REDUCTION IN THE TOXIC EFFECT OF Thio-TEPA ON MICE BY ANTISOMATOTROPIC SERUM

G. M. Sukhin and A. F. Lazarev

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A single injection of antiserum against human growth hormone into mice 1 week before or simultaneously with the beginning of a course of repeated thio-TEPA injections reduced the toxic effect of the compound on the hematopoietic system and considerably increased the survival rate of the experimental animals (to 62.7%) compared with that of the controls receiving normal horse serum and thio-TEPA (13.2-30%) or thio-TEPA only (11.4%).

The high toxicity and inadequate specificity of antitumor preparations substantially detracts from their therapeutic value, limits their use, and at the same time stimulates the search for suitable protective agents [5, 6, 8]. Substances whose protective properties are determined by their effect on metabolism and on the mitotic cycles of vitally important organs and tissues are particularly interesting.

A substance which could fall into this class is antisomatotropic serum (AS), first suggested for the treatment of diseases produced or complicated by pathological secretion of growth hormone [1].

Experiments were carried out on 255 male and female noninbred albino mice weighing 18-20 g. Thio-TEPA was injected intraperitoneally as a single dose of 20-25 mg/kg body weight or daily for 16-18 days in doses of 4 mg/kg, making a total of 72 mg/kg body weight. AS was obtained by immunizing horses with microdoses of antigen (human growth hormone), so that the high specificity of the product and its freedom from contamination by additional antibodies was ensured [2].

The AS was used after purification and standardization [3]. The serum was injected intraperitoneally, and in every case a single dose of 0.5 ml was given 1 week before or on the same day as the administration of thio-TEPA began. In the control series the mice received an injection of 0.5 ml of normal horse serum not containing antibodies against growth hormone.

The total leukocyte count in the peripheral blood, the number of nucleated cells in the femoral bone marrow [7], and the survival rate of the mice were determined.

EXPERIMENTAL RESULTS

A preliminary single injection of 0.5 ml AS into the mice led to a statistically significant decrease in the toxic action of repeated doses of thio-TEPA (Table 1). The survival rate of the animals receiving AS was several times higher than that of controls receiving normal horse serum instead of AS or receiving thio-TEPA alone. At longer intervals after the beginning of thio-TEPA administration the relative survival rate of the animals receiving additional AS increased successively. In one experiment, for instance, when AS and thio-TEPA were given, 75.0±9.7% of the animals survived until the 17th day, compared with 40.0±11.0% in the control series, an almost twofold difference. The difference by the 27th day was fourfold, and by the 37th day of observation 5.5-fold. By this period 55.0±11.1% of animals in the experimental group remained alive but only 10.0±6.7% of the control mice (Fig. 1).

Laboratory of Experimental Therapy of Tumors, Gertsen Moscow Oncological Research Institute.
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TABLE 1. Effect of Antisomatotropic Serum (AS) and Normal Horse Serum (HS) on Toxic Action of Thio-TEPA

Experimental conditions	Number of animals	Survival Rate of mice until 30th day		P
		abs.	%	
AS + thio-TEPA (1)	30	19	62.7	< 0.001
HS + thio-TEPA (1)	30	4	13.2	> 0.5
AS + thio-TEPA (2)	10	6	60.0	< 0.05
HS + thio-TEPA (2)	10	3	30.0	0.2
Thio-TEPA	35	4	11.4	–

Note. AS or HS injected intraperitoneally in a dose of 0.5 ml 1 week before (1) or on day of (2) starting thio-TEPA injections.

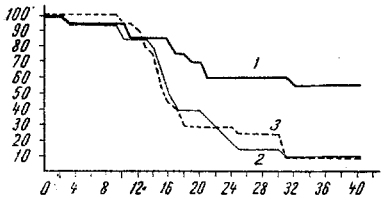

Fig. 1

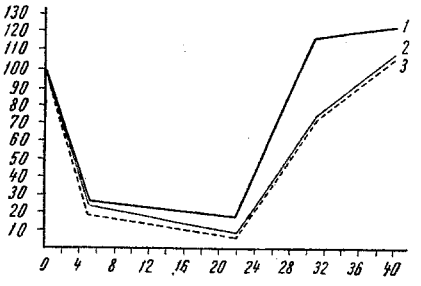

Fig. 2

Fig. 1. Survival rate of mice receiving antisomatotropic serum (1) or normal horse serum (2) 1 week before injection of thio-TEPA in dose of 4 mg/kg daily for 18 days compared with survival of control animals (3) receiving thio-TEPA alone in the same doses. Ordinate, survival rate of mice (in %); abscissa (here and in Figs. 2 and 3), days of experiment.

Fig. 2. Number of nucleated cells in femoral marrow of mice receiving antisomatotropic hormone (1) or normal horse serum (2) 1 week before injection of thio-TEPA and in animals receiving thio-TEPA alone (3). Ordinate, number of nucleated bone marrow cells (in %).

The smallest number of nucleated cells in the femoral marrow of animals receiving AS and thio-TEPA was twice as high as in animals receiving normal horse serum instead of AS or receiving thio-TEPA alone in the same doses (Fig. 2).

Although during the first 13 days of observation the leukocyte count in the circulating blood of all 3 groups of animals fell about equally, restoration of the leukocytes, like that of the nucleated marrow cells, took place fastest and soonest in the animals receiving AS. In those animals the normal leukocyte count was restored by the 32nd day, while in the control animals (receiving HS + thio-TEPA or thio-TEPA alone) the leukocyte count by this time was still only 50% of its initial value (Fig. 3). However, AS in the same dose (0.5 ml) did not protect the mice against single lethal doses of thio-TEPA (LD_{20} and LD_{60}).

The reduction in the toxic action of thio-TEPA brought about by AS must evidently be considered from the standpoint of its specific inhibitory action on production of pituitary growth hormones. AS is known to selectively inhibit the acidophile cells of the pituitary gland which produce this hormone [4], and by contrast with neutralization of growth hormone the inhibitory effect of AS on the acidophile cells is not so strictly specific in character. AS against human growth hormone degranulates the acidophiles and reduces their number not only in man or monkeys, but also in other animals, although not so rapidly and not so intensively. The decrease in growth hormone production under the influence of AS causes a decrease

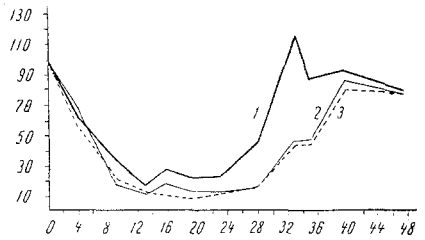

Fig. 3. Leukocyte count in blood of mice receiving antisomatotropic serum (1) or normal horse serum (2) 1 week before administration of thio-TEPA and in animals receiving thio-TEPA alone (3). Ordinate, leukocyte count (in %).

in protein biosynthesis which, in turn, arrests or retards certain phases of the mitotic cycle in cells of various organs and tissues; this is bound to affect the resistance of these cells to the action of thio-TEPA.

AS was also shown to be effective when its inhibitory action on the acidophiles was relatively weak. If homologous AS had been used, it would evidently have been more effective.

In the investigations cited above [4] it was found that AS against human growth hormone produces the strongest possible suppression of the pituitary acidophiles of animals 2-3 weeks after a single injection, whereas in monkeys more marked inhibition of the same cells was observed after an interval of only 4 days. The reason for this could be the absence of a difference in the circulating blood leukocyte counts in the experimental and control animals during the first days of thio-TEPA administration soon after injection of AS. Its therapeutic effect appeared later, when endogenous growth hormone production was more deeply inhibited.

AS thus reduced the toxic action of large doses of thio-TEPA and thus prevented death of the animals.

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