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SIMULTANEOUS APPLICATION OF INFLUENZA CHEMOVACCINE AND "VAXIGRIP" PREPARATIONS WITH BCG

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Summary. — Experiments in guinea pigs and rabbits have shown that simultaneous immunization against influenza and tuberculosis had no effect on the reactogenic properties of both vaccines. No inhibitory effect of the BCG vaccine on the influenza virus antibody production was observed. Production of antituberculosis antibodies was the most intensive following a simultaneous immunization of animals with BCG and inactivated influenza vaccines. Both separate and simultaneous immunization conferred to animals a similar grade of protection against virulent tuberculosis mycobacteria. Application of the combined preparation "Vaxigrip" & BCG (in mixture) caused complicated local reactions and had little immunogenic effect.

Key words: *influenza*; *tuberculosis*; *vaccines*; *antibody*

Introduction

The efforts of investigators in our country and abroad resulted in the development of new inactivated influenza vaccines (Hannoun, 1973; Isupov *et al.*, 1973; Sokolov *et al.*, 1973; Kilbourne, 1975) intended for immunization of adults. At the same time it is known that persons aged 18—19 are subject to compulsory revaccination against tuberculosis. The interval between administration of BCG and any other vaccine must be not less than 2 months. Simultaneous administration of influenza and BCG vaccines would first of all ensure a 100% coverage with influenza immunization of young people in closed communities (military recruits, students, etc.) during the period prior to an influenza epidemic. The present investigations were aimed at studying a Soviet adsorbed chemical influenza (ACI) vaccine and a French preparation "Vaxigrip" in a regimen of simultaneous application with BCG vaccine.

Materials and Methods

Animals. 140 guinea pigs and 24 rabbits were utilized. To compare the efficacy of each influenza vaccine administered either separately or in combination with the BCG vaccine, all animals were divided into equal groups (Table 1). The following preparations were used: 1. Dry BCG vaccine for intracutaneous (i.c.) administration; 2. ACI monovaccine from A/Victoria/35/72

Table 1. Design of vaccination in animals

Vaccination schedule	Animal species	No.	Vaccine			
			Volume (ml)	Route of application	No. of vaccinations	Interval (in days)
ACI-vaccine only	Guinea pigs	20	0.5	s.c.	2	30
ACI-vaccine & BCG (simultaneously)		20	Analogous to separate vaccination			
"Vaxigrip" only		20	0.2	i.c.	2	30
"Vaxigrip & BCG (simultaneously)		20	Analogous to separate vaccination			
"Vaxigrip" & BCG (in mixed preparation)		20	0.3	i.c.	1	In mixture
BCG		20	0.1 mg	i.c.	1	—
Placebo (control)		20	0.5	s.c.	2	30
ACI-vaccine	Rabbits	4	0.5	s.c.	2	30
ACI-vaccine & BCG (simultaneously)		4	Analogous to separate vaccination			
"Vaxigrip"		4	0.5	i.v.	2	30
"Vaxigrip" & BCG (in mixed preparation)		4	1.5	i.v.	1	In mixture
BCG		4	0.5 mg	i.v.	1	—
Placebo (control)		4	0.5	s.c.	2	30

(H3N2) influenza virus, lot No. 37, produced in the I. I. Mechnikov Research Institute, Ufa. One dose of the vaccine contained 2560 haemagglutination units (HAU); 3. Inactivated influenza bivaccine ,,Vaxigrip"-2 (France) purified by zonal centrifugation and containing 17 500 HAU of A/Port Chalmers/1/73 (H3N2) influenza virus and 12 500 HAU of B/Hong Kong/8/73 influenza virus per dose. The "Vaxigrip" vaccine containing no adsorbent has been studied in a regimen of simultaneous and combined administration with BCG. 4. Buffered saline was used as placebo.

Vaccination design. Simultaneous administration of two vaccines was timed to the first immunization. For combined immunization, the two vaccines were mixed in the syringe prior to administration. Guinea-pigs were injected with the vaccines intracutaneously (i.c.) or subcutaneously (s.c.) in the area of carefully depilated abdomen. When the two vaccines were administered simultaneously, one was injected to the right pectoral, another to the left inguinal regions. Rabbits were inoculated intravenously (i.v.) to the right regional vein or s.c. to the left dorsal region.

Testing of immunity. The animals were observed for 8 months. All were examined daily for 5 days following immunization. Intensity of local reactions (erythema, induration) and the size of infiltrations were evaluated. The levels and dynamics of antibody production in blood were determined in haemagglutination-inhibition (HI) test with antigens of influenza A viruses. The levels of antibody to tuberculosis were assayed in indirect HI and complement fixation (CF) tests using polysaccharide, phosphatide and tuberculin antigens.

To evaluate postvaccination immunity, 3 months after immunization the animals were challenged first with influenza virus (A/Victoria/35/72 strain) and 21 day later with tuberculosis mycobacteria. Infectious potency of influenza viruses was 8.0—9.5 lg EID_{50}/0.5 ml. Guinea pigs were inoculated with 0.1 ml influenza virus intranasally, rabbits with 1.0 ml by i.v. route. Human type tuberculosis mycobacteria (strain 4037) were injected to guinea pigs subcutaneously in the dose of 0.1 mg. Rabbits were i.v. challenged with bovine type mycobacteria (strain Valee) in a dose of 0.5 mg. To compare the absolute values, average quadratic error and P were calculated using χ^2 test.

Results

Systemic and local reactions

Groups of guinea pigs and rabbits were observed for 5 days following each immunization; no differences in their behaviour were found. The animals were weighed weekly for 2 months and showed no differences in gaining of weight. Local reactions as induration of 4—10 mm in diameter had disappeared within 3—4 days. Simultaneous injections of two preparations into different regions of the body did not influence the appearance of local reaction (Table 2). The size of infiltrates after injection of the ACI vaccine alone was 5.9 ± ± 1.1 mm, after simultaneous injections of ACI-vaccine & BCG 5.7 ± 0.9 mm. The size of skin lesions after s.c. administration of "Vaxigrip" was 3.3 ± ± 0.7 mm, after "Vaxigrip" & BCG 3.6 ± 1.2 mm. Combined vaccination with "Vaxigrip & BCG injected in a mixture caused larger dense infiltrations 8—9 mm in diameter lasting for 2—3 months in 6 out of 20 guinea pigs. Two-three weeks after s.c. administration of the BCG vaccine alone the induration at the injection site ranged from 4 to 16 mm in diameter showing a small knot in its centre. Local reactions to BCG vaccine disappeared within 2—3 months, with a small scar or pigmentation left.

Influenza antibodies

Two-dose administration regimens of each inactivated vaccine irrespective of the route of application proved to be optimal for immunization against influenza (Table 2). After the first dose, the antibody levels increased 1.7—4.0 times, repeated administration of the vaccine preparation resulted in a 19.7 to 30.0-fold rise.

Table 2. Local reactions to inactivated influenza vaccine and development of influenza antibody in guinea pigs

Vaccination schedule	Induration size (in mm)	Serum antibody geometric mean titre			Antibody increase***
		Before vaccination	After vaccination	After revaccination	
ACI-vaccine	5.9 ± 1.1*	≦ 4.0**	21.1	79.0	19.7
ACI-vaccine & BCG	5.7 ± 0.9	≦ 4.0	19.7	91.0	22.7
"Vaxigrip"	3.3 ± 0.7	4.6	9.2	120.0	26.0
"Vaxigrip" & BCG (simultaneously)	3.6 ± 1.2	≦ 4.0	10.6	120.0	30.0
"Vaxigrip" & BCG (in mixed preparation)	8.4 ± 2.2	≦ 4.0	7.0	7.0	1.7
BCG	—	≦ 4.0	≦ 4.0	≦ 4.0	1.0
Placebo (control)	—	≦ 4.0	≦ 4.0	≦ 4.0	1.0

Note: significance according to the criterion of Wilkoxon: the difference between groups 1 and 2, 3 and 4 is insignificant.

* Day 3 after vaccination.

** Dilution reciprocals.

*** GMT ratio after versus before vaccination.

Comparison of the antigenic potency of influenza vaccines administered either alone, or simultaneously with BCG vaccine, revealed higher antibody levels in animals immunized simultaneously against both influenza and tuberculosis. Thus, if immunization of guinea pigs with ACI-vaccine resulted in a 19.7-fold rise of influenza antibody levels, then administration of this vaccine simultaneously with BCG increased the antibody levels 22.7-fold. Immunization with the "Vaxigrip" vaccine led to 26.0- and 30.0-fold rises of antibody levels, respectively (the difference between groups under comparison is insignificant, $P \leq 0.95$). Immunogenicity of the associated preparation "Vaxigrip" & BCG turned out to be low (1.7-fold rise of antibody levels). Comparison of antigenic potency of ACI-vaccine and "Vaxigrip" revealed that the highest indices were observed with the "Vaxigrip" preparation. Antibody levels to influenza B virus in response to "Vaxigrip" were lower. The dynamics of the influenza antibody increase in rabbits was similar.

Antituberculosis antibodies

The most intense accumulation of antituberculosis antibodies was observed in animals simultaneously immunized with the BCG and inactivated influenza vaccines. Intense development of antipolysaccharide, antiphosphatide and antituberculin antibodies was observed most clearly after simultaneous immunization with the ACI-vaccine and BCG. Antipolysaccharide antibody levels in guinea pigs increased after administration of BCG maximally 5.6 fold, antiphosphatide by 1.7-fold and antituberculin by 8.0 fold. Simultaneous administration of ACI-vaccine and BCG resulted in 32.0-fold, 17.2--fold and 32.0-fold rises in antibody levels, respectively (the difference is significant at $P > 0.99$).

An increase in antipolysaccharide antibody, and to a less extent, in antiphosphatide and antituberculin antibody levels was observed also after simultaneous and combined immunization with the "Vaxigrip" & BCG vaccines. No difference was revealed by comparison of antibody levels developed in response to BCG administration either simultaneously, or in combination with the „Vaxigrip" vaccine. A difference in indices of the elevation of antipolysaccharide and antiphosphatide antibodies (26.0 and 30.0, 3.0 and 2.8) turned out to be statistically insignificant ($P < 0.95$).

Antiprotein antibody levels were higher after simultaneous vaccination as compared to the combined one (16.0-fold and 8.0-fold antibody rises; difference is authentic at $P > 0.95$). Simultaneous vaccination against influenza and tuberculosis revealed similar immunological changes. The most expressed stimulation of accumulation of antipolysaccharide and antiphosphatide antibody was induced by the ACI-vaccine. Unlike guinea pigs, no antiprotein stimulation was detected in rabbits. On i.v. inoculation of rabbits with the associated preparation "Vaxigrip" & BCG indices of antituberculosis antibody levels were practically similar to those induced by BCG alone.

Table 3. Levels of influenza antibody in vaccinated guinea pigs after challenge with the low-passaged influenza virus A/Port Chalmers/1/73 (H3N2)

Vaccination schedule	Antibody geometric mean titres		Increase in antibody levels
	before challenge	after challenge	
ACI-vaccine	70.0*	338.0	+4.8**
ACI-vaccine & BCG (simultaneously)	84.0	338.0	+4.0
"Vaxigrip"	49.0	779.0	+15.9
"Vaxigrip" & BCG (simultaneously)	97.0	1100.0	+11.3
"Vaxigrip" & BCG (in combined preparation)	7.5	309.0	+41.3
BCG	⩽ 4.0	12.1	+3.0
Placebo (control)	⩽ 4.0	19.7	+4.9

* Dilution reciprocals.
** GMT ratio after versus before challenge.

Challenge of animals with influenza viruses and tuberculosis mycobacteria

Since it is impossible to reproduce influenza infection in guinea pigs, they were challenged with the aim to evaluate additional rises in influenza antibody and the effect on the levels of different antituberculosis antibodies. As can be seen from Table 3, intranasal inoculation of guinea pigs with the low-passaged influenza virus A/Port Chalmers/1/73 (H3N2) resulted in additional rises of influenza antibodies, which occurred most of all in animals pre-immunized with "Vaxigrip" or with "Vaxigrip" & BCG. In control animals, which had not received the influenza vaccine, only 3.0-fold or 4.9-fold increase in antibody levels was observed. Challenge with tuberculosis mycobacteria resulted in a decrease of influenza antibody levels in all groups except of controls (Table 4). The decrease in influenza antibody was not

Table 4. Influenza antibody levels in vaccinated guinea pigs after challenge with tuberculosis mycobacteria (Vallee strain)

Vaccination schedule	Antibody geometric mean titre			Changes in antibody levels**	
	before challenge	after challenge 21*	after challenge 50*	21	50
ACI-vaccine	338.0	120.0	158.0	−2.8	−2.1
ACI-vaccine + BCG (simultaneously)	338.0	91.0	275.0	−3.7	−1.2
"Vaxigrip	779.0	779.0	512.0	1.0	−1.5
"Vaxigrip + BCG (in mixed preparation)	309.0	182.0	194.0	−1.7	−1.6
"Vaxigrip + BCG (simultaneously)	1100.0	338.0	362.0	−3.2	−3.0
BCG	12.1	9.2	17.1	−1.3	+1.4
Placebo (control)	19.7	49.0	112.0	+2.5	+5.7

* Days of observation.
** GMT ratio after versus before challenge: decreased (−) or increased (+) in antibody levels.

Table 5. Correlation between survival period of rabbits after challenge with tuberculosis mycobacteria and blood levels of antituberculosis antibodies

Vaccination schedule	Average period of survival	Antibody geometric mean titres		
		Antipoly-saccharides	Antiphospha-tides	Antituber-culins
ACI-vaccine	36.0	256.0	< 16.0	11.3
ACI-vaccine + BCG (simultaneously)	60.7	256.0	11.3	74.0
"Vaxigrip"	25.0	128.0	45.0	16.0
"Vaxigrip" + BCG (in mixed preparation)	35.0	182.0	74.0	45.0
BCG	59.5	156.0	74.0	91.0
Placebo (control)	33.3	256.0	45.0	32.0
Correlarion coefficient (ρ)		0.12	0.22	0.82

Note: 4 animals in each group.

higher than 1.3—3.7-fold and it was similar to that in animals immunized with monopreparations, or with 2 vaccines simultaneously (the differences are statistically insignificant).

Fifty days after challenge of guinea pigs with tuberculosis mycobacteria a tendency towards equalization of influenza antihaemagglutinin antibody levels was observed. After challenge with tuberculosis mycobacteria, the survival of rabbits was the highest in groups of animals which received BCG vaccine both as a single injection or simultaneously with the ACI--vaccine (Table 5). Average period of survival in these groups of animals was 59.5 and 60.7 days. In the group of rabbits immunized with BCG in combination with the "Vaxigrip" vaccine period of survival of the rabbits after challenge with tuberculosis mycobacteria was directly dependent on antituberculosis antibody levels. The highest antibody levels were observed in animals vaccinated with BCG (1:91.0) or with BCG simultaneously with the ACI-vaccine (1:74.0), which corresponds to a longer survival of animals in these groups. Antiphosphatide and antipolysaccharide antibody had no effect on the survival indices of these rabbits.

Discussion

Peculiarities of influenza epidemiology specify the necessity of prophylactic vaccination not later than in October-November. Antituberculosis vaccination of persons called up in autumn for military service to the Soviet Army and students of secondary and higher educational institutions is usually carried out at this time. Coincidence of revaccination with BCG and influenza vaccine makes it expedient to apply them simultaneously. The number of persons subjected to BCG revaccination in the indicated groups of young people being rather significant (40—50%), a simultaneous immunization would sharply increase the level of protection of the indicated contingents of population against influenza.

Much attention has been paid to the use of different viral and bacterial vaccines in connection with a growing increase in the number of vaccine preparations and the necessity to apply them in coinciding regimens. It has been ascertained that with the correct choice of antigens, application of combined preparations or their simultaneous administration turns out to be more preferable than repeated immunization with different antigens. Several authors showed the safety and efficacy of simultaneous vaccination against influenza and intestinal infections, influenza, whooping-cough and diphteria (Nefedova, 1962; Belyakov *et al.*, 1968; Malysheva *et al.*, 1971).

Experience in complex application of BCG with viral vaccines against smallpox, poliomyelitis, tick-borne encephalitis, yellow fever (Aksyenenko, 1971; Galbrait *et al.*, 1971; Rybalko, 1971; Belyakov *et al.*, 1973; Chambon *et al.*, 1973; Felsenfeld, 1973; Samartseva, 1973; Ruben *et al.*, 1974) showed not only the safety, but also the higher efficacy of such vaccination resulting in a stimulating effect of BCG vaccine on antibody formation to other antigens.

There have been so far no data on simultaneous vaccination against influenza and tuberculosis. Simultaneous or combined vaccinations were carried out in experimental animals. According to Belyakov (1968) the term "simultaneous" was used for a vaccination prerformed with two monovaccines administered simultaneously to different body regions. The term "combined vaccination" was used for a vaccination with a mixture of vaccines. Simultaneous administration of two vaccines was timed to the first influenza immunization. For combined vaccination "Vaxigrip" & BCG preparations were mixed in the same syringe prior to administration.

Thus, based on the present work for the first time substantiated the possibility of simultaneous immunization against influenza and tuberculosis using standard inactivated influenza vaccines and BCG. Experiments in animals showed a higher efficacy of ACI-vaccine as compared to the French "Vaxigrip" used in a schedule of simultaneous application. The dynamics of parallel antibody formation by simultaneous development of antituberculosis and influenza immunity in response to BCG and different influenza vaccines was described for the first time.

References

Aksyenenko, G. R. (1971): Simultaneous immunization with TAB_{te}, tick-borne encephalitis, smallpox, tularemia, influenza and BCG vaccines (in Russian). *Zh. Mikrobiol. (Mosk.)* **1971** (12), 41—45.

Belyakov, V. D. (1968): In *Associirovannaya Immunizatsiya i ekstrennaya Profilaktika*. Moskva, 1968.

Belyakov, V. D., Ishkildin, M. I., and Rabinovich, Ya. D. (1973): Simultaneous vaccination of humans against tuberculosis and other infections (in Russian) pp. 40—45. In M. P. Zykov (Ed.): *Problemy Immunologii i Serologii Tuberkuleza*, Leningrad, **65**.

Chambon, M. L., Tommasi, U. B., Barme, M., Robin, Y., and Breul, D. (1971): Combined BCG and Yellow Fever vaccination by PED-O-Jet. *Document WHO/TB/71/88.*

Felsenfeld, O., Wolf, R. H., Gyr. K., Grant, L. S., and Dutta, N. K. (1973): Simultaneous vaccination against cholera and yellow fever. *Lancet* **7801**, 457—459.

Galbrait, N. S., Crosby, G., Karnes, J. M., and Fernandes, R. (1971): Simultaneous immunization with BCG, diphteria-tetanus and oral poliomyelitis vaccines in children aged 13—14. *Brit. med. J.* **2**, 5755, 193—197.

Hannoun, G. (1973): Progress in influenza vaccination. I. A vaccine against future strains. *Nouv. Presse Med.* **2** (18), 1199—1200.

Isupov, F. G. (1973): Preparations of supercapsid and RNP-antigens of viruses and their practical application (in Russian). Essay of thesis for a doctor's degree, Moskva.

Kilbourne, E. D. (1975): New approaches to the prevention of influenza. *Acta med. Scand.*, Suppl., **45**, 576.

Malysheva, A. M., Taros, L. Yu., Balitskaya, S. F., Polyakov, K. B., and Romanov, B. G. (1971): A possibility of simultaneous immunization with live influenza, TAB_{te} and smallpox vaccines (in Russian). The Conference of All-Union Influenza Research Institute, December 1971, Leningrad.

Nefedova, L. A. (1962): Studies of immunogenesis in associated vaccination (in Russian). *Vop. Virus.* **7**, 206—211.

Ruben, F. L., Smith, E. A., Foster, S. O., Casey, H. L., and Pifer J. M. (1974): Simultaneous administration of smallpox, measles, yellow fever and diphteria-pertussis tetanus antigens to Nigerian children. *Bull. Wld. Hlth Org.* **48**, 188—198.

Rybalko, V. V. (1971): Simultaneous immunization of young recruits against tuberculosis, smallpox, typhoid and tetanus. (in Russian). *Vo.-med. Zh.* **1971** (5), 69—74.

Samartseva, V. P. (1973): Characteristics of postvaccination reactions in collective immunization against smallpox and tuberculosis in experiments (in Russian). *Documents of Kirghiz Medical Institute, Frunze*, **84**, 132—134.

Sokolov, N. N., Zhukova, T. E., Malofeeva, L. K., and Geft, R. A. (1973): Improvement of the preparation of influenza donor gamma-globulin. Report 1. Experience in the development of killed influenza vaccine purified and concentrated using gradient centrifugation. In: *Respiratornye virusnye infektsii. Tr. Inst. Epidem. (Leningr.)* **42**, 78—83.