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Short Communication

Optimum scheduling during combination chemotherapy of murine leukemia

Additional examples of schedule-dependent synergism between S-phase-specific antimetabolites and agents inducing mitotic or pre-mitotic (G_2) arrest

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Summary. *In an extension of our prior studies with methotrexate and vinca alkaloids, three additional drug combinations incorporating an S-phase-specific antimetabolite and an agent inducing blockade at mitosis or G_2 were found to exhibit potent schedule-dependent synergism against the L1210 leukemia. Combinations employed include cytosine arabinoside with vindesine and methotrexate with teniposide (VM-26) or the -deaza-pteridine derivative, NSC 181,928. Synergism was observed following sequential administration (antimetabolite given 24 h before the second agent), but effects following simultaneous administration or sequential administration in the reverse order (antimetabolite given 24 h after the second agent) were only additive.*

In earlier studies from our laboratory we documented [1–3] schedule-dependent synergism between methotrexate and vinca alkaloids during combination chemotherapy of the L1210 leukemia. With a sequence of methotrexate followed 24 h later by either vincristine or vindesine, antitumor effects were markedly synergistic, while simultaneous administration of these two agents gave effects which were only additive. With a view of obtaining information on the generality of this potentiation and possible guidance for studies examining its biochemical basis, we studied other combinations of S-phase-specific agents and mitotic inhibitors during therapy of this leukemia. In these studies, similar schedule-dependent synergy was obtained with the combination of methotrexate plus NSC 181,928, a new mitotic inhibitor of the -deaza-pteridine class [10, 11] and with the S-phase-specific agent [4] cytosine arabinoside plus vindesine. We also obtained similar schedule-dependent effects with the combination of methotrexate plus teniposide (VM-26). The latter agent is a semisynthetic podophyllotoxin with antimitotic properties [6]. However, effects on mitosis only occur at concentrations much higher than are needed to inhibit DNA synthesis. It is believed that VM-26 induces cytostasis [6] via inhibition of cell cycle traverse at G_2.

The results of our studies are summarized in Table 1. The S-phase-specific agents were given IP according to a schedule of once every 4 days for a total of five doses starting 2 days after IP transplantation of 10^6 L1210 cells to $BD2F_1$ mice. The second drug of each combination was administered 24 h later, in accordance with the results of our initial studies [1–3], which showed this interval to be optimum for maximum synergy. This schedule of administration was compared with simultaneous administration of both drugs (every 4 days × 5) and also with the same schedule but with the two in the reverse sequence. Details of the methodology used in these experiments have been described [1–3] in earlier reports. Methotrexate and vindesine were given at dosages found in our own studies [1–3] to be the maximum tolerated. The dosage of cytosine arabinoside used (800 mg/kg) was estimated from data in the literature [4, 9] for a slightly different schedule. This was confirmed (see Table 1) as the maximum tolerated dosage in our preliminary experiments for the schedule employed. In view of our lack of experience with the other agents, these were given at more than one dose level to define maximum tolerated dosages for each. We also noted in these experiments and in our earlier [1–3] studies that maximum tolerated dosages were similar whether these agents were given simultaneously or sequentially in the schedule employed.

With combinations including methotrexate with NSC 181,928 and cytosine arabinoside with vindesine, the administration of these agents either simultaneously or in a sequence in which the mitotic inhibitor was given prior to the antimetabolite resulted in therapeutic effects which were no more than additive. Values for increase in lifespan (ILS) based on data for average survival time (AST) were no greater than the sum of values obtained with each given alone. However, when these antimetabolites were given 24 h prior to the mitotic inhibitor synergy was observed, i.e., ILS was substantially greater than the sum of individual effects. In the case of methotrexate with teniposide, marked synergy was observed with the sequence of methotrexate followed by teniposide. Forty percent of the animals in this group were long-term survivors. Again, administration of these agents either simultaneously or in the reverse sequence was no more than additive and no long-term survivors were obtained. Although the results in Table 1 are presented as an average of three separate experiments, evidence for schedule-dependent synergy was consistently obtained in each experiment. Also, these results bring to four the number of combinations of antimetabolites and mitotic inhibitors which exhibit the same schedule-dependent synergy. Similar results were also obtained with a fifth combination, which incorporated an antimetabolite (methotrexate) and an agent (teniposide) whose growth-limiting

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Table 1. Antitumor effects following single and combination drug therapy in leukemic mice

Expt.[a]	Drug A	Dose (mg/kg)	Drug B	Dose (mg/kg)	Schedule	AST ± SE[c] (days)	ILS (%)	90-day survivors	Toxic deaths[e]
I.	Ara C[b]		DVA						
		–		–		7.4 ± 0.5		0/22	0/22
		800		–		20.4 ± 1.8[d]	176[d]	1/24	0/24
		–		0.5		10.3 ± 0.4	39	0/22	0/22
		800		0.5	A plus B	24.1 ± 2.9	226	0/24	0/24
		800		0.5	A before B	31.4 ± 3.5[d]	328[d]	3/24	0/24
		800		0.5	B before A	23.4 ± 2.6	215	0/24	0/24
II.	MTX		VM-26						
		–		–		7.1 ± 0.5		0/17	0/17
		48		–		20.9 ± 0.7	191	0/17	0/17
		–		8		18.6 ± 1.0	160	0/17	0/17
				16		28.3 ± 4.3	298	0/17	6/17
		48		8	A plus B	30.2 ± 2.3	321	0/17	0/17
				16		29.6 ± 5.2	317	0/17	7/17
		48		8	A before B	43.1 ± 3.8[d]	507[d]	8/17	0/17
				16		27.5 ± 5.1	286	0/17	6/17
		48		8	B before A	31.2 ± 3.7	338	0/17	0/17
				16		27.2 ± 6.9	283	0/17	8/17
III.	MTX		NSC181,928						
		–		–		7.2 ± 0.3		0/20	0/20
		48		–		21.0 ± 0.9	193	0/20	0/20
		–		12.5		9.2 ± 0.6	29	0/20	0/20
				25		10.9 ± 0.8	52	0/20	0/20
				50		12.4 ± 1.1	72	0/20	4/20
		48		12.5	A plus B	25.8 ± 2.2	259	0/20	0/20
				25		26.4 ± 2.8[d]	266[d]	1/20	0/20
				50		18.3 ± 3.6	154	0/20	7/20
				12.5	A before B	26.9 ± 3.1[d]	274[d]	1/20	0/20
				25		32.9 ± 2.9[d]	356[d]	2/20	0/20
				50		19.7 ± 3.6	178	0/20	5/20
				12.5	B before A	24.7 ± 2.4	242	0/20	0/20
				25		24.8 ± 2.1	243	0/20	0/20
				50		18.9 ± 3.8	176	0/20	6/20

[a] Treatment schedule employed was one dose every 4 days × 5 IP. Abbreviations: MTX, methotrexate; AraC, cytosine arabinoside; DVA, vindesine; VM-26, tenoposide

[b] A dose of 800 mg/kg was chosen on the basis of preliminary data for toxic deaths obtained with this schedule at 600 (1/10), 800 (1/10), 1,200 (4/10), and 1,600 (6/10) mg/kg in non-tumor-bearing mice using 10 mice per dose

[c] Average survival time ± standard error of the mean in three separate experiments with five to eight mice per group

[d] Long-term survivors not included

[e] Toxic deaths occurred on days 21–28 (Expt. II) and on days 9–22 (Expt. III). In contrast to deaths that were tumor-related, other deaths were classified as drug-related if animals showed profound weight loss and no evidence of ascites or organomegaly

effects are believed to occur at G_2 rather than at mitosis. In addition, we imagine that the agents employed within both categories would be interchangeable and each additional combination would be synergistic with the appropriate schedule.

In view of the diversity in pharmacologic and biochemical properties among this group of agents [4–8] and the schedule-dependency observed, it is reasonable to assume that the synergism obtained with each combination has, in fact, a cytokinetic basis. Additionally, we have shown [8] that in tumor cell populations exposed to these dosages of methotrexate, eventual recovery of DNA synthesis is cyclical, suggesting synchronized resumption of proliferation in the surviving tumor cell fraction. It was also of interest to note here that similar effects were obtained with agents which are believed to achieve their cytotoxic effects through induction of mitotic arrest (vinca alkaloids and NSC 181,928) or by actions during traverse through G_2 (teniposide). However, it has also been shown (reviewed in [6]) that teniposide will induce mitotic arrest at concentrations higher than those required to achieve cytotoxicity. In this regard, it is tempting to speculate that with the sequence of administration shown to be synergistic, an additional effect of teniposide occurs during mitosis. The extent to which biochemical factors might also play a role in the synergism observed must also be considered, since the degree of synergy observed varied considerably even between combinations using [1] related vinca alkaloids. These aspects require further investigation. Also, it will be of interest to examine other categories of agents for this schedule-dependent synergy and the extent to which is might occur in other tumor models. The results of these studies will be presented in a future publication.

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