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CLINICO-IMMUNOLOGIC AND ALLERGOLOGIC STUDIES WITH THE INACTIVATED INFLUENZA VIRUS VACCINE PURIFIED AND CONCENTRATED BY GRADIENT CENTRIFUGATION

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Summary. — Vaccination activity and safety of inactivated influenza centrifugal divaccine have been studied in groups of subjects aged 18 to 22, 15 to 16, and 9 to 14 years. The vaccine tested contained either a standard dose (6—8 μg) or double a dose (12—16 μg) of the haemagglutinin (HA) of influenza viruses A(H1N1) and A(H3N2). The double antigenic load of the vaccine did not enhance its reactogenicity for adults or adolescents aged 15 to 16 years. It enhanced, however, the production of antihaemagglutinine antibodies to the vaccine strains. The range of the antibodies formed and their persistence were independent on the virus dose. The increased dose (12—16 μg) of HA decreased the humoral immune response in school children aged from 11 to 14 years. Neither standard nor double dose of the vaccine caused any changes in the biochemical or haematological parameters of blood and urine. No allergic response was registered by the indirect mast cell degranulation (IMCD) test in rats.

Key words: inactivated influenza vaccine; dosage; allergization; antigenic properties; reactogenicity; safety

Introduction

Inactivated influenza vaccine (IIV) purified and concentrated by gradient centrifugation (IICV) is extensively employed for influenza prevention in adults. Total immunization, however, that would produce immunity in large proportion of the population calls for an extension of the age limits of vaccinated subjects. It is necessary to include school children in the list of vaccinated persons as they are most susceptible to the epidemic outbreak.

Because yearly vaccinations are carried out in the Soviet Union, an optimalization of the antigenic load becomes especially important, i.e. the determination of minimum haemagglutinin content that would provide sufficient immunity, but would not harm the vaccinated subject. The purpose

of this research has been to compare the immunogenicity and safety of two doses of IICV prepared from A(H1N1) and A(H3N2) strains in children and adults.

Materials and Methods

Controlled observations were carried out in organized groups of adults of Leningrad and also in 4 schools, one technical school and one technical college of Donetsk. A total of 2 062 subjects aged from 9 to 22 years was followed. Vaccinations were conducted step by step starting with the oldest group to the youngest, first with a 6—8 μg HA dosage and then with the 12—16 μg dose. Preliminary tests of reactogenicity and safety were carried out in groups of 25 subjects for each HA dose.

Commercial IICV preparation containing in 0.1 ml of 3—4 μg HA of recombinant influenza virus strains A/Kiev/59/79R(H1N1) and A/Leningrad/385/80R(H3N2) was used. Ovalbumin concentration ranged within 1 μg/ml indicating that the degree of purity was more than 99.9%. Double volume of the preparation (0.2 ml) provided a double antigenic dose. Total HA content amounted to 6—8 μg in 0.1 ml and 12—16 μg in 0.2 ml. Apyrogenic saline was used as placebo. The preparations were injected s.c. in the upper third of the arm using an injector BI-3. The vaccinated subjects were observed for 5 days after the vaccination. Every day the temperature was measured, general clinical status, subjective complains and local reaction to the injection were registered. The children who showed local or general reactions to the injection were allowed not to attend school and were observed at home until clinical signs entirely disappeared.

Immunogenic activity of the vaccine was assessed by the antihaemagglutinin antibodies (as measured in HI test). Sera from vaccinated subjects were collected before vaccination and 1, 3, and 12 months after vaccination. For analysis of the range of activity of postvaccinal antibodies inhibitor-resistant strains of influenza vaccine viruses A/Kiev/59/09R(H1N1) and A/Leningrad/385/80R(H3N2) were used; in addition diagnostic strains antigenically related to vaccine strains — A/Khabarovsk/74/77(H1N1) and A/Bangkok/1/79(H3N2), as well as the strain A/Leningrad/X/83(H3N2), an analogue of the epidemically active virus A/Philippines/2/82 (H3N2) were included.

Random haematological and biochemical investigations (total protein, protein fractions, urea, alanine aminotransferase, C-reactive protein, sialic acids) were carried out, as well as routine urine analysis. The materials for laboratory analyses were taken on the vaccination day, then 3 days and 1 month after immunization.

Allergenic reactions to the vaccines were assessed according to the ability of sera of immunized subjects to cause degranulation of rat mast cells in the presence of vaccine preparation (Shpilyuk *et al.*, 1986). The sera were collected before and 30 days after vaccination and stored at −25 °C.

Statistical treatment of the results was carried out using chi-square Pirson's (χ^2) test.

Results

Studies on IICV reactogenic properties demonstrated that a double antigenic load in the vaccine was essentially without effect on the occurrence of general and local reactions and their intensity. The injection of the vaccine caused a slight and shortlasting rise of temperature in 2.9—12.6% subjects given a standard vaccine dose and in 2.7—8.7% of those given a double dose (Table 1). In control groups a rise in temperature up to 37.3 °C also occurred, but not too frequently (4.4—15.1%). In single cases the temperature rose as high as 37.7—37.8 °C without intoxication signs (in 1 adolescent vaccinated with a 0.1 ml dose and in 3 adults given a double dose). It should be noted, however, that in placebo-inoculated subjects moderate temperature increase was also observed in single cases. This seems to be related to

Table 1. Frequency of general and local reactions in subjects aged from 9 to 22 years vaccinated with varying dosages of HCV

Preparation, dosage	Age (years)	No. of subjects	Postvaccination reactions									
			Rise in temperature						Local			
			37.1–37.5 °C		37.6–38.5 °C		38.5 °C and higher		Hyperaemia > 2.5 cm		Infiltrate > 2.5 cm	
			abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Vaccine 6–8 μg HA (0.1 ml)	18–22	185	11	5.9	0	0	0	0	5	2.7	1	0.5
	15–16	226	27	11.9	1	0.4	0	0	4	1.8	0	0
	11–14	182	33	12.6	0	0	0	0	1	0.5	0	0
	9–10	102	3	2.9	0	0	0	0	0	0	0	0
Vaccine 12–16 μg HA (0.2 ml)	18–22	196	17	8.7	3	1.5	0	0	11	5.6	2	1.1
	15–16	207	15	7.2	0	0	0	0	3	1.4	0	0
	11–14	224	6	2.7	0	0	0	0	2	0.9	0	0
Placebo (0.1 ml)	18–22	161	24	14.9	3	1.9	0	0	0	0	0	0
	15–16	113	5	4.4	0	0	0	0	0	0	0	0
	11–14	87	7	8.0	2	2.3	0	0	0	0	0	0
	9–10	55	0	0	0	0	0	0	0	0	0	0
Placebo (0.2 ml)	18–22	143	19	13.3	2	1.4	0	0	0	0	0	0
	11–16	106	16	15.1	1	0.9	0	0	0	0	0	0

the circulation of acute respiratory disease viruses in the groups under observation.

Local reactions were expressed by hyperaemia and infiltrates in the injection site. Slight local reactions expressed by only hyperaemia were found in 0.5—2.7% of the subjects inoculated with a standard vaccine dose, in 0.9—5.6% of those vaccinated with a double dose. Marked local reactions characterized by an infiltrate of more than 2.5 cm diameter were observed only in adults — in 1 subject vaccinated with 0.1 ml (0.5%) and in 2 subjects given 0.2 ml (1.1%). No local reactions were observed in control subjects.

It is noteworthy that, in contrast to adult subjects, increased vaccine dose did not entail enhanced reactogenicity neither in children aged from 11 to 14 years nor in adolescents. Weak reactogenic properties of IICV could be considered for indirect evidence of its safety. It is known that immunization with antiviral vaccines may cause short-term changes in clinical laboratory analyses. No pathologic changes in any of the haematologic or biochemical tests have been seen in any of the three examinations of subjects inoculated with either standard or double dose of the vaccine. Variations of red blood cell count, haemoglobin, leukocytes, formed elements of blood and ESR

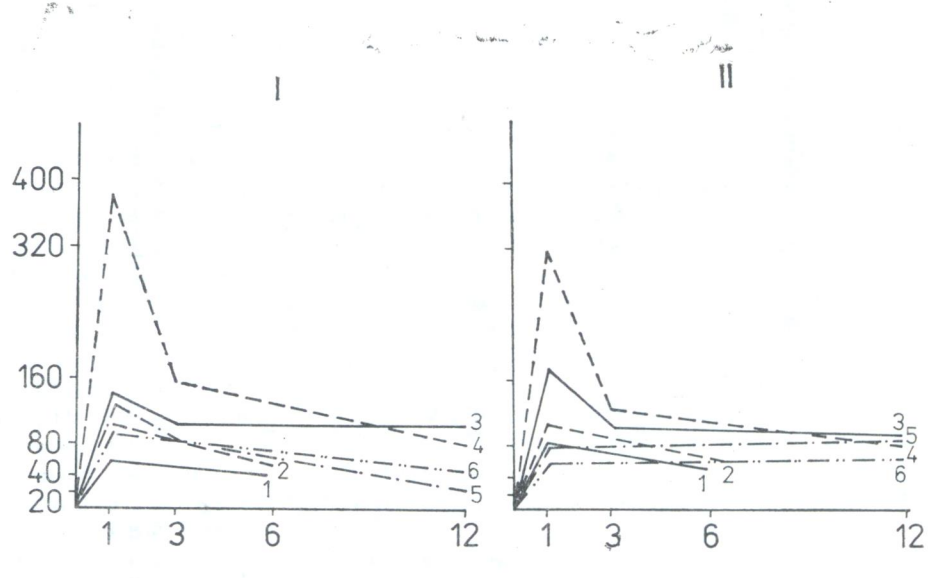

Fig. 1.

Development of antiinfluenza antibodies in sero-negative 11—12-year-old subjects inoculated with different IICV doses

I — to strain A (H1N1), II — to strain A (H3N2).

Abscissa: observation time (months); ordinate: mean geometric antibody titre. 1 — age 18—22 years, dose 0.1 ml; 2 — age 18—22 years, dose 0.2 ml; 3 — age 15—16 years, dose 0.1 ml; 4 — age 15—16 years, dose 0.2 ml; 5 — age 11—14 years, dose 0.1 ml; 6 — age 11—14 years, dose 0.2 ml.

Table 2. Antigenic activity of IICV in seronegative subjects

Preparation dosage	Age (years)	No. of subjects	No. of seronegative subjects to strain		Seroconversion to strain (percentage)		Mean geometric titre of antibodies to strain				Mean increment of antibodies to strain	
			A/Kiev/59/79R (H1N1)	A/Leningrad/385/80R (H3N2)	A/Kiev 59/79R (H1N1)	A/Leningrad/385/80R (H3N2)	A/Kiev/59/79R (H1N1)		A/Leningrad/385/80R (H3N2)		A/Kiev/59/79R (H1N1)	A/Leningrad 385/80R (H3N2)
							I	II	I	II		
Vaccine	18—22	178	131	156	68.7	76.9	8.6	60.0	7.5	84.0	6.9	11.2
6—8 μg HA	15—16	176	176	176	73.3	85.8	13.0	147.0	9.2	169.0	11.3	18.4
(0.1 ml)	11—14	157	135	140	88.2	88.6	8.0	128.0	7.5	79.0	16.0	10.5
	9—10	92	83	37	84.3	83.8	6.5	112.0	14.9	128.0	17.2	8.6
Vaccine	18—22	178	144	159	75.7	83.7	8.6	97.0	7.5	104.0	11.3*	13.8
12—16 μg HA	15—16	186	186	186	83.8*	88.2	13.9	388.0	10.6	315.0	27.9*	29.7*
(0.2 ml)	11—14	170	138	102	73.9*	63.7*	7.5	97.0	10.6	60.0	12.9*	5.7*
Placebo	18—22	244	207	224	20.2	24.5	7.5	13.9	6.5	11.3	1.8	1.7
(altogether	15—16	153	124	140	16.9	15.0	8.0	10.6	6.5	8.6	1.3	1.3
0.1—0.2 ml)	11—14	65	58	60	18.9	26.6	6.5	12.1	7.0	16.0	1.9	2.3
	9—10	55	53	42	5.7	11.9	6.1	7.0	13.9	16.0	1.1	1.2

Note. Asteriks marks significant differences between the indices of increase or decrease in corresponding age groups. I and II designate antibody titres in sera collected before (I) vaccination and 1 month after (II) vaccination.

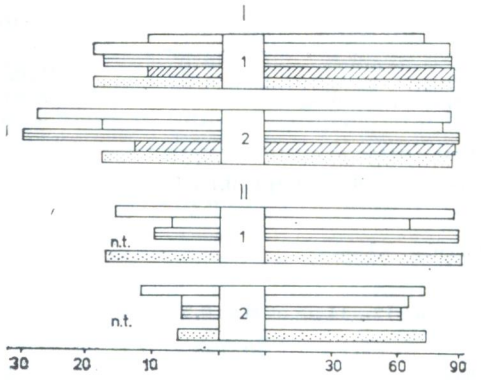

Fig. 2.
Characterization of humoral immune response in sero-negative children and adolescents
I — age 15—16 years, II — age 11—14 years; 1 = 6—8 μg HA; 2 = 12—16 μg HA. Left — increment of antibodies, right — percentage of seroconversions. A/Kiev/59/79R (H1N1) — white columns, A/Khabarovsk/74/77 (H1N1) — black columns, A/Leningrad/385/80R (H3N2) — columns with horizontal section-lining, A/Leningrad/X/83 (H3N2) — dotted columns, A/Bangkok/1/79 (H3N2) — dashed columns
N.t. — not tested.

were within the limits of the age norm. The levels of urea, sialic acids or alanine amino transferase in blood did not undergo significant changes throughout the observation time. No significant alterations of the protein content or protein fractions levels have been observed. An insignificant increase in the content of gamma-globulin and other fractions observed in post-vaccination period was within the limits of physiologic variations. No pathologic changes in urine composition were found in the subjects regardless of the dose injected.

Thus, clinical laboratory analyses indicate the safety of single IICV administration whether the subjects were given 6—8 or 12—16 μg of HA.

While testing the antigenic properties of the vaccine it seemed the most interesting to study the character of humoral response to administration of 2 vaccine strains of influenza virus. Two-fold increase of virus dose in the vaccine appeared to enhance the frequency of immune response in vaccinated subjects by an average of 2.4—10.5%. A significant increase in the number of seroconversions was to vaccine strain A/Kiev/59/79R(H1N1) found in 15—16 years old adolescents only. The intensity of humoral immune response to vaccine strains was largely dependent on the antigen dose. In subjects aged 18—22 and 15—16, who were given a standard vaccine dose, the mean geometric titres of antibodies against A/Kiev/59/79R(H1N1) increased 6.9—11.3-fold, whereas on injection of a double vaccine dose they increased reaching 11.3—27.9 times higher values. The titre of antibodies to strain A/Leningrad/385/80R(H3N2) increased 11.2—18.4-fold and 13.8——18.4-fold respectively.

The effect of the double dose was different in school children aged 11 to 14 years. While production of antihaemagglutinins to injection of standard IICV dose was the same as in adolescents (Table 2), the frequency and intensity of immune response to a double dose were in this age group significantly lower than in other age groups.

The increment of mean geometric titres of antibodies decreased from 10.5—16.0 to 5.7—12.9 times (Table 2). The data indicate caution at vaccination of 9—10 year-old school children with a double dose. It should be

Table 3. IDMC test in rats

Preparation, dosage	Number of vaccinated subjects	Observation time (days)	IDMC	
			negative	positive
Vaccine, 6—8 μg of HA, 0.1 ml	19	0	19	0
		30	19	0
Placebo, 0.1 ml	10	0	9	1 (23.7)*
		30	9	1 (17.7)
Vaccine, 12—14 μg of HA, 0.2 ml	18	0	18	0
		30	18	0
Placebo, 0.2 ml	20	0	19	1 (20.0)
		30	19	1 (21.0)

Note. In parentheses the percentage of degranulated mast cells.

noted that marked stimulation of immune response in 15—16 year-old adults and adolescents achieved by increasing vaccine dose was short--lasting. Three to six months after vaccination the differences in GMT of antibodies were statistically insignificant and after 12 months the titres were essentially identical in all groups compared (Fig. 1). The GMT of antibodies in all the vaccinated groups was, however, higher than the protective effect 1 year after vaccination ($\geqq 1 : 40$).

For the study of the range of protective spectrum of post-vaccinal humoral response the sera of vaccinated adolescents were additionally examined by HI test with a set of influenza virus strains antigenically related to the vaccine strains. Testing of more than 700 paired sera has shown that a double increase of antigenic load in the vaccine up to 12—16 μg of HA content was without effect on the frequency or intensity of antibody production to related influenza virus strains (Fig. 2). High parameters of immunologic protection were also observed with an avid diagnostic strain A/Leningrad/X/83(H3N2), a drift variant isolated later.

The safety of influenza vaccinal preparations can be also confirmed by the absence of allergenic effect on the organism of vaccinated subjects. Testing of sera of subjects vaccinated with either standard or double dose of centrifugal IIV in IDMC has shown that contact of mast cells with sera of most volunteers in the presence of the vaccine failed to enhance their degranulation at all the intervals (Table 3). At the same time, serum samples of 2 subjects of control group collected before and after immunization showed a positive IDMC reaction (17.7—23.7% and 20—21% degranulated mast cells, respectively).

Discussion

IIV purified and concentrated by gradient centrifugation was designed for influenza prevention in adults (Sokolov *et al.*, 1973). Epidemiologic data

obtained in 1978—80 from workers of large industrial enterprises indicate that IICV is areactogenic and has a high preventive activity. Modern complex prophylactic influenza immunization, however (Shadrin *et al.*, 1984), resulted in the appearance of a high proportion of immune subjects and the protection against influenza of adolescents and school children. Thorough safety studies of the vaccine in different age groups should be a prerequisite for substantiation of the feasibility of IICV employment in children and establishment of child-proportioned dosages.

The data obtained indicate that the weak reactogenicity of both doses, i.e. of 6—8 μg of HA and of the double IICV dose are in full accord with the results of American researchers who investigated different age groups (Quinnan *et al.*, 1983; Wright *et al.*, 1983). In the paper of Uno (1985) only a tendency towards a dose-dependent increase in reactogenicity has been established for different influenza vaccines.

According to the results of serologic studies in adults and children inoculated with IICV the increase in antigenic load of the vaccine has some advantages in terms of stimulation of humoral immune response in subjects of 15—22 years of age. These advantages are evident from an augmentation of the increment of GMT of antibody 1 month after vaccination. The studies on the time course of antibody persistence have shown that stimulating effect is short-lasting and already within 3 to 6 months the GMT of antibodies significantly decreased in subjects inoculated with a double dose. Twelve months after immunization the GMT of specific antibodies in subjects vaccinated with either standard or double IICV dose were essentially equal.

La Montagne *et al.* (1983), Cate *et al.* (1983) and Jennings *et al.* (1985) came to analogous conclusions. They reported that two-fold and even four-fold increase of antigenic content in the influenza vaccine preparations failed to enhance significantly the immunologic response in the vaccinated subjects. According to the data of Gremillion and coworkers (1983) the increase in NIV antigenic load resulted in a broad spectrum of antibodies produced owing to the stimulation of specific humoral response to closely related influenza viruses in vaccinated subjects. The rate of seroconversions and the level of GMT of antibodies to the related diagnostic strains were essentially independent of the antigen dose as shown by testing the sera of vaccinated subjects with the diagnostic strains A/Bangkok/1/79 (H3N2), A/Leningrad/X/83 (H3N2) and A/Khabarovsk/74/77 (H1N1). The absence of stimulation of heterologic antibodies seems to be due to the fact that antigen dosages under comparison were within relatively narrow range or that strains used in serologic tests were closely related.

Using IMCD test in rats allowed us to demonstrate that single administration of the vaccine in the doses tested was without allergenic effect. These data are in accord with the results of previous research carried out in limited groups of vaccinated subjects (Perepelkin, 1979; Zosimenko, 1982; Shpilyuk *et al.*, 1986). At the same time, the results indicate that a part of the population was sensibilized to influenza virus or to contaminating components contained in the vaccine.

Thus the observations made in different age groups suggest that single administration of IICV in either standard or double dosage was low reactogenic, safe according to all biochemical and haematologic parameters and caused no instant allergic reactions in rat IMCD test. Injection of a double IICV dose to 15—22-year-old subjects lead to a somewhat more marked stimulation of humoral response. A new "Recommendation for IICV Application" has been compiled and approved on the basis of the data obtained in this paper. In the new version the number of contraindications against vaccinations of adults have been considerably reduced and also the vaccination of children has been authorized (standard dose, starting with 11-year-old children).

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