ANTIVIRAL PROPERTIES OF ADAMANTAMINE

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Adamantamine inhibits propagation of type A influenza virus (strain PR-8) in chick embryos if injected simultaneously with one infecting dose of virus. If the preparation is administered in accordance with a definite scheme, the preparation prolongs life and reduces mortality among mice infected with influenza virus.

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We still have no effective therapeutic preparations for the treatment of virus influenza, or reliable methods of preventing infection during contact with the patient. Vaccination is not always successful because the antigenic properties of the vaccine strain may not correspond to the properties of strains which are the source of infection at that particular time. It is, therefore, important to seek substances among the antibiotics and products of chemical synthesis which will have a therapeutic and prophylactic action in influenza.

A report was published [3] in 1964 of the antiviral activity of adamantamine hydrochloride (amantadin). According to these workers' findings, this preparation has a selective action, depending on its dose, in inhibiting development of influenzal infection in tissue cultures, chick embryos, and mice (viruses A_1, A_2, A_{PR-8}, C, Sendai parainfluenza virus). It was also shown that the activity of the preparation is not associated with direct inactivation of virus, but is the result of disturbance of penetration of virus into the cell.

The prophylactic and therapeutic action of adamantamine was investigated during an influenza epidemic [4]. The efficiency of the preparation was assessed from the frequency and duration of symptoms of the disease and from changes in titer of the serum antibodies. Administration of adamantamine relieved the symptoms of influenza without affecting the duration of the disease or the serum antibody titers.

The object of the present investigation was to study the antiviral properties of adamantamine synthesized at the Institute of Pharmacology and Chemotherapy, Academy of Medical Sciences of the USSR, by A. P. Skoldinov and co-workers [1].

EXPERIMENTAL METHOD AND RESULTS

The effect of adamantamine on propagation of influenza virus was first studied in developing chick embryos. The chick embryos used in the experiments were aged 9-11 days and were infected with one infecting dose of virus. The preparation was injected in a dose of 500 μg into the allantois of the embryos 120 and 30 min before infection, at the same time as the virus, or 30 and 120 min after infection with the virus. Instead of adamantamine, the control embryos received physiological saline at the same time. The action of the preparation was assessed from the number of embryos with a negative hemagglutination reaction (HR), and from the degree of the group HR (determined in a mixture of equal volumes of allantoic fluid from the embryos of that particular group).

The experiments showed (Table 1) that administration of adamantamine inhibits the development of influenza virus only when the preparation and virus are injected at the same time.

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TABLE 1. Effect of Adamantamine on Propagation of Influenza Viruses A (strain PR-8) in Chick Embryos

Time of injection of preparation (in min)	No. of embryos			Group HR
	in expt.	with negative HR		
		%	P	
Control	16	31	>0,05	640
120 before infection	16	50	>0,05	640
30 " "	16	44	>0,05	160
At the same time as infection	16	75	0,008	80
30 after infection	16	50	>0,05	160
120 " "	16	40	>0,05	320

TABLE 2. Effectiveness of Adamantamine in Experimental Influenzal Infection of Albino Mice

Variant of experiment	Group of animals	Mortality*	% of animals dying	P	Mean duration of survival (in days) †
I	Control	22/30	73	0,008	8,9
	Experimental	12/30	40		18
II	Control	24/30	80	0,1	8,5
	Experimental	18/30	60		16

* Numerator – number of dying mice, denominator – total number of animals.
† Calculated by Prigge's method [2].

The effect of size of infecting dose of virus on the activity of adamantamine was next studied. With a tenfold increase in dose of virus (embryos infected with 10 infecting doses of virus) the preparation was inactive.

In the next series of experiments the action of adamantamine was studied on experimental influenzal infection in albino mice.

According to Davis and co-workers [3], LD_{50} of adamantamine for albino mice is 1080 mg/kg. LD_{50} of the adamantamine which we used under the same conditions was 750 mg/kg.

The therapeutic effect of adamantamine in influenzal infection of albino mice was clearly seen when the preparation was injected intraperitoneally in a dose of 50 mg/kg or given by mouth in a dose of 60 mg/kg every 4 h for 2 days (13 times altogether) [2]. We used this scheme (Table 2, variant I), and also another variant of the experiment in which adamantamine was given by mouth in a dose of 60 mg/kg for 3 days, 3 times a day at intervals of 5 h (Table 2, variant II).

Altogether 120 mice weighing 20-22 g were used in the experiment. The animals were infected by instilling type A influenza virus (strain PR-8) into the nose in a dose of 1-10 LD_{50} in a volume of 0.5 ml. The action of the preparation was assessed from the length of survival of the experimental animals and the proportion of animals surviving.

The experiments showed that administration of adamantamine reduces the severity of influenzal infection in albino mice. The substance had a stronger therapeutic action 4-6 days after infection. By this time 21 mice had died in the 2 control groups (7 animals in variant I and 14 in variant II of the experiment); by the same time only 1 mouse had died in each of the experimental groups. By the end of the experiment, on the 14th day after infection, the mortality among the treated mice in variant I of the experiment was 33% below that of the controls, while their survival period was approximately doubled. The difference between

the number of dying mice in the experimental and control groups was not statistically significant for variant II of the experiment. In this case only the duration of survival of the treated mice was increased.

In the final series of experiments the effect of adamantamine on the virus itself – on its infecting titer – was investigated. For this purpose, adamantamine in a concentration of 2-4 mg/ml was mixed with an equal volume of suspension of virus A (strain PR-8; its active concentration was thus 1 and 2 mg/ml), the mixture was incubated at 37° for 3 h, and then used to infect 9-day chick embryos. It was found that adamantamine has no effect on the infecting titer of the virus: the virus was equally active in the experiment and control tests.

The experiments thus showed that adamantamine has an antiviral action on type A influenza virus (strain PR-8), its effectiveness being unconnected with direct action of the substance of the virus.

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