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Comparison of the antitumor activity of DTIC and 1-*p*-(3,3-dimethyl-1-triazeno) benzoic acid potassium salt on murine transplantable tumors and their hematological toxicity

Tina Colombo and Maurizio D'Incalci

Istituto di Ricerche Farmacologiche „Mario Negri", Via Eritrea 62, I-20157 Milan, Italy

Summary. *This study describes a comparison of 1-p-(3,3-dimethyl-1-triazeno)benzoic acid potassium salt (DM-COOK) and imidazole-4-carboxamide,5-(3,3-dimethyl-1-triazeno) (DTIC) with reference to antitumor activity on different murine tumors and hematological toxicity. DM-COOK appeared comparably or slightly more effective in L1210, P388, and M5 tumors in the mouse. However, when the treatment of mice bearing M5 with DM-COOK was combined with surgical removal of the primary tumor, the host's life-span was highly significantly prolonged. The two drugs showed similar activity in an M5 variant selected for resistance to cyclophosphamide. In L1210 Ha, a leukemia that is spontaneously resistant to DTIC, DM-COOK was not effective.*

Both DM-COOK and DTIC caused transient leukopenia with a maximum WBC fall of 57% and 71% compared with control values. DM-COOK's greater chemical stability might be an advantage, as the decomposition of DTIC is thought to lead to products responsible for some toxic effects in humans. Like other phenyldimethyltriazenes DM-COOK, is a good candidate for clinical trials because its water solubility eliminates formulation problems.

Introduction

A number of dimethyltriazenes have been synthesized and tested with the aim of finding DTIC analogs forming fewer of the diazonium ions that are considered to be responsible for toxicity but not for antitumor activity, and thus with a better therapeutic index [1].

One of them, 1-*p*-(3,3-dimethyl-1-triazeno)benzoic acid potassium salt (DM-COOK) [8] appears promising as it shows marked antimetastatic activity in Lewis lung carcinoma of the mouse [7] and, as recently demonstrated by our group [3], significant antitumor and antimetastatic activity in the murine ovarian reticular cell sarcoma of the mouse, M5076 (M5). Preoperative treatment with DM-COOK, combined with surgical removal of the primary tumor, proved therapeutically useful in Lewis lung carcinoma of the mouse [10].

We report here a comparison of the antitumor activity of DM-COOK and DTIC in two murine leukemias currently in use for screening new anticancer agents (L1210, P388), in M5 with or without surgery of the primary tumor, in L1210 Ha, a leukemia spontaneously resistant to the chemotherapeutic activity of DTIC [2], and in M5-CTX-16R, an ovarian reticular cell sarcoma resistant to the chemotherapeutic activity of cyclophosphamide [5]; their hematological toxicity, is also assessed.

Offprint requests to: M. D'Incalci

Materials and methods

Animals and tumors. Male CD2F1 mice (20 ± 2 g) received IP transplants of L1210 or L1210 Ha leukemia cells (10^5 cells/mouse) and P388 leukemia cells (10^6 cells/mouse). Female C57Bl/6J mice (20 ± 2 g) were used for M5076 or M5-CTX-16R ovarian sarcoma (7×10^5 cells/mouse IM).

Drugs. DM-COOK and DTIC were freshly prepared in the dark and injected IP (0.1 ml/10 g). DM-COOK, kindly supplied by Dr L. Lassiani and Dr C. Nisi (University of Trieste, Italy), was dissolved in a solution of 0.1 *M* $NaHCO_3$; DTIC, kindly supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md, was used in a solution of 0.05 *M* citric acid or hydroxypropylcellulose (for larger doses).

Surgical tumor removal. M 5076 ovarian sarcoma was removed surgically 14 days after transplantation. Animals were anesthetized with ether and the tumor-bearing leg was removed. The wound was washed with sterile normal saline and closed with Michell 7-mm wound clips.

Evaluation of antitumor activity. The antitumor activity was evaluated by recording the mean and median survival times according to Geran et al. [6].

Hematological toxicity. Blood cell counts were made in normal female C57Bl/6J mice at various intervals. Blood was taken from the eyes of individual animals (10 mice per group) for several days after treatment and cell counts were made in a Bürker hemocytometer.

Statistical analysis. The statistical significance of the differences between groups was assessed by analysis of variance (Duncan's test).

Results

Table 1 shows the survival of DM-COOK- and DTIC-treated L1210- and P388-bearing mice compared with controls. Both drugs appeared more active in L1210, DM-COOK being

Table 1. Activity of DTIC and DM-COOK against mouse leukemias

Group[a]	Treatment[b] (mg/kg IP)	L1210		P388		L1210 Ha	
		S[c]	T/C%[d]	S	T/C%	S	T/C%
Controls	–	8.2 ± 0.1	–	10.3 ± 0.3	–	9.5 ± 0.3	–
DTIC	100 (daily, 1–6)	12.3 ± 0.2	150.0	13.8 ± 0.7	137	10.9 ± 0.2	122
	200 (day 1 only)	9.2 ± 0.2	112.5	12.2 ± 0.2	114	–	–
	400 (day 1 only)	11.4 ± 0.5	137.5	12.7 ± 0.4	124	6.7 ± 1.2	72
DM-COOK	100 (daily, 1–6)	13.8 ± 0.3**	175.0	14.8 ± 0.3	158	11.1 ± 0.3	122
	200 (day 1 only)	11.8 ± 0.3**	150.0	12.8 ± 0.5	119	–	–
	400 (day 1 only)	13.8 ± 0.9*	169.0	16.4 ± 0.2**	152	9.3 ± 0.7	111

[a] Each group consisted of six animals
[b] 10^5 L1210 cells or 10^6 P388 cells were injected IP to CD2F1 mice. The drugs were injected either daily for 6 consecutive days, starting on day 1 after tumor implant, or else only on day 1 after tumor implant
[c] S, mean survival time (days ± SE)
[d] T/C%, median survival time of treated mice/median survival time of untreated controls × 100
* $P < 0.05$ vs DTIC
** $P < 0.01$ vs DTIC

Table 2. Activity of DTIC and DM-COOK against M5 and M5-CTX-16R ovarian sarcoma

Treatment[a, b]	Tumor	Dose (mg/kg IP)	S[c]	T/C%[d]
Controls	M5	–	32 ± 2	–
Controls + surgery	M5	–	33 ± 2	–
DTIC	M5	36	47 ± 1	164
DTIC + surgery	M5	36	49 ± 11 (9)	162
DM-COOK	M5	40	48 ± 2	148
DM-COOK + surgery	M5	40	74 ± 7 (14)**	250
Controls	M5-CTX-16R	–	28 ± 1	–
DTIC	M5-CTX-16R	36	37 ± 1	131
DM-COOK	M5-CTX-16R	40	35 ± 1	132

[a] Treatment was given daily for 9 consecutive days, starting on day 6 after tumor implant. Surgery was carried out on day 14 after tumor implant
[b] Each group consisted of 10–15 animals
[c] S, mean survival time (days ± SE). In brackets % of animals surviving more than 150 days after tumor transplantation
[d] T/C%, median survival time of treated mice/median survival time of untreated controls × 100
** $P < 0.01$ vs DTIC

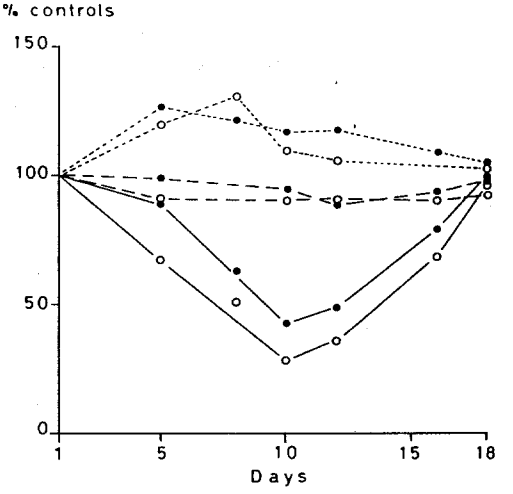

Fig. 1. Leukocyte count (———), platelet count (-------), and hematocrit (– – –) after DM-COOK (●) or DTIC (○) treatment in normal female C57Bl/6J mice (% of control values). Treatments were given for 9 consecutive days (from 1 to 9) (DM-COOK 40 mg/kg IP; DTIC 36 mg/kg IP). The differences between groups were never significant (Duncan's test)

superior with all three dosage schedules employed. DM-COOK was also more active in P388. DM-COOK treatment of L1210 Ha resistant to DTIC (see Table 1, last column), did not extend the survival of animals, suggesting cross resistance between the two drugs. In M5-bearing mice we used lower doses, because these two drugs were more toxic in C57Bl/6J than CD2Fl mice (Table 2). The activity of DTIC and DM-COOK did not appear to be statistically different, but when drug treatment was followed by surgical removal of the primary tumor the therapeutic efficacy, expressed by mean and median survival times of treated animals, was much higher for DM-COOK ($P < 0.01$). The antitumor effect of DM-COOK and of DTIC in M5-CTX-16R, a M5 subline made resistant to cyclophosphamide, was poor. Both drugs increased survival time by about 30%.

As regards hematological toxicity, DTIC and DM-COOK caused leukopenia with nadirs corresponding to a WBC fall of 71% and 57%, respectively compared with control values on the day after the end of the 9th consecutive daily dose cycle. Leukopenia was rapidly reversed in 6–8 days. Platelets or red blood cells appeared unaffected by treatment with either drug (see Fig. 1).

Discussion

The present study provides additional evidence that DM-COOK is an active drug in several rodent tumors. Its activity was comparable to or slightly better than that of DTIC against L1210, P388, and M5, three murine tumor models currently in use for selection of new anticancer agents. DM-COOK appeared to be inactive in L1210 Ha, a line known to be insensitive to DTIC [2]. In M5-CTX-16R, a line made resistant to cyclophosphamide [5], DM-COOK and DTIC showed similar antitumor activity, though this was much less

pronounced than that against the parental M5 tumor. The significant increase in survival time when DM-COOK treatment was followed by surgical removal of the primary M5 tumor is in good agreement with previous data in another experimental tumor [10], which showed the preferential antimetastatic activity of this compound. The hematopoietic toxicity of the two drugs appeared to be similar; neither had any apparent effect on red blood cell or platelet counts in the mouse. Leukopenia was slightly worse with DTIC. These data taken together show that the two drugs have a similar spectrum of activity, thus bearing out the hypothesis of a similar mode of action of DTIC and other dimethyltriazenes, possibly related to the formation of reactive metabolites able to alkylate macromolecules [4, 11]. This point, however, requires further studies, particularly since Sava et al. [9] reported very low *N*-dealkylation of DM-COOK incubated in vitro with rat liver extract, suggesting a lack of correlation between the drug's antitumoral activity and the metabolic pathways usually described for this class of compounds. The much greater chemical stability of DM-COOK than of DTIC may be an advantage, particularly considering the possible role of diazonium ions (formed by photolysis) in the toxicity of DTIC. Though other phenyldimethyltriazenes with a very similar chemical structure are probably equivalent (e.g., carboxamide analog), DM-COOK is a good choice because of its water solubility, which eliminates formulation problems. Since DM-COOK may be a candidate for clinical investigation, we think it worth while to conduct further studies on its mode of action, metabolism, pharmacokinetics, and toxicology so that the first clinical trials can be planned in the safest and most rational way possible.

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