Acta virol. **28**: 69—72, 1984

DEVELOPMENT OF RESISTANCE TO 2-(1-AMINO-ETHYL)-BICYCLO (2.2.1) HEPTANE CHLOROHYDRATE IN INFLUENZA A VIRUS

N. V. GRIBKOVA, N. F. KAZAK, V. I. VOTYAKOV

Byelorussian Research Institute for Epidemiology and Microbiology, 220 050 Minsk, U.S.S.R.

Received September 24, 1982

Summary. — A resistant influenza virus has been obtained during successive passages of influenza A virus in 10 to 11-day-old chick embryos (CE) in the presence of 2-(1′-amino-ethyl)-bicyclo(2.2.1) heptane chlorohydrate possessing a high antiviral activity. The virus resistance to the inhibitor was not lost after one passage in the absence of the drug.

Key words: influenza A virus; resistance; influenza virus inhibitor

Introduction

Appearance of influenza virus variants resistant to defined chemicals was described in several publications. Development of virus stability to rimantadine and amantadine was shown in vitro (Feldblum, 1979*a*; Scholtissek, Faulkner, 1979) and in ovo (Ilyenko, 1975; Feldblum, 1979*b*) experiments. Earlier we showed (Votyakov *et al.*, 1982) that 2-(1′-amino-ethyl)-bicyclo-(2.2.1) heptane chlorohydrate possessed antiviral activity with respect to influenza virus comparable to that of rimantadine. The mechanisms of action of these two drugs, however, appear to be different since the inhibitor under study had a significant effect on reproduction of influenza virus when added to maintenance medium at both early and late stages of the infectious process (Gribkova, Kazak, 1981). Development of resistance to chemical compounds is a rather wide-spread property of viruses (Loddo, 1980), so that studies on appearance of mutants resistant to new antiviral substances seem desirable. Our paper describes the results of experimental in ovo studies on the appearance of influenza A variants resistant to 2-(1′-aminoethyl)--bicyclo(2.2.1) heptane chlorohydrate.

Materials and Methods

Fowl plague virus (FPV) Rostock strain (H7N1) was passaged by inoculation into allantoic cavity of 10 to 11-day-old chick embryos (CE) one hour after addition of 2-(1′-amino-ethyl) -bicyclo(2.2.1) heptane chlorohydrate (Pat. USA N 3 444 3P2) in the experimental group, and of an equal volume of physiological saline in the control group. Each successive passage was carried out by final virus dilution which still caused clear haemagglutination. Infected CE were incubated for 36 hr at 37 °C. Titres of haemagglutinin (HA) and infectivity (using a plaque method, Porterfield and Allison, 1960) were determined in the allantoic cavity of either CE groups.

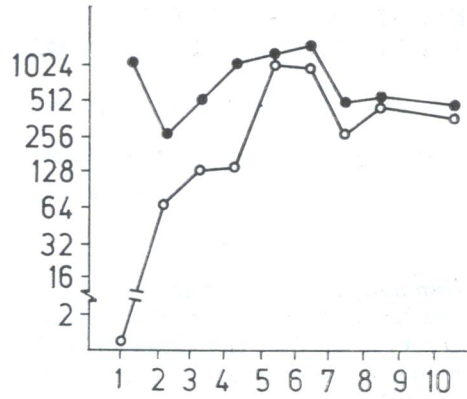

Fig. 1.
Alterations of haemagglutination activity of FPV in the experimental (empty circles) and control (full circles) groups Abscissa: passage number; ordinate: HA units

Results and Discussion

Altogether 10 successive passages in growing CE were made. The initial 8 passages were done in the presence of the drug under study at a dose of 1 mg per CE. In the passages 9—10 it was increased to 2 mg. Fig. 1 shows that HA titres became equal in the experimental and control groups by passage 6. The increase in the dose of the compound to 2 mg did not lead to a decrease in the HA titres at passages 9—10. Studies on the dynamics of development of resistance to the compound in the process of successive passages have shown that infectivity titres became equal in the experimental and control groups by passage 4—5. Further increase in the dose of the compound to 2 mg per CE did not cause a decrease in the virus infectivity in the experimental group as compared to the control one (Table 1).

In the next series of experiments we studied the susceptibility of viruses at different passages in the experimental and control groups to 2-(1′-

Table 1. Infectious activity of FPV passaged in the presence of 2-(1′-amino-ethyl)-bicyclo(2.2.1)heptane chlorohydrate

Passages	Virus titre (PFU/ml)	
	In the presence of the drug	In the absence of the drug
1	1×10^9	3×10^6
2	7.8×10^7	3×10^5
3	1×10^9	1.6×10^6
4	3.7×10^7	1.3×10^6
5	2.6×10^5	1×10^5
6	2×10^7	2.5×10^7
7	2.7×10^6	1×10^6
8	6.1×10^7	5.8×10^7
9	n. t.	n. t.
10	3.2×10^7	1.2×10^7

n. t. — not tested

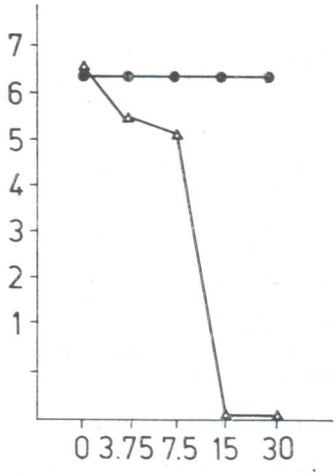

Fig. 2.
Susceptibility to the drug of FPV passaged without the inhibitor
Full circles: treated group, triangles — — control group.
Abscissa: drug dose (μg/ml); ordinate: log PFU/ml

-amino-ethyl)-bicyclo(2.2.1)heptane chlorohydrate. The inhibitor in increasing concentrations was added to the agar overlay. In the control group, i.e. in which the virus was passaged without the inhibitor, it retained a high susceptibility to the compound at all passages, while in the experimental group the susceptibility was gradually decreased. Thus, at passage 4 the viruses in both groups had an approximately similar susceptiblity to the inhibitor doses tested. At passage 7 the virus susceptibility to the compound was 2—3 times lower in the experimental than in the control group. The virus in the experimental group acquired resistance to 2-(1′--amino-ethyl)-bicyclo(2.2.1)heptane chlorohydrate by passage 10, when neither concentration (3.75 to 60 μg/ml) of the drug affected the virus infectivity. The virus of the control group retained a high susceptibility to the substance by passage 10.

A reverse-passage without the inhibitor was carried out in 10 to 11-day-old CE following passage 10. Then the virus susceptibility was tested to the increasing concentrations of the inhibitor. As follows from Fig. 2, the virus from the experimental group retained a high resistance to all doses tested. Our investigations have shown that passaging of FPV in the presence of 2-(1′-amino-ethyl)-bicyclo(2.2.1)heptane chlorohydrate in growing CE leads to a decrease in the virus susceptibility to the compound as early as at passages 4—5. Further passaging of the virus in the presence of the inhibitor under study caused a complete loss of susceptibility of FPV to the compound at passage 10. The acquired resistance to the inhibitor was retained in the process of passages of the resistant mutant without the compound.

Thus, the results obtained as well as the data in literature (Cochran *et al.*, 1965; Ilyenko, 1975; Feldblum, 1979*a*) indicate a rather rapid development of resistance of influenza virus to antiinfluenza compounds.

References

Cochran, K. W., Massaab, H. R., Tsunoda, A., and Berlin, B. S. (1965): Studies on the antiviral activity of amantadine hydrochloride. *Ann. N. Y. Acad. Sci.* **130**, 432–439.

Feldblum, R. L. (1979*a*): Obtaining of fowl plague virus mutant resistant to rimantadine, pp. 67–72. (in Russian) *Antivirusnaya Aktivnost i Mekhanizm Deistviya razlichnykh khimicheskikh Soedinenii* Zinatne, Riga.

Feldblum, R. L. (1979*b*): Development of resistance to rimantadine in A2 Krasnodar influenza virus, pp. 73–75 (in Russian) *Antivirusnaya Aktivnost i Mekhanizm Deistviya razlichnikh khimicheskikh Soedinenii.* Zinatne, Riga.

Gribkova, N. V., and Kazak, N. F. (1981): Comparative studies of the effect of 2-(1′-amino-ethyl) bicyclo(2.2.1)heptane chlorohydrate and rimantadine on the reproduction of influenza A virus, pp. 53–57 (in Russian). *Nukleozidy, Proizvodnye Biciklogeptana i Amantadina, drugiye antivirusnye Veshchestva*, Minsk.

Ilyenko, V. I. (1975): Studies of development of resistance to amantadine and rimantadine in influenza virus variants of A2 subtype (in Russian). *Vop. Virus.* **20**, 199–202.

Loddo, B. (1980): Development of drug resistance and dependence in viruses. *Pharm. Ther.* **10**, 431–460.

Porterfield, J., and Allison, A. (1960): Studies with poxviruses by an improved plaque technique. *Virology* **10**, 233–237.

Scholtissek, C., and Faulkner, G. (1979): Amantadine-resistant and -sensitive influenza A strains and recombinants. *J. gen. Virol.* **44**, 807–815.

Votyakov, V. I., Boreko, E. I., Rusyaev, V. A., Shashikhina, M. N., and Kazak, N. F. (1982): Characterization of the degree and range of activity of 2-(1′-amino-ethyl)-bicyclo(2.2.1)heptane chlorohydrate (in Russian). *Vop. Virus.* **27**, 172–176.