Combined action of ribovirin and rimantadine in experimental myxovirus infection

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Summary. This communication presents the results of the comparative study of the chemotherapeutic activity of ribovirin (virazol) and rimantadine and their combined action on the experimental influenza infection in tissue culture and in vivo.

Numerous attempts to find chemotherapeutic substances active towards the influenza virus have revealed such activity in adamantan derivatives, particularly in rimantadine (α-methyl-1-adamantan-methylamine)[1]. It has also been shown[2,3] that ribovirin (virasol), an anomalous nucleoside, 1-β-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide, which is apparently a structural analogue of xanthilic acid, effectively inhibits the reproduction of ortho- and paramyxoviruses in tissue culture and exhibits a marked protective effect in vivo. The mechanism of the selective antiviral action of ribovirin remains to be elucidated, since the inhibitory effect of the ribovirin-5′-monophosphate on the activity of the inosin-5′-monophosphate dehydrogenase may alter the biosynthesis of the guanyl nucleotides and may damage the biosynthesis of both viral and cellular RNAs[4].

This communication presents the results of the comparative study of the chemotherapeutic activity of ribovirin and rimantadine and of their combined action on experimental influenza infection.

Materials and methods. The toxicity of the both compounds were determined by their introduction into the 48-h-culture of chick embryo fibroblasts grown in the medium 199. The cultures were observed for 2 days. Ribovirin did not cause any cytopathic effects at concentration up to 600 μg/ml. Rimantadine caused the cytopathic effect at the concentration of 50–60 μg/ml.

Experiments were conducted with the Weibridge strain of fowl plague virus[5] (FPV) and the Frunze strain of A2 influenza virus virulent for mice. Chick embryo fibroblast monolayers were infected with FPV, and the antiviral drugs dissolved in the medium 199 were added after absorption of the virus. After 20-h-incubation, infectious and hemagglutinin titers were determined by the routine methods.

Swiss mice (8–10 g) were inoculated by the intranasal route with influenza A2 virus Frunze strain, 5–10 LD_{50}, that caused the death of 60–70% of control mice. The

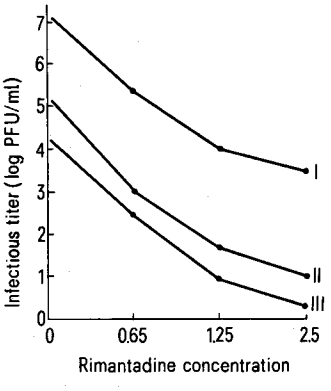

Fig. 1. Action of various concentrations of rimantadine alone (1) and in the combination with ribovirin, 12.5 μg/ml (II) and 25 μg/ml (III) on infectious titer of FPV at m.o.i. of 0.001 PFU/cell 24 h after infection.

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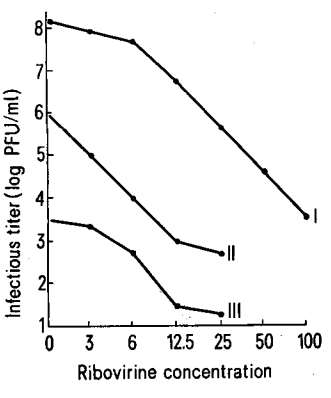

Fig. 2. Action of various concentrations of ribovirin alone (1) and in combination with rimantadine, 0.65 μg/ml (II) and 2.5 μg/ml (III) on infectious titer of FPV at m.o.i. of 0.001 PFU/cell 24 h after infection.

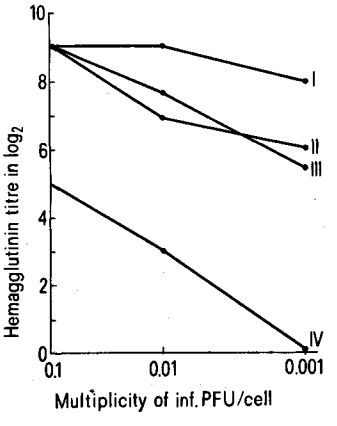

Fig. 3. Combined action of rimantadine and ribovirin on the reproduction of FPV at various m.o.i. I, control; II, ribovirin (25 μg/ml); III, rimantadine (1,25 μg/ml); IV, combination of both compounds at the same doses.

antiviral drugs dissolved in the distilled water (0.2 ml) were given p.o. once 1.5 h before infection. Control animals received water. Ribovirin was kindly given by the Lederle American Cyanamid Company, USA. Rimantadin hydrochloride was kindly given by Dr Y. Y. Polis from the Institute of Organic Synthesis, Latvian Academy of Sciences, Riga, USSR.

Results. It is seen from figure 1 that both rimantadine and ribovirin effectivity inhibit the reproduction of FPV.

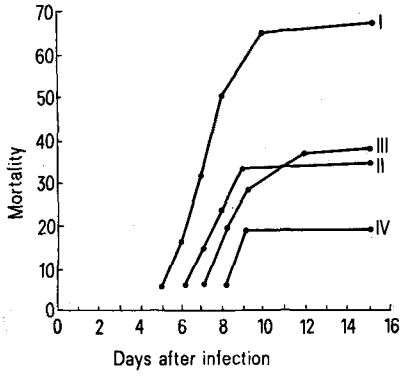

Fig. 4. Protective action of ribovirin and rimantadine on the experimental infection of mice with influenza A_2 virus. I, control group; II, rimantadine 10 mg/kg; III, ribovirin 200 mg/kg; IV, both compounds at the same doses. 40 mice per group up.

Decrease to 1.5–2.0 log PFU/ml is achieved with 12.5 μg/ml (5×10^{-5} M) of ribovirin and 0.65 μg/ml (3.5×10^{-6} M) of rimantadine. Ribovirin, in the concentration of 50 and 100 μg/ml, sharply inhibits the synthesis of hemagglutinin and the infectious virus, even if the m.o.i. is 5–10 PFU/cell (not shown in the figure).

The combined use of both compounds gives an additive effect. As is seen from figure 1, the combination of rimantadine (0.65 μg/ml) and ribovirin (12.5 μg/ml) decreases the infectious titre to 4 log PFU/ml, that is 0.7 log PFU/ml more than the sum of the effects of rimantadine and ribovirin at the given doses. Almost complete inhibition of the virus reproduction is achieved by the combined action of the increased doses of rimantadine (1.25–2.5 μg/ml) and ribovirin (12.5–25 μg/ml).

Figure 2 shows how the addition of rimantadine increases the inhibitory action of ribovirin. It is seen from these data that the combination of ribovirin (3–6 μg/ml) and rimantadine (0.65 μg/ml) gives an inhibitory effect which is more than that of ribovirin in the concentration of 25 μg/ml. It is also seen that the combined effect of both compounds is more than the sum of the effects of both compounds at the given doses.

It is also seen from figure 3 that the combination of both compounds is more effective than the use of them alone, though the effect depends on the m.o.i. The protective effect of the compounds in the experimental infection of mice is shown in figure 4. Again it is seen that the protective effect of the combined action of the compounds is considerably higher that the sum of the effects of both compounds given alone.

Bacterial bioluminescence in chlorophthalmid deep-sea fish: A possible interrelationship between the light organ and the eyes

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Summary. A small perianal light organ was found on a ventral part of the body of Chlorophthalmus spp. Simple tests, histological preparations and bacterial culture of the luminous contents clearly indicated that the luminous substance consists of symbiotic bacteria. The unique eyes of Chlorophthalmus spp. suggests a possible interrelationship between the eyes and the newly observed light organ.

Chlorophthalmus spp. (Iniomi), a benthonic genus, occur at moderate depth (150–750 m) throughout tropical and temperate regions of the world. They are normally under 12 inches long[2]. The eyes of C. agassizi has been described by Denton[3] as possessing bright yellow lens. Somiya and Tamura[4] and Somiya[5] have also reported on the optical properties of the yellow lens and several retinal specializations in the eyes of C. albatrossis.

During the R.V. 'Hakuho Maru' Cruise KH72-1, the author had an opportunity to observe various luminous deep-sea fishes and to examine their eyes. This has led the author to speculate on the possible relationship between the specialized eyes and bioluminescence[6]. The experimental fish were freshly caught Chlorophthalmus albatrossis (figure 1A) and C. nigromarginatus from the Kumano sea (off Owase, Mie prefecture, Japan). Macrourid fish (Coelorhynchus hubbsi) were used as controls. The specimens were observed in a dark-room on board ship. A small light organ was found around the anus on the ventral part of the body of the chlorophthalmid. This small spot like bioluminescence was only visible after about 15 min of dark adaptation on the part of the observer. The luminescence was blue-green and continuous as that of macrourid fishes.

Simple tests[7, 8] for differentiating the nature of luminescence (luminous bacteria or luciferin luciferase) were carried out on fish kept on ice. The results from chlorophthalmid and macrourid fish were the same, as follows: a) Luminous emulsions in sea water were obtained by homogenizing the light organ. 1. With the exception of the upper layer exposed to air the luminescence became quiescent gradually when allowed to stand. When the quiescent emulsion was shaken in air, the luminescence was completely restored again. This emulsion retained the ability to luminesce for many hours. 2. On raising the temperature to around 50 °C (5 min), the emulsion ceased to luminesce and the luminescence could not be restored even when cooled again to 20 °C. If the emulsion was cooled below 5 °C, the luminescence was completely extinguished but it was restored when the emulsion was rewarmed to room temperature. 3. When the luminous emulsion was centrifuged at 300 × g for 15 min at room