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Characterization of the Activity of α/β-Triglycidylurazol (TGU; NSC-332488): a New Antineoplastic Compound*

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Abstract—*The antitumour properties of α/β-triglycidylurazol (TGU) were investigated on various transplantable mouse tumour systems. A high rate of cures of P388 and L1210 leukaemias was obtained with this compound. TGU also had an antitumour effect against B16 melanoma, the colon 38 tumour and the advanced RC renal carcinoma, producing a total regression of the tumour. Finally, the marked* in vivo *activity of TGU against a subline of P388 leukaemia totally resistant to cyclophosphamide (CP), its good water-solubility (7%) and its stability in neutral pH are further elements warrranting clinical studies with this agent.*

INTRODUCTION

THE ANTINEOPLASTIC activity of bifunctional epoxides has been previously reported [1–3]. The antitumour effect of compounds containing higher numbers of epoxide groups has been recently investigated. α-Triglycidyltriazinetrione or TGT (NSC-296934) was the first epoxide evaluated for its antitumour activity and was found to be very active against a wide panel of murine solid tumours and leukaemias [4, 5] as well as against human mammary tumour cell lines *in vitro* [6]. The clinical phase I study showed that thrombophlebitis at the injection site, likely to be due to the poor water-solubility of α-TGT, appeared to be the major limiting factor [7]. On this account we aimed at determining the antineoplastic role of new triepoxides and at selecting an analogue with similar, if not better, anticancer properties to those of α-TGT, with higher water-solubility and not producing the same side-effects.

The racemic compound α/β-triglycidylurazol, or TGU (Fig. 1), was the triepoxide derivative selected to replace α-TGT because of its stability and high water-solubility (7%). The present report describes the antitumour effect of TGU against experimental tumours.

MATERIALS AND METHODS

Drugs

α/βTGU mixtures were supplied by Henkel KGaA (Düsseldorf, F.R.G.). The synthesis was described by Fisher *et al.* [8]. The chemical structure contains one chiral centre at the carbon two of each glycidyl group. Thus several racemic diastereomers are possible. The NSC-332488 is the 50:50 mixture of the diastereomers α and β named TGU. NSC-349440 is the 61:39 mixture and NSC-349441 the 8:92 mixture of the α/β-TGU. A γ isomer (NSC-349442), poorly soluble in water, was also tested. It could be a third diastereomer or most likely another isomer corresponding to the structure in Fig. 2. The α and β forms are both highly water-soluble, which explains why they were not separated. Cyclophosphamide or CP (NSC-26271) was obtained from the Drug Synthesis and Chemistry Branch, National

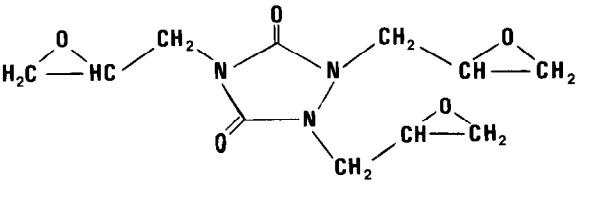

Fig. 1. 1,2,3-Triglycidylurazol or α/β-TGU (NSC-332488).

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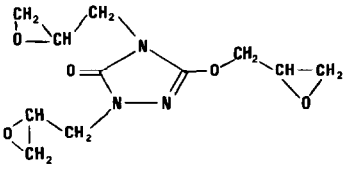

Fig. 2. γ-Isomer.

Cancer Institute. Compounds were dissolved or suspended (for the γ isomer) in saline immediately prior to injection.

Animals and tumours

CDF1 (BALB/c × DBA/2), BDF1 (C57BL/6 × DBA/2), B6C3F1 (C57BL/6 × C3H), C57BL/6 and BALB/c, weighing 20–24 g at the start of the experiment, were supplied by Charles River Breeding Laboratories (Wilmington, MA, USA). All tumour lines were obtained from the Tumour Bank of the National Cancer Institute.

L1210 and P388 leukaemias were maintained in ascitic form by weekly transfers in DBA/2 mice. The Lewis lung carcinoma (LL), B16 melanoma (B16) and the colon 38 (C38) tumour were maintained by s.c. transplantation in C57BL/6 hosts. The resistance of CP-resistant P388 leukaemia (P388/CP) was maintained by weekly s.c. administration of 100 mg/kg, starting 3 days after tumour inoculation. The RC renal adenocarcinoma was maintained in CDF1 mice i.p. serial transfers every 14th day [9]. The percentage increase in lifespan (ILS) or mean tumour weight was calculated according to the NCI protocols [10].

RESULTS

Toxicity of TGU

The LD_{50} and LD_{10} of TGU evaluated over a 14-day observation period were respectively 80 and 65 mg/kg for single i.p. doses in CDF1 mice. In BALB/c mice, these values were respectively 92 and 70 mg/kg.

Antileukaemic activity

In order to obtain initial indications on the antineoplastic activity of the different isomer of TGU, the four different preparations were compared in the P388 system. The results are shown in Table 1. At the optimal i.p. doses of 25 mg/kg injected during five consecutive days, the different α/β-TGU mixtures produced similar high antileukaemic activity evidenced by an increase in lifespan (ILS) of over 200% and by a number of long-term survivors. On the basis of these results, subsequent studies were conducted using the α/β-TGU mixture (50:50) of TGU.

In order to confirm the antileukaemic activity of TGU and to obtain initial indications on the possible cross-resistance between TGU and CP, the effect of these drugs was compared on the P388/CP subline and one illustrative experiment is reported in Table 2. P388/CP was totally resistant to CP: an ILS of 8% only was obtained as compared to the 330% ILS and the 2 cures out of 10 mice obtained when 200 mg/kg CP were given to mice bearing the standard P388 leukaemia. Conversely, TGU was active on both sublines.

The activity of TGU against the L1210 leukaemia and the influence of the schedule of treatment when the drug is given i.p., p.o. and i.v. to CDF1 mice bearing L1210 are indicated in Table 3. It shows that TGU exerted a significant activity with a variety of treatment schedules and routes of administration. The highest number of long-term survivors (over 60 days) was observed with the i.p. administration of a 60 mg/kg single dose 24 hr post-implant.

Activity against solid tumours

In order to ascertain whether the antineoplastic activity of TGU was not limited to murine leukaemias, the effect of the administration of this compound to the i.p. B16 melanoma in BDF1 mice was investigated using standard treatment schedules. TGU was clearly active against this tumour model (Table 4). TGU administered i.p. was inactive against the Lewis lung carcinoma transplanted i.v. into B6C3F1. It had marginal

Table 1. Antileukaemic effect of different isomers of TGU

Doses (mg/kg/day)	ILS(%)* NSC-332488 (α/β 50:50)	NSC-349440 (α/β 61:39)	NSC-349441 (α/β 8:92)	NSC-349442 γ
50	-13	-17	-30	145 (1/6)
25	>200 (4/6)	>200 (4/6)	>200 (4/6)	102
12.5	63 (1/6)	140	125 (1/6)	68

10^6 P388 leukaemic cells were inoculated i.p. on day 0. Drugs were administered i.p. on days 1–5.

( ) = long-term survivors/total number on day 60.

*ILS% = percentage increase in lifespan.

Table 2. Therapeutic activity of TGU against P388 and P388/CP leukaemias in CDF1 mice

Dose (mg/kg/day)	P388		P388/CP	
	ILS(%)	LTS/total	ILS(%)	LTS/total
30 (TGU)	-30	0/10	-26	0/10
20 (TGU)	300	7/10	208	2/10
10 (TGU)	111	0/10	116	0/10
200 (CP)	230	2/10	8	0/10
Controls	0	0/10	0	0/10

Mice were inoculated i.p. with 10^6 leukaemic cells on day 0. The drugs were administered i.p. TGU was injected on days 1-5 and CP only once on day 1. LTS = long-term survivors (>60 days).

Table 3. Influence of the schedule and route of treatment with TGU on the survival of CDF1 mice

Route	Treatment schedule	Dose range (mg/kg/injection)	Optimal dose	ILS (%)	LTS/total L1210-bearing mice	LTS/total Non-leukaemic mice
i.p.	day 1	30–80	60	100	9/10	8/8
i.p.	days 1,9	15–50	50	162	5/10	8/8
i.p.	days 1,5,9	15–50	40	187	4/10	8/8
i.p.	day 1, every 3 hr	1.5–12	6	46	0/10	8/8
i.p.	days 1,5,9. every 3 hr	1.5–12	6	153	1/10	8/8
i.p.	days 1–5	15–60	25	177	4/10	8/8
i.p.	days 1–9	6.25–50	12.5	131	8/10	8/8
p.o.	day 1	160–400	160	75	0/10	8/8
p.o.	days 1–5	30–240	60	82	0/10	7/8
i.v.	day 1	25–80	50	57	0/10	8/8
i.v.	days 1–3	7.5–60	30	71	0/10	8/8
i.v.	days 1,5,9	20–40	40	91	0/10	8/8

Mice were inoculated i.p. with 10^5 leukaemic cells on day 0.
LTS = long-term survivors (>60 days). LTS were excluded from the calculation.

Table 4. Effect of TGU against B16 melanoma

Doses (mg/kg/day)	ILS (%)	LTS
25	-3.5	0
12.5	98	0
6.25	83	0

BDF1 mice received 2×10^6 melanoma cells on day 0. The drug was administered i.p. on days 1-9.

activity against the ependymoblastoma transplanted intracerebrally in C57BL/6 mice (Table 5). A significant reduction in the primary weight of the s.c. C38 tumour was induced by the administration on days 2 and 9 of 50 mg/kg of TGU (Table 6). The lower doses remained significantly active. Both early and delayed administrations of TGU produced a marked effect against the s.c. RC renal adenocarcinoma: a high cure rate was recorded (Table 7). The mean tumour weight at the start of the delayed treatment was 24.8 ± 5.9 and 8 mice out of 10 survived to day 30 with no residual tumour.

DISCUSSION

The results reported here show that TGU has an antineoplastic activity against murine tumours comparable to its analog, α-TGT [4]. Marked prolongations of survival and high numbers of cures (Table 3) in leukaemia-bearing mice were obtained with doses and treatments which were well tolerated in non-leukaemic animals, as evidenced by the absence of lethal toxicity and the very limited body weight loss. Activity was also observed against solid tumours like B16 melanoma and RC renal adenocarcinoma. In the latter, activity was also observed when the tumour was well advanced. A solid tumour known to be relatively resistant to many cancer chemotherapeutic agents such as the colon 38 carcinoma was very sensitive to treatment with TGU. TGU was slightly effective in mice transplanted i.c. with ependymoblastoma, suggesting that the compound may cross the blood-brain barrier. However, further tests need to be performed to confirm the possible exploitation of this property in the treatment of cerebral tumours.

In the L1210 system, TGU given i.p. showed high therapeutic activity with all the schedules used. Although the single-dose treatment induced the highest number of cures, we could not ascertain whether the effectiveness of the drug is schedule-dependent. More particularly, when the drug was administered i.v. the influence of the

Table 5. Effect of TFU against i.c. ependymoblastoma

Doses (mg/kg/day)	ILS (%)
50	lethal
25	25
12.5	31

A 1-mm³ fragment was transplanted i.c. on day 0. The drug was injected i.p. on days 1–4.

Table 6. Effect of TGU on the growth of the colon 38 in B6C3F1 mice

Doses (mg/kg/day)	Tumour growth inhibition on day 20 (%)
50	82
25	75
12.5	75

A 3-mm³ fragment was transplanted s.c. on day 0. The drug was administered i.p. on days 2 and 9.

Table 7. Effectiveness of TGU against s.c. implanted RC renal adenocarcinoma

Early treatment on days 1, 5, 9, 13			Delayed treatment with a single dose on day 10		
Doses (mg/kg/injection)	ILS (%)	LTS/total	Doses (mg/kg/injection)	ILS (%)	LTS/total LTS/total
50	30	4/9	60	>260	10/10*
25	185	8/9			

CDF1 mice received 10^6 RC cells s.c. on day 0. The drug was administered i.p.
LTS = long-term survivors (>90 days).
*A 100% tumour regression was observed in 8 mice. Two LTS had residual tumours.

schedule of treatment on the therapeutic effectiveness was even less obvious.

An additional advantage is that TGU is markedly more water-soluble (7%) than α-TGT (1%). Moreover, the active doses of TGU are approximately half of the active doses of α-TGT. Water-solubility is a favourable property in drug formulation and is one of the factors influencing the activity of any closely related drug [8].

The identical solubility of the α and β isomers of TGU, which explains the presence of the two forms in the preparation, was not a disadvantage since the three mixtures tested containing different proportions of each form were equally active against P388 leukaemia.

The mechanisms by which TGU exerts its cytotoxic activity are not known. However, the presence of epoxide groups suggests an alkylating type of action. In the light of this hypothesis, the high *in vivo* activity of TGU on a subline of P388 leukaemia resistant to CP, one of the most active and widely used alkylating compounds, added to the fact that TGU was more active than melphalan, DTIC and BCNU against human melanoma xenografts [11], appear to be of practical relevance and are further elements warranting clinical studies.

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