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Antitumoral Effect in Mice of a New Triepoxyde Derivative: 1, 3, 5-Triglycidyl-S-Triazinetrione (NSC 296934)*

G. ATASSI,†‡ F. SPREAFICO,§ P. DUMONT,† P. NAYER† and J. KLASTERSKY†

Service de Médecine et Laboratoire d'Investigation Clinique Henri Tagnon, Institut Jules Bordet, rue Héger-Bordet 1, 1000 *Bruxelles, Belgium and §Istituto de Ricerche Farmacologiche Mario Negri, Via Eritrea* 62, 20157 *Milano, Italy*

Abstract—*The antitumor properties of the α and β stereoisomers of* 1, 3, 5-*triglycidyl-s-triazinetrione (TGT) were investigated on various transplantable mouse tumor systems. Although the two stereoisomers displayed a high therapeutic activity against P*388 *and L*1210 *leukemias when administered i.p., α-TGT was superior to the β form in prolonging the lifespan of treated animals and in inducing long-term survival:* 70% *of long-term survivors over* 90 days *of treated animals as compared to controls. α-TGT also demonstrated antitumor effect against advanced L*1210 *leukemia (ILS:* 119% *at* 50 mg/kg × 9) *and was still very active when administered orally against i.v. or ascitic L*1210 *leukemia while its activity when administered i.p. against i.c. L*1210 *was moderate and yet significant. The i.p. treatment with α TGT significantly inhibited the primary tumor growth and lung metastases of Lewis lung carcinoma. Finally, the high* in vivo *activity of α-TGT on normal P*388 *cells and on a subline of this leukemia markedly resistant to cyclophosphamide (Cy) is a further element warranting studies with this agent.*

INTRODUCTION

ALTHOUGH a relatively wide armament of active drugs is currently available to cancer chemotherapists, there is still an urgent need for additional antineoplastic compounds possessing novel cytotoxic modes of action or improved pharmacological properties in the hope of enlarging the spectrum of responsive tumors as well as of ensuring a safer and more effective treatment. In view of the potent activity on both animal and human cancer displayed by many alkylating agents, much effort has been devoted over the past 30 yr to the search for novel compounds possessing this biological activity and exhibiting better therapeutic indexes. The alkylating capacity of epoxy functions is well known [1–3] and the antineoplastic activity of bifunctional epoxides has been reported [4–6]; however, the investigation of active antitumor agents containing higher numbers of epoxy groups in their structure has been very recently performed and only one structure—Tripdiolide (NSC-163063)—is known up to now to contain three epoxy groups [7]. The present report describes initial findings on the high cancer chemotherapeutic activity in experimental systems of 1, 3, 5-triglycidyl-s-triazinetrione (TGT, Fig. 1), a novel chemical containing 3 epoxidic functions in the molecule.

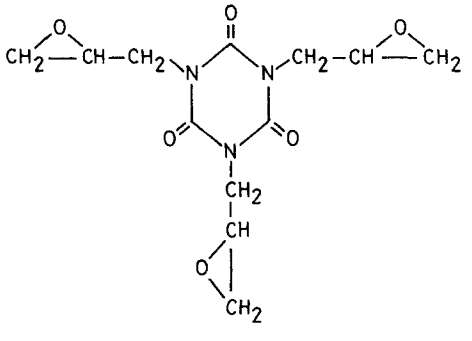

Fig. 1. 1, 3, 5-*Triglycidyl-s-triazinetrione* (*NSC* 296934).

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‡Reprint requests should be addressed to: Dr. G. Atassi, Laboratory for Experimental Chemotherapy and Screening, Institut Jules Bordet, Rue Héger-Bordet 1. 1000 Bruxelles, Belgium.

MATERIALS AND METHODS

Drugs

TGT was supplied by Henkel KGaA, Düsseldorf, Germany. This compound, originally synthetized by Budnowski [8], can be resolved by fractional crystallization in two stereoisomers, α and β, having the configuration (R, R, S/S, S, R) and (R, R, R/S, S, S), respectively. The α (NSC 296934) and β (NSC 296964) forms differ significantly in their melting point (105 and 156°C, respectively) and solubility in water (1.01 and 0.053% at 20°C). Cyclophosphamide (Cy, NSC 26271) was a generous gift of Dr. Narayanan (Drug Synthesis and Chemistry Branch, National Cancer Institute). Compounds were dissolved or suspended in saline immediately prior to injection for which volumes of 0.01 ml/g were used.

Animals and tumors

CDF_1 and C57Bl/6 mice of both sexes, weighing 20–22 g at the start of the experiment, were supplied by Charles River Breeding Laboratories (Wilmington, Mass., U.S.A.) and by Southern Animal Farms (Prattville, Ala., U.S.A.), respectively. The L1210 and P388 leukemias, obtained from Dr. A. Bogden (Mason Research Institute, Worcester, Mass., U.S.A.) were maintained in ascitic form by weekly transfers in DBA/2 mice; cell viability was checked microscopically by dye exclusion before transplantation and only inocula with over 95% viability were accepted.

The Lewis lung (3LL) carcinoma, also obtained from Dr. Bogden, was maintained by the i.m. transplantation of 2×10^5 cells in C57Bl/6 hosts; pulmonary metastasization in the case of the 3LL tumor was assessed as described in [9, 10]. The partially Cy-resistant P388 leukemia subline (P388/Cy) was provided by Dr. F. M. Schabel (Southern Research Institute, Birmingham, Ala., U.S.A.); resistance had been induced in this subline as originally described by Dewys [11] and was maintained by weekly treatments with 100 mg/kg s.c. Cy 3 days after tumor inoculation.

RESULTS

Toxicity of α-and β-TGT

The acute LD50 of α-TGT evaluated over a 14 days observation period was 105 ±20 mg/kg for single i.p. doses and approximately 58 ± 10 mg/kg when a 9 consecutive days treatment was used in normal CDF_1 mice. In these conditions, the median day of death was 8 (range 5–19) and 10 (5–13), respectively. For each schedule, the LD10 was respectively 90 and 45 mg/kg. Because of the poor solubility of β-TGT in saline at concentrations above 0.05%, the acute LD_{50} could not be determined, all normal mice surviving single i.p. doses of 650 mg/kg whereas a 50% mortality was obtained with treatments of 350 mg/kg i.p. × 5.

Antileukemic activity

In order to obtain initial indications on the antineoplastic activity of α- and β-TGT, a first series of experiments was performed in which fixed doses of these compounds were administered i.p. from day 1 to 9 to mice transplanted i.p. on day 0 with 10^6 P388 or 10^5 L1210 leukemic cells. As shown in Table 1, a significant, dose-dependent effect was seen in both systems with both isomers as evidenced by increases in lifespan (ILS) up to 100–145 and 197–215% (range of 3 experiments) in the P388 and L1210 models, respectively, at doses of 50–100 mg/kg. Under these conditions, α-TGT proved to be significantly more potent than β-TGT as revealed by the finding that twice higher doses of the β than those of the α isomer were required to obtain comparable %ILS values specially in the L1210 system.

On the basis of these results, subsequent studies were conducted using preferentially the α isomer and the L1210 model for investigations on the influence of treatment conditions on the antitumoral activity of TGT. As shown by representative data in Table 2, the activity of α TGT was not restricted to the ascitic form of L1210 and to the i.p. route of administration since marked prolongations of survival were also observed in mice transplanted s.c. or i.v. with high numbers of leukemic cells and treated with the compounds by the i.p. or i.v. routes. A high effectiveness was additionally seen when the drugs were administered orally to mice transplanted i.v. with tumor cells (Table 3), and also by this treatment route the efficacy of α-TGT was significantly better than that of β-TGT. Definite increases in survival, of the order of 50–120%, were also seen when i.p. treatments with α-TGT were administered on relatively advanced L1210 (Table 4), better results being observable in the case of the ascitic rather than in that of s.c. tumor implant. On the other hand, only a minor although significant increase in survival was

Table 1. *Effect of α and β-TGT on the survival of CDF$_1$ mice bearing P388 or L1210 ascites*

	α-TGT						β-TGT					
	P388			L1210			P388			L1210		
Dose (mg/kg day)	B.W.C. (g)	Med. S.T. (days)	I.L.S. (%)	B.W.C. (g)	Med. S.T. (days)	I.L.S. (%)	B.W.C. (g)	Med. S.T. (days)	I.L.S. (%)	B.W.C. (g)	Med. S.T. (days)	I.L.S. (%)
200	−5.5		toxic				−4.5		toxic			
100	−2.7	8.3	28	−4.5	8.0	0	−1.5	30	145	−2.5	25.2	215
50	−2.3	24.3	100	−1.8	23.8	197	−1.3	21.3	75	−1.6	16.3	103
25	−0.8	20.3	67	−1.0	14.2	77	−0.6	18.8	54	−0.3	12.2	52
12.5	−0.3	20.0	65	+0.1	11.5	43	0	16.4	34	+0.5	10.2	27
Controls	+1.1	12.1	0	+2.2	8.0	0	+1.1	12.1	0	+2.2	8.0	0

CDF$_1$ mice were inoculated i.p. with leukemic cells (10^6 P388 or 10^5 L1210 cells/mouse) on day 0.
Drugs were administered i.p. in saline on days 1–9.
B.W.C. = body weight change on day 5.
Med.S.T. = median survival time of 10 treated mice and 32 untreated controls.

Table 2. *Effect of α-TGT on the survival of CDF$_1$ mice bearing L1210 s.c. or i.v.*

	L1210 s.c. (treated i.p. on days 1–9)		L1210 i.v. (treated i.v. on days 1–5)	
Dose (mg/kg/day)	Med.S.T. (days)	I.L.S. (%)	Med.S.T. (days)	I.L.S. (%)
50	16.0	72	13.8	115
40	13.3	43	10.7	67
30	11.8	26	7.6	18
Controls	9.3	0	6.4	0

Mice were inoculated s.c. or i.v. with 10^5 leukemic cells on day 0. The drug was administered in saline.
Med.S.T. = median survival time of 10 treated mice and 34 untreated controls.

Table 3. *Effect of α and β-TGT administered p.o. on the survival of CDF$_1$ mice bearing L1210 leukemia i.v.*

	α-TGT				β-TGT			
Doses (mg/kg)	B.W.C. (g)	Med. S.T. (days)	I.L.S. (%)	Dose mg/kg)	B.W.C. (g)	Med. S.T. (days)	I.L.S. (%)
120	−2.0	16.0	150	300	−1.3	11.8	84
100	−1.9	15.0	134	200	−0.8	9.4	46
80	−1.1	14.1	120	100	−0.6	8.8	37
Controls	+0.8	6.4	0	Controls	+0.8	6.4	0

Mice were inoculated with 10^5 leukemic cells on day 0.
The drugs in saline were administered on days 1–9.
B.W.C. = body weight change on day 5.
Med.S.T. = median survival time of 10 treated mice and 34 untreated controls.

Table 4. Effect of α-TGT on the survival of CDF_1 mice bearing advanced L1210 leukemia

Dose (mg/kg)	L1210 i.p. Med. S.T. (days)	L1210 i.p. I.L.S. (%)	L1210 s.c. Med. S.T. (days)	L1210 s.c. I.L.S. (%)
50	18.2	119		
40	16.0	92	14.0	50
30	13.8	66	12.3	32
Controls	8.3	0	9.3	0

Mice were inoculated with 10^5 leukemic cells on day 0.
The drug dissolved in saline was administered i.p. from day 5–13.
Med.S.T. = median survival time of 10 treated mice and 18 untreated controls.

Table 5. Effect of α and β-TGT administered i.p. on the survival of CDF_1 mice bearing L1210 leukemia i.c.

α-TGT Dose (mg/kg)	B.W.C. (g)	Med. S.T. (days)	I.L.S. (%)	β-TGT Dose (mg/kg)	B.W.C. (g)	Med. S.T. (days)	I.L.S. (%)
50	−4.3	10.3	10	90	−2.0	11.1	19
40	−3.5	12.3	32	80	−2.6	9.0	0
30	−3.1	12.6	35	70	−1.6	10.2	9
Controls	+0.7	9.3	0	Controls	+0.7	9.3	0

Mice were inoculated i.c. with 10^5 leukemic cells on day 0.
The two drugs in saline were administered on days 1–9.
B.W.C. = body weight change on day 5.
Med. S.T. = median survival time of 10 untreated mice and 27 untreated controls.

Table 6. Influence of schedule and route of administration of α-TGT on the survival of CDF_1 mice

Route	Treatment schedule	Dose range (mg/kg)	Optimal dose (mg/kg/injection)	I.L.S. (%)	L.T.S. (%) L1210-bearing mice	L.T.S. (%) Non-leukemic mice
i.p.	day 1	50–80	80	119	0	100
i.p.	day 1 every 3 hr	10–40	20	537	40	37.5
i.p.	days 1 and 9	50–300	50	87	0	100
i.p.	days 1 and 9 every 3 hr	10–40	10	76	10	100
i.p.	days 1, 5 and 9	25–200	50	153	30	100
i.p.	days 1, 5 and 9 every 3 hr	5–30	10	112	0	87.5
i.p.	days 1–5	40–70	50	657	50	87.5
i.p.	days 1–9	40–70	40	661	70	100
p.o.	day 1	100–400	400	63	0	100
p.o.	days 1 and 9	100–400	300	85	0	100
p.o.	days 1, 5 and 9	50–300	300	121	0	100
p.o.	days 1–9	12.5–100	100	78	0	87.5

Mice were inoculated i.p. with 10^5 leukemic cells on day 0.
L.T.S. = long-term survivors (>90 days).

Table 7. Influence of schedule and route of administration of β-TGT on the survival of CDF_1 mice

Route	Treatment schedule	Dose range (mg/kg)	Optimal dose (mg/kg/injection)	I.L.S. (%)	L.T.S. % L1210-bearing mice	L.T.S. % Non-leukemic mice
i.p.	day 1	150–450	350	51	0	100
i.p.	day 1 every 3 hr	25–55	25	86	10	100
i.p.	days 1 and 9	150–400	250	60	0	100
i.p.	days 1 and 9 every 3 hr	20–50	30	196	200	100
i.p.	days 1, 5 and 9	100–400	300	122	20	87.5
i.p.	days 1, 5 and 9 every 3 hr	15–45	25	48	50	50
i.p.	days 1–5	50–200	200	97	0	100
i.p.	days 1–9	50–150	75	113	20	100
p.o.	day 1	350–500	450	33	0	100
p.o.	days 1 and 9	350–500	450	23	0	100
p.o.	days 1, 5 and 9	300–450	350	22	0	87.5
p.o.	days 1–9	100–250	200	51	0	100

Mice were inoculated i.p. with 10^5 leukemic cells on day 0.
L.T.S. = long-term survivors (>90 days).

seen when α-TGT was given i.p. to animals transplanted intracerebrally with a high inoculum of leukemia cells, β-TGT being ineffective under the same conditions even at doses three times higher than those of α-TGT producing prolongations of lifespan (Table 5).

The influence of the schedule of administration for TGT given p.o. and i.p. to L1210-bearing CDF_1 mice is presented in Tables 6 and 7. It can be seen that although a significant activity of α-TGT was observed using a variety of treatment schedules, the greatest therapeutic effectiveness was found when this compound was administered i.p. for 9 consecutive days, a dose of 30–40 mg/kg giving 50–70% (range of 3 experiments) long-term (over 90 days) survivors while producing no deaths and very moderate body weight loss (2.0 g) in non-leukemic mice. The lower antineoplastic activity of β-TGT was confirmed also in these tests as evidenced by the fact that consistent proportions of cures (40–50%) were obtained only with treatments resulting in over 40% lethality in non-leukemic hosts. Conversely, treatments which were well tolerated by normal animals produced markedly lower %ILS values than α-TGT and only occasional cures. When oral administrations were used, α-TGT was again superior to the β isomer and, for both drugs, the therapeutic effectiveness was significantly lower than that observed with parenteral administrations. In addition, although repeated doses of α-TGT (e.g., Q4D) were more active than single doses, the therapeutic differential was significantly lower than that seen for this compound with the i.p. route.

In order to obtain initial indications on the possible cross-resistance between α-TGT and Cy, the effect of these drugs was compared on the P388/Cy subline and one representative experiment is presented in Table 8. P388/Cy was markedly, although not totally, resistant to Cy as shown by the obtainment of only 50%ILS as compared to 388% ILS and 20% cures when 180 mg/kg Cy was given to mice bearing the standard P388 leukemia. Conversely, α-TGT was equally active on both sublines and indeed, in both experiments performed, significantly higher %ILS values were seen in the P388/Cy system.

Activity in solid tumors

In order to ascertain that the high antineoplastic activity of α-TGT was not confined to murine leukemias, the effect of treatments with this compound on the LL carcinoma in C57Bl/6 mice was investigated using standard treatment schedules. α-TGT exerted a definite antitumoral activity in the LL model (Table 9), significant reductions in lung metastases number and weight being obtained already

Table 8. Difference in the therapeutic activity of α-TGT against P388 and P388/Cy leukemias

Dose (mg/kg/day)	P388 Med. S.T. (days)	P388 I.L.S. (%)	P388 L.T.S. (%)	P388/Cy Med. S.T. (days)	P388/Cy I.L.S. (%)	P388/Cy L.T.S. (%)
40 (α-TGT)	35.0	250	10	56.0	378	30
30 (α-TGT)	29.0	190	0	32.0	173	0
180 (Cy)	48.8	388	20	17.6	50	0
Controls	10.0	0	0	11.7	0	0

CDF_1 mice were inoculated i.p. with 10^6 leukemic cells on day 0.
The drugs were dissolved in saline and administered i.p. α-TGI was injected on days 1–9 and cyclophosphamide only once on day 1.
Med.S.T. = median survival time of 10 treated mice and 20 untreated controls.
L.T.S. = long-term survivors (>90 days).

Table 9. Effect of α-TGT on Lewis lung carcinoma and lung metastases

Dose (mg/kg/day)	Dead mice before day 11 total	No. of mice with metast. /mice with tumor	Primary tumor weight (g)	Av. No. of metast. (±s.c.)	Av. wt. of metast. (mg)
50	7/10	3/3	2.87±0.7†	10±0.7†	5.38±0.9†
25	0/10	8/8	5.62±0.4*	21±3†	19.0±5.0†
12.5	0/10	9/9	7.63±0.4	26±5†	26.8±7.9†
10	0/10	10/10	7.60±0.4	24±3†	21.2±5.9†
5	0/10	10/10	8.16±0.4	36±3	42.6±12.6
Controls	0/14	14/14	8.49±0.5	48±7.2	63.3±17.9

10^5 cells were implanted i.m. in C57Bl/6 mice on day 0. Animals were sacrificed on day 21.
Treatment was i.p. on days 1–11.
*$P<0.05$; †$P<0.01$.

with a dose of 10–12.5 mg/kg i.p. per 11 days. Treatments with 50 mg/kg × 11 were toxic in this system whereas 25 mg/kg × 11, a schedule not producing early (i.e., before day 11) toxic deaths, produced significant reductions in primary tumor weight and over 50% decreases in the number and weight of pulmonary secondaries.

DISCUSSION

Results presented lead to the conclusion that TGT, a previously unreported chemical characterized by the presence of three epoxide groups in its structure, possessed a high antineoplastic activity in murine tumors. Marked prolongations in survival and high proportions of cures in leukemia-bearing mice could in fact be obtained using this compound in doses and treatments which were well tolerated in non-leukemic animals as evidenced by absence of lethal toxicity and very limited body weight loss. In further confirmation of the high efficacy of TGT, clear activity was also observed in advanced leukemia and in a solid tumor known to be relatively resistant to many cancer chemotherapeutic agents such as the LL carcinoma. In this connection, it is worth noting that tests in progress to be reported elsewhere have given evidence that the compound is therapeutically effective on a wide spectrum of virally or chemically-induced solid rodent neoplasms of different histological origin. Also of interest was the finding that, although the highest activity was found when TGT was administered i.p. on ascitic tumors, the compound was clearly effective not only when given parenterally but also orally, a fact of relevant clinical potential.

Of the two isomers, α-TGT proved definitely superior not only because it produced

significantly greater prolongations in lifespan and higher numbers of long-term survivors at doses being at least one half of the maximum tolerated doses of the β-form in a variety of treatment schedules but also because of the wider interval between effective and toxic doses observed for the α-isomer. In contrast to β-TGT, α-TGT was also slightly but significantly effective in mice transplanted i.c. with high numbers of L1210 cells, suggesting that the compound may cross the blood–brain barrier. However, further tests need to be performed to confirm the practical exploitability of this property in the treatment of cerebral tumors. Of note is additionally the fact that α-TGT is markedly more soluble, a characteristic of importance in drug formulation. In the L1210 system, α-TGT given i.p. was significantly more effective in continuous protracted treatments (e.g., for 5 or 9 consecutive days) than when employed at similar or greater total doses by more spaced schedules. The influence of the schedule of treatment on therapeutic effectiveness was less obvious for β-TGT and for both isomers in the case of the oral route.

The capacity of α-TGT to inhibit the growth of tumor cells *in vitro* as well as its efficacy in immunodepressed hosts (Spreafico *et al.*, in preparation), indicate the *in vivo* antineoplastic activity of this chemical is dependent principally, if not exclusively, on a direct cytocydal effect whose biochemical basis is still formally undetermined. The presence however in TGT structure of epoxide groups, chemical functions known for their potent alkylating capacity [1–3], renders such a mechanism of action plausible. In the light of this hypothesis, the equally high *in vivo* activity of α-TGT on normal P388 cells and a subline of this leukemia resistant to Cy, one of the most active and widely employed alkylating compounds, appears of practical relevance and is a further element warranting additional studies with this agent.

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