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Two antiemetic regimens do not impair chemical xenogenization induced in vivo by 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide

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Summary. The possible interference with 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC)-mediated chemical xenogenization (CX) by antiemetic drugs was studied. DTIC was given alone or in combination with either dexamethasone or metoclopramide plus orphenadrine hydrochloride plus diazepam to CD_2F_1 mice bearing the histocompatible L1210 leukemia. Tumor cells were collected from treated animals and inoculated into histocompatible untreated and drug-treated recipients, for eight transplant generations. More than 50% of intact hosts rejected tumor cells between the fourth and sixth transplant generation, independently of antiemetic treatments. Positive controls treated with DTIC plus quinacrine (QC) confirmed that this antimutagenic compound entirely abrogates CX. The present results point out that the antiemetic regimens investigated in this study do not prevent CX. Since DTIC treatment requires intensive antiemetic support in man, these data are of clinical relevance for CX-oriented immunochemotherapy protocols.

Introduction

A number of triazeno-derivatives can induce strong immunogenicity in neoplastic cells of different murine tumor systems [2–7, 10, 16, 18, 25]. This phenomenon has been termed chemical xenogenization (CX) [12] and is possibly related to drug-mediated somatic mutations of cancer cells [1]. CX can be consistently obtained by giving high-dose DTIC to animals bearing histocompatible non-immunogenic tumors for three to eight transplant generations [3]. Xenogenized cells induce host immune responses, such as production of specific antibodies and graft rejection, similar to those detectable in H-2-incompatible recipients [10, 16, 20–24].

However, treatment with 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC) is highly immunodepressive and enables the outgrowth of H-2-incompatible as well as histocompatible xenogenized tumor cells [9, 13, 19]. Further studies were carried out to overcome this difficulty and a successful immunochemotherapeutic protocol for leukemic mice (i.e., high-dose DTIC followed by restoration of graft responsiveness) was developed in our laboratory [15]. In the hope of extending a similar approach to clinical situations, high priority was given to drug interaction studies to rule out any possible interference with the DTIC-related CX phenomenon by supportive therapy.

Control of nausea and vomiting in patients subjected to high-dose DTIC is a major problem. Since dexamethasone (DXM) or a combination of metoclopramide, orphenadrine hydrochloride and diazepam (i.e., MOD regimen) can be of value in this regard [17], a preclinical study was carried out in mice to test whether CX could be affected by these antiemetic drugs. Results of the present study are encouraging, since no substantial interference with the DTIC-related CX phenomenon by DXM or the MOD regimen was found.

Materials and methods

Mice. (BALB/c Cr × DBA/2 Cr) F_1 (CD_2F_1, H-2/H-2) male mice, 2–4 months old, were obtained from the Mammalian Genetics and Animal Production Section, Division of Cancer Treatment, National Cancer Institute (NIH, Bethesda, Md).

Tumor. L1210 Ha (H-2) ascitic leukemia of DBA/2 origin was obtained from Roswell Park Memorial Institute. The line was passaged weekly by i.p. inoculation of ten cells in sterile Medium 199 (Difco; Detroit, Mich, USA) in a final volume of 0.2 ml.

Drugs. DTIC (100 mg/kg per day. Deticene) was supplied by RBS-Pharma (Milan, Italy). DTIC vials contained DTIC, citric acid, and mannitol (1:1:0.375, by vol.) in a water-soluble preparation. Dexamethasone (DXM, 11 mg/kg per day, Decadron; Merck Sharp and Dhome, Inc., Rahway, NJ), metoclopramide (MP, 10 mg/kg per day, Plasil; Lepetit, Milan, Italy), orphenadrine hydrochloride (OR, 1 mg/day, Disipal; Brocades, Milan, Italy), diazepam (DZ, 0.5 mg/kg per day, Valium; Hoffman-La Roche, Basel, Switzerland) were all commercially available. Quinacrine (QC, 20 mg/kg per day) was kindly supplied by Dr. V. Narayanan (Drug Synthesis and Chemistry Branch, National Cancer Institute, NIH, Bethesda, Md). Drug solutions were prepared in 0.85 *N* sterile saline and injected i.p. in a final volume of 0.1 ml/10 g. DTIC was given as a single daily injection at 10 a.m.; all other drugs were injected as two half-doses daily at 10 a.m. and 4 p.m.

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Table 1. Effect of in vivo treatment of L1210 cells with DTIC, DTIC plus QC, or DTIC plus DXM (i.e., in vivo generation of L1210/DTIC, L1210/DTIC/QC, and L1210/DTIC/DXM lines, respectively) on the survival of non-treated or drug-treated recipient mice challenged with 10^5 leukemic cells i.p.

Transplant generation[a]	L1210/DTIC line[b]					L1210/DTIC/QC line[c]					L1210/DTIC/DXM line[d]				
	non-treated		treated with DTIC[e]			non-treated		treated with DTIC+QC			non-treated		treated with DTIC+DXM		
	MS	D/T	MS	D/T	*P*[f]	MS	D/T	MS	D/T	*P*	MS	D/T	MS	D/T	*P*
1	24	7/7	15	7/7	HS	8	6/6	8	6/6	NS	11	7/7	11	7/7	NS
2	14	4/4	14	4/4	NS	9	6/6	10	6/6	NS	12	7/7	12	6/6	NS
3	44	4/7	15	7/7	HS	15	6/6	13	6/6	NS	29	6/7	14	7/7	HS
4	>60	0/7	16	7/7	HS	18	6/6	15	6/6	NS	>60	3/7	16	7/7	HS
5	>60	0/7	17	7/7	HS	16	6/6	14	6/6	NS	>60	2/7	17	7/7	HS
6	>60	0/6	18	7/7	HS	14	6/6	15	6/6	NS	>60	2/6	15	5/5	HS

[a] At transplant generation 0, all mice were inoculated with non-treated L1210 cells. Non-treated recipients died after a median survival (*MS*) of 8 days; dead over total mice (*D/T*), 8/8. Mice treated with DTIC (100 mg/kg i.p. on days 1–5 after challenge) died after an MS of 9 days; D/T, 7/7. Mice treated with DTIC plus QC (20 mg/kg i.p. on days 1–5) died after an MS of 9 days; D/T, 8/8. Mice treated with DTIC plus DXM (11 mg/kg i.p. on days 1–5) died after an MS of 9 days; D/T, 7/7

[b] Tumor cells obtained from donors treated at the indicated transplant generation with DTIC alone were inoculated into non-treated recipient mice or into mice subjected to the same drug treatment

[c] Tumor cells were collected from donors treated with DTIC plus QC

[d] Tumor cells were collected from donors treated with DTIC plus DXM

[e] Mice immunodepressed with cyclophosphamide (180 mg/kg i.p., 1 day before tumor challenge) and challenged with drug-treated lines at transplant generation 6 died of generalized leukemia

[f] Probability was calculated according to Mann-Whitney U-test analysis: *S*, significant ($P < 0.05$); *HS*, highly significant ($P < 0.01$); *NS*, not significant

Table 2. Effect of in vivo treatment of L1210 cells with DTIC or DTIC plus MOD (i.e., in vivo generation of L1210/DTIC or L1210/DTIC/MOD lines, respectively) on the survival of non-treated or drug-treated recipient mice challenged with 10^5 leukemic cells i.p.

Transplant generation[a]	L1210/DTIC line[b]					L1210/DTIC/MOD line				
	non-treated		treated with DTIC			non-treated		treated with DTIC+MOD[c]		
	MS	D/T	MS	D/T	*P*	MS	D/T	MS	D/T	*P*
1	12	7/7	11	7/7	NS	12	7/7	12	6/6	NS
2	>60	2/5	14	7/7	HS	42	4/7	12	7/7	HS
3	23	4/7	19	7/7	S	15	5/7	15	7/7	NS
4	35	5/7	24	7/7	NS	35	5/6	15	6/6	HS
5	17	7/7	12	7/7	NS	19	4/7	15	7/7	NS
6	>60	0/7	24	6/7	HS	>60	3/7	18	6/7	HS
7	>60	0/7	52	4/7	HS	>60	1/7	17	7/7	HS
8	>60	0/7	24	5/5	HS	>60	2/5	18	6/6	HS

[a] At transplant generation 0, all mice were inoculated with non-treated L1210 cells. Non-treated recipient mice after an MS of 8 days; D/T, 8/8. Mice treated with DTIC alone died after an MS of 8.5 days; D/T, 8/8. Mice treated with DTIC plus MOD died after an MS of 8 days; D/T, 7/7

[b] For all symbols used, see footnotes to Table 1

[c] Donor or recipient mice were treated with MOD; MP (10 mg/kg i.p. on days 1–5) plus OR (1 mg/kg i.p. on days 1–5) plus DZ (0.5 mg/kg i.p. on days 1–5)

Results

Male CD_2F_1 mice (6–8 animals per group) were challenged i.p. with ten L1210 cells on day 0. Recipients were either left untreated or were treated with the drugs under investigation on days 1–5. Ascitic tumor cells were collected on days 7–12 after challenge and inoculated into untreated and drug-treated recipients; this procedure was carried out in six to eight transplant generations. Five drug-treated lines were obtained: two L1210/DTIC lines (from mice treated with DTIC alone); line L1210/DTIC/DXM (from animals treated with DTIC plus DXM); line L1210/DTIC/MOD (from mice treated with DTIC plus MOD); and line L1210/DTIC/QC (from mice treated with DTIC plus QC). The latter line was generated to obtain a positive control for drug-disturbance to CX, since QC has been found to impair DTIC-mediated CX both in vivo and in vitro [14].

All recipient mice treated with DTIC died of generalized leukemia, as shown in the experiments illustrated in Tables 1 and 2. In contrast, non-treated hosts survived beyond the 60-day observation period when inoculated with the L1210/DTIC line at transplant generation 4 (Table 1) or 6 (Table 2). Similarly, the majority of intact mice re-

jected L1210/DTIC/DXM (Table 1) and L1210/DTIC/MOD (Table 2) cells at transplant generations 4 and 6, respectively. According to previous observations [8], L1210/DTIC/QC did not undergo xenogenization, and all recipient mice, whether or not treated with DTIC plus QC, succumbed to generalized leukemia (Table 1).

Discussion

The preliminary results of the present study point out that two antiemetic drug regimens widely used in cancer patients subjected to chemotherapy [17] do not prevent or delay the onset of DTIC-mediated CX of leukemic cells in mice. In addition, the present report confirms previous observations concerning the antagonistic effects of QC on CX, presumably mediated by the antimutagenic activity of the compound [11, 14]. Other studies have shown that inhibitors of drug metabolism by the liver (such as carbon tetrachloride) prevent CX, whereas inducers (such as phenobarbital) accelerate CX generation and increase DTIC toxicity [8]. Moreover, combined or sequential treatment with DTIC and other antineoplastic agents such as vincristine (unpublished observation), cyclophosphamide, or bis-chloroethylnitrosourea does not impair CX [21]. Therefore, two mechanisms of drug interaction could affect DTIC-mediated CX, namely, interaction at the DNA level (e.g., QC) or that at the metabolic level (e.g., carbon tetrachloride).

The antiemetic regimens used in the present study consisted of a corticosteroid or a combination of metoclopramide, orphenadrine hydrochloride, and diazepam. None of these different compounds abrogated DTIC-mediated CX of L1210 leukemia in mice. These results agree with the observation that the antiemetic drugs used in the present investigation do not impair DTIC metabolism (data not shwon) and provide evidence that they do not substantially interfere with DTIC at the DNA level. However, more quantitative studies are required to assess whether a minor influence on CX can be detected following treatment with the antiemetic regimens used in the present investigation.

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