With the recent pandemic of influenza A (H1N1) and vaccine shortages, there has been considerable interest in developing influenza vaccines with reduced antigen content, allowing for increased production capacity. Here we report a prospective, randomized, double-blind, single-center clinical trial of a reduced-dose whole-virion inactivated, adjuvanted influenza vaccine in adult and elderly volunteers. A total of 234 subjects, including 120 adults (18 to 60 years of age) and 114 elderly subjects (>60 years of age) were enrolled to receive either 6 μg of the conventional 15-μg dose of seasonal trivalent influenza vaccines. The subjects were followed for safety analysis, and serum samples were obtained to assess immunogenicity by hemagglutination inhibition testing. The subjects developed antibody responses against the seasonal influenza A virus H1N1 and H3N2 strains, as well as the seasonal influenza B virus included in the vaccines. Single doses of 6 μg fulfilled licensing criteria for seasonal influenza vaccines. No significant differences in rates of seroconversion or seroprotection or in geometric mean titers were found between the two dosage levels. All adverse events were rare, mild, and transient. We found that the present reduced-dose vaccine is safe and immunogenic in healthy adult and elderly subjects and triggers immune responses that comply with licensing criteria.

MATERIALS AND METHODS

Vaccines. The reduced-dose vaccine (Fluval K, an inactivated, whole-virion, trivalent vaccine with 6 μg of HA/strain/0.5 ml content and aluminum phosphate gel adjuvant; lot no. FL-K-004) was produced by Omninvest Ltd. (Budapest, Hungary) as described previously (8, 14–19). With the exception of the antigen amount, the vaccine was prepared by the same method as for the licensed seasonal influenza vaccine Fluval AB (19) and the licensed prepandemic vaccine Fluval H5N1 and the licensed pandemic H1N1 vaccine Fluval P (8, 14–18).

The seasonal vaccine (Fluval AB trivalent inactivated, aluminum phosphate-adjuvanted whole-virion influenza vaccine; lot no. 4807) was also produced by Omninvest, as described in detail elsewhere (19). Like most licensed trivalent inactivated seasonal influenza vaccines, it contained 15 μg of HA/strain/dose. The seasonal vaccine produced by this method has met the requirements of the European Agency for the Evaluation of Medicinal Products (EMEA) for interpackard influenza vaccines each year since 1995, and it has been safely administered to humans in a total of over 18 million cases (19).

Both vaccines contained the A/Solomon Islands/3/2006 (H1N1)-like IVM-145 reassortant strain; the A/Wisconsin/67/2005 (H3N2)-like NYMC X-161B reassortant strain, and the B/Malaysia/2506/2004 strain. The virus strains were chosen according to the European Union recommendations for the seasonal influenza vaccine composition for the season 2007/2008 (4). The seed virus strains were grown in eggs. The HA content was determined before the addition of the aluminum phosphate adjuvant by single radial immunodiffusion test using reagents supplied by the National Institute for Biological Standards and Control (NIBSC), United Kingdom, as described previously (22). Purity was evaluated by the endotoxin content, which was <0.05 IU/dose, and the amount of ovalbumin, which was <5 ng/dose. Both values are much lower than the concentrations considered acceptable by the European Pharmacopoeia, which are 100 IU and 1,000 ng/dose, respectively (6). Aluminum phosphate was used as an adjuvant in the amount of 0.33 mg Al/ampoule, and mertiolate was added as preservative (0.1 mg/ml), meeting the requirements of the European Pharmacopoeia (6).

Participants. Between 11 July 2007 and 5 May 2008, we did a prospective single-center, randomized, double-blind trial at the State Primary Care Center, Pilisvorosvar, Hungary. Patients were recruited by their primary care physicians. A total of 260 healthy volunteers over the age of 18 years were screened, and 234 subjects were enrolled to receive vaccination. Written informed consent was obtained from all potential subjects. A negative pregnancy test on day 0 was required for women of childbearing potential, and the use of an acceptable contraception method was re-

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quired for the duration of the study. Exclusion criteria included immunodeficiency, history of Guillain-Barré syndrome, disease states that may affect immune reactivity (e.g., malignancies, chronic infections [HIV or hepatitis B or C], uncontrolled diabetes mellitus, or autoimmune diseases), use of immunosuppressive medications, conditions precluding compliance, receiving any kind of vaccine 28 days prior to the study, use of influenza vaccines within 6 months, use of investigational agents within 30 days, receipt of blood products or immunoglobulins in the past 6 months, acute febrile illness 1 week before vaccination, nursing, and hypersensitivity to vaccine components.

Based on the stratified randomization method, subjects were stratified according to age: adults (age, 18 to 60 years) and elderly (age, >60 years). The subjects were randomly assigned at a 1:1 ratio to one of the following groups. Subjects in group I received 0.5 ml of the reduced-dose vaccine, while those in group II received 0.5 ml of the regular licensed seasonal vaccine. Based on the subjects’ ages, two subgroups of each group were formed: groups Ia (adults) and Ib (elderly) and groups IIa (adults) and IIb (elderly). The sequence was generated by a statistician who was not involved in the rest of the trial. Assignments were enclosed in sequentially numbered, identical, sealed envelopes. The randomization code was provided to the vaccine administrator, who was aware of study group assignments. Blinding was maintained, as all subjects and investigators who took part in the assessment of safety or immunogenicity were unaware of the assignments.

The study was conducted in compliance with good clinical practice guidelines and the provisions of the Declaration of Helsinki. The protocol was approved by the National Institute of Pharmacy and the Central Ethics Committee for Clinical Pharmacology of the Medical Research Council, Hungary. The study was registered with the European Union Drug Regulatory Authorities, European Medicines Agency (http://eudract.emea.europa.eu/), under clinical trial registration number EUDRA CT 2007-004239-32. The Clinicaltrials.gov registration number is NCT00778297.

**Laboratory Tests.** Serum antibody titers against the vaccine virus strain were measured in duplicates by hemagglutination inhibition (HI) using chicken red blood cells following standard procedures (10). All serological tests were performed at a central laboratory (Department of Virology, National Center for Epidemiology, Budapest, Hungary).

**Interventions.** Baseline evaluations on day 0 included demographic data, medical history, and physical examination. Blood samples were drawn for baseline HI for all three vaccine virus strains. For group I, 0.5 ml of the reduced-dose vaccine was injected into the deltoid muscle, while for group II, 0.5 ml of the regular seasonal vaccine was injected. On day 21 to 28, a medical history and the list of medications used during the days since the last visit was taken, physical examination was performed, and blood samples were collected for HI. The procedures listed for day 21 were repeated on day 110 to 120. Safety variables were collected at the follow-up visits by taking history and performing physical examination and by telephone interviews on days 1, 2, 3, and 7. In addition, subjects were asked to take their temperatures on days 1, 2, and 3 with thermometers supplied by the study center. The definition of fever was temperature of >38°C (100.4°F) orally. Diary cards were provided, and patients were asked to call regarding any side effects.

**Statistical analysis.** All data analyses were carried out according to a preestablished analysis plan. Safety and immunogenicity were prospectively identified as co-primary objectives. The sample size was determined on the basis of the number needed to meet annual licensing requirements for influenza vaccines in the European Union. In accordance with European Committee for Proprietary Medicinal Products (CPMP) criteria, 120 subjects per dose group (60 subjects per age subgroup) were recruited in order to have at least 50 subjects per age subgroup with data that could be evaluated. The power to meet the CPMP criteria for all strains was at least 100% minus the sum of the beta coefficients for each strain and age group. Specifically, if the true seroconversion rate for strains was at least 55% among adults 18 to 60 years of age and at least 45% among those older than 60 years, the study had a statistical power of at least 88% to meet the CPMP criteria with the use of the seroconversion rate alone (5). We summarized results with point estimates and two-sided 95% confidence intervals (CI). We report safety data in terms of the number and proportion of individuals who had reactions in each group, and we used a two-sided Fisher exact test to compare groups when relevant. All reported $P$
TABLE 1 Demographic data for the study groups

| Parameter                              | Value for group: |          |          |
|----------------------------------------|------------------|----------|----------|
|                                        | I (6-μg dose)    | II (15-μg dose) |          |
|                                        | (n = 117)        | (n = 117) |          |
| Mean age, yr (SD)                      | 52.1 (16.8)      | 57.2 (15.2) |          |
| No. (%)                                | 117 (100)        | 117 (100) |          |
| White ethnic background                | 117 (100)        | 117 (100) |          |
| Adult (18–60 yr)                       | 60 (51.3)        | 60 (51.3) |          |
| Elderly (>60 yr)                       | 57 (48.7)        | 57 (48.7) |          |
| Received influenza vaccination previously (>6 months prior to the study) | 13 (11.1) | 15 (12.8) |          |

values are two sided. We used SAS (version 9.1) and MS Excel (version 2003) software.

In the course of tolerability/safety assessment, the frequency, severity, mean time of appearance, and duration of all local and systemic adverse events (AEs) were calculated for all groups in accordance with the CPMP guidelines (5).

We gave hemagglutinin inhibition titers below the limit of detection (1:10) an arbitrary intermediate value of one in five. The geometric mean of duplicate results for each specified time was used for the calculation. The hemagglutinin inhibition endpoints were the geometric mean titers (GMT) at each time point, as well as the variables recommended by European guidelines for seasonal influenza vaccines: (i) postvaccination seroconversion rate (percentage of subjects with titers of ≥40, the titer required by the EU Committee for Human Medicinal Products (CHMP); (ii) the post- to prevaccination GMT ratio; and (iii) the proportion of subjects seroconverting, meaning displaying an at least 4-fold titer increase postvaccination and postvaccination titers of at least 1:40 (5). The hemagglutinin inhibition titer distributions showed no significant differences between the two vaccines for any of the vaccine virus strains (Fig. 2). There were no significant differences between the two vaccine groups in terms of any of the immunogenicity criteria.

RESULTS

A total of 234 subjects, including 120 adult subjects 18 to 60 years of age (mean ± standard deviation [SD], 41.9 ± 11.9 years) and 114 elderly subjects 61 to 84 years of age (mean ± SD, 68.0 ± 6.1 years), were enrolled in the study. Each enrolled subject received vaccination as planned (Fig. 1). One hundred seventeen subjects received the 6-μg dose, and 117 subjects received the 15-μg dose. None of the subjects had participated in influenza vaccine trials before.

Demographic data for the participants are shown in Table 1. There were no significant differences between the two groups in terms of age, ethnicity, previous influenza vaccination status, or baseline hemagglutinin inhibition titers to any of the vaccine virus strains. Two hundred thirty patients attended both follow-up visits and thus were included in the safety and immunogenicity analyses.

Immunogenicity. In group I, administration of the reduced-dose vaccine induced strong immune responses against all seasonal influenza A/H1N1, A/H3N2, and B seed virus antigens, to meet all three Committee for Proprietary Medicinal Products criteria for licensure, 21 to 28 days after immunization in both age groups (Table 2). Similarly, in group II, the regular-dose vaccine induced immune responses meeting or exceeding licensing criteria in both age groups (Table 2). Thus, both vaccines fulfilled immunogenicity criteria for licensing in both age groups at 21 to 28 days after vaccination (Table 2). The hemagglutinin inhibition titer distributions showed no significant differences between the two vaccines for any of the vaccine virus strains (Fig. 2).

Safety and tolerability. All subjects included in the immunogenicity analysis were followed for 120 days to provide safety data. Eight volunteers (3.5%) developed a total of 8 adverse events (Table 3). All adverse events were categorized as mild. Of these, six cases were considered unrelated. One adverse event each in groups I and II was assessed as "probably related." Both cases were pain at injection site, which developed within 24 h after vaccination and resolved in 1 day. Beyond these, no other adverse events were registered.

No other local reaction (induration, redness, swelling, or warmth) was observed. No severe adverse events were observed, and no subject showed vaccine-related systemic adverse events.

TABLE 2 Immunogenicity findings

| Antigen and age group | Group I (reduced dose) (n = 115) |          | Group II (regular dose) (n = 115) |          |
|-----------------------|----------------------------------|----------|----------------------------------|----------|
|                       | Ratio of GMTs after and before vaccination (95% CI) | Seropositivity rate, % (95% CI) | Seroconversion rate, % (95% CI) | Seropositivity rate, % (95% CI) | Seroconversion rate, % (95% CI) |
| H1N1                  |                                  |          |                                  |          |
| Adults                | 5.9 (4.4–7.9)                    | 94.9 (85.9–98.9) | 76.3 (63.4–86.4)                  | 6.1 (4.6–8.1) | 94.8 (85.6–98.9) | 70.7 (57.3–81.9) |
| Elderly               | 4.5 (3.4–5.9)                    | 92.9 (82.7–98.0) | 60.7 (46.8–73.5)                  | 4.9 (4.1–5.9) | 96.5 (87.9–99.6) | 78.9 (66.1–88.6) |
| H3N2                  |                                  |          |                                  |          |
| Adults                | 6.6 (4.7–9.1)                    | 94.9 (85.9–98.9) | 69.5 (56.1–80.8)                  | 6.4 (4.7–8.6) | 89.7 (78.8–96.1) | 70.7 (57.3–81.9) |
| Elderly               | 5.3 (3.8–7.3)                    | 92.9 (82.7–98.0) | 67.9 (54.0–79.7)                  | 6.1 (4.7–8.0) | 96.5 (87.9–99.6) | 80.7 (68.1–90.0) |
| B                     |                                  |          |                                  |          |
| Adult                 | 7.6 (6.1–9.6)                    | 94.9 (85.9–98.9) | 84.7 (73.0–92.8)                  | 9.6 (7.9–11.6) | 91.4 (81.0–97.1) | 87.9 (76.7–95.0) |
| Elderly               | 7.0 (5.4–9.0)                    | 92.9 (82.7–98.0) | 82.1 (69.6–91.1)                  | 7.7 (6.4–9.3) | 96.5 (87.9–99.6) | 87.7 (76.3–94.9) |

*Each value met licensing criteria, as follows. For adult subjects, (i) the number of seroconversions or significant (i.e., ≥4-fold) increases in HI antibody titer should be >40%, (ii) the postvaccination/prevaccination geometric mean titer ratio (increase) should be ≥2.5-fold, and (iii) the proportion of subjects achieving an HI titer of ≥40 should be ≥70%.

For elderly subjects, (i) the number of seroconversions or significant (i.e., ≥4-fold) increases in HI antibody titer should be >30%, (ii) the postvaccination/prevaccination geometric mean titer ratio (increase) should be ≥2.0-fold, and (iii) the proportion of subjects achieving an HI titer of ≥40 should be ≥60%.
DISCUSSION

Seasonal trivalent influenza vaccines are unadjuvanted split-virion or subunit vaccines and contain 15 μg of hemagglutinin/strain (7, 21). We previously reported the results of several clinical trials showing convincing immunogenicity and even cross-reactive immunity in adult and elderly patients after one injection of a 6-μg dose of a licensed monovalent prepandemic influenza A virus H5N1 vaccine (Fluvax H5N1; Omninvest, Hungary) or a licensed monovalent pandemic H1N1 vaccine (Fluvax P; Omninvest, Hungary) produced by essentially the same methods as reported for this trial (8, 14–18). Thus, the findings of this trial accord with our previous work with whole-virion, inactivated, adjuvanted prototype prepandemic and pandemic influenza vaccines (8, 14–18). We now extend the findings of strong immunogenicity with only 6 μg of hemagglutinin to trivalent seasonal vaccines as well, as we found that the administration of the reduced-dose seasonal trivalent influenza vaccine containing 6 μg of hemagglutinin/strain was safe in adult and elderly patients and met international criteria for licensing (5). Administration of the smallest possible amount of the viral antigen is crucial in dose-sparing strategies (11).

New guidelines with extended recommendations for influenza vaccination and recent vaccine shortages make the development of dose-sparing strategies more important than ever (1). Only a few clinical trials have addressed the immunogenicity of reduced-dose seasonal influenza vaccination in randomized controlled clinical trial settings (3, 12, 13). Palache et al. found that a 10-μg dose provided seroprotection against influenza A virus that was
similar to that provided by the 15-μg dose, at least in a primed population (12). Two studies, although not double blinded, concluded that half-dose vaccination may be an effective strategy for healthy adults younger than 50 years in the setting of an influenza vaccine shortage (3, 13). Our findings confirm this and suggest that further dose reduction is possible with the use of adjuvants. Furthermore, we extend these findings to elderly subjects. Including elderly participants in influenza vaccine trials is important, since influenza-associated morbidity and mortality increase with age, and influenza vaccination is associated with a reduced risk of mortality in elderly people living in communities (20). However, the immunization of elderly patients can be challenging, and therefore, an increased-dose (60 μg HA/strain) vaccine has recently been tested and was approved in 2010 for the elderly population (21). Instead of increasing the dose, another promising approach to improve responses to influenza vaccination in the elderly could be the use of adjuvants or whole-virion vaccines with acceptable safety profiles, as in the present study. This even allows dose sparing to 6 μg HA/strain in adult and elderly patients, as seen in this trial. Intradermal vaccine systems appear to work well in adults but have not been approved for elderly patients (9).

Our findings have shown that administration of the reduced-dose Fluvax K seasonal trivalent influenza vaccine was safe and well tolerated by all groups. We noted no clinically significant changes in the physical condition of the volunteers and no vaccine-related moderate or any serious adverse events. Side effects were rare and mild, and no medical intervention was necessary. Notably, the safety and tolerability results agree with previous clinical trials of a prepandemic H5N1 vaccine and a licensed pandemic H1N1 vaccine produced by the same methods and with the same antigen content as the reduced-dose vaccine in this clinical trial (8, 14–18). Furthermore, we found a similarly low rate of vaccine-related adverse events with the regular-dose vaccine during the past 13 years (19).

A potential limitation of the study is that it enrolled white patients only. Although no known differences in immune responses to influenza vaccines have been reported before, we believe that studies involving participants of different ethnic backgrounds are desirable. Similarly, trials involving patients with impaired immunity, pregnant women, and children will be necessary to develop the best possible vaccination policies. Another limitation of the study is the relatively low number of subjects, particularly for detecting adverse effects. Importantly, our aim was not to prove noninferiority to a licensed seasonal influenza vaccine. Instead, we simply studied whether the reduced dose would still fulfill immunogenicity licensing criteria with an acceptable safety profile.

We are currently conducting a clinical trial with intracutaneously administered influenza vaccines for further dose sparing.

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We declare that we have no conflict of interest. All authors are government employees. The funding source had no role in the conduct of the study or the preparation of this report.

All authors contributed to the content of the manuscript, had full access to all study data, and vouch for the completeness and accuracy of the data. The corresponding author had the final responsibility to submit for publication. Z.V. wrote the report, analyzed and interpreted data, and did the literature search. F.T. did laboratory analyses, did the literature search, interpreted data, and was responsible for study design. F.T. collected data and recruited patients.

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TABLE 3 Adverse events

| Group and no. (%) of cases | Day of onset | Description | Assessment | Grade |
|---------------------------|-------------|-------------|------------|-------|
| I                         |             |             |            |       |
| 1 (0.9)                   | 2           | Cold symptoms, sore throat | Not related | 1     |
| 1 (0.9)                   | 118         | Sore throat | Not related | 1     |
| 1 (0.9)                   | 1           | Pain at injection site | Probably related | 1     |
| II                        |             |             |            |       |
| 1 (0.9)                   | 115         | Cold symptoms, runny nose | Not related | 1     |
| 1 (0.9)                   | 109         | Sore throat, runny nose | Not related | 1     |
| 1 (0.9)                   | 107         | Dry cough, sore throat | Not related | 1     |
| 1 (0.9)                   | 11          | Diarrhea, fever | Not related | 1     |
| 1 (0.9)                   | 1           | Pain at injection site | Probably related | 1     |

a Not related, another cause of the event is most plausible, a clinically plausible temporal sequence is inconsistent with the onset of the event and the study drug administration, and/or a causal relation is considered biologically implausible. Possibly related, an event that follows a reasonable temporal sequence from administration of the study drug or follows a known or expected pattern of response to the suspected drug but that could readily have been produced by several other factors. Possibly related, an event follows a reasonable temporal sequence from administration of the study drug, there is a biologically plausible mechanism for the study drug causing or contributing to the adverse event, and the event could not be reasonably explained by the known characteristics of the patient’s clinical state. Additionally, the relation might be confirmed by improvement on stopping and reappearance of the event on repeated exposure.

b Grade 1, mild (transient or mild discomfort, no limitation in activity, some assistance or therapy needed); grade 2, moderate (mild to moderate limitation in activity, some assistance might be needed, no or minimal medical intervention or therapy needed); grade 3, severe (substantial limitation in activity, some assistance usually needed, medical intervention or therapy needed with hospital admission possible).
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