington strain.

Methods

Study design

This trial was an open-label, non-controlled multicentre trial conducted in two centres each in Adelaide and Sydney, Australia, in accordance with the Declaration of Helsinki, Good Clinical Practice and local regulatory requirements. Independent approval of the trial protocol was obtained from the ethics committee of each centre before enrolment. All subjects gave their written informed consent before enrolment. The study was designed to describe the immune response to the inactivated, split-virion influenza vaccine in two age groups, each comprising at least 50 evaluable healthy individuals: 18- to 60- and ≥60-year-olds. There were no other objectives.

Population

The planned population was two groups of healthy subjects: 60 'young adults' aged 18–59 years and 60 'older adults' aged 60 years and over. The principal exclusion criteria were febrile illness (oral temperature ≥37·5°C) on the day of vaccination; congenital or acquired immunodeficiency; immunosuppressive therapy within the preceding 6 months; long-term systemic corticosteroid therapy; hypersensitivity to any vaccine component or to egg or chick proteins; receipt of blood or blood-derived products in the previous 3 months; vaccination in the previous

4 weeks or planned in the three following weeks; influenza vaccination in the previous 6 months. Breastfeeding women, pregnant women and those at risk of becoming pregnant were also ineligible.

Study procedures

After inclusion on D0, a pre-vaccination blood sample was obtained. Subjects then received a single 0·5-ml dose of influenza vaccine intramuscularly into the deltoid and were kept under observation for 30 minutes following vaccination to monitor immediate adverse reactions. At the second and last visits, 3 weeks after vaccination (D21), a post-vaccination blood sample was obtained. Adverse events spontaneously reported by the subjects were investigated and documented.

Vaccine

The vaccine used was the southern hemisphere 2005 formulation of the trivalent, inactivated, split-virion influenza vaccine, Vaxigrip® (Sanofi Pasteur), manufactured as previously described.[14] Such vaccines usually contain 15 μg of HA from each of the three recommended strains per dose. An error in the reconstitution of the reference reagent for the A/Wellington/1/2004 (H3N2)-like strain meant that the 2005 southern hemisphere vaccine contained a low dose of HA for this strain. The batch chosen for this trial had a potency (9·16 μg of HA for H3N2 per 0·5-ml dose) that was lower than the mean potency of the tested batches (9·36 μg of HA for H3N2 per dose). This was to allow the immunogenicity results to be extrapolated to all vaccine batches. The two other strains – A/New Caledonia/20/99(H1N1)-like strain and the B/Shanghai/361/2002-like strain B/Jiangsu/10/2003 – had the required 15 μg of HA per 0·5-ml dose.

Immunogenicity

Pre- and post-vaccination sera were stored and transferred frozen at –20°C or less to Sanofi Pasteur's Global Clinical Immunology laboratory (Swiftwater, PA, USA). Haemagglutination inhibition (HAI) antibody titres against the vaccine strains were determined in duplicate on paired pre- and post-vaccination sera using the WHO HAI reference technique.[15] For each vaccine strain, HAI titres were summarized by subject as the individual geometric mean (GM) of duplicates and expressed as an inverse dilution (1/dil). A titre of five 1/dil was assigned if the sample had a titre below the ten 1/dil detection limit of the assay. In accordance with the European Committee for Proprietary Medicinal Products (CPMP) guidance,[16] results are expressed for each age group and for each strain in terms of: (a) the rate of seroconversion or significant increase (at least fourfold) in titre between D0 and D21; (b) the mean geometric increase between D0 and D21; and (c) the preva-

lence of seroprotection on D21. These results were then compared with the CPMP requirements for each endpoint.[16]

Statistical methods

The study was descriptive based on the use of the CPMP endpoints for inactivated influenza vaccine immunogenicity assessment described above. Additionally, pre- and post-vaccination geometric mean titres, and 95% confidence intervals (CI) for all endpoints were calculated.

Results

Subject disposition

A total of 140 subjects (75 young adults and 65 elderly adults) were included in the study and vaccinated over 4 days between 21 and 23 March 2005. All 140 completed the study, 21 days later. The mean age (±SD) in years was 39·9 (±12·95) in the young adult group and 65·5 (±4·24) in the elderly adult group. More women were included in the younger age group (male/female ratio: 0·39) and a similar proportion of men and women (ratio: 0·9) were included in the older age group.

Forty-three young adults (57%) and 53 older adults (82%) had a history of influenza vaccination, most of whom (68/96, 71%) were vaccinated in 2004 [26/43 (60%) young adults, and 42/53 (79%) elderly adults]. No serious adverse event was reported during the trial.

Immunogenicity

In both the younger and older age groups, HAI titres against A/Wellington/1/2004(H3N2) were high even before vaccination: 64% (48/75) of young adults and 68% (44/65) of older adults had seroprotective titres. These rates were lower against the A/H1N1 (respectively 33% and 46%) and B (17% and 23%) strains. Furthermore, only 8% of subjects in each age group (six young adults and five older adults) were seronegative against H3N2 (titre <ten 1/dil) before vaccination. Among subjects with seroprotective titres against A/H3N2, 45·8% (22/48) of 18- to 59-year-olds and 70·5% (31/44) of ≥60-year-olds were vaccinated in 2004.

Twenty-one days after vaccination, the HAI response against A/Wellington/1/2004(H3N2) surpassed all three CPMP criteria in both age groups (Tables 1 and 2). The GMT had increased more than sevenfold from 47 to 331 in the younger group, and more than fourfold from 57·2 to 232 in the older group. The proportion seroprotected after vaccination was in excess of 90% in both groups. The proportion of young and older adults seroconverting or showing a significant rise (≥fourfold) in titre was, respectively, 56% and 42%. Among the initially seronegative subjects, four (of six) young and four (of five) older adults seroconverted. Fourfold or higher titer increases among the initially seropositive (titer >ten 1/dil) subjects were 55% and 38% in the two groups respectively.

All three CPMP immunogenicity criteria were also surpassed in both age groups for the A/New Caledonia/20/99(H1N1)-like strain and B/Jiangsu/10/2003 strains (Tables 1 and 2). The HAI GMT against H1N1 increased from 21·5 to 201 in the younger age group, and from 24·5 to 80·9 in the older age group. The GMT against the B strain increased from 11·81 to 97·6 and from 13·7 to 54·5 in the two age groups respectively.

Table 1. Immunogenicity of the 2005 southern hemisphere formulation of Vaxigrip® in 75 healthy adults aged 18–60 years

	A/Wellington/ 1/2004 (H3N2)	A/New Caledonia/ 20/99 (H1N1)	B/Jiangsu/ 10/2003
Percentage of seroconversion or significant increase in titre on D21*	**56·0**	**66·7**	**66·7**
95% confidence interval	44·1–67·5	54·8–77·1	54·8–77·1
Geometric mean increase between D0 and D21†	**7·03**	**9·32**	**8·26**
95% confidence interval	4·99–9·90	6·53–13·29	6·18–11·05
Percentage of seroprotected subjects on D21‡	**96·0**	**92·0**	**78·7**
95% confidence interval	88·8–99·2	83·4–97·0	67·7–87·3

Bold typeface indicates compliance with Committee for Proprietary Medicinal Products (CPMP) recommendations for the immunogenicity of an inactivated influenza vaccine.
*Proportion of subjects with a pre-vaccination titre <10 (1/dil) and a post-vaccination titre ≥40 (1/dil) or with titres ≥10 before vaccination and ≥fourfold increase in the titre.
†Geometric mean of individual ratios (post-/pre-vaccination titres).
‡Proportion of subjects achieving a post-vaccination titre ≥40 (1/dil).

Table 2. Immunogenicity of the 2005 southern hemisphere formulation of Vaxigrip® in 65 healthy adults aged ≥60 years

	A/Wellington/ 1/2004 (H3N2)	A/New Caledonia/ 20/99 (H1N1)	B/Jiangsu/ 10/2003
Percentage of seroconversion or significant increase in titre on D21*	**41·5**	**40·0**	**47·7**
95% confidence interval	29·4–54·4	28·0–52·9	35·1–60·5
Geometric mean increase between D0 and D21†	**4·06**	**3·3**	**3·98**
95% confidence interval	2·96–5·59	2·4–4·54	2·98–5·31
Percentage of seroprotected subjects on D21‡	**93·8**	**78·5**	**67·7**
95% confidence interval	85·0–98·3	66·5–87·7	54·9–78·8

Bold typeface indicates compliance with Committee for Proprietary Medicinal Products (CPMP) recommendations for the immunogenicity of an inactivated influenza vaccine.
*Proportion of subjects with a pre-vaccination titre <10 (1/dil) and a post-vaccination titre ≥40 (1/dil) or with titres ≥10 before vaccination and ≥fourfold increase in the titre.
†Geometric mean of individual ratios (post-/pre-vaccination titres).
‡Proportion of subjects achieving a post-vaccination titre ≥40 (1/dil).

Discussion

The 2005 southern hemisphere inactivated influenza vaccine Vaxigrip® was erroneously formulated with a potency below specifications for the strain A/Wellington/1/2004(H3N2) strain. Potency met EP and WHO specifications for the other two strains. This study was therefore performed to evaluate the impact of this reduced dose of haemagglutinin (approximately 9 μg instead of 15 μg per dose) on immunogenicity. Specifically, we investigated whether the haemagglutinin inhibition (HAI) immune response to this strain in adult and elderly populations met the European Committee for Propriety Medicinal Products (CPMP) requirements. Serum HAI immune response, expressed in terms of mean geometric increase, seroconversion and seroprotection, is the standard primary endpoint for evaluating influenza vaccine.[17–19] An HAI titre of 40 is used as a cut-off level for protection based on the correlation with a reduction in influenza-like illness: a population HI titre of 1:40 is considered to be indicative of a 50% reduction in the risk of contracting influenza.[17,20,21] Our results showed the immune response to be satisfactory, enabling the vaccine to be used to protect people in the southern hemisphere against influenza during the 2005 winter season.

Assessing the immunogenicity of reduced doses of antigen from published studies is complex as factors, such as age, influenza infection and vaccination history, relative immunogenicity of different strains, and differing manufacturing processes (e.g. whole virus versus split vaccines), must be taken into consideration. Two papers looking at large numbers of patients provide interesting information on reduced dose influenza vaccines. A meta-analysis of 20 studies (1978–1991) comparing 10 and 15 μg doses of HA in various age groups was published in 1993.[22] The authors concluded that the results did not justify the expectation that a vaccine dose of 15 μg of HA per strain would be clinically superior to a 10 μg dose. In the 2000–2001 influenza season, in the context of vaccine shortages in the USA, the immunogenicity of half-strength inactivated influenza vaccine was evaluated in comparison with full-strength vaccine in 1009 healthy adults.[23] Although the observed GMTs and seroprotection rates in the half dose group were high and differences between groups were small, differences in GMT were nevertheless statistically significantly lower with the half-strength vaccine. The current consensus is that the immune response achieved by available influenza vaccines formulated at 15 μg, needs improvement, particularly in the elderly. For example, a recent quantitative review of antibody responses to influenza vaccination in the elderly concluded that there is a need for more immunogenic formulations for the elderly.[24] A routine reduction in the dosage of the influenza vaccine from 15 μg of HA per strain would not help achieve this goal.

In our study, mean geometric increases, seroprotection and seroconversion rates after vaccination met the requirements in both age groups. The response in elderly subjects also met the higher requirements defined for younger adults. Post-vaccination antibody responses against A/Wellington/1/2004(H3N2) were therefore satisfactory, despite the reduced vaccinating dose of HA. However, the seroprotection rate and GMT were high even before vaccination. The prevalence of protective titres against the A/H3N2 strain prior to vaccination is explained by two factors: the extensive circulation of A/H3N2 strains in Australia in

recent years, especially in 2004, and the high rate of previous influenza immunization in the study population.

In conclusion, despite the reduced antigen content of the A/Wellington strain, the southern hemisphere 2005 formulation of the trivalent inactivated, split-virion influenza vaccine, Vaxigrip®, both age groups of vaccinated subjects displayed satisfactory immune responses as all immunogenicity requirements of the CPMP recommendations for each age group were satisfied for all three influenza strains. However, these results should be interpreted with caution. They were obtained in favourable circumstances (healthy, ambulant population, high pre-vaccination seroprotection rate and extensive recent circulation of A/H3N2 strains similar to the A/H3N2 component of the vaccine). It is not clear whether a 9-μg dose of HA would induce similar results in other, less favourable circumstances or in the case of the other two vaccine strains.

Addendum

The study investigators CB, RB, NW, AME, DT, KW, WL and AM were responsible for the recruitment, vaccination and follow-up of subjects. CB was the overall coordinating investigator; DT, AM, RB were the principal investigators at their respective centres. MS was the sponsor's clinical team leader and responsible medical officer and SPC was the clinical scientist; both designed the study protocol, oversaw the study monitoring, data collection and analysis. All authors were involved in the preparation of this manuscript and approved the final version.

Acknowledgments

References

1 Nicholson KG, Wood JM, Zambon M. Influenza [review]. Lancet 2003; 362:1733–1745.
2 Izurieta HS, Thompson WW, Kramarz P et al. Influenza and the rates of hospitalization for respiratory disease among infants and young children. N Engl J Med 2000; 342:232–239.
3 Wood SC, Alexseiv A, Nguyen VH. Effectiveness and economical impact of vaccination against influenza among a working population in Moscow. Vaccine 1999; 17(Suppl. 3):S81–S87.
4 Nichol KL, Mallon KP, Mendelman PM. Cost benefit of influenza vaccination in healthy, working adults: an economic analysis based on the results of a clinical trial of trivalent live attenuated influenza virus vaccine. Vaccine 2003; 21:2216–2226.
5 Barker WH, Mullooly JP. Influenza vaccination of elderly persons: reduction in pneumonia and influenza hospitalization and death. JAMA 1980; 244:2547–2549.
6 Patriarca PA, Weber JA, Parker RA et al. Efficacy of influenza vaccine in nursing homes: reduction in illness and complications during an influenza A (H3N2) epidemic. JAMA 1985; 253:1136–1139.
7 Gross PA, Quinnan GV, Weksler ME et al. Immunization of elderly people with high doses of influenza vaccine. J Am Geriat Soc 1988; 36:209–212.
8 Nichol KL, Margolis KL, Wuorenma J, Von Sternberg T. The efficacy and cost effectiveness of vaccination against influenza among elderly persons living in the community. N Engl J Med 1994; 331:778–784.
9 Nichol KL, Margolis KL, Murdoch M et al. The effectiveness of vaccination against influenza in healthy, working adults. N Engl J Med 1995; 333:889–893.
10 World Health Organisation. WHO Technical Report Series, No. 814, 1991 – annexe 2. Requirements for influenza vaccine (inactivated). Requirements for Biological Substances No. 17, revised 1990.
11 European Pharmacopoeia 5.0, Monograph 0158.
12 WHO. Recommended composition of influenza vaccines for use in the 2005 influenza season. Wkly Epidemiol Rec 2004; 79:369–373.
13 National Notifiable Diseases Surveillance System (NNDSS). Department of Health and Ageing, Australian Government. National influenza surveillance 2004. Influenza Report 6 January 2005 (No. 15). Available at: http://www.health.gov.au/internet/wcms/Publishing. nsf/Content/cda-surveil-ozflu-flucurr.htm#report (last access 6 April 2005).
14 Lina B, Fletcher MA, Valette M et al. A triton X-100-split virion influenza vaccine is safe and fulfills the Committee for Proprietary Medicinal Products (CPMP) recommendations for the European Community for Immunogenicity, in Children, Adults and the Elderly. Biologicals 2000; 28:95–103.
15 Palmer DF, Dowie WR, Coleman MT, Schild GC. Advanced laboratory technicals for immunological diagnostic. in: US Department of Health (ed). Atlanta: Welfare PHS.Immunology Ser. No. 6, Procedural Guide, Part 2: Haemagglutination – Inhibition Test, 1975, 25–62.
16 European Committee for Proprietary Medicinal Products. Note for guidance on harmonisation of requirements for influenza vaccines (CPMP/BWP/214/96). European Agency for the Evaluation of Medicinal Products, 1997.
17 Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum hemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg 1972; 70:767–777.
18 Wesselius de Casparis A, Masurel N, Kerrebijn KF. Field trial with human and equine influenza vaccine in children; protection and antibody titers. Bull World Health Organ 1972; 146:151.
19 Longini IM, Koopman JS, Haber M, Cotsonis GA. Statistical interference for infectious diseases. Risk-specific household and community transmission parameters. Am J Epidemiol 1988; 128: 845–859.
20 Al-Khayatt R, Jennings R, Potter CW. Interpretation of responses and protective levels of antibody against attenuated influenza A viruses using single radial haemolysis. J Hyg 1984; 93:301–312.
21 Delem A. Serum SRH antibody level as a measure of the immunity against natural and artificial A/Victoria/3/75 infections. Dev Biol Stand 1977; 89:391–396.

22 Palache AM, Beyer WE, Luchters G *et al.* Influenza vaccines: the effect of vaccine dose on antibody response in primed populations during the ongoing interpandemic period. A review of the literature [review]. Vaccine 1993; 11:892–908.

23 Treanor J, Keitel W, Belshe R *et al.* Evaluation of a single dose of half strength inactivated influenza vaccine in healthy adults. Vaccine 2002; 20:1099–1105.

24 Goodwin K, Viboud C, Simonsen L. Antibody response to influenza vaccination in the elderly: A quantitative review [review]. Vaccine 2006; 24:1159–1169.