

COMPARATIVE STUDY ON THE INHIBITORY EFFECT OF RIMANTADINE ON THE REPRODUCTION OF EPIDEMIC STRAINS OF INFLUENZA A VIRUS H3N2 AND H1N1

N. L. PUSHKARSKAYA, N. D. LVOV, *Z. N. KOCHMASOVA

The D. I. Ivanovsky Institute of Virology, U.S.S.R. Academy of Medical Sciences, 123098 Moscow; and *Chair of Microbiology, The I. M. Sechenov Ist Moscow Medical Institute, Moscow, U.S.S.R.

Received November 28, 1981; revised March 18, 1981

Summary. — The sensitivity to rimantadine of the reproduction of influenza virus A/Texas/1/77 (H3N2) and A (H1N1) isolates Nos 897, 778 and 782, isolated from patients during an epidemic influenza outbreak in Moscow in the winter of 1977/1978 was compared. A (H3N2) virus proved to be more sensitive than the A (H1N1) viruses tested, as manifested by decreases of the infectious titre in ovo and of the haemagglutinin titre in MDCK cells.

Key words: influenza A virus; rimantadine; virus inhibition

It has been clearly demonstrated that such highly specific preparations with practical importance like rimantadine and amantadine inhibit the reproduction of influenza A viruses which show basic antigenic differences (Pushkarskaya *et al.*, 1977a, b; Oxford and Schild, 1977; Grunert and Hoffmann, 1977; Galegov *et al.*, 1979). We carried out a comparative study on the effectiveness of rimantadine against influenza A viruses H3N2 and H1N1.

We used the reference influenza virus strain A/Texas/1/77 (H3N2), obtained from Dr. M. A. Yakhno, Ivanovsky Institute of Virology, Moscow, and 3 isolates of influenza virus A/1/77 (H1N1), namely Nos 782, 778 and 897, isolated during an influenza outbreak in the winter of 1977/1978 from influenza patients at the clinic of the Ivanovsky Institute of Virology, Moscow, and identified in the Laboratory of ecology of this Institute.

Table 1. Effect of rimantadine on the titres of A/Texas/1/77 virus and isolates related to A/U.S.S.R./1/77 (H1N1) virus

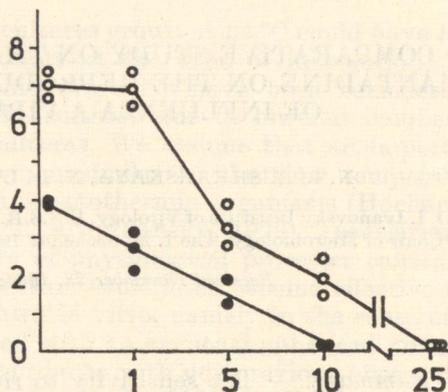
Virus	Virus titre (M ± m) in log EID ₅₀ values		Difference
	I	II	I—II
A/Texas/1/77	8.2 ± 0.45	4.0 ± 0.24	4.2
No. 897	6.3 ± 0.86	3.0 ± 0.50	3.3
No. 778	6.7 ± 0.56	4.0 ± 0.56	2.7
No. 782	7.0 ± 0.33	3.5 ± 0.31	2.5

I — Titres in control embryos; II — titres in the presence of rimantadine (2 mg/embryo).

Fig. 1.
The effect of rimantadine on the reproduction of influenza viruses A/Texas/1/77 (H3N2) (●) and A/782/77 (H1N1) (○) in MDCK cells

The cultures were inoculated with 10^6 EID₅₀.

Abscissa: rimantadine concentration ($\mu\text{g}/\text{ml}$); ordinate: haemagglutinin titre (\log_2 values)



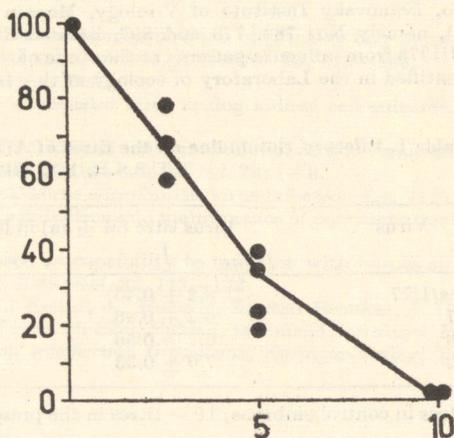
The sensitivity of the viruses to rimantadine was assayed by three methods: (1) inhibition of infectious virus in ovo; (2) inhibition by various rimantadine concentrations of the replication of A/Texas/1/77 and No. 782 viruses in Madin-Darby canine kidney (MDCK) cells maintained in Eagle's medium (Difco) supplemented with $2 \mu\text{g}/\text{ml}$ crystalline trypsin (Worthington, U.S.A.); and (3) plaque inhibition test (Appleyard, 1977) with A/Texas/1/77 virus in chick embryo cell (CEC) cultures; rimantadine was added to the solid overlay consisting of Eagle's medium (Difco) in double concentration and containing 0.6 % agarose (Bio-Rad, U.S.A.) and $2 \mu\text{g}/\text{ml}$ crystalline trypsin (final concentration).

In the presence of 2 mg rimantadine per embryo, the titre of A/Texas/1/77 virus in the allantoic fluid decreased by 4.2 log EID₅₀ while that of H1N1 virus isolates by 2.5–3.3 log EID₅₀ (Table 1).

Both A/Texas/1/77 virus and No. 782 isolate reproduced in MDCK cells with a cytopathic effect and haemagglutinin formation. Haemagglutinin production by A/Texas/1/77 virus was completely inhibited by $10 \mu\text{g}/\text{m}$ rimantadine while for the inhibition of H1N1 virus $25 \mu\text{g}/\text{ml}$ rimantadine were needed (Fig. 1). The latter concentration was not cytotoxic.

Fig. 2.
Inhibition of plaque formation by A/Texas/1/77 virus in CEC cultures

Abscissa: rimantadine concentration ($\mu\text{g}/\text{ml}$) in agarose overlay; ordinate: plaque formation in %. The inoculum was 100–150 PFU per culture.



Only A/Texas/1/77 virus produced plaques in CEC cultures under agarose overlay. The addition to the overlay of 2.5 and 5 $\mu\text{g/ml}$ rimantadine reduced plaque formation by 30 and 80 %, respectively (Fig. 2). Plaque formation was completely inhibited by 10 $\mu\text{g/ml}$ rimantadine.

Our experiments thus showed that both the reference strain A/Texas/1/77 and three isolates of H1N1 influenza virus proved to be sensitive to rimantadine in two parallel test systems. In embryonated eggs, H3N2 virus was more sensitive to rimantadine than H1N1 virus. The same tendency was manifested also in MDCK cell cultures.

Influenza viruses A/Texas/1/77 (H3N2) and H1N1 continued to play a major role as epidemiologically actual strains in epidemic outbreaks in the period from 1977—1979. The sensitivity of epidemic strains to rimantadine under experimental conditions justifies the assumption that chemotherapy and chemoprophylaxis of influenza might be effective in humans. This assumption is supported by the fact that the results concerning the inhibitory effect of rimantadine *in vitro* have been in complete accordance with its efficiency in clinical epidemiological investigations.

References

- Appleyard, G. (1977): Amantadine resistance as a genetic marker for influenza viruses. *J. gen. Virol.* **36**, 249—255.
- Galegov, G. A., Pushkarskaya, N. L., Smirnova, N. R., Lvov, N. D., Yatsina, A. A., and Zhdanov, V. M. (1979): Rimantadine as inhibitor of reproduction of influenza virus A/USSR/77. *Lancet* **i**, 269—270.
- Grunert, R. R., and Hoffman, C. E. (1977): Sensitivity of influenza A/New Jersey/8/76 (Hsw1N1) virus to amantadine HCl. *J. inf. Dis.* **136**, 297—300.
- Oxford, J. S., and Schild, G. C. (1977): Inhibition of swine influenza virus A/New Jersey/76 (Hsw1N1) multiplication and polypeptide synthesis by amantadine. *FEMS Microbiol. Letters* **1**, 223—226.
- Pushkarskaya, N. L., Blinova, V. K., Podchernyaeva, R. Ya., Obrosova-Serova, N. P., Galegov, G. A., Lvov, D. K., and Zhdanov, V. M. (1977a): Study on the sensitivity to rimantadine of influenza virus A/New Jersey/8/76 (in Russian). *Vop. Virus.* **22**, 369—371.
- Pushkarskaya, N. L., Obrosova-Serova, N. P., Shenderovich, S. F., Zakstelskaya, L. Ya., and Galegov, G. A. (1977b): Study on the sensitivity to rimantadine of various influenza A2 virus strains (in Russian). *Vop. Virus.* **22**, 421—423.