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Effects of Priming Dose Schedules in Cytoxan Treatment of Mouse Leukemia L1210 (37967)

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In previous studies utilizing the mouse leukemic L1210 system, it was demonstrated that longer survivals occurred with methotrexate (MTX,NSC-740) therapy when treatment was initiated with a high priming dose followed by lower doses (1). The optimal priming dose was at a level approximating the optimal single dose.

Similarly, when one of a series of scheduled doses of MTX was substituted by a high dose of MTX or Cytoxan (Cyt), the earlier the substitution the longer was the survival (2). On the other hand, if a regularly scheduled dose of MTX was omitted, earlier omission was disadvantageous (3). These data (1–3) suggested that MTX, as a cell-cycle-stage specific agent (CCSS), kills a smaller percentage of tumor cells as the population of tumor cells increases. It was indicated that the early high-priming therapy lowers the population of tumor cells sufficiently for subsequent doses of MTX to kill more effectively. These experiments emphasized the importance of early institution of maximally effective regimens of MTX when employed singly or in combination therapy.

It had been generally considered that for cell-cycle-stage nonspecific agents (CCSN), tumor cell kill followed first-order kinetics (4), i.e., the percent kill of tumor cells is constant irrespective of population size. If this were so, one would not anticipate an advantage to therapy by employing high-priming-dose schedules with CCSN drugs.

However, there has been recent evidence to suggest that Cyt, considered to be a CCSN agent (5), may in fact be partially cell cycle dependent (6, 7). Also, Valeriote *et al.* have indicated that at higher doses of Cyt, the percentage kill of malignant cells decreased (8). Van Putten and Lelieveld have suggested that Cyt may be more active against the more rapidly proliferating hemopoietic stem cells (9).

Valeriote and Tolen further demonstrated that of a number of alkylating agents Cyt was the most cycle-specific agent tested in the spleen colony assay (10). Cycle specificity for Cyt was also suggested in a study in which one of a series of doses of Cyt was substituted by a high dose of BCNU (11). As in the MTX experiment (2), the earlier the substitution the longer was the survival.

We have reported studies in which markedly increased survivals were observed employing loading-dose schedules of Carbazilquinone (NSC-134679) (12). This drug may possess alkylating properties as does Cyt (13, 14).

Therefore, this study was conducted to determine whether the clinically effective CCSN alkylating agent Cyt would demonstrate any change in therapeutic effectiveness when utilized in a loading dose schedule as compared with standard schedules. Other treatment-schedule designs were employed in an attempt to elucidate on a kinetic basis any observed differences in survival of mice on the standard and loading-dose schedules.

Materials and Methods. Stock tumor of lymphoid leukemia L1210 was carried in this laboratory by weekly intraperitoneal

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TABLE I. Comparison of the Influence of Loading-Dose and Constant-Dose Schedules of Cytoxan (Cyt) on Survival Time.[a]

Q2 day dose Cyt mg/kg from Day 6 to death	Median survival time (days)									
	Initial dose of Cyt mg/kg Day 4									
	337.5	225	150	100	66.7	44.4	29.6	19.7	13.1	8.7
150			9.5	10	10	11.5	11	12	11	11
100		12.5	14.5	19.5	18.5	19	21	14	11.5	14.5
66.7	10	24	23.5	21	18.5	14	13	12	11.5	10.5
44.4	22.5	22.5	22.5	21	16.5	12	10.5	10.5	10	10.5
29.6	23	19	17.5	15	14	12	10.5	10	10	10
19.7	26.5	18.5	16.5	14.5	12.5	11.5	10	10	9.5	9.5
13.1	10[b]	19.5	16	13	12	11	10	9	9	9
8.7	28.5	18	15	13	12	11	9.5	9	9	9

[a] CDF_1 male mice weighing 19–25 g were injected intraperitoneally with 10^5 L1210 ascites tumor cells on Day 0. Median survival time (MST) of tumor control mice was 8 days. There were 8 mice per group. The MST of groups of mice on constant-dose schedules are joined by a diagonal line.

[b] 5 of 8 mice in this group died by Day 10 of toxicity.

passage in DBA/2 male mice. Ascites tumor cells were counted in a hemocytometer, diluted in Hank's Balanced Salt Solution to 1×10^6 cells/ml, and the suspension kept in an ice bath. 1×10^5 cells (0.1 ml) of tumor cell suspension were injected intraperitoneally into CDF (BALB/cAnCrx DBA/2Cr)F_1 male mice weighing 19–25 g. Mice were randomized and housed in a constant-temperature facility and provided with water and laboratory chow ad lib. Cyclophosphamide (Cytoxan, Cyt, from Mead Johnson, Lot #2 man 121) NSC-26271, was freshly prepared at each injection period. It was dissolved in 0.85% saline and injected intraperitoneally in a volume of 0.01 ml/g body wt.

Results. Comparison of the influence of loading-dose schedules and constant-dose schedules on survival time. Three experiments were conducted with Cyt in which the interval between doses was 2, 5, and 7 days (Tables I–III) respectively. The median survival time (MST) of control groups in all experiments was 8 days.

Of the groups of mice receiving Cyt every 2 days (Q2d) (Table I), the group that survived longest had an MST of 28.5 days. It had received 337.5 mg/kg Cyt on Day 4 and 8.7 mg/kg Q2d from Day 6. Another group which received 337.5 mg/kg initially, followed by 19.7 mg/kg Q2d, survived the next longest; MST 26.5 days. Four of the total of 16 mice in these two groups died early of toxicity. In the group which received 337.5 mg/kg initially followed Q2d by 13.1 mg/kg, the MST was only 10 days, as 5 of 8 mice died by Day 10. The groups with the next longest survival times (22.5–24 days) all received high initial doses of Cyt, in the range of 150–337.5 mg/kg. The group that survived longest on a constant dose schedule received 100 mg/kg Q2d; MST 19.5 days. Thus, on a loading-dose schedule, groups of mice survived as much as 9 days longer than the longest surviving group on a constant-dose schedule. In total, there were eight loading-dose groups with survival times greater than the longest surviving group on a constant-dose schedule.[2]

On an every-5-day (Q5d) (Table II) schedule, the longest surviving group was on a constant-dose schedule, 225 mg/kg (MST 23.5 days). The next longest sur-

[2] The MST's of the two groups which survived longest were significantly greater than the MST of the longest surviving group on a constant dose schedule ($P \leqq 0.05$).

TABLE II. Comparison of the Influence of Loading-Dose and Constant-Dose Schedules of Cytoxan (Cyt) on Survival Time.[a]

	Median survival time (days)					
	Initial dose of Cyt mg/kg Day 4					
Q5 day dose	337.5	225	150	100	66.7	44.4
337.5	14	17	17	17	18	9.5
225.0	13.5	23.5	19.5	23	16.5	10.5
150.0	15.5	21	22.5	21	16.5	10
100.0	18	23	21.5	20	15.5	12
66.7	12.5	20	19	15	14.0	10
44.4	15.5	17	18	14.5	13.5	10.5
29.6	17.5	18	14.5	14.5		
19.7	17.5	19.5	15			

[a] CDF_1 male mice weighing 19–25 g were injected intraperitoneally with 10^5 L1210 ascites tumor cells on Day 0. Median survival time (MST) of tumor control mice was 8 days. There were 8 mice per group. The MST of groups of mice on constant-dose schedules are joined by a diagonal line.

TABLE III. Comparison of the Influence of Loading-Dose and Constant-Dose Schedules of Cytoxan (Cyt) on Survival Time[a]

	Median survival time (days)				
	Initial dose of Cyt mg/kg Day 4				
Weekly dose Cyt mg/kg from Day 11 to death	337.5	225.0	150.0	100.00	66.7
337.5	15.5	19	18.6	17	12
225.0	14	23.5	17.5	15	12
150.0	17	21	21	21	12
100.0	11.5	14.5	18.5	18	11.5
66.7	14	21	18	17	12
44.4	13.5				

[a] CDF_1 male mice weighing 19–25 g were injected intraperitoneally with 10^5 L1210 ascites tumor cells on Day 0. Median survival time (MST) of tumor control mice was 8 days. There were 8 mice per group. The MST of groups of mice on constant-dose schedules are joined by a diagonal line.

viving group received 225 mg/kg initially followed by 100 mg/kg; MST 23.0 days. Similarly on a weekly schedule (Q7d) (Table III), the longest MST (23.5 days) was obtained on a constant schedule (225 mg/kg Q7d).

Thus when the interval between treatments was increased to 5 or 7 days, there was no advantage to therapy in the priming-schedule design. For the 3 schedules studied (Tables I–III), the longest surviving groups were on loading-dose schedules with Q2d intervals.

Effect of single treatment with Cyt on the survival time and lethal toxicity for leukemic mice. The single-dose levels of Cyt on Day 4 which resulted in the greatest increases in survival time of L1210 leukemic mice were 337.5 and 225 mg/kg (Table IV). However, at the former level, 43.7% of the mice died of toxicity.[3]

[3] Animals which died prior to Day 8 or with definite weight loss (>10%) when accompanied by no evidence of ascites were considered as toxic deaths.

TABLE IV. Effect of Single Treatment of Cyt on the Survival Time and Lethal Toxicity for Leukemic Mice.[a]

Cyst mg/kg Day 4	MST (days)	Mean survival[b] (days)	% Dying of toxicity
337.5	18.5	19.3	43.7
225	18	18.7	6.7
150	15	15.5	0
100	13	12.9	0
66.7	12	12.0	0
44.4	11	10.6	0
29.6	10	9.9	0
19.7	10	9.4	0
13.1	9	9.3	0
8.7	9	9.0	0

[a] 10^5 L1210 cells were injected intraperitoneally on Day 0, Median survival time (MST) of untreated control mice was 8 days. 8–16 mice were used in each treatment group.

[b] Mean survival time as well as median is included because the mean may enable us to make finer distinctions in survival time not possible with medians.

The initial loading dose of the longest surviving group in each of the first three experiments was in the range of 225–337.5 mg/kg (Tables I–III), which corresponds with the optimal single doses (Table IV).

Relationship of percent kill by Cyt to tumor cell population. (A) Comparison of survival time using multiple-treatment schedules of Cyt at varying tumor inoculum levels. When Cyt 50 mg/kg was administered Q4d from Day 3 to mice inoculated with log dilutions of tumor, the increase in lifespan of treated groups over controls (T–C) increased substantially at lower tumor inoculum levels (Table V). The T–C of groups inoculated with 10^5 tumor cells was 6 days. The difference was increased to 11.5 days in groups inoculated with 10^3 cells.

(B) Comparison of survival time using increasing numbers of treatments following a loading dose of lower initial dose. A comparison was made of the effect of the total number of treatments with Cyt on the lifespan of leukemic mice (Fig. 1). The MST's of mice which received one dose of Cyt 125 mg/kg averaged 2 and a ¼ days longer than that of the groups which received 50 mg/kg. However, beyond 4 doses of drug, the groups with the low initial dose had no further increase in lifespan. In groups which received the high initial dose, lifespan continued to increase until 6 doses were administered, so that after 6 doses the MST's of corresponding groups were about 4 days apart.

Discussion. In the current study, therapeutic effectiveness was increased by the employment of a loading-dose schedule of Cyt when the interval between doses was small (2 days). For this schedule, since the recovery time is not extensive, the optimal constant dose was relatively low (100 mg/kg). The ability to employ an initial high dose (approximately an optimal single dose) (337.5 mg/kg or 225 mg/kg) on this schedule, even though followed by subsequent doses that were substantially reduced, permitted the observation of improved therapeutic effectiveness.

In contrast, at longer intervals between treatments (Q5d and Q7d) where the recovery time was greater, the optimal priming dose and optimal constant dose were the same and equivalent to the optimal single dose. The results of this study are similar to those with MTX (1) in that the optimal priming dose for all schedules approximated the optimal single dose.

This study indicates that the use of loading doses may alter the optimal scheduling of a drug. For Cyt, with constant-dose schedules, longer intervals (Q5d and Q7d) were most effective. This is in agreement

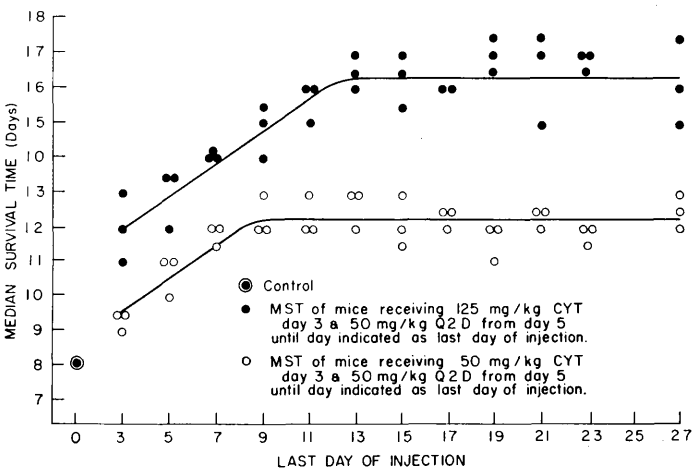

FIG. 1. Comparison of survival time using increasing numbers of treatments of MTX following a loading dose or low initial dose of Cyt. 10^5 L1210 ascites tumor cells were inoculated intraperitoneally on Day 0. Treatment with Cyt was intraperitoneal, beginning on Day 3. Each point represents the MST of 6 mice.

with previous studies (15, 16). However, on loading-dose schedules, the longest surviving groups were observed when treatment was Q2d. It is possible that with a high loading dose, those cells not killed directly may be sublethally damaged (6). A subsequent small dose of drug such as 8.7 mg/kg (Table I) if administered before repair occurs might then suffice to kill the damaged cells. When 8.7 mg/kg was administered Q2d without a prior loading dose, there was almost no increase in survival time. A single dose 337 mg/kg increased MST over controls by 10.5 days (Table IV). The same loading dose followed by 8.7 mg/kg Q2d increased MST to 20.5 days.

The data in Table V and Fig. 1 provide evidence that Cyt kills a diminished percentage of tumor cells at increased populations. A schematic representation of the results of Fig. 1 is presented in Fig. 2. In this schema, the percent kill is constant until the tumor is very large, at which point it diminishes. Such a change in percent kill is consistent with the finding of Valeriote *et al.* (8) and DeWys and Knight (6) in that cell kill with Cyt did not follow first-order kinetics in relation to drug concentration.

Several possibilities may be presented which could account for varying percent kill with Cyt. (a) MTX, as a CCSS agent, may be expected to demonstrate a reduced percent tumor cell kill for larger tumors, if there is a decreased growth fraction (GF) (17), with progressive tumor growth. Cyt may kill cells in any phase of the cell cycle (18). The drug's cell-cycle-dependence (preferential kill for proliferating cells) is implied by Valeriote's data (10). As a tumor grows and more cells go into the G_0 (resting or nonproliferating) stage, the

TABLE V. Comparison of Survival Time Using Multiple Treatment Schedules of Cytoxan at Varying Tumor Inoculum Levels.

	Median survival time (days)		
	L1210 inoculum size intraperitoneally Day 0		
	10^5	10^4	10^3
Treated[a]	16	19	23.5
Controls	10	10.5	12
T–C[b]	6	8.5	11.5

[a] Treated groups received cytoxan 50 mg/kg Q4d from Day 3 to death.

[b] Treated minus controls.

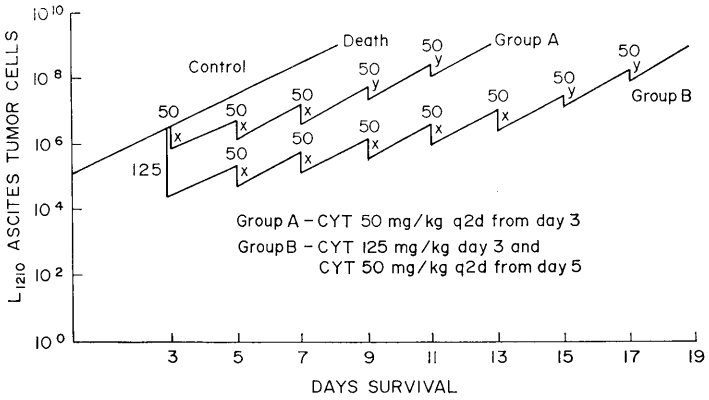

FIG. 2. Diagramatic representation of tumor cell kill of Cyt using increasing numbers of treatments following a loading dose or low initial dose. Death is assumed to occur at 10^9 cells. Changes in generation time of tumor cells are not considered. The loading dose of Cyt, 125 mg/kg, kills approximately 2 logs of tumor cells, and 50 mg/kg kills (X) approximately 2/3 log of tumor cells. For the two doses subsequent to the initial dose in both groups, 50 mg/kg Cyt demonstrates a constant percent tumor cell kill. At a critical level of tumor cells, $>10^7$, the log kill (Y) for 50 mg/kg is diminished. This occurs by the 4th dose in group A and by the 7th dose in group B.

tumor would become less sensitive to a cell-cycle-dependent agent. (b) As most tumors grow, the mean generation time (T_c) increases (1, 18–21). If Cyt is more effective during certain phases of the proliferative cycle (6), and if the duration of the sensitive phase does not increase proportionally as much as does T_c with tumor growth, then Cyt would kill a smaller percentage of cells with tumor growth. (c) Cyt may be more effective against the rapid proliferating cells (9). The percentage of these cells in a tumor are reduced as growth occurs. (d) Another possible reason for diminished percent tumor cell kill would be that with larger tumors, the increased population of dead cells and decreased vascularity may interfere with the ability of the drug to kill tumor cells.

The present study with Cyt indicates the need for further studies designed to elucidate the kinetics of tumor cell kill. In particular, age-response experiments (22) may elucidate the differential cell kill of Cyt for the various phases of the cell cycle. Kinetic data may provide insights into the design of more effective clinical drug schedules.

Summary. The purpose of this study was to determine whether priming-dose schedules of Cytoxan (Cyt) could result in increased antileukemic effectiveness in L1210 leukemic mice. In three experiments, each initial dose of Cyt was followed by various schedules of Cyt. When the interval between doses was short (2 days), the optimal treatment was found to consist of a high priming dose followed by markedly reduced regular doses. At longer intervals (5 and 7 days), the optimal modality was a constant-dose schedule. On constant-dose schedules, a longer interval between treatments was advantageous. When loading-dose schedules were included, the longest surviving group was observed when a shorter interval was employed.

The results of an experiment in which mice were inoculated with log dilutions of L1210 and treated with a course of Cyt suggest that the percentage of tumor cells killed by Cyt may diminish with larger tumor-cell populations. It is further suggested that the diminished percent kill may become evident only after the tumor is very large.

The current observations support the

concept that Cyt is at least partially cell-specific.

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