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Experimental Inhibition of the Virus-Induced Rauscher Leukemia of the Mouse

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Murine virus-induced leukemias are not commonly used as testing systems for chemotherapeutic agents and were not advantageous compared to transplantable leukemias as for instance L 1210. Only Friend-virus induced splenomegaly is well known in screening systems.

We made some experiments with Rauscher virus-induced leukemia (Rauscher, 1962) which will be reported here in preliminary results. Hematology of Rauscher leukemia has been described in a most detailed study by Boiron *et al.* (1965), which will not be referred to in this paper. In earlier reports Chirigos *et al.* (1963) showed good antileukemic effectivity of 6-Mercaptopurine (6-MP), 5-Fluorouracil (5-FU), and TEM, treating five times over a period of five successive days and noting extent of splenomegaly and survival times.

Like Siegel *et al.* (1964) we found an increase in nucleated cell count in peripheral blood, continuing without remarkable remission until the death of the animal. This we thought could be taken as parameter for the development of leukemia and we based our therapy thereon, regarding cell counts, body weight and general condition, according to treatment of human leukemia. Remission of nucleated cell count was expected and after therapy covering several weeks a drug resistance could occur.

Material and method

Balb/c mice of both sexes weighing 17-18 g received 0.2 ml i.p. of a cell-free 10% suspension in 0.9% saline of leukemic spleens (approximately 10^5 inducing doses). Therapy was started 9, 10, and 13 days later. Drugs were suspended in saline and injected subcutaneously. In most weekly intervals nucleated cells were counted by punction of the retroorbital sinus and only animals with more than 10000 cells/mm^3 were treated. Body weight was determined twice weekly. Drugs employed were 6-MP, 5-FU, Melphalan and Trenimon(R).

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Results

The increase of nucleated cell count of 10 untreated control animals of a former experiment is shown in Tab. 1. Only on three occasions did a slight remission occur.

Prolongation of survival time by 5-FU, Trenimon, and 6-MP at the present state is shown in Figs. 1-3 and exceeds 100 per cent in all groups. In one group (Fig. 2) therapy was initiated by 6-MP and continued by Melphalan and showed the high toxicity of this combination. About 10 days after the first dosage of Melphalan several animals died from infection. Only Melphalan was tested in a third experiment and also proved to be effective (Fig. 4).

The influence of chemotherapy on leukemia (cell count) and body weight is demonstrated more exactly in the next figures. Limitations in cell counting for the diagrams were 12 000, 25 000, 75 000, and more cells/mm^3.

Most distinct was the effect of the first treatment compared with controls on day 17, when nearly all untreated animals of the first and the second experiment had severe leukemia (*cf.* Fig. 5: only 1 resp. 2 were under 12 000, 5 resp. 3 under 25 000, 8 resp. 2 under 50 000, 2 resp. 5 under 75 000, and 4 resp. 8 had even more cells/mm^3). Only some animals of the experimental groups show leukemia. But especially those treated with 6-MP were found to be almost free.

In the further course of therapy (Figs. 6-10) remarkable remissions with simultaneous loss of body weight were induced by 6-MP. Trenimon produced similar results. 5-FU was a little less effective. This is illustrated for 6-MP in Fig. 8 on day 38, five days after the last treatment, and on day 53. For Trenimon see the same on day 38 in Fig. 10. In experiment I the effect on nucleated cell count was at first more pronounced, but the animals lost weight and the period of survival was decreased. In experiment II lower initial doses permitted quicker repetition of treatment and later on produced better results with both drugs. Application of drugs was always repeated when the experimental group had increased again in body weight, but animals with less than 10 000 cells/mm^3 were not included.

Conclusions

Peripheral blood cell count seems to be a good parameter of Rauscher leukemia on both control and treated animals. The frequent punction (8 times until now!) seems to have no effect on cell count.

Prolongation of survival time already exceeds 100 per cent with all drugs used. Drug resistance may be the reason of the unsuccessful effect of the repeated therapy with 5-FU (*cf.* Fig. 6). In the therapy with 6-MP and Trenimon during our experiments no drug resistance appeared. Both of these substances induced remarkable remissions of the number of nucleated cells.

It should be emphasized that remission can not be used as the single criterion for effectivity. It is also necessary to consider the toxicity of the drug. In our test

Tab. 1. Nucleated cell count of 10 untreated animals with Rauscher leukemia. Spontaneous remissions did occur only in 3 steps

Days after infection		13	16	23	31
Animal	1	8900	27800	+	
	2	33500	137000	+	
	3	5600	17200	44700	+
	4	13200	76800	48000	+
	5	8200	60400	51600	+
	6	8100	26000	50400	+
	7	14900	47000	47000	+
	8	8800	21400	51800	56600
	9	25000	78000	40600	69200
	10	9800	41400	64800	142600

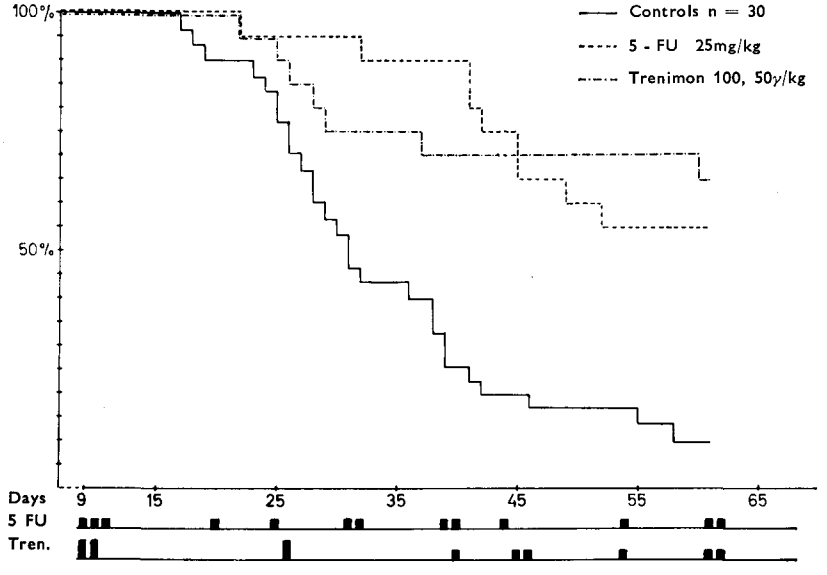

Fig. 1. Exp. I: Survival time of controls (n = 30) and 20 animals with 5 - FU resp. Trenimon under repeated therapy. Indication for treatment: *cf.* text

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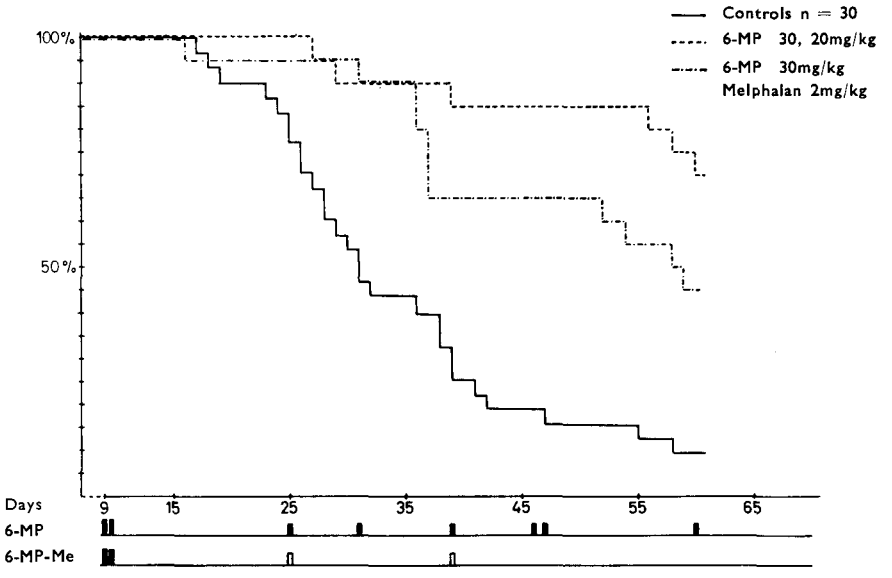

Fig. 2. Exp. I continued: Treatment with 6-MP and 6-MP, continued with Melphalan. Explanation: *cf.* text

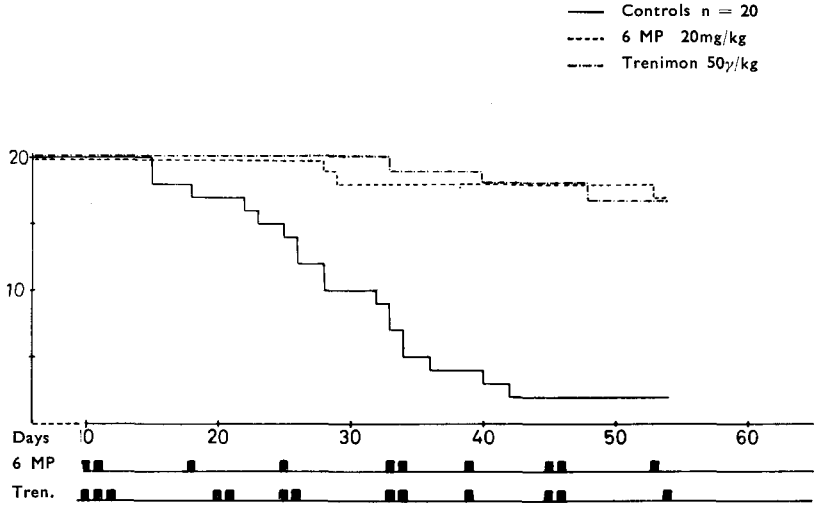

Fig. 2. Exp. II: Survival time of controls (n = 20) and animals trated with 6-MP and Trenimon. Therapy was initiated with lower doses than in exp. I

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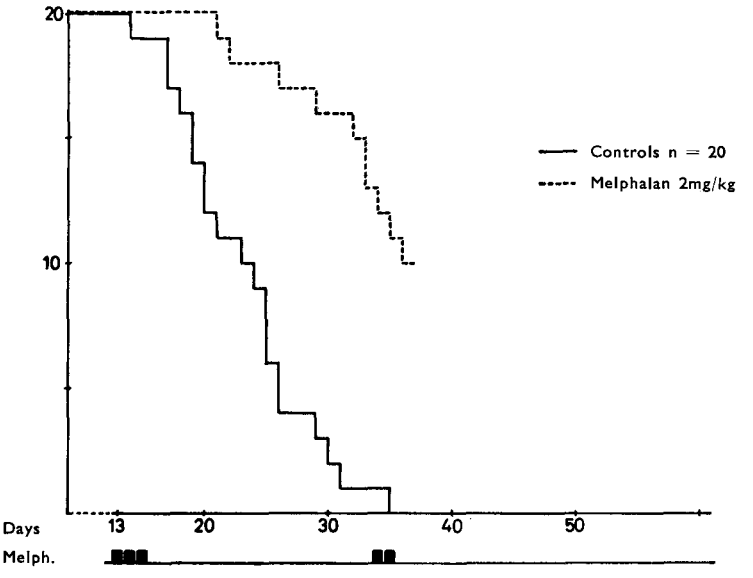

Fig. 4. Exp. III: Survival time of controls and animals treated with Melphalan

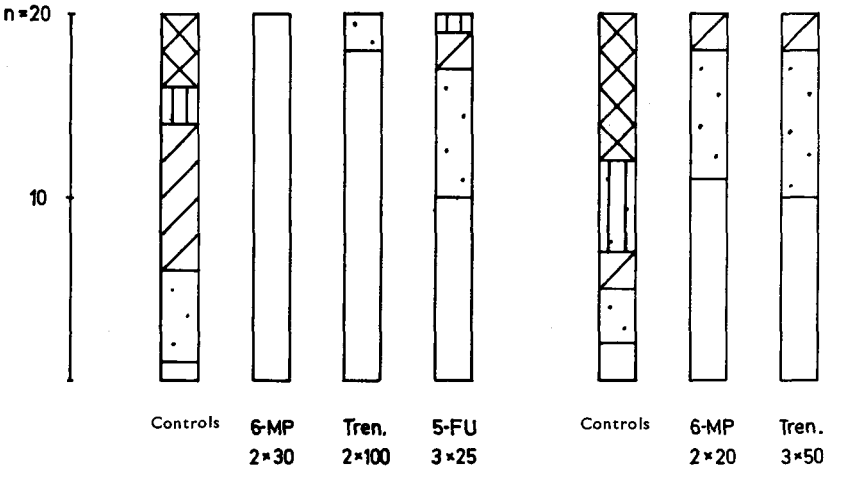

Fig. 5. Degree of leukemia (indicated by cell count) at day 17 of exp. I (left) and exp. II right) after the first therapy with controls. □ under 12 000 cells/mm³; ⁚ under 25 000; ▯ : under 50 000; ▯ under 75 000; ▯ : more than 75 000 cells/mm³. See almost total absence of increased cell counts in the treated groups

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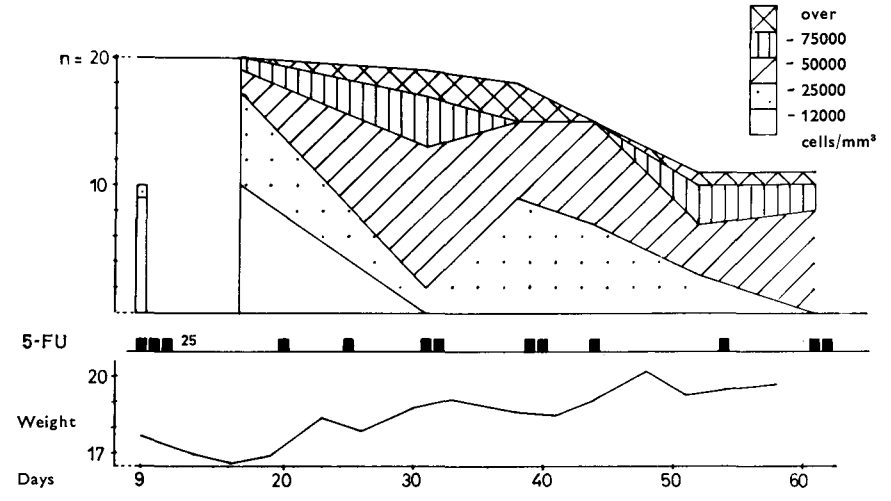

Fig. 6. Exp. I: Development of leukemia (indicated by cell count) of initially 20 animals under repeated therapy with 5-FU. See loss of body weight after each dosage and only slight remission at day 38

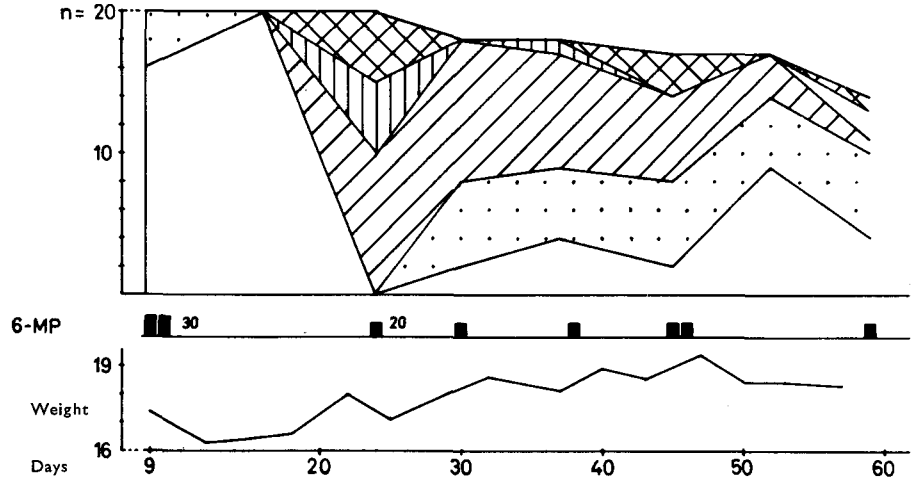

Fig. 7. Exp. I: Development of leukemia under 6-MP

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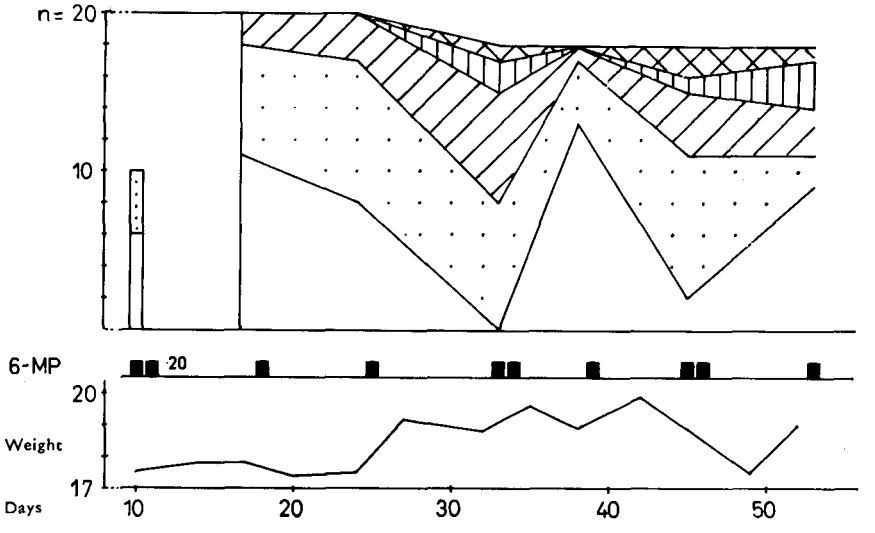

Fig. 8. Exp. II: Development of leukemia under 6-MP in lower doses at the beginning and quicker repetition than in exp. I

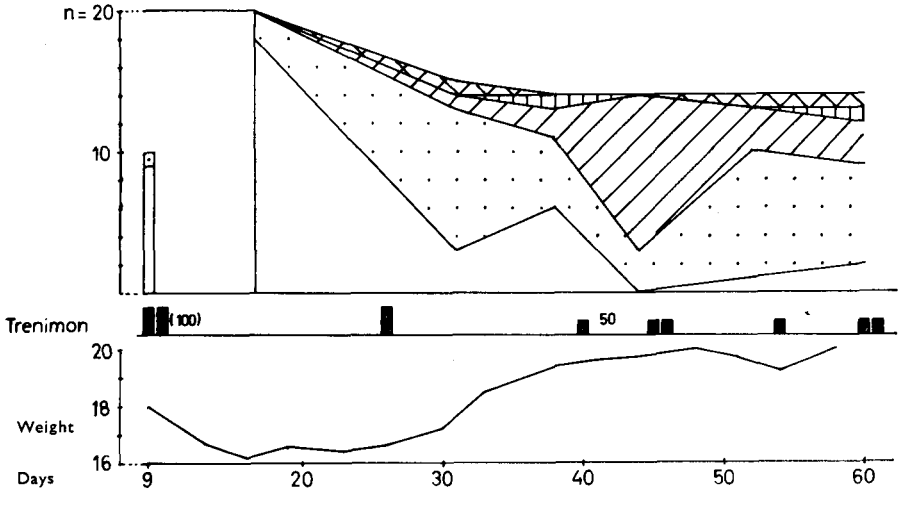

Fig. 9. Exp. I: Development of leukemia under Trenimon

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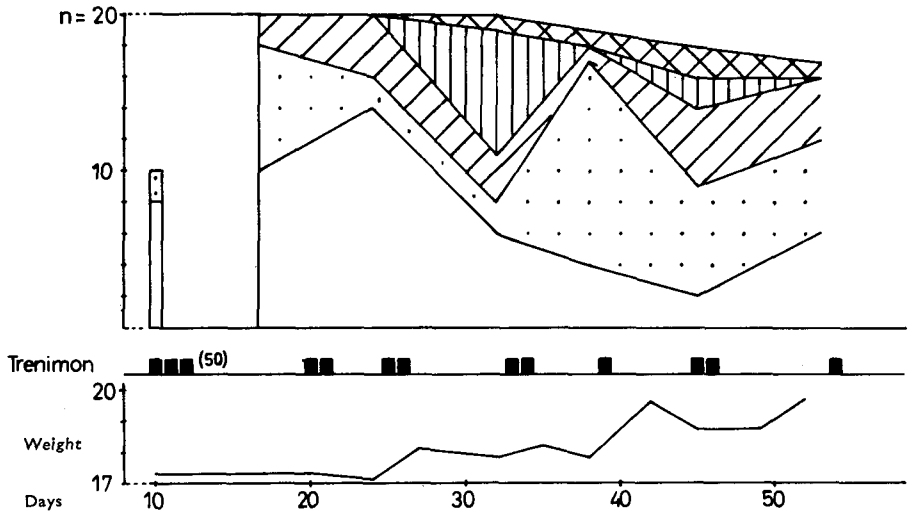

Fig. 10. Exp. II: Development of leukemia under Trenimon in lower dosage at the beginning and quicker repetition than in exp. I

system this toxicity can be seen by the decrease of the body weight, and furthermore, repetition of treatment gives information about regression of toxicity in comparison to the therapeutic effect.

This test is closer to the clinical therapy of human leukemia which has also the necessity to find the way between toxic and therapeutic effects of a drug. That seems to be a real advantage.

Summary

Chemotherapy of murine Rauscher virus-induced leukemia using Trenimon, 6-Mercaptopurine, 5-Fluorouracil and Melphalan is described. Therapy is based on nucleated cell counts in peripheral blood and body weight of the animals. Remarkable remissions of the number of nucleated cells — not occurring in untreated animals — are induced by Trenimon and 6-Mercaptopurine. Prolongation of survival times exceeds 100% in the groups treated with Trenimon, 6-Mercaptopurine, and 5-Fluorouracil.

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RIASSUNTO

Viene descritta la chemioterapia delle leucemie indotte dal virus di Rauscher con Trenimon, 6-Mercaptopurina, 5-Fluorouracile e Melphalan. La terapia si basa su conteggi delle cellule nucleate nel sangue periferico e sul peso corporeo degli animali. Notevoli riduzioni del numero di cellule nucleate (che non si verificano negli animali non trattati) vengono indotte dal Trenimon e dalla 6-Mercaptopurina. Il prolungamento dei tempi di sopravvivenza supera il 100% nei gruppi trattati con Trenimon, 6-Mercaptopurina e 5-Fluorouracile.

RÉSUMÉ

La chimiothérapie des leucémies induites par le virus de Rauscher avec Trenimon, 6-Mercaptopurine, 5-Fluorouracil et Melphalan est décrite. La manière de traitement se dirige suivant le nombre des cellules nucléées dans le sang périphérique et suivant le poids des animaux. On atteint des réductions remarquables du nombre de cellules avec Trenimon et 6-Mercaptopurine — qui n'existe pas chez les animaux non traités. La prolongation de survivance dépasse 100% chez les groupes traités avec Trenimon, 6-Mercaptopurine et 5-Fluorouracil.

ZUSAMMENFASSUNG

Die Chemotherapie der durch das Rauscher-Virus induzierten Mäuseleukämie mit Trenimon, 6-Mercaptopurin, 5-Fluorouracil und Melphalan wird beschrieben. Die Behandlungsweise richtet sich nach der Zahl der kernhaltigen Zellen im peripheren Blut und dem Körpergewicht der Tiere. Deutliche Remissionen der Zellzahl, wie sie bei unbehandelten Tieren nicht vorkommen, werden mit Trenimon und 6-Mercaptopurin erzielt. Die Verlängerung der Überlebenszeit überschreitet 100% bei den mit Trenimon, mit 6-Mercaptopurin und bei den mit 5-Fluorouracil behandelten Gruppen.