EXPERIMENTAL BASIS FOR THE CHEMOPROPHYLAXIS OF SMALLPOX

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The great progress made in the field of virology has hardly affected the section of the chemotherapy and chemoprophylaxis of virus infections. Against the overwhelming majority of virus diseases no antibiotics or chemotherapeutic preparations are available, administration of which gives rise to a prophylactic or therapeutic effect. However, the attention of investigators has recently been attracted to reports of the prophylactic activity of thiosemicarbazones of the isatin series in relation to infections caused by viruses of the variola group [3-5, 9].

The object of the present investigation was to study the prophylactic action of β-thiosemicarbazone of N-methylisatin in experimental smallpox.

EXPERIMENTAL METHOD

A chorioallantoic culture of natural smallpox virus, strain "All-55" of the 15th passage was used. The dose of virus was determined by titration in chick embryos by the method described in the literature [10]. The titer was expressed as the number of pox-forming units in 1 ml of the virus-containing suspensions (PFU/ml). The β-thiosemicarbazones of N-methylisatin (TS) which was used is an amorphous light powder, orange in color, and almost insoluble in water, alcohol, and other organic solvents. It decomposes at a temperature of 243-245°. A suspension of TS in 1% starch enema was used.

The dose of TS was calculated per kg body weight and injected in a volume of 0.02 ml subcutaneously in the dorsal region once or twice daily on five successive days.

The authors previously showed that as a result of the intracerebral injection of natural smallpox virus into albino mice under ten days old, a generalized infection develops with a primary and secondary viremia and multiplication of the virus in the internal organs, equivalent to some extent to natural smallpox infection [1]. This model of smallpox infection was chosen to test the efficacy of the preparation mentioned above.

Newborn mice were kept in the same cage as the mother animals throughout the period of observation (14 days). Before the experiment the animals were weighed and 100 LD_{50} of natural smallpox virus was injected into the brain by the usual method. For a comparative study of the character of the infection in the treated and untreated animals, some mice from both groups were exsanguinated and their brain, lungs, and kidneys extracted after certain intervals of time. The amount of virus in the blood and organs was determined by titration in chick embryos by the method described above.

EXPERIMENTAL RESULTS

In the experiments of series I the toxicity of TS for albino mice aged 9-10 days was investigated. The results of observations over a period of three weeks on animals receiving TS subcutaneously in the dorsal region twice daily for five days showed that with a daily dose of 200 mg/kg (total for the course 1,000 mg/kg) TS had no general toxic action: the animals appeared healthy, gained weight satisfactorily, and were not retarded in development by comparison with the controls.

Efficacy of β-Thiosemicarbazone of N-Methylisatin in Experimental Smallpox in Albino Mice

Time of injection of preparation	Dose (in mg/kg)			Results
	individual	daily	total during course	
7 h and 30 min before infection	100	200	200	1/19
7 h before infection	100	100	100	1/36
Immediately after injection of virus	25	25	25	1/16
	50	100	500	1/24
	25	50	250	0/10
	12,5	25	125	0/10
	6,25	12,5	62,5	0/9
Control	Not injected			28/29

Note. Numerator—number of dying animals, denominator—number of infected animals.

The prophylactic value of TS was then investigated in relation to infection of albino mice caused by intracerebral injection of natural smallpox virus. It is clear from the table that two injections of the preparation into albino mice, each of 100 mg/kg, 7 h and 30 min before infection, prevented death of 18 of the 19 infected animals. A single injection of the same dose 7 h before infection gave similar results: of the 36 infected mice, 1 died, while in the control series 28 of the 29 infected animals died. When the dose of the preparation was reduced to one-quarter (to 25 mg/kg), the same result was obtained.

Injection of TS immediately after infection likewise proved effective, whereas in the control series nearly all the animals died (see the table). Despite the wide range of doses used in these experiments—from 500-62 mg/kg per course of treatment—no gradient in the effectiveness of TS could be discovered. However, this result is in agreement with reports in the literature [6]: death of animals was prevented by the use of 50 mg/kg of an analogous chemotherapeutic preparation per course of treatment given by the same scheme of administration.

In the next experiments the character of the infection process was studied in animals receiving TS in a single dose of 100 mg/kg 7 h before infection and in untreated animals. As a result of the quantitative determination of the virus in the organs of both groups of mice it was found that, whereas in the untreated animals (third day) at the time of maximal severity of the symptoms of infection in the internal organs (brain, kidneys, lungs) and the blood, because of proliferation the virus was present in these quantities (2.2×10^6, 1.2×10^4, 1.4×10^4, 1.8×10^3 PFU/ml respectively), in the mice receiving TS the virus was detected only in the brain tissue, and it was either absent from the lungs, kidneys, and blood or was present there in negligible amounts.

So far as the presence of virus in the brain tissue of the mice receiving TS is concerned, no substantial increase in titer could be detected by the third day after infection, whereas in the mice not receiving TS the titer of virus was increased on the third day (3.7×10^4 PFU/ml 5 min after injection of virus and 2.2×10^6 PFU/ml 3 days after injection). By the seventh day the amount of virus in the brain of the animals receiving TS was very small, and none could be isolated from the other organs.

An attempt was made to determine the therapeutic effect of TS—treatment began 12 or 24 h after infection. Some promising results were obtained, although before a more definite decision can be made regarding the efficacy of this preparation in later stages of the disease further observations are necessary.

Hence, these investigations confirm the prophylactic activity of the Soviet preparation β-thiosemicarbazone of N-methylisatin in experimental smallpox infection. The results obtained are in agreement with findings reported in the literature [6].

It was found that generalization of the infection, which is always observed in mice infected intracerebrally with natural smallpox virus, did not take place in the treated animals. The absence of secondary viremia and of accumulation of virus in the internal organs of these animals evidently shows that TS prevents the development of the virus in the susceptible tissues of the infected animals. Because of this, no severe lesions develop in the internal organs of the treated animals, as occurred in the untreated animals, where they were responsible for their death.

No investigations of the mechanism of action of TS on smallpox infection in vivo could be found in the literature. The conclusion reached by those workers who have studied this question in vitro (2, 7, 8) are in agreement in principle with the results obtained during the present investigation.

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