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A Characterization of the Activity of α-1,3,5-Triglycidyl-s-Triazinetrione, a Novel Antineoplastic Compound

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Summary. *To extend initial results on the antineoplastic activity of α-1,3,5-triglycidyl-s-triazinetrione (TGT, NSC 296934), a novel triepoxidic derivative, this compound was tested in a series of murine transplantable tumors. Repeated daily treatments with well-tolerated systemic doses of this chemical produced substantial retardation in tumor growth and significant prolongation of survival in the line 16 mammary, M5067 ovarian, and Madison 109 lung carcinomas and in mFS6 fibrosarcoma. Very marked activity was also seen in the P815 mastocytoma, B16 melanoma, line 38 colon carcinoma, and an intracerebrally transplanted ependymoblastoma, with high proportions of cures after one or two injections in IP transplanted SL2 lymphoma and line 26 colon carcinoma. It is concluded that the high level of antineoplastic effectiveness and the wide spectrum of TGT activity together with its novel structural characteristics could be of clinical significance.*

Introduction

Initial evidence on the in vivo antineoplastic activity in murine model systems of α-1,3,5-triglycidyl-s-triazenetrione (TGT, NSC 296934, Fig. 1) has recently been reported [1]. This previously undescribed chemical, characterized by the presence of three epoxy groups in the molecule, was in fact found to be active in P388 and L1210 leukemias and the Lewis lung carcinoma, its administration inducing either prolonged increases in survival or, depending on the model used and the treatment conditions employed, cures in up to 70% of tumor-bearing hosts.

On the basis of these initial results, and considering that only one other chemical characterized by three epoxy functions has been described that possesses antitumor activity, namely tripdiolide (NSC 163063) [4], it was of interest to extend testing of this compound, with the aim of obtaining a fuller characterization of its spectrum of antineoplastic activity. This report describes results obtained with TGT in a series of mainly solid rodent tumors, presenting data which further establish the high therapeutic potential of this chemical possessing novel structural characteristics.

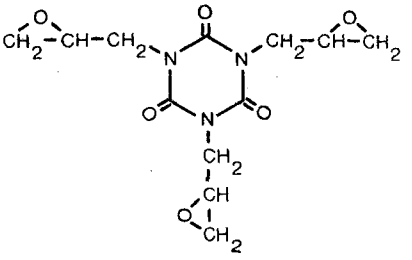

Fig. 1. α-1,3,5-Triglycidyl-s-triazinetrione (TGT, NSC 296934)

Materials and Methods

Drugs

TGT was obtained from Henkel Cie, Frankfurt, Germany, in whose laboratories the compound was originally synthesized [2]. The drug was dissolved in saline immediately prior to administration in volumes of 0.01 ml/g body weight.

Animals and Tumors

Mice (20–22 g at the start of experiments) were obtained from Charles River Italy S.p.A. (Calco, Italy). The Madison 109 lung carcinoma, originally obtained from Dr. A. Bogden (Mason Research Institute, Worcester, Mass., USA) was maintained in Balb/c mice and transplanted IM in compatible $CD2F_1$ male mice in inocula of 2×10^5 viable cells. Male $CD2F_1$ mice were also used as compatible recipients for the SL2 lymphoma (10^5 cells IP) and

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P815 mastocytoma (10^6 cells IP), whereas the mFS6 fibrosarcoma [5] was maintained and transplanted IM in syngeneic male C57BL/6 mice in inocula of 10^5 viable cells. Female C57BL/6 mice were used for the syngeneic M5076 ovarian carcinoma (10^6 cells IM), originally obtained from the Mason Research Institute. The line 16 mammary carcinoma (Dr. D. P. Griswold, Southern Research Institute, Birmingham, Ala.) was maintained and transplanted in female C3H mice (10^5 cells IM), whereas compatible $B6D2F_1$ hosts were used as recipients for the line 26 and line 38 colon carcinomas [3], the inocula used consisting of 0.5 ml 1:100 tumor homogenate IP and 70-mg tumor fragments SC, respectively. The Zimmerman-Arnold ependymoblastoma [8], originally obtained from Dr. R. Geran (DCT, NCI, NIH), was transplanted intracerebrally (IC) in the right cerebral hemisphere of lightly anesthetized male $B6C3F_1$ mice, 1-mm^3 tumor fragments being used.

Table 1. Effect of TGT on the M 5067 ovarian carcinoma[a] in C57BL/6 mice

Exp. group	Dose[b] (mg/kg IP)	Tumor diameter ($cm^{-1} \pm$ SE) on day					
		12	17	20	24	27	31
Control	–	0.88 ± 0.05	1.18 ± 0.08	1.36 ± 0.08	1.47 ± 0.07	1.51 ± 0.12	1.72 ± 0.10
TGT	50	–	–	–	–	–	–
	25	–	–	–	0.65 ± 0.03[d]	0.74 ± 0.11[d]	0.98 ± 0.09[d]
	12.5	–	0.77 ± 0.05[d]	0.90 ± 0.07[d]	0.98 ± 0.11[d]	1.20 ± 0.07[d]	1.15 ± 0.13[d]

Exp. group	Tumor diameter ($cm^{-1} \pm$ SE) on day					MST (days)	LTS[c]
	34	38	41	45	49		
Control	1.98 ± 0.13	1.89 ± 0.09	2.03 ± 0.14	–	–	41.3 ± 4.1	0/15
TGT	–	–	–	–	–	11.4 ± 1.4	0/10
	1.27 ± 0.09[d]	1.37 ± 0.10[d]	1.38 ± 0.20[d]	1.65 ± 0.13	1.75 ± 0.16	51.6 ± 6.0[d]	2/10
	1.60 ± 0.13	1.68 ± 0.11	1.60 ± 0.14[d]	1.78 ± 0.25	–	46.7 ± 5.8	1/10

[a] 10^6 cells were injected IM on day 0
[b] Drug treatment was from day 1 to day 11; dose is per injection
[c] Long-term survivors (over 120 days)
[d] $P < 0.05$ vs controls

Table 2. Effect of TGT on line 16 mammary carcinoma[a] in C3H mice

Exp. group	Dose[b] (mg/kg IP)	Tumor diameters ($cm^{-1} \pm$ SE) on day				
		15	19	22	26	29
Control	–	0.78 ± 0.05	1.52 ± 0.04	1.81 ± 0.03	2.00 ± 0.05	2.11 ± 0.09
TGT	50	–	–	–	–	0.62 ± 0.02[d]
	25	–	–	–	0.83 ± 0.05[d]	1.02 ± 0.05[d]
	12.5	–	0.62 ± 0.02[d]	0.86 ± 0.04[d]	1.31 ± 0.07[d]	1.53 ± 0.06[d]
	5	0.74 ± 0.03	1.36 ± 0.06	1.65 ± 0.04	1.97 0.06	1.95 ± 0.05

Exp. group	Tumor diameters ($cm^{-1} \pm$ SE) on day				MST (days)
	33	36	40	43	
Control	–	–	–	–	28. ± 1.6
TGT	0.81 ± 0.09	–	–	–	32.1 ± 5.6
	1.29 ± 0.06	1.65 ± 0.07	1.77 ± 0.04	2.03 ± 0.06	47.2 ± 1.4[c]
	1.57 ± 0.05	1.80 ± 0.09	1.99 ± 0.11	2.07 ± 0.08	43.7 ± 2.6[c]
	2.13 ± 0.09	–	–	–	36.3 ± 2.2[d]

[a] 10^5 cells were transplanted IM on day 0
[b] Drug treatment was from day 1 to day 11
[c] $P < 0.01$ vs controls
[d] $P < 0.05$ vs controls

Data presented are representative of at least three experiments performed with at least ten animals per group; statistical significance was assessed by Dunnett's test.

Results

Representative results obtained with TGT treatment on the IM M5076 ovarian carcinoma in C57BL/6 mice are presented in Table 1. It can be seen that a dose of 25 mg/kg IP per injection administered from day 1 to day 11 caused a significant retardation in tumor progression, with a 10- to 12-day delay in its clinical appearance and a 9- to 13-day prolongation of mean survival time; this treatment schedule also consistently produced 20%–30% long term (i.e., over 120 days) survivors. Significantly reduced tumor growth and modest increases in survival time (5–7 days) were also observed with a dose of 12.5 mg/kg IP × 11, a treatment which, however, only inconsistently

Table 3. Effect of TGT on the mFS6 fibrosarcoma[a] in C57BL/6 mice

Exp. group	Dose[b] (mg/kg IP)	Tumor diameters ($cm^{-1} \pm SE$) on day			
		13	16	20	23
Control	–	1.01 ± 0.02	1.17 ± 0.03	1.41 ± 0.03	1.70 ± 0.06
TGT	25	0.60 ± 0.03[c]	0.69 ± 0.06[c]	1.05 ± 0.08[c]	1.39 ± 0.07[c]
	12.5	0.80 ± 0.03[c]	0.91 ± 0.03[c]	1.20 ± 0.03[c]	1.55 ± 0.04

Exp. group	Tumor diameters ($cm^{-1} \pm SE$) on day				MST (days)
	27	30	34	38	
Control	1.84 ± 0.05	1.91 ± 0.06	–	–	30.6 ± 0.8
TGT	1.53 ± 0.11[c]	1.59 ± 0.09[c]	1.66 ± 0.12	1.78 ± 0.11	42.6 ± 2.2[c]
	1.74 ± 0.04	1.89 ± 0.03	2.07 ± 0.06	–	34.9 ± 0.5

[a] 10^5 cells were transplanted IM on day 0
[b] Drug treatment was from day 1 to day 11
[c] $P < 0.05$ vs controls

Table 4. Effect of TGT on the Madison 109 carcinoma[a] in $CD2F_1$ mice

Exp. group	Dose (mg/kg IP)	Tumor diameter ($cm^{-1} \pm SE$) on day				
		10	13	17	20	23
Control	–	0.70 ± 0.02	0.93 ± 0.04	1.26 ± 0.04	1.37 ± 0.04	–
TGT	35	–	–	0.85 ± 0.05[c]	0.98 ± 0.06[c]	1.19 ± 0.04
	25	–	0.68 ± 0.03[c]	0.93 ± 0.05[c]	1.06 ± 0.03[c]	1.31 ± 0.05
	15	–	0.80 ± 0.02[c]	1.04 ± 0.03[c]	1.28 ± 0.03	1.45 ± 0.03
	10	0.68 ± 0.03	0.90 ± 0.03	1.16 ± 0.03	1.21 ± 0.08	1.38 ± 0.10

Exp. group	Tumor diameter ($cm^{-1} \pm SE$) on day				MST (days)
	27	30	34	37	
Control	–	–	–	–	23.2 ± 0.3
TGT	1.34 ± 0.03	1.58 ± 0.05	1.76 ± 0.06	1.83 ± 0.07	39.3 ± 1.2[b]
	1.38 ± 0.05	1.57 ± 0.03	1.76 ± 0.09	–	35.4 ± 0.6[b]
	1.55 ± 0.04	1.65 ± 0.07	1.98 ± 0.05	–	35.6 ± 0.8[b]
	1.38 ± 0.08	1.62 ± 0.10	1.85 ± 0.22	–	32.5 ± 2.5[b]

[a] 2×10^5 cells were transplanted IM; drug was administered from day 1 to day 11
[b] $P < 0.01$ vs controls
[c] $P < 0.05$ vs controls

Table 5. Effect of TGT on the survival of mice bearing IC ependymoblastoma[a], IP P 815 mastocytoma[b], or IP B16 melanoma[b]

Tumor system	Exp. group	Dose[c] (mg/kg IP)	MST (days)	T/C %	BWC[d] (g)
Ependymo-blastoma	Control	–	19.3 ± 1.4	–	+ 1.5
	TGT	40	31.0 ± 4.2	160	− 2.7
		30	32.0 ± 3.5	165	− 2.1
		20	23.1 ± 1.7	119	− 0.6
P 815	Control	–	11.0 ± 0.9	–	+ 1.4
	TGT	40	23.0 ± 3.6	209	− 2.6
		20	18.0 ± 2.4	163	− 1.1
		10	16.5 ± 2.1	150	− 0.3
B16	Control	–	15.8 ± 1.2	–	+ 1.3
	TGT	50	37.2 ± 4.7	234	− 2.3
		25	36.0 ± 2.8	227	+ 0.2
		12.5	29.8 ± 2.6	188	+ 1.2

[a] 1-mm^3 tumor fragments were transplanted intracerebrally on day 0 in $B6C3F_1$ mice
[b] 10^6 cells transplanted IP on day 0 in $CD2F_1$ and $B6C3F_1$ mice, respectively
[c] Drug was administered IP from day 1 to day 9
[d] Body weight change determined on day 5

Table 6. Effect of TGT on the survival of mice transplanted with the SL2 lymphoma[a] or colon adenocarcinoma 26[b]

Tumor	Exp. group	Dose (mg/kg IP)	MST (days)	T/C %	LTS[c]
SL2	Control	–	11.5 ± 1.3	–	0/30
	TGT	100 × 1	20.0 ± 6.5	173	2/20
		70 × 1	23.5 ± 6.3	200	4/20
		60 × 1	20.6 ± 2.3	179	3/20
		50 × 1	21.1 ± 1.5	183	1/20
		40 × 1	18.5 ± 1.2	160	0/20
Colon 26	Control	–	25.3 ± 2.8	–	0/20
	TGT	100 × 3	49.6 ± 5.3	193	4/10
		50 × 3	90	360	10/10
		25 × 3	58.2 ± 6.9	237	6/10

[a] 10^5 cells IP on day 0 in $CD2F_1$ mice; drug on day 1
[b] 0.5 ml of a 1 : 100 tumor homogenate IP on day 0 in $B6C3F_1$ mice; drug on days 1, 5, and 9
[c] Long-term (i.e., over 120 days) survivors

Table 7. Effect of TGT on line 38 colon carcinoma[a] in $B6C3F_1$ mice

Exp. group	Dose (mg/kg IP)	BWC[b] (g)	MTW[c] (mg ± SE)	% Reduction
Control	–	+ 2.5	512 ± 48	–
TGT	25	+ 1.3	279 ± 41[d]	46
	50	+ 2.2	153 ± 23[d]	71
	100	toxic		–

[a] 70-mg tumor fragments SC on day 0; drug on days 2 and 9
[b] Body weight change on day 5
[c] Mean tumor weight on day 20
[d] = $P < 0.05$ vs controls

produced long-term survivors, whereas a dose of 50 mg/kg × 11 was toxic in these conditions. With the same treatment schedule TGT was also definitely active in the line 16 mammary carcinoma in C3H mice (Table 2), the injection of 25 mg/kg × 11 causing delays of 9–12 days in tumor appearance and increases in survival in the 80%–105% range. Clear antitumor activity was also seen in this model with a dose of 12.5 mg/kg × 11, whereas a dose of 5 mg/kg given according to the same schedule was marginally active and 50 mg/kg for 11 daily doses was poorly tolerated, as evidenced by shorter survival times than were seen with lower doses.

Results similar to those just described were observed in the mFS6 fibrosarcoma system in C57BL/6 mice, in which treatments with 50 mg/kg IP for 11 successive days were again poorly tolerated, as evidenced by survival times not better than in controls, and doses of 12.5 mg/kg × 11 were of borderline therapeutic effectiveness (Table 3). Conversely, decreases of 30%–40% in tumor diameters throughout the observation period were seen with a dose of 25 mg/kg × 11, which in addition produced a 10- to 14-day increase in mean survival time (MST). This treatment also reduced the proportion of animals with pulmonary metastases to 10%–20% as against 40%–65% (range of ten experiments) in untreated controls. TGT also proved to have antitumor activity in the Madison 109 carcinoma, as shown by increases in MST by 12–17 days obtained with doses of 25–35 mg/kg × 11 (Table 4); in these conditions significant prolongation of survival and reduction in tumor size were also found with injections of 10 mg/kg daily for 11 days.

To determine the spectrum of its antineoplastic activity in greater detail, TGT was subsequently tested in IC ependymoblastoma-bearing $B6C3F_1$ mice. As shown in Table 5 by representative data, the drug was clearly active in this tumor model also, doses of 40 mg/kg × 9 giving T/C% values of 153–174. The same table shows that the compound was additionally markedly effective on P 815 mastocytoma in which 40 mg/kg on each of 9 consecutive days produced T/C% values of 192–221 (range of three experiments), definite prolongations in survival also being observed with a dose of 10 mg/kg × 9. Essentially comparable results to those in P 815 were seen in mice that had received IP transplants of B16 melanoma cells (Table 5). Activity in these systems, however, was clearly inferior to that seen in the SL2 lymphoma (Table 6), in which single IP doses of 60–70 mg/kg produced a doubling in lifespan with a small (10%–20%) but consistently observed proportion of cures. Table 6 also shows that a course of three injections of 25 or 50 mg TGT/kg produced 60%–100% long-term survivors in mice that received IP transplants of colon carcinoma 26. TGT also proved highly effective in the SC transplanted line 38 colon carcinoma; as shown in Table 7, two injections, on days 2 and 9, of 25 or 50 mg/kg produced very marked reductions in day-20 primary tumor weight; on the other hand, 100 mg/kg × 2 in these conditions was toxic.

Discussion

Initial results obtained in two murine leukemias and one solid tumor model, the Lewis lung carcinoma, permitted the tentative conclusion that TGT, a chemical possessing novel structural characteristics, was an active antineoplastic agent [1]. Data here recorded in a larger series of mainly solid experimental tumors and showing that treatment with this compound can produce clear reductions in tumor growth resulting in significant increases in lifespan and, in certain systems, in consistent proportions of long-term survivors, amply confirm the earlier conclusion.

On the basis of available evidence, two features of this drug appear to be of special interest, namely its high level of antineoplastic effectiveness and its large spectrum of activity. As previously documented for the P388 and L1210 leukemias, in which percentage cure rates of up to 80% could be obtained with early treatments and survival times were more than doubled in advanced, widely dissseminated tumors, in this study too the degree of antineoplastic effectiveness observed with TGT was substantial. In fact, in the mFS6 fibrosarcoma, the least responsive of the panel of tumors tested, prolongation of survival by at least one-third was seen with the treatment conditions employed, whereas over 60% increases in lifespan were seen in IC ependymoblastoma and low (20%) but consistent cure rates were found in the M5076 ovarian carcinoma. Single drug doses could also produce cures in the SL2 lymphoma system. Since the main aim of these experiments was to confirm the antineoplastic activity of TGT in a larger series of neoplasms, fixed treatments employing essentially continuous daily schedules were employed. This choice was based on results in the L1210 system showing that such a schedule was more effective than an intermittent (i.e., every 4 days) treatment [1]. Similarly, a daily treatment was more effective in animals with transplanted P815 mastocytomas (data not shown). However, in both systems the therapeutic differential between continuous and intermittent schedules was not very marked; in addition, there are significant differences in factors

that can critically affect the effectiveness of chemotherapeutic agents (e.g., cytokinetics and/or drugs availability at target sites) between ascitic and solid tumors. Accordingly, no claims are advanced that the treatment schedules used in this study are the optimal ones, and it is possible that better therapeutic results might have been obtained with other schedules of drug administration. On-going studies on the hematotoxicity of TGT and its pharmacokinetics and disposition should help in clarifying this problem.

It is also of note that in all systems investigated in this and a previous study, marked activity was seen with TGT doses which were well tolerated by tumor-bearing hosts as judged by decreases in body weight, which never exceeded 5% in any of the conditions in which this admittedly crude parameter was measured, and by the absence of early toxic deaths. Although a detailed picture of the toxicity following acute and repeated TGT administration is still unavailable, it can be mentioned that when the LD_{50} of this chemical was determined all mice succumbed not later than day 15, and that cured animals were routinely observed for at least 120 days without delayed deaths or, indeed, signs of overt residual toxicity being seen.

Another aspect emerging from this study and of possible clinical significance is the apparently wide spectrum of antineoplastic activity displayed by TGT. This drug has in fact been found effective in each of the 13 tumor models tested in this and a previous report [1] encompassing neoplasms differing widely in origin, histology, growth rate, tendency to metastasize, immunogenicity, and sensitivity to chemotherapy. In addition, indications of definite effectiveness have recently been obtained in a further series of murine (ADJ/PC6A plasmocytoma, TLX5 lymphoma, C22LR osteosarcoma) and rat tumors (K5222 leukemia and Yoshida sarcoma) (EORTC Screening and Pharmacology Group, unpublished data). Furthermore, activity has been observed in a proportion of human head and neck tumors xenografted in nude mice, these xenografts being resistant to a number of other concomitantly tested cancer chemotherapeutic agents (F. Sneeuwloper, personal communication). Relevance is attached not only to the observation of TGT efficacy in animal tumors that are models of neoplasms frequently encountered clinically and for many of which (e.g., colon) currently available agents provide only limited therapeutic benefit, but also to the drug's clear effectiveness on an IC transplanted tumor. This finding confirms previous indications in leukemic mice [1] of the ability of TGT (and/or its biotransformation products) to reach therapeutic concentrations in intracranial tumors, and constitutes a further reason for interest in this compound.

The biochemical basis of TGT cytotoxic acitivity is still unknown. Considering the presence in the structure of this chemical of three epoxy groups and the well-known alkylating capacity of such functions [6, 7], such a mechanism appears likely, although formal proof is still lacking. In the light of this hypothesis, our previous demonstration [1] of a comparable in vivo effectiveness of TGT in a 'wild' and a cyclophosphamide-resistant L1210 leukemia subline could potentially be of clinical use. It is also not known whether the compound is active per se and/or through biotransformation products whose existence is also suggested by preliminary findings indicating a short half-life of the compound in the circulation. The nature and relative cytotoxic capacity of TGT metabolites are currently being investigated.

In conclusion, the high effectiveness of TGT consistently observed in a wide range of experimental leukemia lymphomas and solid tumors, and its hydrosolubility, chemical stability, and novel structural characteristics, appear to justify active interest in this compound.

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