EFFECT OF ESCULAMINE ON EXPERIMENTAL INFLUENZAL INFECTION

(UDC 616.921.5-085.3 : 577.164.3]-092.9)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 6,
pp. 65-68, June, 1966
Original article submitted September 19, 1964

Changes in vascular and cellular permeability are of great importance for the penetration of tissues and cells of the host organism by microorganisms and viruses. An important role in this process belongs to the state of the cell membrane and also of the intercellular substance, the components of which include mucopolysaccharides, and especially hyaluronic acid; after depolarization of the latter, the permeability of the tissue barrier is increased.

It has also been shown [2, 3] that substances with vitamin P activity (rutin, citrin, quercetin, esculamine) possess an antihyaluronidase property, thereby protecting the hyaluronic acid and other mucopolysaccharides from the depolarizing action of hyaluronidase, the amount of which increases during infections. The beneficial effect of flavonoids in certain infections has been reported [6, 9] and the inhibitory action of quercetin and, to a lesser degree, of rutin on the growth of various bacteria in vitro has been described [7, 12]. In some cases, however (for example, in influenza), quercetin and rutin had no therapeutic action [9], and several authors [8, 10, 11] have likewise reported that they have no antibacterial and antiviral action in vitro.

Esculamine (the hydrochloride of $\beta\beta^1$-dihydroxydiethylaminomethyl-4-methylesculetin)—a substance with vitamin P activity—has been synthesized in the Institute of Pharmacology and Chemotherapy, AMN SSSR [1]. Besides its other properties, it has the ability to depress tissue and vascular permeability and it possesses an antihyaluronidase action [5].

The object of the present investigation was to study the action of esculamine in experimental virus influenza.

EXPERIMENTAL METHOD AND RESULTS

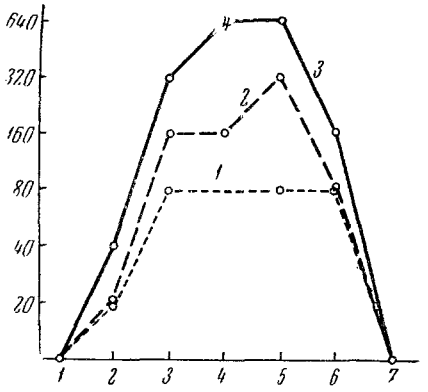
In the experiments of series I the action of esculamine was investigated on multiplication of influenza virus in developing chick embryos. Esculamine, in the maximal tolerated dose (1 mg per embryo) in 0.2 ml physiological saline, was injected into the allantoic sac of 10-day chick embryos 10 and 60 min before infection or 180 min after injection. The embryos were incubated at 37° for 48 h. The action of esculamine was tested on 1 and 10 infecting doses of virus. When the action of the preparation was assessed, attention was paid to the number of embryos with a negative hemagglutination reaction (HR) for each group of embryos (8 embryos in each group) and also the group HR (mixture of equal volume of allantoic fluid of embryos of the particular group in a dilution of 1:10). The HR was performed with a 1% suspension of chick erythrocytes.

The results of these experiments are given in Table 1.

It is clear from Table 1 that administration of esculamine 60 min before and 180 min after infection had no effect on multiplication of the influenza virus in the chick embryos. An antiviral action was observed when the preparation was given 10 min before injection of the virus, but only when the embryos were infected with one infecting dose of virus.

TABLE 1. Effect of Esculamine on Multiplication of Influenza Virus A (strain PR-8) in Chick Embryos

Experimental conditions	Amount of virus (in infecting doses)	No. of embryos		Arithmetical mean titer of HR	Group HR
		total	with negative HR		
Control	1	6	1	168	320
	10	8	0	1125	1160
Injection of esculamine:					
10 min before infection	1	6	5	54	40
	10	8	1	305	320
60 min before infection	1	8	0	160	160
	10	8	0	483	320
180 min before infection	1	8	0	120	160
	10	8	0	868	640



Effect of esculamine on reproduction of virus in lungs of albino mice with experimental influenzal infection. 1) Mice receiving preparation in a dose of 100 mg/kg by mouth; 2) mice receiving preparation in a dose of 2 mg/kg intraperitoneally; 3) control animals. Along the axis of ordinates—titer of HR; along the axis of abscissas—days of observation.

In the next series of experiments the action of esculamine was studied on the course of experimental influenza pneumonia in albino mice.

The experiments were carried out on 150 albino mice weighing 20-22 g. The animals were infected intranasally under superficial ether anesthesia with virus A (strain PR-8) adapted to mice, in a dose of 10 LD_{50} in a volume of 0.5 ml. Esculamine was injected in a dose of 2,000 mg/kg intraperitoneally (close to the maximal tolerated dose) once or given in a dose of 100 mg/kg by mouth daily for 5 days (in this dose esculamine depressed tissue and vascular permeability in experimental animals) [4].

To assess the action of esculamine, attention was paid to the duration and rate of survival of the animals and to the dynamics of development of the infection. For this purpose, control and experimental mice were sacrificed at various times after infection (4 mice from each group daily after 1-8 days), and the degree of the macroscopic changes in the lungs was recorded by + signs and the reproduction of the virus in the lungs was determined by the HR between a suspension of the lungs and a 1% suspension of chick erythrocytes. Each variant of the experiment and control series was carried out on 50 mice, the survival rate was determined in 10 animals, reproduction of the virus in 20 animals, and the macroscopic and microscopic changes were observed in 20 animals. The results of these experiments are given in the figure and in Tables 2 and 3.

The figure shows that administration of esculamine to mice before infection with influenza virus depressed reproduction of the virus in the lungs of these animals, and this was particularly marked after repeated administration of the preparation. Similar results were obtained during the macroscopic investigation of the changes in the lungs (Table 2). The hemorrhagic manifestations in the lungs of the mice receiving esculamine were much less than in the control animals.

Administration of esculamine to the animals prolonged their survival and reduced their mortality. In the control group, for example, 5 mice died in the first 5 days compared with only one in the experimental group. Observation of the animals for 14 days showed that their survival was increased in the group of mice receiving esculamine 5 times in a dose of 100 mg/kg (in the control group all animals had died at this time, but only half in the experimental group; see Table 3).

TABLE 2. Effect of Esculamine on Changes in Lungs (macroscopic picture) of Albino Mice with Experimental Influenzal Pneumonia

Experimental conditions	Mouse no.	Intensity of lung lesion at various times after infection						
		1 day	2 days	3 days	4 days	5 days	6 days	7 days
Control	1	—	—	+	+++	++++	++++	++++
	2	—	+	+	++++	++++	++++	++++
Injection of esculamine:								
Once	1	—	—	—	+++	++	+	++
	2	—	—	—	++	+	+++	++
Repeatedly	1	—	—	—	+	++	++	++
	2	—	+	+	+	+	+++	+++

TABLE 3. Effect of Esculamine on Survival of Albino Mice with Experimental Influenzal Pneumonia

Experimental conditions	Mouse no.	Number of mice dying at the following times after infection									Total
		1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9-14 days	
Control	10	0	0	0	1	4	2	0	3	0	10
Injection of esculamine:											
Once	10	0	0	0	1	0	3	1	4	0	9
Repeatedly	10	0	0	0	0	1	2	2	0		5

The results demonstrate some inhibition of development of influenza virus in chick embryos and in the lungs of albino mice.

It was also important to determine whether in these conditions esculamine affected the virus directly or whether the action of the preparation was mediated through the tissues and cells of the host. To solve this problem, experiments were carried out in vitro. Esculamine in a concentration of 1 mg/ml was mixed with an equal volume of suspension of virus in a dilution of 10^{-3} (the infecting titer of the virus was 10^{-8}). The mixture was kept at room temperature for 2 h and then diluted 1,000 times, and injected in a volume of 0.25 ml into the allantoic sac of 9-day chick embryos. This dose of the preparation was tested on 10 embryos. When evaluating the action of esculamine, attention was paid to the titer of the HR of the allantoic fluid of each embryo (with a 1% suspension of chick erythrocytes) and the mean titer of the HR for each group of embryos. The infected embryos were incubated for 48 h at 37°. The results of these experiments showed that esculamine in a concentration of 1 mg/ml did not inactivate influenza virus.

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