COMBINED USE OF INTERFERON AND CERTAIN STYRYLQUINOLINES IN CELL CULTURING AND IN EXPERIMENTAL ARBOVIRUS INFECTION

N. A. Leonteva, et al

Army Medical Research Institute of Infectious Diseases
Frederick, Maryland

1973

AD

UDC 616.988.25.092.9-085.37:576.858.095.383

COMBINED USE OF INTERFERON AND CERTAIN STYRYLQUINOLINES IN CELL CULTURING AND IN EXPERIMENTAL ARBOVIRUS INFECTION

[Paper by N. A. Leont'yeva, A. N. Fomina, Z. V. Idrisova, A. K. Shubladze and G. A. Galegov, D. I. Ivanovskiy Institute of Virology, USSR Academy of Medical Sciences, Moscow; Voprosy Virusologii (Problems of Virology), 17 April 1972, pp 482-485]

The effect of the combined use of interferon and certain preparations of the styrylquinoline group on the reproduction of the viruses of Venezuelan equine encephalomyelitis (VEE) and Western equine encephalomyelitis (WEE) was studied. The preparations R-29P, R-42P and R-43P in combination with interferon in chicken embryo fibroblast cultures showed antiviral activity in a reduction of VEE and WEE virus titers by 6.6 and 7.5 log, respectively. Preliminary treatment of white mice with WEE virus, followed by two injections of R-42), led to increased protective antiviral activity and to prevention of development of the disease in 60-75% of the test animals, depending on the infectious dose.

At the present time a number of methods are known for the prevention or limitation of arbovirus infections. These include immunization with antiviral vaccines, the administration of specific γ-globulins or immune serums, and the application of prophylactic measures. As regards treatment of viral infections, no effective remedies against arboviruses within the infected organism are yet known. The search for effective prophylactic, and especially therapeutic means for use in arbovirus infections is therefore an urgent task.

Antiviral activity of the styrylquinolines in the case of myxo- and arboviruses is described in the medical literature in only two reports [3, 6].

Several investigators have demonstrated that interferon has a definite prophylactic and therapeutic effect during recuperation of animals from arbovirus infections [1, 2, 4, 7]. A number of investigators, in order to produce increased antiviral activity of synthetic compounds, have employed a combination of a given synthetic preperation with interferon [5, 8, 9, 10].

The object of the present study was to investigate the prophylactic and

chemotherapeutic value of the combined use of preparations of the styrylquinoline series and exogenous interferon in cell cultures and also in white mice infected with Western encephalomyelitis virus.

TABLE 1

Combined Effect of Styrylquinolines and Interferon on the Reproduction of the Viruses of Venezuelan and Western Encephalomyelitis in Cell Cultures

Preparation	Concentration of preparation (μg/ml)	VEE Infection titre (in biol. toxic units/ml)			WEE Infection titre (in biol. toxic units/ml)		
		A	B	C	A	B	C
Virus control	0	9.6	5.6	4.0	8.1	3.9	4.2
R-29P	10	7.3	3.3	6.3	6.1	0.6	7.5
R-42P	10	7.0	3.0	6.6	6.6	1.1	7.0
R-43P	10	7.2	3.2	6.4	5.6	0.6	7.5

A - in the absence of interferon; B - in the presence of interferon; C - degree of inhibition with combined administration

NOTE: Multiplicity of infection, 0.001 biol. toxic units/cell. Interferon concentration, 16 immun. units.

Material and methods. Viruses of Venezuelan (VEE) and Western equine (WEE) encephalomyelitis were used. Tests were run on chicken embryo fibroblasts obtained by trypsination of 10- to 11-day-old embryos. Medium No. 199 with 2% of ox serum served as a culture medium.

The authors studied the antiviral activity of compounds of the styrylquinone group, synthesized by the All-Union Scientific-Research Chemico-Pharmaceutical Institute imeni S. Ordzhonikidze. The preparations were dissolved in medium No. 199 and placed in flasks with embryo fibroblast cultures following 1 hour's adsorption of the virus at 37°C. Titration of the infection virus and hemagglutinins, and microscopy of the fibroblast cultures, were carried out 24 hours following infection, at the moment of maximal accumulation of virus, with multiplicity of 0.001 biol. toxic units/cell.

In the tests in vivo the preparations were dissolved in distilled water and administered intra-abdominally in the amount of 0.1 ml per animal. Experimental encephalomyelitis was induced in white mice of 5-6 g weight by subcutaneous injection and intra-abdominal doses of 100, 10 and 1 LD_{50} per 50 ml.

Interferon used in the cell culture tests was obtained by infection of chicken embryo fibroblast cultures with Eastern equine encephalomyelitis virus; in the in vivo tests, the authors used serum mouse interferon induced by the virus of Newcastle disease injected intravenously in mice of 18-20 g weight.

Results and discussion. The marked correspondence between the special feathers of antiviral action of the styrylquinolines and their chemical structure had been previously established. Of the 20 compounds tested, the most active were those containing a methoxyl group or a haloid atom in the quinoline component. Thus, thiophosphate hydrate 2-(4'-bromostyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinoline (R-29P), thiophosphate hydrate 2-3'-methoxy-4'oxystyryl)-4-(δ-diethylamino-α-methylbutylamino)-6-methoxyquinoline (R-43P), and thiophosphate hydrate 2-(3'-methoxy-4'-oxystyryl)-4-(δ-diethylamino-α-methylbutylamino)-7-chloroquinoline (R-42P), in concentrations of 20 μg/ml, retarded the development of the cytopathological effect for 24 hours, reduced the infection of the VEE and WEE virus by 2.0 and 2.6 log, respectively, and completely suppressed the accumulation of hemagglutinins. To study the combined effect of interferon and the styrylquinolines on the reproduction of VEE and WEE viruses, a chicken embryo fibroblast culture was treated with interferon for 24 hours, the interferon was removed, and the cells were infected with the virus. Following 1 hour's contact with the virus, the culture was washed with a supporting medium containing 10 μg/ml of the corresponding preparation.

In Table 1 above are given summary data for the experiments. It is evident that preliminary treatment of the cell cultures with interferon, with subsequent use of a chemical preparation, leads to marked synergistic action, evidenced by a pronounced lowering of the infection titers of VEE and WEE viruses (by 6.6 and 7.5 log, respectively). Of the number of most active preparations tested by the authors, R-42P was selected for use in tests on white mice.

In a subsequent series of tests the authors studied the effect of interferon on the course of acute encephalomyelitis in mice. The comparison of data obtained with subcutaneous and intra-abdominal means of administering interferon at various times before and after infection with the virus showed that the most effective technique is subcutaneous injection not earlier than 24 hours before, or 2-4 hours following, infection with the virus.

As is well known, combined use of various chemical preparations, antibiotics and specific serums and preparations of interferon, in certain cases produces a synergistic and additive antiviral effect. As was shown by subsequent tests, the combined use of interferon and a preparation from the group of styrylquinolines in the presence of acute encephalomyelitis in mice was accompanied by a synergistic effect, as expressed in a significant degree of antiviral action.

TABLE 2

Effect of Styrylquinoline R-42P Alone and in Combination on the Survival of White Mice Infected with MEE Virus

	Dose (mg/kg)	Multiplicity of infection: 10 LD_{50}/0.03 ml A	B	C	D	100 LD_{50}/0.03 ml A	B	C	D
Virus contro, virus + prep.*		220	20	9.1	4.7	210	0	0	4.5
	18					210	21	10	7.5
	9	200	71	35.0	8.3	210	63	30	7.7
	4.5					210	8	3.8	7.1
Virus + interferon; virus + prep. + interferon		200	70	35.0	5.6	120	36	30	6.1
	9	120	90	75.0	9.5	200	120	60	8.1

*LD_{50} of preparation is 36 mg/kg

A - Total infected; B - Number of survivors in absolute numbers; C - Number of survivors in percents; D - Mean survival period, in days.

N O T E S. 1. The preparation of styrylquinoline was administered 2 and 24 hours following intra-abdominal infection. 2. The interferon in the amount of 250 inf. units per 0.2 ml was injected subcutaneously 24 and 2 hours before, and 2 hours following infection with the virus. 3. With combined use, the interferon was injected 24 and 2 hours before, and 2 hours following, infection. 4. The preparation of styrylquinoline in dosage of 9 mg/kg was administered intra-abdominally 2 and 24 hours following injection with virus.

As is evident from Table 2, preliminary double injection of white mice with homologous interferon 24 hours before infection with WEE virus and a subsequent double injection with preparation R-42P in concentration of 9 mg/kg lead to reinforcement of protective antiviral action. Such combination of prophylactic and therapeutic use of interferon and styrylquinoline prevented the development of generalized infection in 60-75% of the test animals, depending on the infection dose, the method and time of administration of the interferon at the same concentration of the chemico-therapeutic preparation.

The results obtained demonstrate the possibility of the combined use of interferon and synthetic preparations for experimental arbovirus infection. It should be emphasized, however, that the most reliable results were obtained in tests run on animals infected with minimal doses of the virus.

The positive results of the experiments conducted indicate the promising character of further tests in the development of prophylactic and therapeutic preparations in connection with infections produced by arboviruses of the genus Alfavirus.

B I B L I O G R A P H Y

1. Balezina, T. I., Korabel'nikova, N. I., Pyrikova, A. P. et al.; Antibiotiki (Antibiotics), 1968, No. 2, p. 176.

2. Grokhovskaya, A. A. Vopr. virusol. (Problems of Virology), 1966, No. 2, p. 163.

3. Zeytlenok, N. A., Nesterova, G. F., Krigsgaber, M. R. et al.; Tezisy dokladov 9-go Mezhdunarodn. kongressa po mikrobiologii (Abstracts of Reports Given at the Ninth International Congress on Microbiology), Moscow, 1966, p. 551.

4. Chumakov, M. P., Vil'ner, L. M., Zeytlenok, N. A. et al. Vzaimootnosheniye virusa i kletki (Interrelationship of Virus and Cell), Riga, 1965, p. 4.

5. Bauer, D. J. Brit. J. Exp. Path., 1966, Vol. 36, p. 105.

6. Chan, J. C. and Gadebush, H. H., Experientia, 1969, Vol. 25, p. 329.

7. Finter, N. B., Brit. J. Exp. Path., 1966, Vol. 47, p. 361.

8. Furusawa, E., Cutting, W., Buckley, P. et al. Proc. Soc. Exp. Biol. (N.Y.) 1964, Vol. 116, p. 938.

9. Furusawa, E., Furusawa, S. and Cutting, W., Ibid., 1965, Vol. 118, p. 394.

10. Lavrov, S. V. Eremkina, E. I., Orlova, T. G. et al. Nature, 1968, Vol. 217, p. 856.

COMBINED USE OF INTERFERON AND CERTAIN STYRYLQUINOLINES IN CELL CULTURE AND IN EXPERIMENTAL ARBOVIRUS INFECTION

N. A. Leontieva, A. N. Fomina, Z. V. Idrisova, A. K. Shubladze, G. A. Galegov

The effect of combined use of interferon and some drugs of the styrylquinoline group on reproduction of Venezuelan equine encephalomyelitis (VEE) and Western equine encephalomyelitis (WEE) viruses was studied. The drugs designater as P-29П, P-42П, P-43П in combination with interferon were shown in chick embryo fibroblast cultures to possess antiviral activity which was manifested in reduction of infectious titers of VEE and WEE viruses by 6.6 and 7.5 lg, respectively.

Pretreatment of white mice with interferon before the infection with WEE virus followed by two injections of P-42П drug resulted in enhancement of the protective antiviral effect and prevented development of encephalomyelitis in 60%—75% of animals, depending upon the infectious dose.