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Effective combination therapy of metastatic murine solid tumors with edatrexate and the vinca alkaloids, vinblastine, navelbine and vindesine

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Abstract. Studies are described in which a new folate analogue, edatrexate (EDX), in combination with the vinca alkaloids, vinblastine (VBL), navelbine (NVB) or vindesine (DVA) was evaluated against E0771 mammary adenocarcinoma, T241 fibrosarcoma and the Lewis lung tumor. Each of the four agents when given individually to animals 3 days after transplant of these tumors resulted in increases in survival of 53–143%. The relative effectiveness of these agents was (in increasing order) VBL, NVB ≃ DVA, EDX, with no long-term survivors obtained with any. Combination therapy with EDX and vinca alkaloids required dosage attenuation but was still markedly more effective. Treatment of E0771 and T241 tumors with EDX and either NVB or DVA increased survival 3- to 4-fold compared with therapy with individual agents and yielded 40–70% long-term survivors, while EDX with VBL increased survival 2- to 3-fold and yielded 20–40% long-term survivors. Simultaneous or sequential (EDX given 24 h before vinca alkaloid) administration of combined therapy was equally effective. Sequential administration of these agents at the same doses in the reverse order was highly toxic and required further dosage attenuation which compromised efficacy. Effects of these combinations against the Lewis Lung tumor were not as pronounced and were somewhat schedule-dependent. Simultaneous administration of EDX with VBL, NVB or DVA increased survival 2- to 3-fold over that obtained with single agents alone and yielded 10–40% long-term survivors, while sequential administration increased survival <2-fold over that obtained with single agents and yielded 0–20% long-term survivors. These results suggest that combined therapy with these agents in patients may have appreciable utility and provide a basis for further clinical trials.

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Introduction

Edatrexate (EDX) is a member of the 10-deaza-aminopterin class of 4-aminofolate analogues [1–3] targeted to dihydrofolate reductase. This analogue exhibited markedly enhanced antitumor activity compared with MTX (methotrexate) in animal tumor models, including ascitic and solid murine tumors [1–3] and a group of human tumor xenografts [4] in mice that are relatively resistant to MTX. This analogue has also been shown to induce an appreciable number of responses in patients with a variety of neoplasms, particularly non-small-cell lung cancer [5], breast cancer [6, 7] and malignant fibrous histiocytoma [8]. EDX is now under study in patients in combination with a variety of DNA-damaging agents [9] and mitotic inhibitors [9, 10]. To assist in the design of these clinical trials, prior laboratory studies in animals of EDX in various combinations have been carried out [11]. In the current paper, results are presented from an evaluation of the effectiveness and schedule-dependence of combinations of EDX with one of three vinca alkaloids, vinblastine (VBL), navelbine (NVB) and vindesine (DVA) in murine solid tumor models. These results demonstrate that combinations of EDX with each one of these mitotic inhibitors are well tolerated, markedly improve median survival and achieve high rates of apparent cures in these model systems.

Materials and methods

The tumors used for these studies were the E0771 mammary adenocarcinoma, T241 fibrosarcoma and Lewis lung carcinoma. The individual tumors were maintained by s.c. transplantation [11] in female BD2F1 (C57BL × DBA/2F$_1$) mice purchased from the National Cancer Institute. New tumor lines were initiated in mice from a bank of frozen cells prepared earlier from a subline determined to be malignant and maintained for only 10–12 transplant generations. For therapeutic experiments, a brei containing 10^6 viable tumor cells in suspension was transplanted [1, 4, 11, 12] i.p. into mice. This amount of tumor cells was at least 10^5-fold higher than the minimum number of cells required to obtain a successful transplant 100% of the time. This was determined in preliminary experiments (data not shown) with different

Table 1. Maximum tolerated doses for edatrexate and various vinca alkaloids administered individually or in combination to BD2F1 mice

R_x[a]	MTD[b]	
	EDX (mg/kg)	Vinca (mg/kg)
EDX	120	
VBL		0.5
NVB		4.0
DVA		1.5
EDX + VBL	80	0.4
EDX → VBL	80	0.4
VBL → EDX	40	0.2
EDX + NVB	80	3.0
EDX → NVB	80	3.0
NVB → EDX	40	1.5
EDX + DVA	80	1.0
EDX → DVA	80	1.0
DVA → EDX	40	0.5

[a] EDX given together with (EDX + vinca), 24 h before (EDX → vinca) or 24 h after (vinca → EDX) vinca alkaloid

[b] Maximum tolerated dose estimated from a dose-versus-toxicity plot obtained following treatment of mice with varying doses on a schedule of q3-4d × 5. Standard error of the mean was <14% in 2–3 experiments

numbers of tumor cells in the transplant. For untreated animals rapid disease progression is observed with frank metastasis to the lung within a few days. Median survival of mice receiving 10^6 E0771, T241 and Lewis Lung tumor cells was 11±1, 17±2 and 13±2 days, respectively. Implanted mice were randomly distributed among treated and untreated groups, and therapy was initiated 3 days later. EDX was administered s.c. in the flank and the vinca alkaloids were administered i.p. For these experiments, a schedule of drug administration of twice (every 3–4 days) weekly for a total of five doses was used. EDX and each vinca alkaloid were given either simultaneously or sequentially (EDX or vinca alkaloid followed 24 h later by vinca alkaloid or EDX). EDX, NVB and DVA were provided by Ciba-Geigy. Antitumor effects were expressed [11] as percent increased life span (ILS) based upon the median survival time (MST) of treated versus control groups (5–10 mice per group). Toxicity was monitored [11] by weight loss and by drug-induced deaths in normal mice or in mice with no evidence of tumor. Whenever possible, mice identified as profoundly moribund were humanely terminated. These deaths were recorded for calculation of MST as day of death plus one. Otherwise, the care of animals and conduct of these experiments were in accordance with institutional guidelines. Tolerance for each drug or combination of drugs was obtained in preliminary experiments examining animals for the effects of various doses of each. Tolerances to these agents was similar in tumor-bearing and non-tumor-bearing mice. Mice scored as long-term survivors were autopsied at 60 days to verify their status as tumor free. Analysis for statistical significance was carried out using the Chi-square test described by Zar [13].

Results and Discussion

Preliminary toxicology

The maximum tolerated dose (MTD) of EDX and the three vinca alkaloids were determined in preliminary experiments (see Table 1) comparing different doses of each in non-tumor-bearing BD2F1 mice. The dose eventually selected as the MTD for each agent resulted in approximately 10% weight loss during treatment, and there was only an occasional drug-induced death. On a schedule of twice weekly (every 3–4 days) × 5, the MTD for EDX was 120 mg/kg, while the corresponding values for the vinca alkaloids were 0.5 (VBL), 40 (NVB) and 1.5 (DVA) mg/kg. In similar experiments (Table 1), EDX and the vinca alkaloids in combination required attenuation of the MTD for individual administration. In actual practice, we found it necessary to reduce the dose of each agent in the combination to approximately three-quarters of the MTD when given alone on the same schedule. Sequential administration of these agents at these doses with EDX given first and followed 24 h later by the vinca alkaloid was also similarly well tolerated with only an occasional drug-induced death.

Table 2. Antitumor effects of EDX and various vinca alkaloids alone or in combination against E0771 mammary adenocarcinoma

R_x	Dosage[a]		Combination schedule	MST[b] (days)	ILS (%)	60-day[c] survivors number/total
	EDX (A) (mg/kg)	Vinca (B) (mg/kg)				
				11±1		0/21
EDX	120			27±2	+145	0/21
VBL		0.5		21±2	+ 91	0/15
NVB		4.0		26±3	+136	0/15
DVA		1.5		25±2	+127	0/15
EDX+VBL	80	0.4	Simultaneous	51±5	+364	7/15
	80	0.4	A before B	44±5	+300	3/15
	40	0.2	B before A	15±3	+138	0/10
EDX+NVB	80	3.0	Simultaneous	58±8	+427	7/15
	80	3.0	A before B	53±6	+382	7/15
	40	1.5	B before A	15±2	+138	0/10
EDX+DVA	80	1.0	Simultaneous	>60	>+445	9/14[d]
	80	1.0	A before B	59±9	+436	7/15
	40	0.5	B before A	24±3	+185	0/10

[a] EDX was given s.c. twice weekly ×5, starting 3 days after i.p. tumor implant. Vinca alkaloids were given i.p. on the same schedule, either together or sequentially starting on day 3 (EDX 24 h before vinca or vinca 24 h before EDX)

[b] Median survival time in days ± SEM (three separate experiments with 5–7 mice per group)

[c] Survivors were tumor-free at 60 days as determined by autopsy

[d] Toxic deaths not included in the determination of MST

Table 3. Antitumor effects of EDX and various vinca alkaloids alone or in combination against T241 fibrosarcoma

R_x	Dosage[a]		Combination schedule	MST[b] (days ± SEM)	ILS (%)	60-day[c] survivors (number/total)
	EDX (mg/kg)	Vinca (mg/kg)				
				17 ± 2		0/21
EDX	120			39 ± 4	129	0/20[d]
VBL		0.5		26 ± 3	53	0/21
NVB		4.0		29 ± 3	71	0/21
DVA		1.5		36 ± 4	112	0/21
EDX+VBL	80	0.4	Simultaneous	53 ± 6	212	5/15
	80	0.4	Sequential	53 ± 6	212	7/15
EDX+NVB	80	3.0	Simultaneous	>60	>253	11/15
	80	3.0	Sequential	>60	>253	8/15
EDX+DVA	80	1.0	Simultaneous	>60	>253	7/14[d]
	80	1.0	Sequential	>60	>253	9/14[d]

[a] EDX was given s.c. twice weekly × 5 starting 3 days after i.p. tumor implant. Vinca alkaloids were given i.p. on the same schedule either together with (simultaneous) or 24 h after (sequential) each dose of EDX

[b] Median survival (MST) in days ± SE of the mean (3 separate experiments with 5 mice per group)

[c] Survivors were tumor free at 60 days as determined by autopsy

[d] Toxic deaths not included in the determination of MST

Table 4. The antitumor effects of EDX and various vinca alkaloids alone or in combination against Lewis lung carcinoma

R_x	Dosage[a]		Combination schedule	MST[b] (days ± SEM)	ILS (%)	60-day[c] survivors (number/total)
	EDX (mg/kg)	Vinca (mg/kg)				
				13 ± 2		0/21
EDX	120			28 ± 3	115	0/20[d]
VBL		0.5		21 ± 2	62	0/14[d]
NVB		4.0		24 ± 3	85	0/15
DVA		1.5		25 ± 3	93	0/15
EDX+VBL	80	0.4	Simultaneous	37 ± 4	185	1/14[d]
	80	0.4	Sequential	31 ± 4	138	1/15
EDX+NVB	80	3.0	Simultaneous	44 ± 5	238	5/15
	80	3.0	Sequential	34 ± 5	162	0/15
EDX+DVA	80	1.0	Simultaneous	42 ± 6	223	5/14[d]
	80	1.0	Sequential	36 ± 4	177	3/15

[a] EDX was given s.c. twice weekly × 5 starting 3 days after i.p. tumor implant. Vinca alkaloids were given i.p. on the same schedule either together with (simultaneous) or 24 h after (sequential) each dose of EDX

[b] Median survival time (MST) in days ± SE of the mean (3 separate experiments with 5–7 per group)

[c] Survivors were tumor free at 60 days as determined by autopsy

[d] Toxic deaths not included in the determination of MST

By comparison, the sequential administration of these doses in reverse order, i.e. vinca alkaloid followed 24 h later by EDX, was extremely toxic, necessitating further reduction in dosage (see Table 1). This schedule was used in only a limited number of experiments.

Therapy studies

The effects of EDX and the vinca alkaloids administered either individually or in combination against three murine solid tumors are shown in Tables 2–4. EDX was administered s.c. in these experiments. Since these vinca alkaloids were essentially ineffective against all three tumors when administered s.c. to mice (data not shown), we changed over to i.p. administration of these agents. Against the E0771 mammary adenocarcinoma (Table 2), s.c. administration of EDX alone was more effective than any of the vinca alkaloids given i.p. The relative effectiveness was (increasing order) VBL, NVB ≃ DVA and EDX. There were no long-term survivors following treatment with any one of these agents alone. Combined therapy with EDX and a vinca alkaloid with simultaneous or sequential (EDX given 24 h before vinca) administration was highly effective compared with therapy with the individual agents alone ($P < 0.001$). The combination of EDX with VBL was least effective in terms of increased survival, but as many as 20–40% long-term survivors were obtained. EDX with either NVB or DVA was more effective ($P = < 0.05$). Greater increases in survival were obtained and long-term survivors derived were in the range of 40–60% after treatment. With the possible exception of EDX with VBL, the therapeutic effects of combined therapy with these agents was similar with either simultaneous or sequential (EDX before vinca alkaloid) regimens of administration. Sequential administration of these agents in the reverse

order (vinca alkaloid given 24 h before EDX) was least effective overall against this tumor. Because of the toxicity of this schedule of administration much greater attenuation of dosage was required. This attenuation had a severe negative impact on survival (>2-fold reduction compared with the other two schedules), and there were no long-term survivors.

In the case of the T241 fibrosarcoma the results (Table 3) were somewhat similar, but there were greater numbers of long-term survivors with combination therapy. Against this tumor, the effectiveness of each agent alone was again in the increasing order of VBL, NVB≃DVA and EDX. No long-term survivors were obtained with any agent alone. The combination of EDX with VBL was somewhat less effective in increasing survival ($P = >0.01$) than the other two combinations, but 30–40% long-term survivors were still obtained. In contrast, for EDX with NVB or DVA, there were 40–70% long-term survivors. Against this tumor, all three combinations were approximately equally effective with either simultaneous or sequential (EDX given 24 h before vinca alkaloid) regimens of administration. In view of the results obtained with the E0771 tumor, sequential administration of EDX and vinca alkaloid in the reverse order was not tested.

The results in the Lewis lung tumor were less impressive than those obtained with the E0771 and T241 tumors, but still noteworthy. Responsiveness to the individual agents remains in the increasing order of VBL, NVB≃DVA and EDX, and no long-term survivors were obtained with any agent alone. The combination of EDX with VBL was less effective ($P = <0.05$) than the other combinations containing EDX, but still more effective than therapy with either of the individual agents alone. However, very few long-term survivors were obtained. For EDX with NVB and EDX with DVA the results showed greater effectiveness and as many as 30% long-term survivors were obtained. Interestingly, in this group of experiments with this tumor, at least for EDX with NVB or DVA, simultaneous administration appeared to be more effective ($P = <0.05$) than sequential (EDX given 24 h before vinca alkaloid) administration. Sequential administration of EDX and vinca alkaloid in the reverse order was not tested in view of its toxicity and poor therapeutic effect in the case of the E0771 tumor.

In summary, these results show that the effects of EDX against E0771, T241 and Lewis lung tumors were modestly or substantially better than any of the vinca alkaloids, even though i.p. administration of the latter would be expected to favor their activity over EDX. Among the vinca alkaloids, NVB and DVA appeared to have equivalent action against these tumors, while VBL was less active. None of these agents alone resulted in long-term survivors in tumor-bearing mice. The results also show that combination therapy with EDX and vinca alkaloids was by comparison markedly more effective even though some attenuation of dosage was necessary in each combination to avoid unacceptable toxicity. Combinations of EDX and either NVB or DVA were more effective than EDX with VBL, and with the E0771 and T241 tumors appeared not to be schedule-dependent: simultaneous and sequential (EDX 24 h before vinca alkaloid) administration gave comparable results. Combination therapy with EDX and VBL was more effective against E0771 and T241 tumors than against Lewis lung tumors, but in the latter the results depended on which of the two schedules was used. The administration of EDX and vinca alkaloid in the reverse order was severely compromised by the greater toxicity of these agents, which made marked attenuation of the dosage necessary. The basis of this schedule-dependent increase in toxicity is not understood.

These results differ somewhat from much earlier findings [14–16] obtained in the author's laboratory, which documented the effectiveness of similar combined therapy in tumor-bearing mice. In those experiments, when MTX combined with either VCR or DVA was tested against L1210 leukemia, greater therapeutic effects of the combination than of either agent alone were obtained when MTX was administered 24–48 h before VCR or DVA. Administration of the combinations in the reverse sequence was not carried out. The basis of the differences observed in these and the current studies is unknown. However, in these earlier studies, all the agents were administered i.p. to an i.p. ascites tumor, which has a much more rapid growth rate than the solid tumors used here. In addition, in the earlier studies an antifolate (MTX) was used that is accumulated to a lesser extent in tumor cells and is a relatively poor substrate for the polyglutamylating enzyme, folylpolyglutamate synthetase, and considerably less therapeutically effective overall than EDX [1–4, 10, 17, 18]. The role these factors might play in the interaction with a vinca alkaloid in this setting is unclear. A comparison of these antifolates in the context of the present studies seemed inappropriate in view of the consistently documented superiority of EDX [2–4, 10, 18, 19] in numerous animal studies. Moreover, the solid tumor models selected for these studies are only minimally susceptible [2–4, 10, 18, 19] to MTX.

From the data presented, it is not possible to determine whether the results of these experiments reflect synergistic interactions or merely additivity in the effects of these agents in each combination. The extensive dose-effect relationships that must be documented [19] before such analyses can be carried out are impractical at the in vivo level and would very probably raise ethical concerns. Moreover, such an analysis is precluded in those instances where there is an extremely high frequency of long-term survivors only following combined therapy. This issue would be more appropriately pursued in a retrospective study [20] in cell culture, in which bolus dosing in animals or patients would need to be simulated. However, in view of the magnitude of the effects of combined therapy compared with single-agent therapy and the fact that the former occurred following attenuation of dosage, it seems likely that these results reflect some degree of potentiation between the agents in each combination. In any event, the results obtained in these studies are suggestive of an appreciable clinical utility of these combinations and should be helpful in planning additional studies of their use in patients.

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