

# Effects of Priming Dose Schedules in Methotrexate Treatment of Mouse Leukemia L1210

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## SUMMARY

This study was conducted to determine whether priming dose schedules of methotrexate (MTX) could result in increased antileukemic effectiveness. CDF<sub>1</sub> mice received i.p. inoculations of 10<sup>5</sup> L1210 ascites tumor cells. Beginning 2 days after leukemic inoculation (i.p.), the animals were treated with MTX (i.p.) on a series of dose schedules. In each experiment, a number of geometrically spaced doses of MTX were used at the first treatment, and each was followed by various geometrically spaced levels of regularly scheduled MTX. For each of the schedules used, the optimal treatment consisted of a relatively high priming dose approximating the optimal single dose of MTX, followed by reduced regular doses. For the 5 schedules studied, the longest surviving group had a median survival time 2.5 to 6.0 days longer than that of the optimal group on a constant dose schedule.

The results of experiments in which mice received log dilutions of L1210 and were treated with a course of MTX, and in which treatment with MTX was terminated at varying intervals, suggest that MTX has a greater percentage of tumor cell kill at lower tumor cell populations. A cell cycle stage-specific agent such as MTX may kill a small percentage of tumor cells as the number of cells increases if there is either a corresponding decrease in growth fraction or an increase in mean generation time. In either case, a priming dose of MTX then may lower the tumor cell population sufficiently for subsequent low doses of MTX to kill more effectively.

## INTRODUCTION

Anticancer drugs have exhibited schedule dependency, in both experimental tumor systems and in the clinic, and this has stimulated a strong focus, in laboratory studies, on the determination of optimal dosage schedules (4-7, 9, 14, 15, 17, 18, 20-23). Goldin *et al.* (4, 6, 7) and Venditti *et al.* (21), using the L1210 *in vivo* tumor system, showed that the therapeutic efficacy of a drug depended on such factors as time of initial treatment, total dosage, duration of treatment, interval between the administration of individual doses, number of doses, and route of administration. Skipper *et al.* (17, 18) and Schabel (14) developed treatment schedules based on considerations of tumor cell kinetics and obtained increases in the life-span of leukemic animals. However, in

most studies, irrespective of drug, tumor system, or approach to schedule design, the schedules have incorporated fixed dosage levels of drugs with uniform intervals between treatments.

The studies reported here were designed to determine whether systematic alteration of the initial dosage level of a drug in relation to subsequent doses might lead to increase in therapeutic effectiveness. Specifically, the hypothesis was tested that longer survivals could be obtained, as compared with standard schedules, if an initial loading dose was followed by lower doses. A kinetic approach was used in an attempt to elucidate any observed differences in survival of mice subjected to the standard and loading dose schedules.

In the current investigations, MTX<sup>1</sup> was chosen as representative of a drug of clinical importance for which there has been broad experience in the L1210 ascites system (23).

## MATERIALS AND METHODS

A stock tumor of lymphoid leukemia L1210 was carried i.p. in strain DBA/2 male mice. Ascites tumor cells were counted in a hemocytometer and diluted in Hanks' balanced salt solution to  $1 \times 10^6$  cells/ml, and the suspension was kept in an ice bath. CDF<sub>1</sub> male mice weighing 18 to 25 g received 0.1-ml i.p. injections of tumor cell suspension. Mice were randomized and housed in plastic cages in a constant temperature facility and were provided with water and laboratory chow *ad libitum*.

MTX (amethopterin; NSC 740) was prepared from Lederle Lot No. 1260x8105. It was dissolved in 2% sodium bicarbonate and injected i.p. in a volume of 0.01 mg/g body weight. It was prepared every 4 days and refrigerated in an opaque jar. Serial dilutions were prepared at 0.6 intervals over a range of 100 to 0.22 mg/kg. All treatments were begun 2 days after tumor inoculation.

## RESULTS

### Comparison of the Influence of Loading Dose and Constant Dose Schedules on Survival Time

The MST of control groups in all experiments was 8 days. In 5 experiments conducted with MTX, the intervals between

<sup>1</sup>The abbreviations used are: MTX, methotrexate; MST, median survival time; CCSS, cell cycle stage specific; GF, growth fraction; CCNS, cell cycle stage nonspecific.

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doses were 1, 2, 3, 4, and 5 days, respectively (Table 2). Regardless of the interval between doses, the life-span of the mice was longest when they received an initial dose of highly concentrated MTX, followed by lower doses (Tables 1 and 2).

Of the groups of mice receiving MTX daily (Table 1), the group that survived longest had an MST of 25.0 days. This group had received 21.6 mg/kg on Day 2 and 0.60 mg/kg daily from Day 3. The group that survived longest on a constant dose schedule received 1.01 mg/kg daily with a MST of 19.0 days. Thus, on a daily schedule, the former group had an additional 6-day increase in MST. Similarly, on an every-2-, -3-, -4-, or -5-day schedule, the longest surviving groups received a loading dose schedule (Table 2).

For the 5 schedules studied, the longest surviving group had a MST 2.5 to 6.0 days greater than the optimal group on a constant dose (Table 2). The best survivals for all groups were obtained following loading doses, at daily and every-2-day intervals. In each experiment, the total dosage of MTX in the group showing maximum survival on a loading dose schedule was greater than the total dosage for the longest surviving group on a constant dose schedule.

**Effect of Single Treatment of MTX on the Survival Time and Lethal Toxicity for Leukemic Mice**

The single dose of MTX given on Day 2 which resulted in the greatest increase in survival time of leukemic mice was 60 mg/kg, and this was followed closely by 36 mg/kg (Table 3). Nevertheless, there was evidence of lethal toxicity at these drug levels. The criteria for toxic death were no evidence of ascites at death and either a 10% body weight loss or death before Day 8 (the day of tumor control deaths). On this basis, doses of 60 and 36 mg/kg were lethal to 10.8 and 6.0% of the mice, respectively. The 100 mg/kg dose was lethal for 56% of the mice.

The initial loading dose of the longest surviving group in

each of the first 5 experiments was in the range of 21.6 to 60.0 mg/kg (Table 2). This tended to approximate the optimal single dose (Table 3).

**Experimental Design to Determine the Relative Log Kill of MTX at Varying Tumor Cell Populations**

**Comparison of Survival Time with 1 or 2 Doses of MTX at Varying Tumor Inoculum Levels.** Although the survival time increase was greater with 2 MTX treatments than with 1, the increase in life-span over controls for these dose regimens did not appear to be influenced by the concentration of the leukemic inoculum (Chart 1).

**Comparison of Survival Time with Multiple MTX Treatment Schedules at Varying Tumor Inoculum Levels.** When a greater number of MTX injections were given to mice receiving inoculations of log dilutions of tumor, the increase in life-span was significantly greater at lower tumor inoculum levels (Table 4). The increase in life-span over controls of groups given inoculations of 10<sup>5</sup> tumor cells was 11.5 days, and this was increased to 20 days in groups given inoculations of 10<sup>3</sup> cells.

**Comparison of Survival Time with Increasing Numbers of Treatments Following a Loading Dose or Low Initial Dose.** A comparison was made of the effect of the total number of treatments with MTX on the life-span of leukemic mice. As the number of doses (at fixed intervals) was increased, the survival time of groups with the high initial loading dose increased more rapidly than that of groups with the lower initial dose (Chart 2). Similar results were obtained in a duplicate experiment.

**DISCUSSION**

The current studies indicate that with MTX it is possible to obtain longer survival of leukemic animals if the schedule

Table 1

*Comparison of the influence of loading dose schedules and constant dose schedules of MTX on survival time*

CDF<sub>1</sub> male mice weighing 18 to 25 g received i.p. injections of 10<sup>5</sup> L1210 ascites tumor cells on Day 0. The MST of untreated control mice was 8 days, range 8 to 9. There were 8 mice per group. The MST of groups of mice on constant dose schedules are joined by a diagonal line.

Daily dose of MTX from Day 3 to death (mg/kg)	MST (days)											
	Initial dose of MTX on Day 2 (mg/kg)											
	100 mg/kg	60 mg/kg	36 mg/kg	21.6 mg/kg	12.96 mg/kg	7.77 mg/kg	4.66 mg/kg	2.80 mg/kg	1.68 mg/kg	1.01 mg/kg	0.60 mg/kg	0.36 mg/kg
2.80											10	11
1.68	7	13	9	10	13	12.5	13	9	12	17.5	(9-12) <sup>a</sup>	(8-14)
1.01	(6-8)	(6-19)	(6-14)	(6-14)	(10-30)	(9-20)	(9-17)	(8-19)	(10-16)	(10-27)	14.5	14
0.60	7.5	10.5	14.5	11	17.5	16.5	19.5	16.5	19	19 <sup>c</sup>	13.5	19
0.36	(6-20)	(7-29)	(7-25)	(6-29)	(11-27)	(7-29)	(13-23)	(13-23)	(14-21)	(15-23)	(11-20)	(14-23)
0.22	14	8.5	22	25 <sup>b</sup>	19.5	20.5	21.5	20	18	18	17.5	16
	(6-23)	(6-23)	(14-29)	(11-27)	(17-26)	(11-30)	(12-25)	(16-23)	(9-35)	(14-34)	(15-20)	(15-18)
	7	19	18.5	17	18	17	18	15	15	16	14	13
	(6-19)	(9-23)	(9-20)	(9-21)	(16-23)	(16-27)	(15-20)	(12-19)	(12-26)	(13-19)	(11-16)	(13-14)
	15.5	18	16	15	15	14.5	14	13				
	(6-19)	(6-20)	(10-18)	(14-16)	(14-18)	(13-20)	(13-15)	(11-14)				

<sup>a</sup> Numbers in parentheses, range of survival time.  
<sup>b</sup> Maximum MST, all dose schedules used.  
<sup>c</sup> Maximum MST, constant dose schedules only.

**Table 2**  
 Comparison of the influence of loading dose schedules and constant dose schedules of MTX on survival time (summary of Experiments 1 to 5)

CDF<sub>1</sub> male mice weighing 18 to 25 g received i.p. injections of 10<sup>5</sup> L1210 ascites tumor cells on Day 0. MST of untreated control mice in each experiment was 8 days.

Interval between doses of MTX (days)	Optimal constant dose results		Optimal loading dose results		
	Optimal constant dose of MTX beginning on Day 2 (mg/kg)	MST (days)	Initial loading dose of MTX on Day 2 (mg/kg)	Subsequent constant dose of MTX (mg/kg)	MST (days)
1	1.01	19 (15-23) <sup>a</sup>	21.6	0.6	25 (11-27)
2	4.66	22.5 (18-28)	60	2.8	25 (7-30)
3	12.96	18 (12-21)	60	7.77	21.5 (7-26)
4	21.6	16 (10-21)	36	12.96	20 (15-25)
5	21.6	16 (12-17)	60	21.6	19.5 (6-21)

<sup>a</sup> Numbers in parentheses, range of survival time.

**Table 3**

Effect of single treatment of MTX on the survival time and lethal toxicity for leukemic mice

L1210 cells (10<sup>5</sup>) were injected i.p. on Day 0. MST of untreated control mice was 8 days. From 25 to 50 mice were used in each treatment group.

MTX on Day 2 (mg/kg)	MST (days)	Mean survival time (days) <sup>a</sup>	Mice dying of toxicity (%)
100	8.5	10.4	55.9
60	12.0	11.9	10.8
36	11.0	11.7	6.0
21.6	11.0	11.0	5.2
12.96	11.0	10.8	0

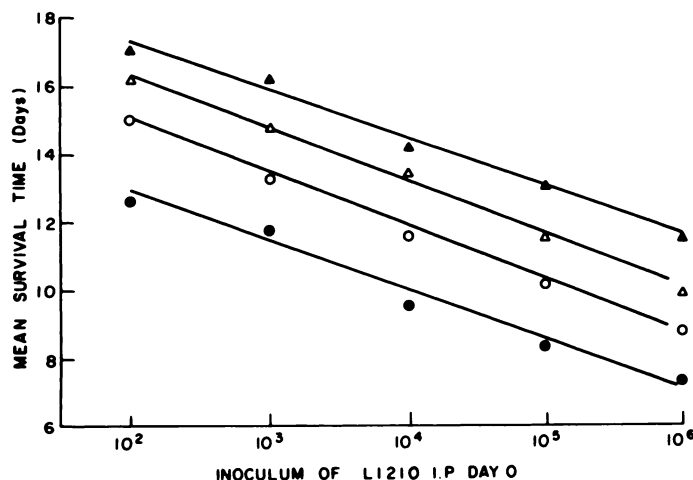
<sup>a</sup> Mean survival time as well as MST is included because the mean may enable us to make finer distinctions in survival time not possible with medians.

**Table 4**

Comparison of survival time with multiple treatment schedules of MTX at varying tumor inoculum levels

	MST (days)		
	Inoculum size i.p. (injected at Day 0)		
	10 <sup>5</sup>	10 <sup>4</sup>	10 <sup>3</sup>
Treated <sup>a</sup>	19.5	27.5	31.0
Controls	8.0	10.0	11.0
Difference	11.5	17.5	20.0

<sup>a</sup> Treated groups received MTX, 3.3 mg/kg, on an every-2-day schedule from Day 2 to death.



**Chart 1.** Comparison of survival times with 1 or 2 doses of MTX at varying tumor cell populations. L1210 ascites tumor cells (10<sup>5</sup>) were inoculated i.p. on Day 0. MTX treatment was i.p. Each point represents the mean of 12 to 26 mice. Schedule with MTX: Δ, 36 mg/kg on Day 2; ○, 3.3 mg/kg on Day 2; ▲, 36 mg/kg on Day 2 and 3.3 mg on Day 4; ●, controls.

design includes a varying dose regimen. Regardless of the interval between doses, the increase in life-span was longest when an initial high dose of MTX was followed by lower doses (Tables 1 and 2). A pattern was indicated, in that the optimal group in each experiment received initial doses in the range of 21.6 to 60 mg/kg, which approximated the optimal single dose (Tables 2 and 3).

It would appear that the loading dose schedule on which mice survived longest optimizes tumor cell kill. The current studies suggest the possibility that the percentage of tumor cell kill of MTX, a CCSS agent, increases as the tumor cell population decreases. This evidence is provided by the experiments shown on Chart 2 and Table 4. If this were so, the initial loading dose, by lowering the population of tumor cells more extensively than would a small dose, would result in subsequent doses causing a greater percentage of kill for a given dosage of drug. In tumor cell kill, the advantage gained in lowering the tumor cell population with a high initial dose would be greater than any loss incurred by having to use smaller doses subsequently.

In the current experiments in which the rapidly proliferating L1210 ascites tumor was used, the life-span of mice treated with 1 or 2 doses of MTX was increased over controls by a constant amount over 5 log dilutions of tumor (Chart 1). This observation is in agreement with the earlier reports involving single-dose treatment of leukemia L1210 (14,

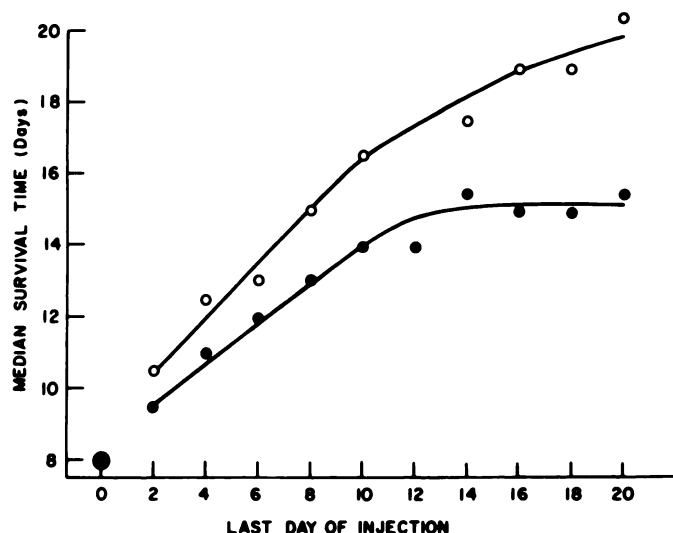


Chart 2. Comparison of survival times with an increasing number of treatments of MTX after a loading dose or low initial dose of MTX. L1210 ascites cells ( $10^5$ ) were inoculated i.p. on Day 0. MTX treatment (i.p.) began on Day 2. Each point represents the mean of 8 mice. ●, control; ○, MST of mice receiving 30 mg/kg of MTX on Day 2 and 3.0 mg/kg of MTX on an every-2-day schedule from Day 4 until the indicated last day of injection; ●, MST of mice receiving 3.0 mg/kg of MTX on Day 2 and 3.0 mg/kg MTX on an every-2-day schedule from Day 4 until day indicated as last day of injection.

18). It appears, however, that a system for measuring a difference in log kill of L1210 ascites tumor at various log dilutions of tumor with 1 or 2 doses of MTX may not be sufficiently sensitive to identify possible varying percentages of kill of leukemic cells.

However, in leukemic mice treated with multiple doses of MTX, sufficient increases in survival time were observed to permit statistical evaluation. With multiple-dose MTX therapy in mice receiving log dilutions of L1210, the increases in survival time were greater at lower tumor inoculum levels (Table 4). Furthermore, an experimental protocol involving discontinuation of MTX treatment at various times also indicated that, following an initial loading dose, a greater percentage of tumor cell kill is achieved as the result of a reduced tumor cell population. In this study, as the number of treatments was increased, the increased therapeutic response in the groups receiving loading dose therapy also became more evident. Thus from the above studies involving multiple doses of MTX, one may infer that increased percentage kill, although not readily evident, may occur at decreasing tumor cell populations of L1210 following even a single dose of MTX.

Schabel (15) suggested that as tumors increase in size the GF of viable tumor cells that are in the cell division cycle decreases. As the GF decreases, the sensitivity to anti-metabolite drugs would then decrease.

An alternative explanation, which would not require GF change for a decreased percentage of tumor cell kill of MTX at increasing tumor sizes, could be that the mean cell cycle generation time ( $T_C$ ) of tumors increases as the tumor cell population increases (10, 11, 13, 16, 19, 24, 25). Changes in the S phase portion of the cycle with tumor growth are not as

well established as the  $T_C$  increase (11, 19, 25). If a cell has to produce a given amount of DNA during a cycle in order to double, and the cycle lengthens, 2 possible types of adjustments may occur. Either the S wave may remain the same shape but occupy a smaller segment of the cycle, or it may lengthen and flatten (Chart 3). In either case, if lengthening of  $T_C$  does occur, it would provide an additional rationale for MTX, a DNA inhibitor (1, 2) killing fewer cells at greater tumor cell populations. A CCSN agent such as Cytosan might be expected to elicit cell kill, regardless of whether there are increasing  $T_C$  and S-phase changes.

Either the GF or "mean generation time" explanation may account for CCSS agents becoming less effective as tumors increase in size and for CCSN agents maintaining their percentage kill. The difference in the way in which the 2 classes of drugs (CCSS and CCSN) kill in relation to tumor size has application in the planning of single-drug, sequential, and combination chemotherapy. Goldin *et al.* (3, 8) pointed out that 1 type of combination treatment involves the use of priming therapy to decrease the number of tumor cells. This in turn permits the drug which is used sequentially to be more effective. In this study, 1,3-bis(2-chloroethyl)-1-nitrosourea (a CCSN agent) was followed by 1- $\beta$ -D-arabinofuranosylcytosine (a CCSS agent). Schabel (15) and Laster *et al.* (12) similarly proposed a rational chemotherapeutic approach involving sequential therapy with a CCSN followed by a CCSS agent. In other experiments in this laboratory, it was demonstrated that, when Cytosan and MTX are used sequentially in the L1210 system, optimal results are obtained if Cytosan is given first (M. J. Straus, N. Mantel, and A. Goldin, unpublished data). Thus, in the present experiments with MTX, the initial loading dose acts similarly to the priming CCSN agent in sequential chemotherapy.

MTX has unusual characteristics. It may not be a typical S-phase inhibitor in that it also may inhibit progression through the cell cycle (1). Also MTX has a high 50% lethal dose level for a single-dose treatment but has a low 50% lethal dose level for daily treatment (22-24). Other drugs must be evaluated on loading dose schedules to determine whether the results of this study are unique to MTX.

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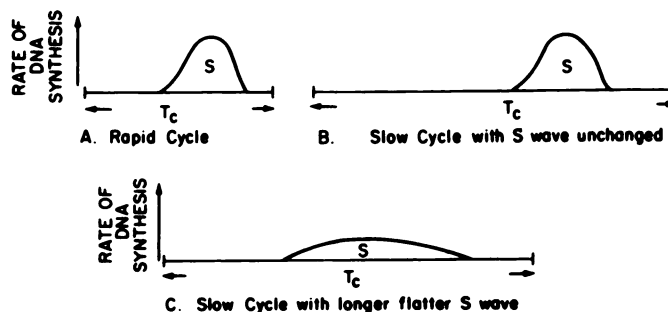


Chart 3. Possible changes in the S-phase portion of the cell cycle when mean generation time increases.

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