Influence of the Route of Administration on the Relative Effectiveness of 3′,5′-Dichloroamethopterin and Amethopterin against Advanced Leukemia (L1210) in Mice*

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SUMMARY

3′,5′-Dichloroamethopterin (DCM) and amethopterin (MTX) were compared with respect to their ability to prolong the lifespan of mice with advanced leukemia (L1210) on subcutaneous and oral administration. In agreement with previous studies, subcutaneous DCM was markedly more effective than subcutaneous MTX in increasing the survival time of the mice. On oral administration, the antileukemic activity of both drugs was reduced. The reduction in the effectiveness of DCM via the oral route was so extensive that oral DCM was no more effective than oral MTX in increasing the survival time of the mice.

Whereas subcutaneous DCM was able to produce complete regression of the local tumor without body weight loss, reduction of the local tumor during oral DCM treatment was seen only in conjunction with severe body weight loss.

The advantage of the subcutaneous route over the oral route for DCM or MTX did not appear to be influenced by the schedule of treatment employed.

Previous studies had shown that substitution of halogens in the benzene ring of amethopterin substantially increased its antileukemic activity (5, 9). For example, the increase in the lifespan of mice with systemic leukemia (L1210) produced by subcutaneous daily treatment with 3′,5′-dichloroamethopterin (DCM) or 3′-bromo-5′-chloroamethopterin (BCM) was over 4 times as great as the increase provided by similar treatment with amethopterin (MTX) (5). In addition, treatment with DCM or BCM was able to produce survivors of 6 months or longer among mice bearing advanced systemic leukemia (4, 5). Moreover, a high percentage of these apparently cured animals were immune to subsequent reinoculation with leukemic cells (4). With the parent compound, MTX, tumor-free survivors could be obtained only when treatment was begun prior to the development of systemic disease (6).

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The improvement in the therapy of systemic leukemia in mice with halogenated derivatives of MTX and the clinical interest in these compounds suggested that their pharmacologic characteristics be studied further. The current experiments were conducted to determine the influence of the route of drug administration on the relative antileukemic effectiveness of DCM and MTX.

MATERIALS AND METHODS

The experiments were conducted with 21- to 29-gm. CDBA, BDF_1, or D_2BC mice.[1] The strain and sex used in each experiment are reported in the table and charts. The mice were given inoculations, in the right hind leg, of 0.1 or 0.2 ml. of a saline suspension of leukemic cells prepared from the spleens of DBA or CDBA mice bearing lymphoid leukemia L1210. The leukemic inoculum for the various experiments ranged from 1 to 1.2 million cells per mouse. Treatment was initiated when the local tumor at the site of leukemic inoculation

[1] CDBA = (BALB/c × DBA/2)F_1.
BDF_1 = (C_{57}Bl/6 × DBA/2)F_1.
D_2BC = (ZBC × DBA/2)F_1.

was at least 7 mm. in diameter. This was 6 or 7 days after tumor inoculation (3 or 4 days prior to the median day of death of untreated controls). In each experiment, the leukemia had become systemic by the day of treatment initiation. This was evidenced by its transmissibility to normal mice on transplantation of spleens from mice selected at random from the population to be treated.

MTX and DCM were dissolved in 2 per cent aqueous sodium bicarbonate.[2] The drugs were administered on a daily schedule in the constant volume of 0.01 ml. of drug solution per gram of body weight. The routes of drug administration for each experiment are indicated in the table and charts. Parenteral treatments were given by the subcutaneous route. Oral treatments were by gavage, with the use of a 2-inch, 18-gauge bent needle with a rounded blunt tip attached to a 1-cc. tuberculin syringe.

RESULTS

Table 1 summarizes the results of six experiments which illustrate the advantage of the subcutaneous over the oral route in the daily treatment of systemic leukemia L1210 with DCM or MTX. Comparison of the increase in median survival time provided by the optimal daily dose of MTX via the two routes shows that oral MTX was only 55–77 per cent as effective as subcutaneous MTX in prolonging the lifetime of the mice. Of special significance in the current experiments, however, was the observation that the previously reported (5, 9) superiority of DCM over MTX, by the subcutaneous route, was lost when the drugs were given orally. In agreement with the previous studies (5, 9), the current experiments show that subcutaneous DCM was approximately 2–5 times more effective than subcutaneous MTX in prolonging the lifetime of the mice (Table 1). In contrast, when the drugs were given orally, DCM was no more effective than MTX, being 41–88 per cent as effective as subcutaneous MTX (Table 1).

The diminution in the antileukemic effectiveness of DCM or MTX on oral administration was observed over a range of schedules of treatment. For example, in the experiment summarized in Chart 1, subcutaneous DCM, at its optimal dosage levels, increased the median survival time of the mice from 11 days (untreated controls) to 86.5 days when given twice daily, to over 90 days when given daily, and to 56.5 days when given every 3d day. In contrast, optimal levels of oral DCM elicited median survival times of 22.5, 17.5, and 16.5 days when given twice daily, daily, and every 3d day, respectively. Moreover, a total of sixteen mice receiving subcutaneous DCM on the three schedules employed survived beyond day 90, whereas no mice receiving oral DCM on any schedule survived beyond day 36. Although treatment every 3d day with subcutaneous DCM appeared to be less effective than treatment daily or twice daily, it is noteworthy that the every-3d-day schedule still provided a high degree of activity against systemic L1210. Previous studies (8) in this laboratory had shown that subcutaneous MTX given every 3 or 4 days was relatively ineffective against the advanced stages of this disease. For the treatment schedules employed, the diminution in antileukemic effectiveness for the oral route resulted in the same range of activity for DCM and MTX (Chart 1).

Representative data on the relative effects of subcutaneous and oral DCM on the growth of the local tumor at the site of leukemic inoculation and on the host body weight are summarized in Chart 2. In this experiment, daily treatment was begun 6 days after tumor inoculation and terminated on day 50. DCM was given over a series of daily doses ranging from 27 to 125 mg/kg subcutaneously and from 27 to 208 mg/kg orally. Included in Chart 2 are the results of treatment at three selected dosage levels including the optimal dose for each route of administration. Untreated controls succumbed with a median survival time of 10 days. At two dosage levels of subcutaneous DCM (45 and 75 mg/kg/day) eight of nine mice survived beyond day 70 (20 days following the final treatment). At these optimal daily treatment levels, subcutaneous DCM produced complete regression of the local tumor in the surviving animals without concomitant loss in body weight (Chart 2). Subcutaneous DCM at 125 mg/kg/day produced considerable body weight loss, and the mice succumbed relatively early.

Oral DCM, at its optimal daily dose (45 mg/kg), increased the median survival time of the mice to only 25 days, and no animal receiving oral DCM survived beyond day 30 (Chart 2). At the optimal level of treatment and at the higher doses (75 and 125 mg/kg/day) oral DCM failed to inhibit the growth of the local tumor initially, and the animals showed considerable body weight loss (Chart 2).

To investigate the extent to which drug toxicity to normal mice limits the therapeutic usefulness of DCM or MTX on subcutaneous or oral administration, an experiment was conducted in which normal mice without tumor as well as leukemic mice were treated via the two routes (Chart 3).

[2] 3′,5′-Dichloroamethopterin and amethopterin (Lederle Methotrexate) were obtained from the Lederle Laboratories Division, American Cyanamid Company, Pearl River, N.

Daily treatment was begun 7 days after tumor inoculation and terminated on day 67.

Of significance in this experiment was the observation that on subcutaneous treatment the median survival time (61 days) of leukemic mice receiving the optimal daily dose of DCM (75 mg/kg) was not appreciably different from the median survival time of normal mice (63.5 days) receiving the same treatment. In contrast, when DCM was given orally, the median survival time (21 days) of leukemic mice at the optimal daily dose (75 mg/kg) was at least 50 days less than the median survival time of normal mice at the same dosage level.

Examination of the results of treating normal mice with DCM shows that, at the higher dosage levels, mice receiving the drug orally tended to live longer than mice receiving equivalent treatment subcutaneously.[3] At the lower doses by either route the toxicity for normal mice diminished sharply so that it was no longer a limiting factor.[4]

Thus, these data suggest that, when given subcutaneously, optimal levels of DCM display an extensive antileukemic activity which may be limited eventually by the toxicity commensurate with prolonged treatment. In contrast, the anti-

[3] In subsequent experiments the decrease in the toxicity of DCM to normal mice on oral administration was more extensive than in the present experiments.

[4] Deaths occurring at the lower doses of oral DCM (27 and 45 mg/kg) may be a reflection of accidental damage incurred during continuous forced feeding. Three of eight normal mice receiving the vehicle for the drugs orally died during the course of the experiment. In general, deaths among mice receiving the low doses of DCM or the vehicle did not occur before day 50. In subsequent experiments, normal mice receiving the vehicle orally twice a day for 50 days failed to show evidence of toxicity.

TABLE 1

INFLUENCE OF THE ROUTE OF ADMINISTRATION ON THE RELATIVE EFFECTIVENESS OF 3′,5′-DICHLOROAMETHOPTERIN (DCM) AND AMETHOPTERIN (MTX) AGAINST ADVANCED LEUKEMIA L1210

Experiment no.	Mice (strain and sex)	Day of treatment initiation	Treatment	Daily dose range (mg/kg)	Optimal daily dose (mg/kg)	Median survival time (days)	Relative increase in median survival time over controls (subcutaneous MTX increase = 100)
1	BDF_1 male	7	MTX, subcutaneous	0.38–1.5	1.5	26	100
			MTX, oral	0.75–3.0	1.5	20.5	66
			DCM, subcutaneous	38–150	38	56	288
			DCM, oral	75–300	75	19.5	59
			Controls			10	
2	D_2BC male	7	MTX, subcutaneous	0.23–5.0	0.65	24	100
			MTX, oral	0.39–8.3	1.8	19	64
			DCM, subcutaneous	14–180	39	32	157
			DCM, oral	14–300	65	19	64
			Controls			10	
3	CDBA female	7	MTX, subcutaneous	0.27–3.5	0.76	27	100
			MTX, oral	0.45–9.7	0.76	20.5	59
			DCM, subcutaneous	27–125	27	>90	>494
			DCM, oral	27–208	75	17.5	41
			Controls			11	
4	CDBA female	7	MTX, subcutaneous	0.27–3.5	0.45	21	100
			MTX, oral	0.27–9.7	1.25	18.5	77
			DCM, subcutaneous	27–208	45	34	218
			DCM, oral	27–208	75	18	73
			Controls			10	
5	CDBA male	6	MTX, subcutaneous	0.75	0.75	27	100
			DCM, subcutaneous	27–125	45, 75	>70	>352
			DCM, oral	27–208	45	25	88
			Controls			10	
6	CDBA male	7	MTX, subcutaneous	0.27–3.5	0.76	30	100
			MTX, oral	0.27–5.8	3.5	21.5	55
			DCM, subcutaneous	27–125	75	61	263
			DCM, oral	16–208	75	21	53
			Controls			11	

No. of mice: ten per treated group, twenty untreated controls (Exps. 1, 2, 4, and 6); eight per group (Exp. 3); nine per group (Exp. 5).

leukemic effectiveness of oral DCM or of MTX via either route was not sufficient to raise the median survival time of leukemic mice to the same level as that of normal mice receiving equivalent treatment (Chart 3).

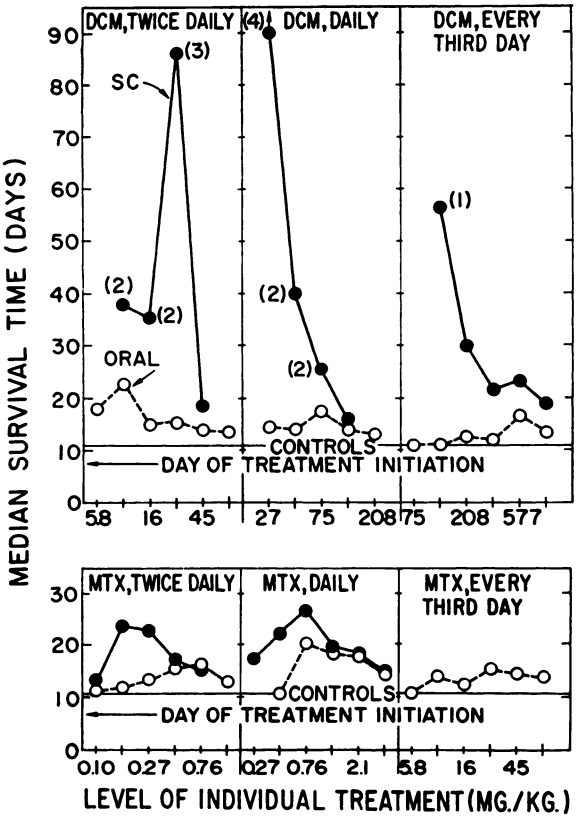

CHART 1.—Influence of the schedule of treatment on the antileukemic (L1210) effectiveness of subcutaneously or orally administered 3',5'-dichloroamethopterin or amethopterin. Eight CDBA female mice per group. Treatment was begun 7 days after tumor inoculation and continued on the schedules indicated until day 64. Numbers in parentheses indicate the number of 90-day survivors. The results of daily treatment are also shown in Table 1, Experiment 3.

DISCUSSION

The current study corroborates former reports (5, 9) with respect to the superiority of DCM over MTX in the subcutaneous treatment of systemic mouse leukemia (L1210). Since the profound antileukemic activity of DCM in mice suggested this compound for clinical trial, the question of the influence of the route of administration on its therapeutic efficacy became one of importance. The current experiments show that the capacity of both DCM and MTX to prolong the lifetime of mice with advanced leukemia L1210 is reduced on oral administration. Of significance was the observation that the reduction in DCM effectiveness was considerably greater than the reduction in MTX effectiveness with the result that, on oral administration of the drugs, the therapeutic advantage of DCM over MTX was lost. The marked diminution in DCM activity via the oral route was also reflected in its diminished capacity to induce regression of the local tumor growth at the site of inoculation. Whereas subcutaneous DCM at appropriate dosage levels was able to produce complete regression of the local tumor without host body weight loss, reduction in local tumor size as a result of oral DCM treatment was invariably accompanied by a severe body weight loss.

Previous studies have shown that many factors pertaining to the host-tumor-drug relationship may influence the antitumor specificity of a drug. Among such factors are the schedule of treatment employed (8), the time in the development of the disease when treatment is initiated (8), and the use of metabolites with antimetabolites (7). The route of administration may also be an important factor in modifying the activities of various antineoplastic agents including 6-mercaptopurine (3), 6-thioguanine (2), 5-fluorouracil (1), and various alkylating agents (10).

With respect to the current observations, the cause of the diminution in the antileukemic effectiveness of DCM and of MTX on oral administration is not known. The observation that subcutaneous DCM markedly inhibits the growth of the local tumor, while oral DCM does not, suggests that on oral administration the drug fails to reach its site of action in a concentration sufficient to achieve its maximum potential. The observation (12) that the capacity of both DCM and MTX to inhibit the incorporation of formate-C^{14} into the acid-soluble adenine of leukemic spleens of mice is markedly reduced on oral administration supports this view. Moreover, Rall *et al.* (11) have demonstrated that the maximum plasma concentrations of DCM after oral administration to dogs and humans was substantially lower than the plasma concentrations after parenteral injections of the drug.

Diminished absorption of DCM or MTX on oral administration cannot of itself account for the lesser therapeutic efficacy of the drugs. Such diminution alone would reduce the toxic effects equally for both tumor and host. In this event one might have expected the oral route to provide increases in the survival time of leukemic mice as great as those seen on subcutaneous treatment, but at higher dosage levels.

It is not clear to what extent oral administration of the antifolic drugs may evoke toxic activity in leukemic mice without a corresponding increase in

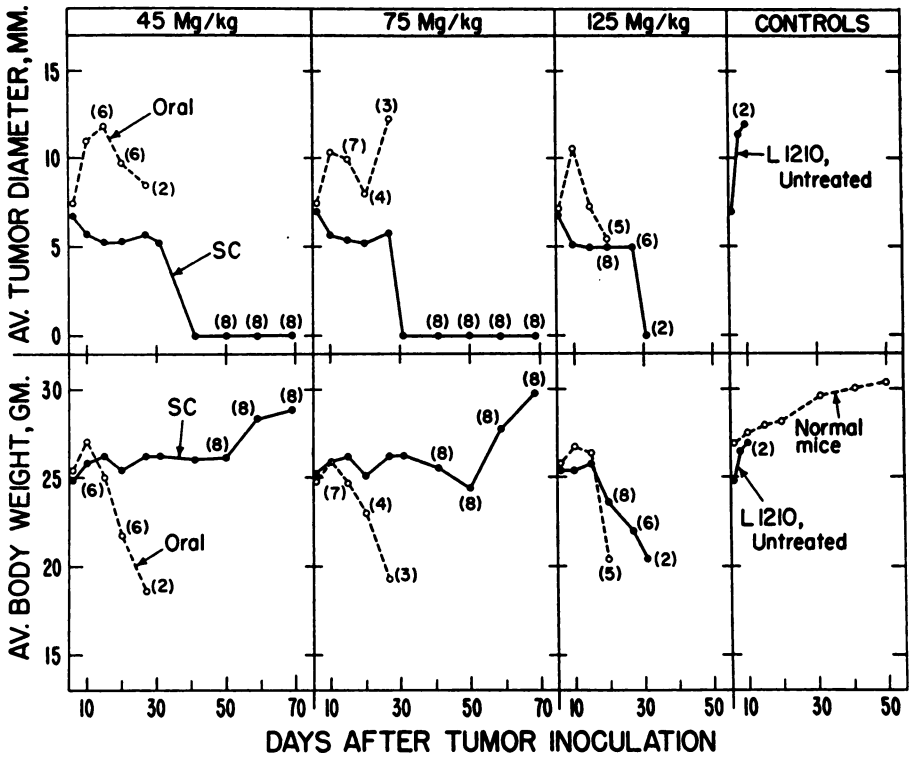

CHART 2.—Relative effectiveness of subcutaneous and oral administration of 3′,5′-dichloroamethopterin on the local tumor diameter, host body weight, and survival time of mice with advanced leukemia L1210. Nine CDBA male mice per group. Numbers in parentheses indicate the number of survivors. Treatments were given daily from day 6 through day 50 at a series of doses ranging from 27 to 208 mg/kg/day (orally) and 27 to 125 mg/kg/day (subcutaneously). The increases in median survival time provided by the optimal daily doses of subcutaneous or oral DCM are shown in Table 1, Experiment 5. Data at three daily dose levels for each route are shown. The normal mice received 2 per cent aqueous sodium bicarbonate orally in the constant volume of 0.01 ml/gm of body weight daily.

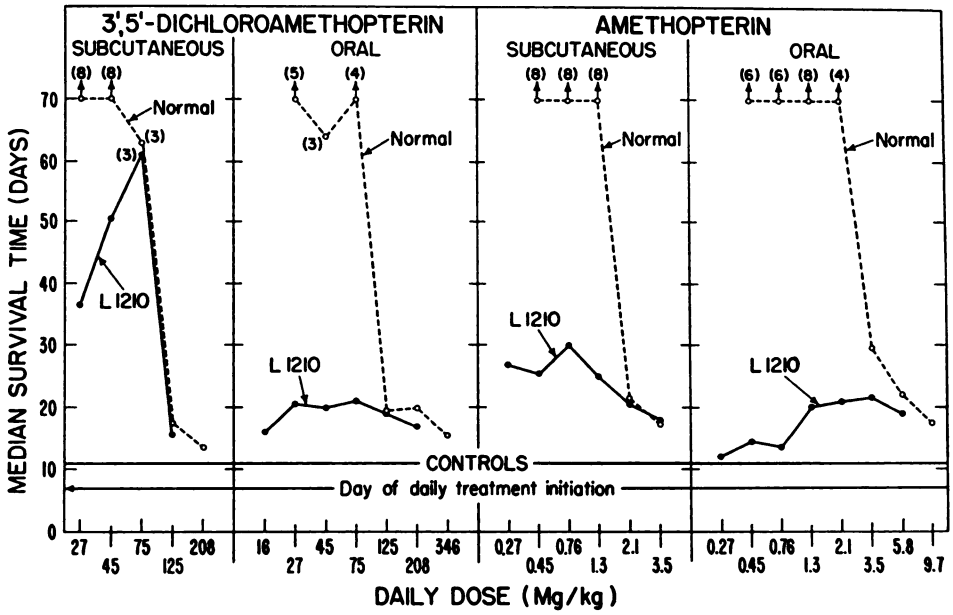

CHART 3.—Comparison of the effects of subcutaneous and oral administration of 3′,5′-dichloroamethopterin and amethopterin on the survival of mice with systemic leukemia and on normal mice. CDBA male mice; ten per leukemic group, eight per normal mouse group. Daily treatments were given from day 7 through day 67. Numbers in parentheses indicate the number of survivors on day 70. The results of the treatment of leukemic mice at the optimal daily doses are also shown in Table 1, Experiment 6.

inhibitory effect on the tumor. Such toxicity, for example, could be reflected in damage to the gastrointestinal mucosa or to the intestinal flora.

Whatever the underlying cause for the diminution in the antileukemic effectiveness of antifolics in mice on oral administration, the current experiments do show that, when DCM or MTX is administered orally, there is a marked reduction in their effect against the tumor, without a proportional reduction in host toxicity, resulting in diminished antitumor specificity.

The present study further emphasizes the need for the quantitative exploration of the influence of factors such as the route of administration which may modify the usefulness of therapeutically active compounds.

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