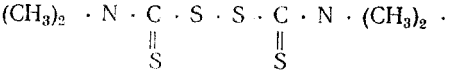
THE EFFECT OF SODIUM NUCLEATE ON THE COURSE AND ISSUE OF LEUKOPENIA INDUCED BY TETRATHIONE (TETRAMETHYLTHIURAM DISULFIDE)

M. V. Korablev

Department of Pharmacology (Head—Docent V. I. Zavrazhnov)
of the Voronezh Medical Institute
(Presented by Active Member AMN SSSR, V. V. Parin)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50, No. 12,
pp. 58-61, December, 1960
Original article submitted December 10, 1959

In the past few years, the arsenal of therapeutic agents available for the treatment of leukoses has grown considerably (nitrous analogs of yperite, AFK* myelosan (1, 4-dimethanosulfonylhydroxybutane), 6-mercaptopurine, ethylenimines, et al.). Nevertheless, the question of the treatment of leukoses is not yet solved. Research in this field, therefore, continues.

As we have already demonstrated, sulfides of the thiuram group (Antabuse, Tetrathione and sodium dimethyldithiocarbamate) act to inhibit leukopoiesis. Tetrathione (tetramethylthiuram disulfide, TMTD):



is the most active in this respect.

TMTD is a powder insoluble in water. It has long been used by industry to vulcanize resin [8]. Agriculture is now beginning to use it extensively as a fungicide, seed-dip and antioxidant in the storage of products [2, 3, 9-12].

The lethal dose of TMTD (introduced into the stomach once) is 700 mg/kg for rats and 1250 mg/kg for guinea pigs [1, 4, 5]. We have established that TMTD, introduced in a dose of 40 mg/kg or more repeatedly into the stomach, usually induces in rabbits leukopenia of the agranulocytic type which is not spontaneously resolved. Leukopenia develops as the result of the inhibition caused by the preparation of the myeloblastic hemopoietic function. In an absolute majority of animals, the hemoglobin and number of erythrocytes increase, as does the number of erythroblastic forms in the bone marrow [4, 5].

In this work, we studied the effect of sodium nucleate on the course and issue of leukopenia induced by TMTD. This is a question of real interest in view of the irreversible nature of such leukopenia, the introduction of TMTD into agriculture (where accidental poisoning is possible) and the fact that it has been recommended that the drug be clinically tested.

METHOD

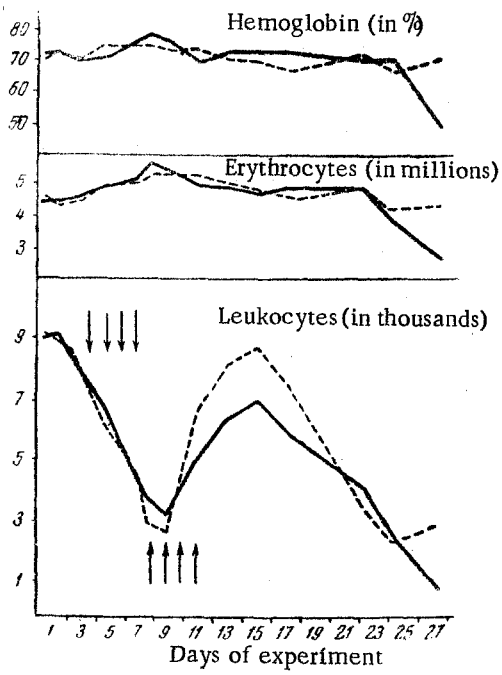
The experiments were performed on 15 male rabbits weighing 1.9-3.5 kg. After establishing the original "background" of the peripheral blood, we introduced TMTD (suspension in a 2% starch paste) in a dose of 40 mg/kg into the stomach of the animals once a day for four successive days in order to induce leukopenia.

*AFK is transliteration of Russian.

Arithmetic Mean Indices of Hemoglobin, Erythrocytes, Absolute Number of Lymphocytes and Pseudoeosinophils in the Rabbits' Blood

Day of experiment	Control				Experiment			
	hemoglobin (in %)	erythrocytes (in millions)	lymphocytes	segmented pseudoeosinophils	hemoglobin (in %)	erthrocytes (in millions)	lymphocytes	segmented pseudoeosinophils
1 st	72	4 671	6 397	2 420	71	4 500	6 002	2 671
2 nd	72	4 364	6 518	2 412	73	4 492	5 903	2 831
3 rd	71	4 554	6 258	2 175	70	4 592	5 298	2 830
5 th	75	4 998	4 977	1 409	71	4 995	4 763	1 797
7 th	75	5 085	4 308	961	77	5 367	3 122	1 547
8 th	75	5 337	2 408	534	79	5 724	2 485	642
9 th	74	5 324	2 146	521	77	5 524	2 746	565
11 th	74	5 321	4 802	1 588	70	5 078	3 859	1 116
13 th	71	5 000	4 935	2 952	73	5 000	4 183	3 467
15 th	70	4 890	4 483	3 933	73	4 767	3 698	2 956
17 th	67	4 671	4 510	2 778	73	4 900	3 797	1 950
22 nd	72	5 096	3 017	942	71	4 951	3 292	1 931
24 th	67	4 252	2 377	569	71	3 868	2 286	376
27 th	72	4 330	—	—	49	2 652	—	—

Note. TMTD was administered on the 4th, 5th, 6th, and 7th days, sodium nucleate on the 8th, 9th, 10th, and 11th days.



Dynamics of change in hemoglobin, erythrocytes and leukocytes in rabbits with leukopenia treated with sodium nucleate (average data). Key: ——) experiment; - - -) control; ↓) days of tetramethylthiuram disulfide administration; ↑) days of sodium nucleate administration.

Twenty-four hours after the fourth administration of TMTD, when leukopenia had developed in the animals, the rabbits were divided into two groups. The first, or experimental, group (eight rabbits) received sodium nucleate subcutaneously in a dose of 30 mg/kg once a day for four days in succession; the second, or control, group (seven rabbits) were subcutaneously injected with distilled water. First to establish the "background" and then during the development of leukopenia, the peripheral blood was examined every day for three days, after which it was examined every other day in most cases. We determined the hemoglobin, erythrocytes, total number of leukocytes, leukocyte formula and absolute number of lymphocytes and segmented pseudoeosinophils in the peripheral blood. Punctates were taken twice (during leukopenia and after treatment with sodium nucleate) from the epiphysis of the animals' tibia. We determined the total number of nucleated cells (myelokaryocytes) in the bone marrow. The customary experimental and clinical methods were used to examine the peripheral blood and the bone marrow. The efficiency of sodium nucleate was gauged according to the increase in the total number of leukocytes and myelokaryocytes, the normalization of the blood formula and the times at which the animals died in the control and in the experiment. The results of the experiments were recorded, and the average data were then computed from them. The table and the figure show average data from observations on seven experimental and seven control rabbits.

RESULTS

The figure shows that the total number of leukocytes in the blood of the experimental rabbits decreased, after the fourth administration of TMTD, from 8954 (initial norm) to 3171 per 1 mm^3 (eighth day of experiment). The absolute number of lymphocytes decreased 2.3 times (from 5734 to 2485), and the segmented pseudoeosinophils decreased 4.3 times (from 2777 to 642).

Treatment with sodium nucleate was undertaken on this background. After four injections of the leukopoiesis stimulator, the number of leukocytes in the rabbits had increased on the 15th day to 7000 per 1 mm^3 (see figure), the increase consisting mostly in granulocytes. Subsequently, in spite of the treatment administered, the number of leukocytes began to decrease gradually, constituting 925 per 1 mm^3 on the day of the last blood examination. A sharp decrease in the number of granulocytes was observed during this period in all the rabbits.

The leukocytes of the control rabbits showed similar changes, although the increase in their number (succeeding the primary leukopenia) was more pronounced (see table and figure). TMTD caused the total number of leukocytes in the blood of the control rabbits to decrease from the original norm of 9020 to an average of 2992 per 1 mm^3. Then, after spontaneously increasing to 8764 (on the 15th day of the experiment), their number gradually decreased, constituting 3066 per 1 mm^3 on the last day of the investigation (see figure). As in the experiment, the granular leukocytes were largely responsible for these changes (see table).

The data obtained indicate that sodium nucleate does not promote an increase in the number of leukocytes in the blood of rabbits with experimentally induced "Tetrathione" leukopenia. This was further confirmed by the results of the bone marrow investigations.

For example, we counted an average of 35,830 myelokaryocytes per 1 mm^3 of bone marrow from the experimental rabbits during the leukopenia period, and an average of 41,520 after treatment with sodium nucleate. The number of myelokaryocytes counted in the control animals during these same two periods of the investigation was respectively 32,820 and 50,640 per 1 mm^3. If the final results are disregarded, no differences were observed in the dynamics of the erythrocytes and hemoglobin in the experimental and control rabbits.

As the table shows, the administration of TMTD in both cases was attended by an increase in the number of erythrocytes and amount of hemoglobin per unit of blood volume. At the end of the experiments, we observed a decrease in the hemoglobin and erythrocytes of four out of seven experimental rabbits, and this decrease, naturally, was reflected in the average indices; in the control group, however, we only observed a slight increase in the amount of hemoglobin and number of erythrocytes in two rabbits, which did not affect the character of the average data.

All the control and experimental animals died. One rabbit of the experimental group died after the second injection of the leukopoiesis stimulator (data obtained from this animal were not in computation of the averages); 12 rabbits died between the 24th and 27th days of the experiment and two (one experimental and one control) died two months after the observations commenced.

The investigations described allow one to assume that sodium nucleate, subcutaneously administered in a dose of 30 mg/kg daily for four consecutive days does not eliminate leukopenia caused by TMTD. Indeed, the decrease in the erythrocytes and hemoglobin and the smaller increase in the leukocytes and myelokaryocytes observed in the experimental rabbits compared with the control would seem to indicate that sodium nucleate has a negative effect on hemopoiesis when administered on a background of the action of TMTD. TMTD leukopenia can be used as a "model" in the hunt for new stimulators of leukopoiesis.

SUMMARY

Tetrathione introduced into the stomach of rabbits daily for four consecutive days, in a dose of 40 mg/kg, provokes a granulocytic type of leukopenia which fails to disappear spontaneously. Sodium nucleate, injected subcutaneously for four consecutive days, once daily, in a dose of 30 mg/kg, does not improve the state of leukopenia provoked by Tetrathione.

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