EFFECT OF PHENYLALANINE MUSTARD ON FRIEND VIRUS AND LEUKEMIA INDUCED BY IT

G. N. Platonova, E. S. Revazova, and L. F. Larionov

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Friend virus, previously treated with a highly active antitumor preparation of phenylalanine mustard, induces leukemias in mice later than normally and not in all animals. The use of phenylalanine mustard in Friend leukemia in vivo considerably increases the life span of the mice with this leukemia.

The virus nature of mouse leukemias is now firmly established and several leukemogenic viruses (Friend, Mazurenko, Moloney, Rauscher, etc.) have been studied in considerable detail from different standpoints.

In the last decade there has been increased attention to this study of the action of chemotherapeutic antitumor substances not only on the developed leukemias but also on the viruses inducing them.

Investigations [2-9] have shown that TET, endoxan, melfalan, 6-mercaptopurine, antifolic acid preparations, and 5-fluorouracil considerably increase the survival time of mice with Moloney's Rauscher's, and Friend's leukemias but do not cure the leukemias in these mice and do not affect proliferation of leukemogenic viruses. Despite the fact that many antitumor compounds have already been studied, no reports

TABLE 1. Action of Phenylalanine Mustard on Friend's Virus and Leukemia Induced by It Studied by the Contact Method

Day of sacrifice	Treatment	Results of infection of mice with virus*	P	Day of sacrifice	Treatment	Results of infection of mice with virus*	P
14	Control	9/10	—	21	Control	9/9	—
	Phenylalanine mustard:				Phenylalanine mustard:		
	1 MTD	2/10	0,016		1 MTD	2/10	0,00055
	0.2 MTD	1/10	0,006		0.2 MTD	1/10	0,0027
	0.1 MTD	1/10	0,0006		0.1 MTD	2/9	0,016
	0.025 MTD	2/10	0,055		0.025 MTD	4/10	0,1
	0.01 MTD	10/10	No effect		0.01 MTD	10/10	No effect

*Numerator shows number of mice developing leukemia, denominator number of mice infected with Friend's virus.

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TABLE 2. Action of Phenylalanine Mustard on Friend's Virus and Leukemia Induced by It in Vivo

Experiment No.	Treatment	Number of mice	Mean life span (in days)	Increase in mean life span (in percent)	P
1	Control	20	35,2 (21—44)	—	—
	Phenylalanine mustard 1 mg/kg for 15 days at intervals of 24 h	20	52,5 (24—66)	49,2	0,0001
2	Control	20	29,8 (26—34)	—	—
	Phenylalanine mustard 1 mg/kg for 13 days at intervals of 24 h	15	65,8 (43—90)	121	0,0001
	Phenylalanine mustard 3 mg/kg for 5 days at intervals of 72 h	15	70 (46—98)	135	0,0001

could be found in the literature of the action of the well-known compound phenylalanine mustard on Friend's leukemia.

The object of this investigation was to study the effect of phenylalanine mustard, which has a cytotoxic action, on a leukemogenic virus.

EXPERIMENTAL METHOD

Friend virus, obtained through L. A. Zil'ber from its author and maintained for 4 years in BALB/c mice in the Laboratory of Virology of Leukemias at the authors' Institute, was chosen as the experimental model. A cell-free supernatant of a 20% homogenate of leukemic mouse spleen was used in the experiments. The viricidal action of phenylalanine mustard was studied by the contact method and in experiments in vivo. In the contact method various concentrations of phenylalanine mustard were added in equal volumes to the virus in a titer of 10^{-5}, and the samples were incubated at 37°C for 3 h. The mixture of virus with phenylalanine mustard was then injected intraperitoneally into BALB/c mice in a dose of 0.5 ml per mouse so that all mice of both experimental and control groups (virus without sarcolysin) received the same dose of virus with a final dilution of 1:50. In the experiments in vitro the mice were infected intraperitoneally with Friend's virus in a titer of 10^{-4}, in a volume of 0.25 ml per mouse with a final dilution of 1:100. Intraperitoneal injections of phenylalanine mustard began 24 h after infection and continued for 13-15 days. The results were assessed from the mean life span, the weight of the spleen, and the incidence of leukemia. The results were analyzed by the Kolmogorov-Smirnov method. The results of treatment with phenylalanine mustard were regarded as successful if $P < 0.01$ [1].

EXPERIMENTAL RESULTS

Results of the treatment of Friend's virus with phenylalanine mustard in doses varying from the maximal tolerated dose (1 MTD) to 0.01 MTD are given in Tables 1 and 2. As a result of incubation of the virus for 3 h with sarcolysin in doses of 1 MTD, 0.2 MTD, and 0.1 MTD considerable weakening of the leukemogenic action of the Friend's virus was obtained. Whereas in the control group of mice infected with virus untreated with sarcolysin leukemia developed in 90-100% of cases, in the experimental groups leukemia developed in only 10-20% of the mice. Equal results were obtained whether the animals were sacrificed on the 14th or the 21st day after infection. As regards differences between the groups receiving different concentrations of phenylalanine mustard, none were found between 1 MTD and 0.25 MTD. Leukemia developed in 100% of the animals, just as in the control, only in the group receiving 0.01 MTD, when only traces of phenylalanine mustard were present.

Consequently, by the contact method of investigation phenylalanine mustard induces distinct weakening of the leukemogenic properties of Friend's virus, as reflected in a decrease in the incidence of leukemia.

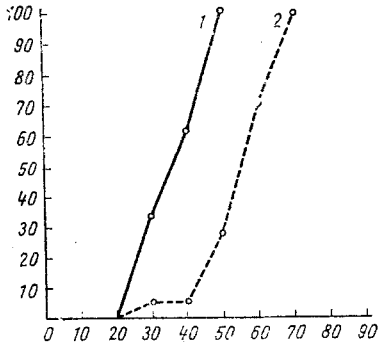

Fig. 1.

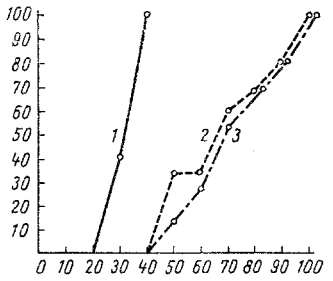

Fig. 2.

Fig. 1. Change in life span of mice with Friend's leukemia following daily injections of phenylalanine mustard: 1) control; 2) phenylalanine mustard; Abscissa, life span (in days); ordinate, overall mortality (in percent).

Fig. 2. Change in life span of mice with Friend's leukemia after administration of phenylalanine mustard in several ways: 1) control; 2) phenylalanine mustard daily; 3) phenylalanine mustard once every 72 h. Abscissa, life span (days); ordinate, overall mortality (%).

The results of experiments in vivo are shown in Table 2. They show that during treatment with phenylalanine mustard in a dose of 1 mg/kg daily for 15 days an increase in the life span of the mice infected by Friend's virus on the average by 17.3 days was obtained, i.e., an increase of 49.2% compared with the control group of untreated mice. This effect of phenylalanine mustard was accompanied by inhibition of development of splenomegaly by 65.9%. In another experiment an even greater increase in the life span of the mice with Friend's leukemia was obtained, namely, by 121 and 135%. In this experiment, however, the increase in the life span was accompanied by less marked inhibition of splenomegaly (by 24-32%). The significance of these results is clearly demonstrated by the graphs in Figs. 1 and 2.

These experiments thus showed that both after contact between virus and phenylalanine mustard in vitro and after exposure of the virus to phenylalanine mustard in vivo leukemia eventually develops, but it does so in fewer cases than in the control, or after longer intervals of time.

Consequently, the results, together with data in the literature, indicate that several cytostatic antitumor compounds exert an antiviral action, but the degree of their effect on the virus is insufficient to rid the mice of leukemia. Bearing this in mind it will be evident that in future attempts to find antileukemic substances it will be worth while using combinations of cytostatic drugs with antiviral agents so that action can be directed simultaneously on the virus and the leukemic cell.

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