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# SUBUNIT INFLUENZA VIRUS VACCINE GRIPPOVAC SE-AZH (VACCINATION OF ADULTS)

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*Summary.* — Grippovac SE-AZh a polytype, subunit influenza virus vaccine containing H1N1 and H3N2 influenza A virus strains and one influenza B virus strain has been tested in 80 volunteers. The trials demonstrated the harmlessness, the absence of adverse reactions, and except of the B type, the high antigenicity of vaccine preparation when administered in two injections. The optimal dose of each viral strain haemagglutinin (HA) was 15 μg in 0.5 ml.

*Key words: influenza A Grippovac subunit vaccine; testing in volunteers; antigenicity*

## *Introduction*

A complex program for mass protection of the population in large cities and country regions employing influenza virus vaccines and chemotherapeutic agents has been elaborated in the U.S.S.R. (Karpukhin *et al.*, 1978). The absence of reliable means of protection of children, particularly those suffering from various chronic diseases, is a considerable complication implementing the elaborated program.

From this standpoint the development of harmless and effective means preventing influenza in children, not comprising 17.0% of the population in the U.S.S.R., appears exceptionally urgent.

Studies in abroad demonstrated a greater reactogenicity of corpuscular vaccines and this stimulated the work on creation of desintegrated and subunit vaccines (Parkman *et al.*, 1977; Wise *et al.*, 1977). The purpose of the present study was to employ healthy adult volunteers to investigate the harmlessness, reactogenicity and antigenic activity of experimental series of a novel influenza virus subunit vaccine named Grippovac SE-AZh, developed at the Research Department of the Institute of Poliomyelitis and Viral Encephalitides, U.S.S.R. Academy of Medical Sciences, Moscow.

## *Materials and Methods*

*The first 3 series of the trivaccine* (Nos 01, 04, and 07) have been tested in 80 healthy volunteers aged between 18 and 25. The vaccines differed from each another by the haemagglutinin content of the three influenza viruses: A/Khabarovsk/74/77(N1N1), A/Texas/1/77(H3N2) and B/Hong

Kong/8/73. The series 01, 07 and 04 contained 7.5, 15 and 20 μg of HA in the vaccination dose of 0.5 ml.

*The volunteers* were divided into 4 groups: 20 persons were vaccinated parenterally with vaccine 01; 10 of them received 0.5 ml of the vaccine subcutaneously into the upper third of the shoulder and 10 received intramuscular injections (1.0 ml of the vaccine). Another group of 20 persons was vaccinated with the 04 series subcutaneuosly (0.5 ml), the third group received subcutaneous injections of the 07 series vaccine (also 0.5 ml), and the forth group of 20 persons received 0.5 ml of apyrogenic physiological saline subcutaneously (control group). Revaccination was performed as the first vaccination by 30 days later.

*Clinical examination* after the first vaccination was carried out for 5 days under hospital conditions. After revaccination the examination continued for 3 days in an outpatient department. The examinations were performed daily and consisted of measuring the pulse, respiration frequency, arterial pressure, and body temperature. Local reactions were recorded 6, 24, 48 and 72 hr after vaccination. The sera obtained from the volunteers before vaccination, before revaccination and 21 days after revaccination were examined using the standard haemagglutination inhibition (HI) test with antigens of the three viruses.

## *Results*

### *Reactogenic properties*

Clinical observations indicated that some volunteers displayed a slight transient increase of body temperature during the first 6—48 hr after vaccination. In the group that received the 01 series subcutaneously only, slight febrile reaction was recorded in one case. Two persons showed weak febrile reaction among those injected with the same series intramuscularly. The groups that received the 04 and 07 series showed in 4 and in 8 cases weak febrile reactions, respectively. Such clinical reactions were also noted for 7 volunteers of the control group. The reactions persisted for no longer than one day.

Local reactions in the form of hyperaemia and infiltrate at the site of injection, classified as weak (degree 1) were observed in all groups. Only two persons in the 04 series group showed moderate local reactions (infiltrate sizes of 25 and 35 mm). The reactions appeared during the first 6 —48 hr after vaccination (peak at 24 hr) and then gradually declined in the course of 3 days. In the rest of volunteers the local reaction consisted of hyperaemia or infiltration not larger than 25 mm which lasted for no more than 2 days (weak reaction). A complex of clinical-instrumental and laboratory investigations following vaccination or revaccination did not reveal any signs indicative of side-effects of the preparations.

The biochemical markers studied (alanine aminotransferase, alkaline phosphatase, urea and total protein) were the same before and after vaccination and did not differ from those in the control group. None of the volunteers showed a positive reaction when examined for the presence of C-reactive protein.

Haematological values (leukocytes, basophils, lymphocytes, monocytes, erythrocyte sedimentation rate) as recorded in vaccinated persons and in the control group remained the same in the course of the study and did not differ from the norm.

**Table 1. Antigenic activity of the Grippovac vaccine**

| Series | Administration route | No. of volunteers 1st* vaccination | No. of volunteers 2nd** vaccination | B/Leningrad/369/75 1st No. | B/Leningrad/369/75 1st % | B/Leningrad/369/75 2nd No. | B/Leningrad/369/75 2nd % | A/Khabarovsk/74/77 1st No. | A/Khabarovsk/74/77 1st % | A/Khabarovsk/74/77 2nd No. | A/Khabarovsk/74/77 2nd % | A/Texas/1/77 1st No. | A/Texas/1/77 1st % | A/Texas/1/77 2nd No. | A/Texas/1/77 2nd % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 01 | s.c. | 10 | 9 | 3 | 30 | 4 | 44 | 8 | 80 | 8 | 88 | 10 | 100 | 9 | 100 |
| | i.m. | 10 | 7 | 3 | 30 | 3 | 42.8 | 7 | 70 | 5 | 71.4 | 6 | 60 | 5 | 71.4 |
| 04 | s.c. | 20 | 14 | 7 | 35 | 6 | 42.8 | 12 | 60 | 10 | 71.4 | 15 | 75 | 14 | 100 |
| 07 | s.c. | 19 | 15 | 5 | 26.3 | 5 | 33 | 13 | 68.4 | 12 | 80 | 15 | 78.9 | 12 | 80 |
| Placebo | s.c. | 20 | 13 | 0 | — | 0 | | 2 | 10 | 3 | 23.07 | 1 | 5 | 2 | 15.3 |

* first vaccination; ** revaccination; s.c. = subsutaneous; i.m. = intramuscular

**Table 2. Antiheamagglutinin indices in seronegative volunteers vaccinated with the Grippovac SE-AZh vaccine**

| Series | Administration route | Mean HI antibody titres to viruses B/Leningrad/369/75 I | II | III | A/Khabarovsk/74/77(H1N1) I | II | III | A/Texas/1/77 (H3N2) I | II | III | Average ratio of titre increase B/Leningrad 369/75 II | III | A/Khabarovsk/74/77 II | III | A/Texas 1/77 II | III |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 01 | s.c. | 7.5/17.1/30 | | | 6.1./ 42/42 | | | 8.6/158/239 | | | 2.3.4.0 | | 6.9/ 6.9 | | 18.4/27.1 | |
| 01 | i.m. | 8.6/19.7/19.7 | | | 5.7/ 26/ 69 | | | 6.1/ 69/138 | | | 2.3/1.3 | | 4.6/12.1 | | 11.3/22.6 | |
| 04 | s.c. | 6.5./19.7/17.1 | | | 6.1/120/182 | | | 5.7/ 91/112 | | | 3. 3/2.7 | | 19.7/29.8 | | 16.0/19.6 | |
| 07 | s.c. | 7.0/10.6/24.2 | | | 6.1/ 74/112 | | | 6.1/112/223 | | | 1.57/3.5 | | 12.1/18.4 | | 18.4/36.5 | |
| Placebo | s.c. | 7.5/9.8/9.8 | | | 6.1/8.0/8.2 | | | 7.5/8.6/11.3 | | | 1.3/1.3 | | 1.3/1.5 | | 1.1/1.73 | |

Notice: The oblique stroke (I) separates values before (I), after first (II) and second vaccinations (III).

*Antigenic activity of the vaccine*

All of the Grippovac series studied (07, 04 and 01) showed high antigenic activity and induced seroconversion with the A(H1N1) virus in 80.0, 60.0 and 68.4 % of vaccinated individuals (88.0, 71.4 and 80.0 % after revaccination), and with the A(H3N2) virus in 100.0, 75.0 and 78.9 % of vaccinated individuals (100.0, 100.0 and 80.0 % after revaccination — Table 1). Seroconversion after intramuscular vaccination was lower, i.e. 70.0—71.5 % with A(H1N1) virus and 60.0 —71.4 % with A(H3N2) virus. Thus, revaccination changed the seroconversion only slightly. As regards the B influenza virus, the highest serological characteristics after the first immunisation were obtained with the 04 series which had the highest haemagglutinin content (35.0%). After revaccination the seroconversion index varied from 33.0 to 44.0%.

A comparison of geometric mean titre and the ratio of the antibody increase indicated that the highest response occurred in the groups vaccinated with series 04 and 07 (20 and 15 mg of each HA), especially regarding to the antibody against the A(H1N1) virus (Table 2). A similar pattern was observed after the first and second vaccinations. The antibody titre and its increase ratio after first vaccination ranged from 1 : 74 to 1 : 120 and 12.1—19.7, respectively; after revaccination between 1 : 112—1 : 118 and 18.4—29.8. The mean antibody titres were the highest against the A(H3N2) virus; 1 : 69—1 : 158 after vaccination and 1 : 112—1 : 239 after revaccination. The ratio of antibody titre increase to the latter virus ranged between 11.3—18.4 and 19.6——36.6, respectively.

The lowest immunological response was observed for the influenza B virus. The response did not depend on the route of vaccine administration (intramuscular or subcutaneous) or the content of haemagglutinin in the dose (the mean antibody titre was not higher than 1 : 30 and the titre increase rate was equal or below 4.0).

## *Discussion*

Grippovac proved to be harmless for humans. The weak general and local reactions suggest low reactogenicity of the preparations containing 7.5. and 15 μg of HA. In the case of 20 μg HA (series 04) 10% of vaccinated persons showed moderate local reactions, which did not meet the specifications for the vaccine in question. The antibody response after vaccination was high and increased after revaccination. In agreement with the data of other workers who used the subunit Sandovac vaccine (Kunz, 1977; Lutz *et al.*, 1978; Ferry *et al.*, 1978; Kuwert, 1979), the response to influenza A virus antigens was higher than to the influenza B antigens.

Concerning the evaluation of the Grippovac vaccine and the decision of whether it should be introduced into the health care practice, Chumakov (1980) proposed to take into account the following: 1) glycoprotein subunit vaccines allow a considerable (3 to 5-fold) increase of the quantity of specific antigen in a minimum (0.25—0.5 mm) dose volume without taking the risk of adverse-reactions increasing above the acceptable level; 2) repeated (annual)

vaccination of nonspecific antigens from the vaccine; 3) stocks of subunit vaccines can be made beforehand and multitype preparations containing 3 or more antigens of the most topical viruses can be manufactured.

In view of the WHO recommendations and the vaccine characteristics outlined above, trials should follow on younger children representing the population most susceptible to influenza. In compliance with these recommendations, the vaccine is polyvalent and contains an influenza B component which is important for children. A two-step administration of the preparation, as suggested in the instruction, should be regarded for additional factor of stimulating immunity in children who had not encountered this virus type before.

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