III. Prophylactical problems

ON SOME BIOLOGICAL PROPERTIES OF INFLUENZA VIRUSES TYPE A ISOLATED DURING THE PERIOD 1981-1983

G. Kaprelyan, L. Karaivanova, N. Nikolova, V. Rusev, A. Saruyan, V. Lyutzkanova

Key-words: influenza virus type A — antigenic structure — biology — Varna city

A basic and unique feature of influenza viruses type A is their unceasing antigenic instability (antigenic drift or shift) which has an effect on their biological properties (1—6). It is found out that the activity of the epidemic process is determined by the appearance and distribution of viral varieties with altered envelope antigens (7—11). Recently, two antigenic subtypes of influenza virus type A are in an epidemic circulation, namely A (H1N1) and A(H3N2). Their simultaneous circulation suggests the possibility for the origin of recombinants or, at least, for reciprocal influence of some biological properties.

The present work is a stage of an overall and systemic investigation of the circulation of influenza viruses in respect to the etiologic prognostication of influenza infections and to an eventual selection of vaccinal strains.

Material and methods

The following main properties of influenza viruses type A isolated in 1981-1983 were studied: isolation ability on chick embryo model; antigenic structure; sensibility to nonspecific inhibitors; elution activity; hemagglutinin thermostability; infectiousness and toxicity; and cell culture adaptation ability. The methods used were previously described (4, 6, 8, 12).

Results and discussion

Influenza virus isolation from nose-throat washing of patients with clinical diagnosis "influenza" and "acute respiratory disease" showed certain differences. The highest isolation rates were in 1981 — 11,78 per cent, followed by these in 1983 — 3,44 per cent, and in 1982 — 1,2 per cent. It could be due to the difference of epidemic waves intensity and of antigenic structure of predominant influenza virus strains in these years. Influenza viruses with an antigenic formula A(H1N1) were more easily isolated than these with a formula A(H3N2). Our studies demonstrated that influenza viruses type A(H1N1) predominated in the district of Varna in 1981 and 1983.

We examined the antigenic structure and some basic biological properties of a certain part of the isolated viruses. It was shown that influenza viruses circulating in the district of Varna during this period were antigenically most closely related to the standard strains A(Texas) 1/77(H3N2) and A(Brazil) 1/78(H1N1) (table 1). Newly isolated strains with an antigenic formula A(H1N1) receded from

the standard ones — A(Klim)49 and A(USSR)90/77 which were antigenically very closely related with the circulating influenza viruses in the district of Varna during the period from 1978 till 1980. Similar data were reported by the National influenza centre when other districts in our country were concerned.

Table 1

Antigenic structure of influenza virus strains isolated in the Varna district in 1981-1983

Standard antisera / Influenza virus strains	Antigenic structure	A (Klim) 49	A (USSR) 90/77	A (Brazil) 78	A (Singapore) 57	A (Hong Kong) 1 68	A (Victoria) 3/75	A (Texas) 77	A (Bang Cock) 79	A (SW) 1/80	B (Hong Kong) 5/72	B (Singapore) 79	C (USA) 12 13/47	Sendai 1	NS from guinea pig	NS from horse
Homologous serum		800	800	400	200	6400	6400	400	400	400	3200	200	800	400	—	—
A Vn/748/81	H3N2	1/8	1/4	1/8	0	0	0	0	0	0	0	0	0	0		
A Vn/67/81	H1N1	0	0	0	0	1/8	1/32	1/4	1/16	0	0	0	0	0	0	0
A Vn/1147/81	H1N1	0	0	0	0	1/16	1/32	1/4	1/32	0	0	0	0	0	0	0
A Vn/759/81	H1N1	1/4	1/2	1/2	0	0	0	0	0	0	0	0	0	0	0	0
A Vn/69/81	H3N2	0	0	0	0	1/4	1/16	1/2	1/4	0	0	0	0	0	0	0
A Vn/35/81	H3N2	0	0	0	0	1/8	1/32	1/4	1/16	0	0	0	0	0	0	0
A Vn/47/83	H3N2	0	0	0	0	1/16	0	1/2	1/8	0	0	0	0	0	0	0
A Vn/52/83	H3N2	0	0	0	0	1/32	1/32	1/2	1/16	0	0	0	0	0	0	0
A Vn/143/83	H1N1	1/32	1/16	1/4	0	0	0	0	0	0	0	0	0	0	0	0
A Vn/149/83	H1N1	0	1/16	1/2	0	0	0	0	0	0	0	0	0	0	0	0
A Vn/151/83	H1N1	1/32	1/8	1/2	0	0	0	0	0	0	0	0	0	0	0	0

Table 2

Results from the hemagglutination activity of influenza viruses isolated in 1981-1983

Strain	Antigenic formula	1 % erythrocyte suspension from								
		Guinea pig	Rabbit	Pigeon	Rat	Lamb	Swine	Human	Cattle	Ram
A Vn/35/81	H3N2	1/256	0	1/64	0	0	0	0	0	0
A Vn/67/81	H3N2	1/32	0	1/32	0	0	0	0	0	0
A Vn/48/81	H1N1	1/128	0	1/32	0	0	0	0	0	0
A Vn/6/82	H3N2	1/64	1/4096	1/16	1/4096	—	1/4096	1/1024	1/4096	1/2048
A Vn/10/82	H3N2	1/128	1/4096	1/128	1/4096	—	1/4096	1/512	1/4096	1/128
A Vn/116/82	H3N2	1/128	1/4096	1/128	1/4096	—	1/4096	1/2048	1/4096	1/1024
A Vn/47/83	H3N2	1/128	1/4096	1/64	1/4096	—	1/128	1/4096	1/8	1/1024
A Vn/143/83	H1N1	1/1024	1/4096	1/1024	1/4096	—	1/32	1/4096	1/4096	1/128
A Vn/149/83	H1N1	1/256	1/4096	1/64	1/4096	—	1/256	1/4096	1/64	1/256

The hemagglutination activity (HA) of the strains increased (table 2), i. e. the hemagglutination spectrum broadened. There was no direct correlation between HA and antigenic formula of influenza virus strains. Influenza virus HA can be suppressed by non-specific inhibitors from the serum of various animal species. Virus sensitivity increase towards nonspecific inhibitors is considered a signal for forthcoming antigenic shift. The inhibitor sensitivity of the strains for the

Table 3

Hemagglutinin thermostability of influenza viruses isolated in 1981-1983

Influenza virus strains	Antigenic formula	Initial titer	Hemagglutination titer after			
			15 min	30 min	60 min	120 min
A Vn/35/81	H3N2	1/512	1:128	1:64	1:64	1:64
A Vn/67/81	H3N2	1/256	1:128	1:128	1:64	1:32
A Vn/748/81	H1N1	1/512	1:256	1:128	1:64	1:64
A Vn/6/82	H3N2	1/512	1:256	1/64	1:64	1:64
A Vn/10/82	H3N2	1/1024	1:256	1:128	1:64	1:64
A Vn/116/82	H3N2	1/1024	1:256	1:128	1:128	1:128
A Vn/47/83	H3N2	1/256	1:128	1:128	1:64	1:32
A Vn/143/83	H1N1	1/1024	1:512	1:128	1:128	1:128
A Vn/149/83	H1N1	1/256	1:128	1:64	1:64	1:64
A Brazil/1/78	H1N1	1/256	1:128	1:128	1:128	1:128
A Texas/1/77	H3N2	1/256	1:64	1:32	1:32	1:32

Table 4

Results from the hemagglutination activity of influenza viruses isolated in 1931—1983

Influenza virus strains	Antigenic structure	Heated or non-heated	Initial titer	Elution activity in saline at 37°C for				
				15 min	30 min	60 min	120 min	180 min
			HA	HA	HA	HA	HA	HA
A Vn3/3/5/81	H3N2	yes	1/128	0	0	0	0	1/4
		no	1/512	1/128	1/128	1/128	1/256	1/256
A Vn/67/81	H3N2	yes	1/64	1/64	1/64	1/2	1/2	1/2
		no	1/512	1/256	1/256	1/128	1/128	1/256
A Vn/748/81	H1N1	yes	1/128	1/8	1/16	1/32	1/64	1/128
		no	1/512	1/128	1/256	1/256	1/512	1/512
A Vn/6/82	H3N2	yes	1/64	1/16	1/32	1/32	1/64	1/64
		no	1/128	1/64	1/64	1/64	1/64	1/64
A Vn/10 82	H3N2	yes	1/64	0	0	0	0	0
		no	1/128	1/32	1/32	1/32	1/32	1/32
A Vn/116/82	H3N2	yes	1/512	1/256	1/256	1/256	1/256	1/128
		no	1/2048	1/256	1/512	1/512	1/512	1/256
A Vn/47/83	H3N2	yes	1/128	1/2	1/4	1/8	1/8	1/8
		no	1/256	1/128	1/256	1/256	1/256	1/256
A Vn/143/83	H1N1	yes	1/512	1/64	1/64	1/64	1/64	1/64
		no	1/1024	1/512	1/512	1/512	1/512	1/512
A Vn/149/83	H1N1	yes	1/64	1/16	1/8	1/8	1/8	1/8
		no	1/128	1/64	1/128	1/128	1/128	1/128

period 1981-1983 was examined by using of sera taken from humans (blood group "0"), guinea pigs, pigeons, rabbits, fowls, rats, swines, and rams. The strains w ere

heterogenous concerning this property. It was to be noted that inhibitor-resistant prevailed in contrast to those isolated in the past (1972—1977) characterized as inhibitor-sensitive influenza virus strains. There were no differences in inhibitor sensitivity between the strains belonging to both serosubtypes influenza viruses type A. It is probably an expression of the interaction between the viruses of both subtypes in the course of their parallel circulation.

Hemagglutinin thermosensitivity (HT) is one of the important biological properties of influenza viruses reflecting the antigenic drift. According to their different relation to chick erythrocytes after heating at 56 °C for 15 min, 30 min, 60 min, and 120 min influenza virus strains are divided into two types: with thermostable and with thermolabile hemagglutinin. Table 3 shown the results of the examination of influenza virus HT concerning the viruses isolated in our country in 1981—1983. A relatively good HT was established that allowed us to consider the strains examined relatively thermostable. However, there was no direct correlation between HT and antigenic structure of influenza virus strains

Table 5

Results from the infectious activity of influenza viruses isolated in 1981—1983

Influenza virus strains	Antigenic formula	Initial virus titer	Infectious titer
A Vn/35/81	H3N2	1/256	6,83
A Vn/67/81	H3N2	1/256	3,33
A Vn/748/81	H1N1	1/256	3,66
A Vn/6/82	H3N2	1,256	3,0
A Vn/10/82	H3N2	1/1024	1,66
A Vn/116/82	H3N2	1/1024	3,66
A Vn/47/83	H3N2	1/128	3,33
A Vn/143/83	H1N1	1/1024	4,33
A Vn/149/83	H1N1	1/512	3,55

isolated. It is namely a property of the strain proper and amids the strains which were recently isolated these with thermostable hemagglutinin prevail.

The property elution of influenza viruses from erythrocyte surface is due to the presence of their surface antigen neuraminidase. Influenza virus strains isolated and studied in 1981 differed in elution properties but this variety was not related with a difference in their antigenic structure (table 4). Similar results were obtained when the next years were concerned. Most probably, hemagglutinin participates in the process of elution, too, i. e. neuraminidase is the reason for elution but elution properties depend also on the strength of the link between hemagglutinin and erythrocytes as well as on other factors. That is why namely the elution properties of the strains with different antigenic structure could not be distinguished.

On table 5 the infectious activity of influenza virus strains examined during this period is presented. It varies in broad limits — from 1,66 up to 6,83 log LD_{50}. The results show that most isolated strains possess a well-expressed infectious activity which is important in influenza infection dissemination.

The investigations of the pathogenic and toxic properties of influenza viruses isolated in 1981—1983 show that these properties are slightly expressed which corresponds with the clinical severity of the epidemic waves. These properties are not related with strain immunogenicity. The immunization of cocks, mice, rabbits with influenza viruses isolated by ourselves induces high-titer immune sera. This fact allows us to suggest a possibility for asymptomatic course of the influenza infection in a part of the population.

The experiments for adaptation of the isolated influenza virus strains to primary trypsinized cell cultures of human embryonal kidney (HEK) and chick embryonal kidney (CEK) showed a positive reaction of hemadsorption only after 2—3 passages for HEK and after 4—5 ones for CEK.

On the basis of the results obtained the following main conclusions could be drawn:

1. Influenza virus isolation ability varies in 1981—1983 and depends on the antigenic structure and epidemic wave intensity. The percentage of isolated viruses is higher when strains with an antigenic formula A(H1N1) prevail in comparison with that of virus type A(H3N2) circulation.

2. The study of the antigenic structure of newly-isolated influenza virus strains shows that there is still a parallel circulation of subtypes A(H1N1) and A(H3N2). There is a receding of the viruses type A(H1N1) from the standard strain A(USSR)90/77.

3. The isolated strains are characterized with well-expresed HA, HT, a relative inhibitor resistance and a good elution activity. There is no direct correlation between strain differences of biological properties and the antigenic formula. It is probably due to the interaction of both virus serosubtypes during their parallel circulation.

4. The infectious, pathogenic and toxic activity of the isolated strains is not high which corresponds with the dissemination and the clinical course of influenza epidemic waves in 1981—1983. These properties do not effect on the immunogenicity of the same strains that is high enough to stimulate antibody formation without any apparent manifestations in the clinical picture.

REFERENCES

1. Бароян, О. В., Р. А. Рвачев. *Вопр. вирусол.*, 1978, № 2, 131—137. — 2. Жданов, В. М., С. С. Ямникова, В. А. Исаченко, и др. *Вопр. вирусол.*, 1981, № 6, 657—664. — 3. Закстельская, Л. Я., З. Николова, М. Яхно, и др. *Вопр. вирусол.*, 1982, № 6, 671—676. — 4. Мури, М. Варна, Канд. дис., 1982, 138 л. — 5. Николова, З., Р. Коцева. *Епидемиол., микробиол. и инфекц. болести*, 1979, № 1, 8—15. — 6. Николова, З., Р. Коцева, Г. Капрелян, В. Арнаудова, Б. Мандулов, и др. — В: Нац. конгр. инфекц. и паразит болести, V. София, 1982 г. Резюмета, с. 15. — 7. Jewdwall, J. W., R. G. Webster, W. U. Gerhard. *Nature*, 279, 1979, 246—248. — 8. Kaprelyan, G., et al. In: Sem. med. Balkan., XVI. 7—13. IX. 1980, p. 46. — 9. Köppler, T., et al. *Immunobiology*, 157, 1980, 320—330. — 10. Kuzmov, K., et al. In: Internat. Congr. Infect. Parasit. Dis., VII. Varna, 1978, 1, 314—318. — 11. Laver, W. G., G. M. Air, R. G. Webster, et al. *Virology*, 98, 1979, No 1, 226—237. — 12. Muri, M., G. Kaprelyan. In:Internat. Congr. Virol., V. Strasbourg, 1981. Poster session 43, orthomyxoviridae, p. 134.

О НЕКОТОРЫХ БИОЛОГИЧЕСКИХ СВОЙСТВАХ ГРИППОЗНЫХ ВИРУСОВ ТИПА А, ИЗОЛИРОВАННЫХ С 1981 ПО 1983 Г.

Г. Капрелян, Л. Караиванова, Н. Николова, В. Русев, А. Саръян, В. Люцканова

РЕЗЮМЕ

Изучена антигенная структура и некоторые биологические свойства штаммов гриппозных вирусов за период с 1981 г. по 1983 г. Изолируемость вирусов не одинакова и показывает варьирование в зависимости от антигенной формулы преобладающих эпидемических штаммов и интенсивности эпидемических вспышек. Свежеизолированные штаммы обладают высокой гемагглютинационной активностью, гетерогенностью по отношению к ингибиторчувствительности, а также по отношению к термостабильному гемагглютинину. Прямой зависимости между антигенной структурой изолированных штаммов и исследованными биологическими свойствами не было установлено.

Свежеизолированные штаммы гриппозных вирусов обладают хорошо выраженной инфекционной активностью и иммуногенностью и слабо выраженными патогенными и токсическими свойствами, что соответствует клинический тяжести вспышек эпидемии в течение указанного периода.