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Testing the Safety and Effectiveness of Oral Administration of a Live Influenza Vaccine *

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Tests to determine the reactogenicity and immunogenicity of a live influenza vaccine when administered by mouth showed that the new method caused no reactions in adults and children and was adequately immunogenic, the results being in no way inferior to those obtained by intranasal administration. These results provide a basis for the wider use of this simpler and more convenient method of administration.

Oral administration of the live influenza vaccine was followed by reproduction of the vaccine virus. The rapid elimination of catarrhal symptoms existing before vaccination indicates intensive production of interferon in the nasopharynx of the vaccinated persons.

The standard influenza vaccine for adults induced strong reactions in children, even when the less dangerous oral route of vaccination was used. For oral vaccination of children it is thus essential to use the variant of the live influenza vaccine made from further-attenuated, cold-adapted vaccine strains of influenza virus. This vaccine is quite safe and effective whether administered intranasally or by mouth.

Investigations by Selivanov, Kugel' & Skrjabina (1961) showed that live polyvalent adenovirus vaccine made from serotypes 3, 4 and 7 was highly immunogenic when given orally.

The clinical reactions to vaccine that develop regularly when similar vaccine strains are administered by the respiratory route were completely eliminated. These results have been confirmed by Couch et al. (1962) and Chanock et al. (1966). Since 1967, live orally-administered adenovirus vaccine and live influenza vaccine have been studied together. At first an enteric-coated preparation designed to allow the virus to pass freely through the stomach and to enter the small intestine was used (Selivanov, Gipp & Lozanovskaja, 1967). In view of the low immunogenicity of the influenza vaccine when administered by this route, a liquid influenza vaccine, also administered orally, was subsequently used with success (Aleksandrova et al., 1968). Oral administration of a live influenza vaccine (tissue-culture type) has been studied independently at the Institute for Research on Virus Preparations, Moscow (Alekseeva et al., 1968).

The present paper records the results of study of intranasal and oral administration of live allantoic-fluid influenza vaccine to adults and children.

MATERIALS AND METHODS

The vaccine

Adults were vaccinated with a freeze-dried live influenza vaccine prepared at the Leningrad Research Institute for Vaccines and Sera from strains A2/21, B/95 and B/Lih which have been used in the USSR to manufacture a standard preparation for immunizing the population over 16 years of age. The experimental batches were prepared from strains A2/133/65 and B2/67. The vaccine for oral administration was used either in the dried state (for preparing an enteric-coated preparation) or in the liquid state after the dried preparation had been dissolved in a volume equal to the initial volume of allantoic fluid. Some of the experiments were carried out with the original allantoic fluid obtained from developing chick embryos infected with vaccine strains of influenza virus.

Children were vaccinated with a vaccine manufactured from variants of the vaccine strains A2/21/17 and B/14/17 that had been further attenuated.

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All these vaccines were prepared in developing chick embryos on the basis of instructions approved by the Vaccines and Sera Committee of the Ministry of Health of the USSR. Tests were made with the A2 and B monovalent vaccines or the A2+B divalent vaccine obtained by pooling equal volumes of the liquid monovalent vaccines immediately before administration.

The enteric-coated vaccine was prepared by filling gelatine capsules with 0.5 g of dried vaccine. To prevent the capsules dissolving in the mouth and stomach they were processed with a 10% solution of cellulose-acetate-phthalate in acetone.

Method of vaccination

Two groups of healthy adults aged 20–45 years and 2 groups of children aged 3–7 years were selected by random sampling. The persons in the first group were given the live influenza vaccine and those in the second group (the controls) were given a placebo (normal allantoic fluid from developing chick embryos), which was administered in the same volume and by the same method as the vaccine. For intranasal vaccination the vaccine was diluted immediately before use in 5 times its volume of physiological salt solution and 0.25 ml of the diluted vaccine was sprayed into each nasal passage from a glass Smirnov spray. For oral vaccination a teaspoonful of the liquid preparation (2.0 ml) was given on each occasion. The vaccination was repeated 2 or 3 times at intervals of 7–10 days.

For 5 days following vaccination, the vaccinated persons were kept under medical supervision. Every day they were examined and their temperatures were taken: in some cases the observations were supplemented by laboratory tests.

Virus isolation from the throats of vaccinated persons

Throat swabs were taken 24 h, 48 h, 72 h and 96 h after vaccination and the material was emulsified in 2 ml of a buffer solution. After being centrifuged for 15 min at 5000 rev/min the clarified material was injected in volumes of 0.5 ml into the allantoic cavity of chick embryos 10–12 days old. After incubation at 33°C for 48 h–72 h the allantoic fluid was subjected to the haemagglutination test with chick erythrocytes. If the result of the haemagglutination test was negative a further passage of the allantoic fluid in fresh embryos was carried out. If the result of the haemagglutination test was positive, the strains isolated were identified by means of the haemagglutination-inhibition test with specific immune sera against the vaccine strains used.

Isolation of the virus from the faeces of vaccinated children

Faecal specimens were collected 48 h, 72 h and 96 h after vaccination and placed in flasks. The content of each flask was mixed with quartz sand, antibiotics were added (2000 IU/ml of penicillin and 1000 μg/ml of streptomycin) and the material was emulsified in 5 ml–7 ml of physiological saline and then shaken on a shaker for 15 min. The emulsion was then centrifuged and injected into the chick embryos in the same way as for isolation of virus from throat swabs.

The immunogenic potency of the influenza vaccine was determined on the basis of changes in the antihaemagglutinin level in paired sera from the inoculated persons, the first taken before the first vaccination and the second 20–30 days after the final vaccination.

Method of carrying out the haemagglutination-inhibition test

Specimens of freshly obtained allantoic fluid from developing chick embryos infected with inhibitor-resistant strain A2/21/17 and with viruses B/14/17, B/95 and B/2/67 served as antigens in this test.

The test sera were heated for 30 min at 58°C to remove any non-specific thermolabile inhibitors of haemagglutination. The heat-stable inhibitors of A2 influenza virus were not removed, since an inhibitor-resistant variant was being used in the test. Two-fold serial dilutions of the serum were made, ranging from 1 : 8 to 1 : 512, and placed in wells cut in Perspex panels; a dose of 4 haemagglutinating units of the working dilution of viruses A2 and B was then added in each well. The results of the test were read after 18 hours' contact at 4°C. In titrating the antibodies to the A2 virus, human red cells of the O group were used. Chick red cells were used in titrating antibodies to the B virus.

RESULTS

The reactogenicity of orally administered live influenza vaccine

Oral administration of liquid or enteric-coated allantoic-fluid vaccine proved fully safe for adults and caused no clinical reactions to vaccination. A total of 528 persons was vaccinated but in no case

did the temperature rise above 37.5°C. The occasional cases of slight temperature reactions (up to 37.5°C) were not accompanied by catarrhal symptoms or intoxication.

Clinical laboratory tests of the urine from vaccinated persons showed no change. In the peripheral blood there was occasionally moderate neutrophilic leucocytosis and less often a tendency towards leucopenia; the white blood cell picture remained substantially unchanged. Very occasionally there was aneosinophilia, relative lymphocytosis and the occurrence of single plasmacytes. The erythrocyte counts and haemoglobin values and the erythrocyte sedimentation rates were within normal limits. The activity of alanine and aspartate transaminases in the blood serum of the vaccinated persons also remained within the normal range.

The testing of the reactogenicity of orally administered influenza vaccine among children started in boarding schools and was continued in kindergartens in pre-school-age groups of children aged 3–7 years. The most detailed test was concerned with the reactogenicity for children of the further-attenuated forms of live influenza vaccine made from cold-adapted vaccine strains developed by the authors in 1962–67 and which had proved harmless for children when administered intranasally (Aleksandrova, 1961, 1965, 1968; Smorodintsev, 1965). Table 1 shows the results of clinical examination of 256 vaccinated children aged 3–7 years and 171 children in a similarly constituted control group, drawn from the same children's establishments. Oral administration of the live vaccine to children of pre-school age proved completely harmless, even when a dose of vaccine 20 times as large as that used for intranasal administration was employed. There were only rare cases among the vaccinated children of slight reactions to vaccination in the form of a transient rise in temperature. The difference in the frequency of the more marked feverish reactions (rise of temperature to above 37.5°C) in the groups of vaccinated and unvaccinated children did not exceed 0.5%–1.5%.

Oral vaccination of children with the further-attenuated variant of the influenza vaccine was not accompanied by any intoxication or disturbance in the general state of health. Catarrhal symptoms in the form of a cough or a cold in the nose occurred rarely and were extremely slight and short-lived. In some cases slight local inflammatory reactions developed in Waldeyer's tonsillar ring and in the eyes (injection of the scleral vessels, marginal conjunctivitis, congestion of the faucial mucosa, granulation in the posterior wall of the pharynx, and the palatoglossal arches and sometimes swelling of the tonsils). It should be noted that these symptoms also occurred quite frequently in the unvaccinated children but their intensity was more varied.

Laboratory examinations of material from the vaccinated children showed the absence of any pathological changes in the urine and only slight deviations in the peripheral blood (a moderate fluctuation in the total leucocyte count without any essential change in the white blood cell picture). The indices of transaminase activity remained at their initial level. In some cases during the post-vaccination period, a slightly positive reaction to carbon-reactive protein was discovered.

All these changes in the blood were short-lived. Thus the special variant of the live influenza vaccine proved completely harmless for children aged 3–7 years. On the other hand oral administration to children of the standard live influenza vaccine used for immunizing adults led to frequent and intensive vaccination reactions in 3%–16% of the children (Table 1).

The most characteristic clinical response to oral vaccination in children was a slight and transient enlargement of the submandibular lymph nodes. This symptom occurred in 30%–70% of vaccinated children, 1 or 2 days after vaccination (Table 2). The intensity and frequency of the occurrence of this symptom were directly related to the type of influenza vaccine and were more marked with the standard vaccine.

Enlargement of regional lymph nodes may be ascribed to resorption of the influenza toxin as a result of the intensive multiplication of the virus in Waldeyer's pharyngeal lymphatic ring: the virus was isolated regularly from the nasopharynx in the 4 days following the beginning of oral vaccination (Table 3).

Attempts to isolate the vaccine virus from the faeces of persons to whom the liquid or enteric-coated preparation had been administered were not successful. This indicated that the virus does not reproduce actively in the intestines.

Oral administration of the special variant of the live influenza vaccine to children stimulated interferon production. This was shown not only by the regular discovery of interferon in throat swab material but also by the interesting fact that catarrhal symptoms present in the children before vaccination were frequently eliminated. Thus in one children's

TABLE 1

INDICES OF REACTOGENICITY OF THE LIVE INFLUENZA VACCINE FOR CHILDREN 3–7 YEARS OF AGE FOLLOWING ORAL ADMINISTRATION

Strain	No. of children	Intensity of the temperature reaction								Frequency of various symptoms											
		No reaction		Weak reaction (<37.5°C)		Moderate reaction (37.6°C–38.5°C)		Strong reaction (≥38.5°C)		Conjunctivitis		Rhinitis		Congestion of faucial tissues		Congestion of posterior wall of pharynx		Changes in indices of pulmonary function		Symptoms of intoxication	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Further-attenuated live influenza vaccines (for children)																					
A2/21/17	62	48	77.4	13	21.0	1	1.6	0	0	3	4.8	7	11.3	16	25.8	9	14.5	6	9.7	0	0
Placebo	29	20	69.0	8	27.6	1	3.4	0	0	1	3.4	4	13.8	6	20.7	0	0	2	6.8	0	0
B/14/17	41	36	87.8	5	12.2	0	0	0	0	2	4.9	7	17.1	8	19.5	5	12.2	6	14.6	0	0
Placebo	17	15	88.3	2	11.8	0	0	0	0	3	17.7	3	17.6	6	35.3	1	5.9	2	11.8	0	0
A2/21/17 + B/14/17	111	85	76.6	23	20.7	2	1.8	1	0.9	8	7.2	17	15.3	28	25.2	8	7.2	5	4.5	0	0
Placebo	71	58	81.8	12	16.9	1	1.4	0	0	10	14.1	12	17.0	18	25.4	3	4.2	4	5.6	0	0
Standard vaccines (for adults)																					
A2/21	18	11	61.1	6	33.3	1	5.6	0	0	3	16.7	1	5.6	2	11.1	1	5.6	1	5.6	1	5.6
Placebo	40	31	77.5	8	20.0	1	2.5	0	0	2	5.0	1	2.5	1	2.5	0	0	1	2.5	0	0
B/Lih + B/95	24	15	62.5	5	20.8	0	0	4	16.7	4	16.7	6	25.0	6	25.0	1	4.2	2	8.3	4	16.6
Placebo	14	9	64.3	5	35.7	0	0	0	0	2	14.3	4	28.5	5	35.7	0	0	1	7.1	0	0

TABLE 2

ENLARGEMENT OF REGIONAL SUBMANDIBULAR LYMPH NODES FOLLOWING ORAL VACCINATION OF CHILDREN AGED 3–7 YEARS

Name of vaccine strain	Children given vaccine by mouth				Unvaccinated children		
	No. of children in group	With enlarged lymph nodes			No. of children in group	With enlarged lymph nodes	
		No.	%	95 % Confidence limits		No.	%
Further-attenuated live influenza vaccines							
A2/21/17	51	22	43.1	29.35–57.75	29	0	0
B/14/17	41	12	29.3	16.13–45.54	17	0	0
A2/21/17+B/14/17	111	57	51.4	41.9 –60.7	71	0	0
Standard vaccines							
A2/21	18	12	66.67	40.99–86.66	40	0	0
B/95+B/Lih	24	17	70.8	48.91–87.38	14	0	0

TABLE 3

ISOLATION OF THE INFLUENZA VIRUS FROM THE NASOPHARYNX OF CHILDREN VACCINATED ORALLY OR INTRANASALLY WITH MONOVALENT VACCINES A2/21/17 AND B/14/17, 48–72 HOURS AFTER VACCINATION

Method of vaccination	Vaccine serotype	No. of children vaccinated	Indices of virus isolation		
			No.	%	95 % Confidence limits
Oral	A2	19	13	68.4	43.45–87.42
	B	22	12	54.51	32.21–75.61
Intranasal	A2	50	23	46.0	31.81–60.68
	B	50	21	42.0	28.19–56.79

establishment where vaccination coincided with increased morbidity from acute respiratory infections a swift elimination of catarrhal symptoms occurred in 20 out of the 29 children vaccinated, whereas among 14 children in the control group the intensity of those symptoms increased in 6 children (Table 4).

Immunogenicity of the vaccine when administered by mouth

The vaccine made from further-attenuated strains of influenza virus was shown to be highly immunogenic whether administered to children intranasally or by mouth. When children were given the monovalent vaccine A2/21/17, or the divalent vaccine made up of strains A2/21/17 and B/14/17, by mouth an increase of 4-fold or more in the level of antibodies to the A2 vaccine variety occurred in all the serologically negative children. An increase in antibodies to the B vaccine occurred in 50% of the serologically negative children vaccinated with the monovalent vaccine B/14/17 or with the divalent A2 and B vaccine (Table 5).

Table 6 summarizes the results of comparative tests in adults of the immunogenicity of the standard live influenza vaccine administered intranasally or by mouth. When the live influenza vaccine was administered as an enteric-coated preparation, and thus did not come into contact with the tissues of the upper portion of the digestive tract, the oesophagus or the stomach, it showed markedly less immunogenic potency than when given intranasally. Oral administration of the liquid preparation, however, led to intensive production of antihaemagglutinins

TABLE 4

EFFECT OF ORAL VACCINATION WITH LIVE INFLUENZA VACCINE ON CATARRHAL SYMPTOMS IN CHILDREN

Groups of children	No. with catarrhal symptoms before vaccination	Intensity of catarrhal symptoms in 5 days after vaccination					
		Disappeared		Intensified		No change	
		No.	%	No.	%	No.	%
Vaccine given by mouth	29	20	64.0	1	3.4	8	27.6
Unvaccinated	14	2	14.3	6	42.9	6	42.9

TABLE 5

IMMUNOGENICITY OF THE FURTHER-ATTENUATED LIVE INFLUENZA VACCINES FOR CHILDREN 3–7 YEARS OF AGE WHEN THE VACCINE WAS GIVEN ORALLY OR INTRANASALLY [a]

Type of vaccine	Method of vaccination	Virus used in haemagglutination-inhibition test	Serologically negative persons				Average index of increase in antibody level	Mean antibody titre after vaccination
			Total no.	With 4-fold or more increase in antibodies				
				No.	%	95 % Confidence limits		
Divalent vaccine A2+B	Intranasal	A2/21/17	28	25	89.3	71.77–97.73	6.5	69.0
		B/14/17	154	94	61.0	53.2 –68.8	4.0	24.2
	Oral	A2/21/17	25	25	100.0	86.28–100.0	9.8	91.0
		B/14/17	70	31	44.3	32.41–56.66	2.5	13.0
Monovalent vaccine A2	Intranasal	A2/21/17	43	37	86.0	72.07–94.70	11.3	97.0
	Oral	A2/21/17	12	12	100.0	73.54–100.0	52.0	388.0
Monovalent vaccine B	Intranasal	B/14/17	30	20	66.67	47.19–82.71	7.0	42.0
	Oral	B/14/17	28	14	50.0	30.65–69.35	2.7	8.6

[a] Based on the haemagglutination-inhibition test with paired sera.

in the blood of 53 %–68 % of the persons vaccinated.

These results give grounds for supposing that the tissues in the upper portion of the digestive tract are considerably more sensitive to the development of vaccine infection and the reproduction of the influenza virus than the intestinal tract. For that reason the oral method of administering the live influenza vaccine deserves special attention because of its great simplicity and its immunogenic effectiveness.

DISCUSSION

The effectiveness of the live influenza vaccine largely depends on the method of administration. The generally adopted intranasal method of administering a sufficiently high dose of attenuated virus stimulates intensive production of anti-influenza virus-neutralizing antibodies, not only in the blood, but, what is more important, in the nasopharynx of the vaccinated person, thus leading to the development of local immunity to influenzal infection in the respiratory tract.

The work reported in this paper showed the effectiveness of oral administration of live influenza vaccine; this was demonstrated in adults by intensive antibody production in the blood and the complete absence of clinical reactions following vaccination. The effect was considerably weaker following vaccination with an enteric-coated preparation. This finding indicates that the vaccine virus multiplies directly in the upper portion of the digestive tract, and this is confirmed by the frequent enlargement

TABLE 6

IMMUNOGENICITY OF LIVE INFLUENZA VACCINES FOR ADULTS WHEN ADMINISTERED INTRANASALLY OR ORALLY

Type of vaccine	Method of vaccination	Virus used in haemagglutination-inhibition test	Total no. vaccinated	Serologically negative persons			
				Total no.	With 4-fold or more increase in antibodies		
					No.	%	95 % Confidence limits
A2/133 + B/2	Oral (in capsules)	A2/133	260	108	26	24.1	15.7 –32.5
		B/2	265	64	6	9.4	3.52–19.30
	Intranasal	A2/133	204	118	52	44.1	35.1 –53.1
		B/2	204	127	90	70.9	62.90–78.9
A2/21 + B/95 + BLih	Oral (liquid vaccine)	A2/21	190	25	17	68.0	46.50–85.05
		B95	186	30	16	53.3	34.33–71.66
	Intranasal	A/21	150	60	27	45.0	32.12–58.39
		B95	149	89	53	59.6	48.62–69.83

of the submandibular lymph nodes in children vaccinated with the vaccine for adults and by the ease and frequency with which the vaccine virus can be isolated from the nasopharynx of vaccinated subjects.

The tests in children of the live influenza vaccine made from further-attenuated vaccine strains of A2 and B influenza established the safety and high degree of immunogenicity of that type of vaccine when given intranasally or by mouth. The standard live influenza vaccine, however, caused quite a few reactions in children even when given by mouth, although the reactions were then milder than when it was given intranasally.

These results make it clear that the testing of oral live influenza vaccines in the form of a monovalent preparation or in association with other virus vaccines should be extended to determine their effectiveness in the prevention of naturally developing outbreaks of influenza. Standard egg-adapted influenza vaccine can be used for oral vaccination and as the production of this is already well established no new techniques such as the tissue cultures used by Alekseeva et al. (1968) would be required.

RÉSUMÉ

ÉVALUATION DE L'INNOCUITÉ ET DE L'EFFICACITÉ DE L'ADMINISTRATION PAR VOIE BUCCALE D'UN VACCIN ANTIGRIPPAL VIVANT

On a administré à des adultes âgés de 20 à 45 ans un vaccin antigrippal vivant du type habituellement utilisé en URSS, et à des enfants de 3 à 7 ans un vaccin vivant suratténué, en utilisant soit la voie buccale soit la voie intranasale. Des groupes témoins ont reçu un placebo.

Donné par voie buccale, le vaccin n'a provoqué aucune réaction postvaccinale chez les adultes.

Chez les enfants, seuls des symptômes très bénins — élévation passagère de la température — ont été observés, même lorsque la dose a été portée à 20 fois la dose administrée par voie intranasale. On n'a constaté aucune altération notable de l'urine et du sang. Du point de vue clinique, la réaction la plus caractéristique a consisté en une hypertrophie légère et transitoire des ganglions lymphatiques sous-maxillaires. Ce signe, observé chez 30 à 70% des enfants, est attribué à une résorption de toxine, conséquence de la multiplication intensive du virus au niveau du cercle amygdalien de Waldeyer. Le virus a été isolé régulièrement du nasopharynx pendant les 4 premiers jours suivant la vaccination.

La vaccination par voie buccale a suscité une forte production d'interféron, avec disparition fréquente des symptômes catarrhaux présents chez certains enfants, et une réponse immunitaire aussi satisfaisante que la vaccination par voie intranasale.

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