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High-dose 1,2,4-triglycidylurazol given in regimens preparatory to bone marrow transplantation*

A preclinical pharmacology study

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Summary. To elucidate its potential role in the framework of bone marrow transplantation, we studied the toxicologic and pharmacologic properties of high doses of the triepoxide derivate 1,2,4-triglycidylurazol (TGU) in a preclinical dog model. Dose-dependent and dose-limiting gastrointestinal toxicity occurred in a dose range between 40 and 75 mg/kg, with the lethal dose for 50% of animals (LD_{50}) being estimated at 60 mg/kg. Severe and life-threatening hematologic toxicity developed at all dose levels examined but was generally reversible. The combination of TGU and total-body irradiation produced synergistic gastrointestinal toxicity, necessitating reductions of the TGU dose by 50% as compared with the single-agent dose. In contrast, the combination of TGU and high-dose busulfan resulted in no apparent nonhematologic synergistic toxicities. The immunosuppressive properties of TGU given in this combination enabled sustained histocompatible allogeneic marrow engraftment in three of four animals. The pharmacokinetics of TGU were not influenced by prior total-body irradiation or high-dose busulfan. We conclude that the myelotoxic, pharmacologic and immunosuppressive properties of high-dose TGU observed in this preclinical model seem to render the drug particularly suitable for use in regimens preparatory to bone marrow transplantation.

Introduction

The racemic compound α/β-1,2,4-triglycidylurazol (TGU; NSC 332488), a triepoxide with alkylating properties, has shown a broad spectrum of anticancer activity in animal models, including the L1210 and the P388 murine leukemias as well as the L5222 rat leukemia (reviewed in [6]). In a cyclophosphamide-resistant P388 leukemia subline, TGU exerted high antileukemic activity, which suggested that the compound might be non-cross-resistant to this group of alkylating agents.

A number of clinical phase I/II trials using TGU in patients with a variety of solid tumors clearly demonstrated that its dose-limiting toxicity was myelosuppression, with a steep dose-toxicity curve being found [1–5, 7, 9]. Nonhematologic toxic effects of TGU included nausea and vomiting, local thrombophlebitis, acute gastrointestinal toxicity and (possibly) impairments of liver function [9]. Its pharmacokinetic profile (with rapid plasma clearance in humans and animal models) together with its dose-limiting myelotoxic properties implies that TGU might be useful in settings of high-dose therapy with bone marrow rescue, as has been proposed elsewhere [2].

To elucidate its potential role in bone marrow transplantation (BMT), we performed dose-escalation and pharmacokinetic studies of TGU in a preclinical dog model. The primary purpose of these studies was to determine whether the maximum tolerated TGU dose with regard to acute nonhematologic toxic effects could induce irreversible marrow aplasia in this model. To obtain information about potential synergistic toxicities, we used combinations of TGU and either total-body irradiation (TBI) or high-dose busulfan (HD-BU) in dogs receiving allogeneic bone marrow rescue. Since HD-BU alone does not enable sustained allogeneic marrow engraftment in this model, it also appeared important that we determine whether TGU was sufficiently immunosuppressive to enable engraftment when given in conjuction with busulfan.

Materials and methods

Dogs. Beagle dogs purchased from the animal breeding resources of the University of Heidelberg were dewormed and vaccinated against distemper, leptospirosis, hepatitis, and parvovirus. The dogs were 18–33 months old and weighed 11–21 kg. Animals were kept in single

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cages during experiments. Supportive therapy consisted of gut decontamination, systemic antibiotics, parenteral fluids, electrolyte supplementation and platelet or whole-blood substitution when indicated. All blood products used for transfusions were irradiated in vitro (15 Gy). Research was conducted according to Article 9 of the Statutes for the Prevention of Cruelty Against Animals in the Federal Republic of Germany.

Treatment. TGU was kindly supplied by Asta-Werke (FRG) as a lyophilized powder containing 20 mg D-mannitol/100 mg drug. It was reconstituted in 0.9% saline to yield a final concentration of 10 mg TGU/ml solution. The required dose was given as a bolus injection over 1 min in the right internal jugular vein. TBI was given in two daily fractions of 2 Gy over 3 days (total dose, 12 Gy) using a source of cobalt 60 (dose rate, 6 cGy/min). During irradiation, dogs were anesthetized with intravenous ketamine, xylazine and atropine. Oral busulfan was given as an aqueous suspension of pulverized tablets at a dose of 4 mg/kg daily in four divided doses over 4 days. Adequate busulfan ingestion and absorption were controlled by the measurement of busulfan plasma levels twice daily during the treatment period.

Bone marrow was harvested from anesthetized dogs by bilateral multiple aspirations from the humerus heads. For removal of fat and bone particles, the marrow cell suspension was first filtered. After centrifugation at 1,000 *g* for 20 min, a nucleated cell-enriched buffy coat was extracted and resuspended in culture medium to a volume of 100 ml. The final cell yield constantly exceeded 4×10^8 cells/kg body weight of the recipient animal. The marrow cell suspension was infused into the internal jugular vein over 15 min. For prophylaxis of acute graft-versus-host disease, all recipients were treated with oral cyclosporin. In all animals receiving combinations of TGU with either TBI or HD-BU, TGU was started 1 day after termination of the respective pretreatment (Table 1).

Toxicologic evaluation. For estimation of acute toxic effects on hematopoiesis, evaluation of kidney and liver functions and white blood cell (WBC) and platelet counts as well as determination of blood urea nitrogen (BUN), creatinine, transaminases and alkaline phosphatase (AP) levels were carried out on all weekdays during treatment. Evaluation of gastrointestinal (GI) toxicity was based on the frequency of vomiting and diarrhea. GI toxicity was classified according to a graded toxicity scale [8]. Moreover, complete autopsies including histologic examinations were performed on all dogs that died.

Chimerism. In recipient dogs, hematopoietic chimerism was evaluated by sex chromosome differences using cultured bone marrow cells.

Pharmacokinetic study. Blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) from a heparin lock placed in a leg vein at 0, 1, 2, 5, 10, 15, 30, 45 and 60 min and at 1.5, 2, 4, 6, 12 and 24 h. Specimens were chilled at –1° C immediately after collection and then centrifuged at 1,270 *g* for 10 min, and the plasma was stored at –18° C until analysis. TGU was measured in plasma and organ extracts using high-presure liquid chromatography (HPLC) as previously described [10, 11]. The HPLC system consisted of a Rheodyne 7125 injector (Rheodyne, Inc. Berkeley, Calif.) with a 100-µl loop, a precolumn Waters C_{18} Guardpak (Waters Associates, Milford, Mass.), a Waters µBondapak C_{18} column (inside diameter, 300 × 3.9 mm; particle size, 10 µm), a Waters 45 solvent delivery system set at a flow rate of 1 ml/min, a Waters model 481 detector set at 216 nm, and a Servogor 120 strip-chart recorder (BBC Metrawatt, Vienna, Austria). The solvent system was 15% acetonitrile and 85% HPLC-grade water (v/v; Merck Darmstadt, FRG). Plasma (1 ml) was added to 9 ml chloroform in a test tube, vortex-mixed for 10 s, shaken for 10 min, then centrifuged at 1,270 *g* for 10 min, and the supernatant was removed. The chloroform layer was evaporated to dryness at 40° C. The residue was redissolved in 100 µl methanol, and a 20-µl aliquot was injected onto the HPLC column. Animal predose blood or urine samples did not contain interference at the retention volume of TGU, and the lower limit of sensitivity (twice the normal background) was 10 ng/ml plasma.

Statistical analysis. Blood plasma concentration-time data were analyzed using the AUTOAN computer program (supplied by Dr. J. G. Wagner, Upjohn Center for Clinical Pharmacology, Ann Arbor, Mich.). The program used curve-stripping techniques to choose the most appropriate pharmacokinetic model. Plasma pharmacokinetic parameters (AUC, clearance, volume of distribution and $t_{1/2}$ values) were computed for individual animals. For detection of differences in the pharmacokinetic profile of TGU in animals that received the compound alone vs those that were treated with combinations of TGU and either TBI or HD-BU, pharmacokinetic parameters in the different treatment groups were compared using Wilcoxon's rank-sum test. These parameters were additionally compared in animals with and those without lethal GI toxicity. Data-base management and descriptive and comparative statistics were performed on an IBM PS/2 model 80 computer using SAS software (FSP, UNIVARIATE, NPAR1WAY procedures; SAS Insitute Inc., Cary, N. C.).

Table 1. Dose schedules of TGU alone and combinations of TGU with TBI or HD-BU followed by allogeneic bone marrow rescue

Total TGU dose (mg/kg)	Dose/fraction (mg/kg)	Fractions (*n*)	Animals (*n*)
40	40	1	4
50	25	2	4
60	60	1	1
60	30	2	7
75	25	3	4
20+ TBI[a]	20	1	4
40+ TBI	40	1	6
40+ HD-BU[b]	40	1	8

[a] TBI, Total-body irradiation delivered by a source of cobalt 60 in two daily fractions of 2 Gy (dose rate, 6 cGy/min) over 3 days

[b] HD-BU, 1 mg/kg oral busulfan every 6 h over 4 days

Results

Toxicology

The major adverse effects of TGU observed in this animal model involved GI and hematologic toxicity. GI toxicity occurred within 2–6 days after administration of the compound and manifested as moderate to severe enteritis associated with serous or bloody diarrhea. For this adverse effect, we found a steep dose-response relationship, with an estimated LD_{50} in the range of 60 mg/kg TGU occurring when the drug was given in two dose fractions of 30 mg/kg daily for 2 days. A single dose of 60 mg/kg, however, was followed by a generalized capillary-leakage syndrome, which led to death at 6 h after TGU administration in one animal. The maximal tolerated single dose of TGU was 40 mg/kg, and dose fractions of 25 mg/kg daily enabled dose escalation to a maximal cumulative dose of 50 mg/kg without producing lethal GI toxicity. All animals receiving a total dose of 75 mg/kg TGU in three fractions of 25 mg/kg daily died of GI toxicity. Histopathologic changes in the gut walls included areactive necrosis and denudation of the epithelium with concomitant thrombosis of submucosal veins and atrophy of lymphatic follicles. In addition, TGU led to dose-dependent toxic effects on liver function, with a 3 to 4-fold increase in AP concentrations occurring in the dose range between 40 and 75 mg/kg. Moderate elevations of BUN and creatinine plasma levels

Table 2. Nonhematologic toxicity of TGU and combinations of TGU with TBI or HD-BU

TGU dose (mg/kg) Additional treatment	40	50	60	75	20 TBI	40 TBI	40 HD-BU
Animals (*n*)	4	4	7	4	4	6	8
Gastrointestinal:							
Diarrhea[a]	2–3	2–3	3–4	4	2–3	3–4	2–3
Lethal toxicity (%)[b]	0/4 (0)	0/4 (0)	4/7 (57)	4/4 (100)	0/4 (0)	4/6 (66)	0/8 (0)
Liver:							
GOT (Iu/l)[c]	32 (24–75)	41 (29–75)	30 (22–140)	108 (49–187)	22 (16–103)	30 (15–291)	24 (16–34)
GPT (Iu/l)[c]	87 (32–180)	64 (41–132)	86 (39–256)	189 (77–269)	61 (39–216)	142 (31–1,179)	52 (14–147)
AP (Iu/l)[c]	419 (231–5,280)	290 (101–325)	1,042 (109–3,624)	1,238 (887–2,917)	203 (135–208)	846 (168–2,224)	204 (102–678)
Kidney:							
Creatinine (mg/dl)[c]	1.1 (0.9–3.2)	0.7 (0.6–1)	0.8 (0.7–3.9)	1.8 (1.3–2.9)	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.7 (0.7–1.2)
BUN (mg/dl)[c]	21 (18–69)	17 (14–25)	18 (15–55)	64 (22–79)	24 (10–26)	26 (18–66)	20 (15–24)

[a] Severity of organ involvement (graded toxicity scale)
[b] Proportion of animals with lethal gastrointestinal toxicity as a consequence of intolerable necrotizing enteritis
[c] Median peak plasma concentrations (range)

Table 3. Hematologic toxicity of TGU

TGU dose (mg/kg)	40	50	60	75
Animals (*n*)	4	4	7	4
WBC nadir ($\times 10^9$/l)				
Median	0.1	0.15	0	0.1
Range	0–0.2	0.1–0.4	0–0.1	0.1–0.1
WBC nadir (day)				
Median	4	5	4	3
Range	4–7	4–5	3–6	3–6
WBC recovery (day)				
Median	25	28	28	NE
Range	16–35	22–30	25–33	NE
Platelet nadir ($\times 10^9$/l)				
Median	4	9	18	NE
Range	4–7	5–12	10–35	NE
Platelet nadir (day)				
Median	11	16	7	NE
Range	1–13	1–28	4–18	NE
Platelet recovery (day)				
Median	23	30	23	NE
Range	19–34	26–31	20–29	NE

NE, Not evaluable. All animals died of therapy-related toxicity before hematopoietic recovery could occur

Table 4. Cytogenetic analysis of bone marrow cells in animals receiving sex-mismatched DLA-identical marrow grafts after a preparative regimen of HD-BU and 40 mg/kg TGU

Dog number	Sex		Interval between BMT and analysis (days)	Karyotype
	Donor	Recipient		
87/159	F	M	63	XX (10)
			99	XX (8), XY (2)
			273	XX (10)
88/49	F	M	158	XX (4), XY (6)
88/54	M	F	131	XY (10)
88/58	M	F	173	XY (10)

Numbers in parentheses represent numbers of metaphases analyzed

occurred in some animals as a consequence of dehydration due to diarrhea (Table 1). Renal, cardiac, pulmonary or other nonhematologic complications attributable to drug-related toxic effects were not observed.

Hematologic toxicity in animals surviving GI toxic effects was severe and life-threatening, and necessitated frequent blood-product substitution due to anemia and thrombocytopenia. Marrow aplasia and complete atrophy of the lymph node and spleen tissue was found at autopsy in all animals that died of GI toxicity. No clear-cut dose-response relationship existed between the TGU dose and the duration of leuco-or thrombocytopenia (Table 3). Although prolonged pancytopenia occurred in a dose range between 40 and 60 mg/kg TGU, all surviving dogs showed complete hematopoietic recovery.

The maximum tolerated single TGU dose given in combination with TBI and allogeneic dog leucocyte antigene (DLA)-identical marrow rescue was reduced by 50% to 20 mg/kg TGU as compared with the single-agent dose. Again, a steep dose-response relationship was found between the TGU dose and GI toxicity, with an estimated LD_{50} in the range of 30 mg/kg (Table 2). Furthermore, a 2-fold increase in AP plasma levels was observed in animals receiving 40 mg/kg TGU in conjunction with TBI as compared with 40 mg/kg TGU alone. Thus, the combination of TGU and TBI unequivocally showed cumulative toxic effects on both the GI tract and liver function.

In contrast, the combination of a single dose of 40 mg/kg TGU with HD-BU and allogeneic marrow rescue was not associated with increased cumulative GI or liver toxicity (Table 2). Two of eight dogs (20%) died of marrow aplasia without showing significant histopathologic signs of organ toxicity, whereas the surviving six animals displayed complete hematopoietic recovery. Two dogs receiving DLA-nonidentical, unrelated marrow transplants rejected their grafts. In three of four dogs with DLA-identical, related marrow donors, sustained engraft-

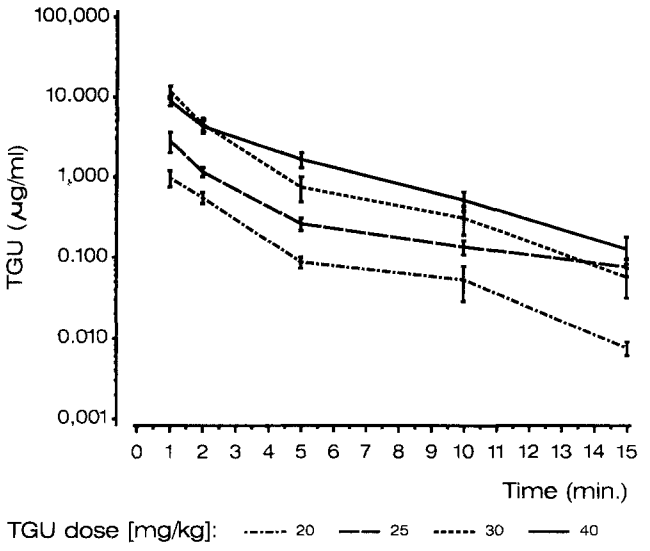

Fig. 1. Plasma TGU concentrations in animals treated with 20 ($n = 4$), 25 ($n = 8$), 30 ($n = 7$) or 40 mg/kg ($n = 18$) drug. In animals receiving more than one fraction of TGU, calculations for the plasma concentration versus time plot were based on the first fraction given. Values represent means and standard errors

ment could be demonstrated by sex chromosome analysis (Table 4). Since DLA-identical marrow engraftment cannot be attained using HD-BU alone in this model, it can justifiably be assumed that the immunosuppressive properties of TGU enabled engraftment.

Pharmacokinetic study

The plasma pharmacokinetic parameters of TGU are shown in Table 5. The best fit to the data was obtained using a one-compartment open-system model with an exponential decay (Fig. 1) Prior treatment with TBI or HD-BU had no significant influence on these parameters in animals treated with 40 mg/kg TGU. Animals with lethal GI toxicity, however, showed markedly increased peak plasma levels and AUC values as well as reduced plasma clearance values as compared with those that survived GI toxic effects (Table 6). This may reflect the contribution of interindividual differences in drug metabolism to the severity of such adverse effects. Neither the parent compound nor any metabolites were detected in any urine samples. Low, albeit significant, TGU concentrations could be extracted from organs at up to 7 days after admin-

Table 5. Plasma pharmacokinetic parameters for animals treated with different doses of TGU

TGU dose (mg/kg)	Fractions (n)	Peak plasma concentration (µg/ml)	AUC (µg 10^{-2}/ml h)	Clearance (ml/min)	Volume of distribution (l)	$t_{1/2}$ (min)
20	4	0.96 (0.22)[a]	4.33 (0.38)	109,432 (8,443)	137,7 (88.7)	2.7 (0)
25	12	3.15 (0.99)	10.41 (1.86)	88,846 (12,939)	165.7 (86.5)	2.9 (0.1)
30	14	11.57 (2.08)	38.37 (7.04)	31,773 (5,844)	154.4 (40.9)	3.1 (0.3)
40	18	8.00 (1.31)	39.57 (9.9)	51,078 (7,795)	123.2 (29.2)	3.1 (0.1)
40	4	7.65 (1.42)[b]	35.63 (14.19)	58,106 (22,707)	148.3 (87.8)	2.9 (0.1)
40+TBI	6	7.8 (2.59)	44.20 (22.76)	59,168 (14,661)	161.8 (58.9)	2.8 (0.1)
40+HD-BU	8	8.37 (1.54)	36.92 (9.93)	39,475 (7,405)	72.1 (6.4)	3.5 (0.3)

[a] Data represent the mean (± SE) values calculated for each individual dose fraction. The number of fractions on which these calculations were based is indicated.

[b] No significant differences were found between peak plasma concentrations, AUC, clearance, volume of distribution or half-life values in animals given 40 mg/kg TGU alone or in combination with either TBI or HD-BU

Table 6. Comparison of plasma pharmacokinetic parameters in animals with vs without lethal toxic enteritis

TGU dose[a] (mg/kg)	Outcome[b]	Peak plasma concentration[c] (µg/ml)	AUC[c] (µg 10^{-2}/ml h)	Clearance[c] (ml/min)	Volume of distribution[c] (l)	$t_{1/2}$[c] (min)
30 (2)	Alive (3)	5.6 (1.02)	22.54 (6.25)	46,343 (8,574)	248.8 (77.9)	3.5 (0.8)
30 (2)	Dead (4)	16.06*[1] (2.62)	50.25*[2] (9.66)	20,846*[3] (5,641)	84.4*[3] (22.6)	2.8*[4] (0)
40 (1)+ TBI	Alive (2)	3.95 (0.95)	9.04 (1.81)	99,854 (10,745)	399.4 (42.9)	2.8 (0)
40 (1)+ TBI	Dead (4)	11.91 (4.47)	80.2 (39.41)	26,688 (13,021)	107.1 (52.4)	2.8 (0.1)

[a] Values in parentheses represent the number of fractions of the indicated TGU dose given to each animal

[b] Values in parentheses represent the number of animals in which pharmacokinetics were studied

[c] Data represent the means (± SE) of plasma pharmacokinetic parameters calculated for each individual dose fraction in animals with vs without lethal toxic enteritis. P values were derived from comparisons of these parameters by Wilcoxon's two-sample rank-sum test.

*[1] $P = 0.006$, *[2] $P = 0.06$, *[3] $P = 0.02$, *[4] $P = 0.04$

Table 7. TGU content of organ extracts from animals with lethal therapy-related complications

Organ system	Day after last TGU application	TGU dose (mg/kg)	Organ weight (g)	Concentration ($\mu g \times 10^{-3}\ g^{-1}$)	TGU content	
					Total ($\mu g \times 10^{-3}$)	% dose
Gastrointestinal tract:						
Duodenum	1	60	475	66	31,350	0.003
	4	60	100	2,050	205,000	0.04
	6	40	60	289	17,340	0.003
	7	60	150	1,346	201,900	0.04
Jejunum	1	60	475	58	27,550	0.002
	6	40	305	189	57,645	0.01
	6	60	475	147	69,825	0.01
	7	60	555	711	394,605	0.08
Ileum	4	60	30	164	4,900	0.001
Cecum	4	60	30	2,106	63,180	0.01
Liver	1	60	620	130	80,600	0.01
	6	40	640	388	248,320	0.04
Bile bladder	6	60	40	38	1,520	0.0002
Spleen	4	60	55	120	6,600	0.001
	6	60	25	173	4,325	0.001
Heart	1	60	170	190	32,300	0.003
	6	60	230	84	19,320	0.003
Urinary tract:						
Kidney	1	60	60	38	2,280	0.0002
	6	60	55	85	4,675	0.001
Urinary bladder	1	60	10	33	330	0.00003
	4	60	40	390	15,600	0.003
	7	60	25	760	19,000	0.004

istration of the drug, indicating extensive binding of the drug to tissues (Table 7).

Discussion

Previous animal studies in a wide range of experimental leukemias, including those resistant to cyclophosphamide, have suggested that TGU may exert high antileukemic activity [6]. The present study in a preclinical dog model dealt with the question as to whether TGU may be a useful agent in settings of high-dose radiochemotherapy that necessitate bone marrow rescue. Our results clearly demonstrate that the compound has dose-dependent and dose-limiting toxic effects on the GI tract under conditions of intensive supportive care, resulting in an estimated LD_{50} in the range of 60 mg/kg (corresponding to 2,500 mg/m^2) given in two consecutive daily fractions of 30 mg/kg. This toxic effect was also characterized by a steep dose-response relationship. Animals with lethal GI toxicity showed increased peak plasma levels and AUC values as compared with those that did not develop lethal GI side effects, suggesting that interindividual differences in the metabolism of the compound contributed to the severity of this adverse effect.

As estimated by nadir values for WBC and platelets as well as the time required for recovery of peripheral blood counts, myelosuppression was severe, life-threatening, at all doses examined. Hematologic toxicity, however, was not clearly dose-dependent, which contrasts with the results of one clinical trial in which TGU showed dose-dependent myelosuppression in a dose range between 480 and 1,250 mg/m^2 given in five fractions over 5 days [9]. Despite limitations in converting dosages between different species, TGU doses used in the present study were 2.5- to 3.5-fold those in the report cited above, which may in part explain the lack of a dose-toxicity relationship in this model. Complete, albeit prolonged, hematologic recovery occurred in all animals that survived GI toxic effects, which indicates that the maximum tolerated TGU dose was not irreversibly marrow-ablative.

When TGU was combined with the modalities of myeloablation most frequently used prior to bone marrow transplantation, i.e. TBI or HD-BU, cumulative GI toxicity was restricted to the combination with TBI, which necessitated reductions of the TGU dose by 50% to mitigate this adverse effect. The increase in GI toxicity was not dependent on changes in the pharmacokinetic profile of the compound due to prior treatment with TBI.

The immunosuppressive properties of TGU in this model were impressive, since complete and sustained allogeneic DLA-identical marrow engraftment was attained in three of four recipient animals that had been pretreated with HD-BU, a compound with negligible immunosuppressive efficacy in settings of allogeneic BMT. Because this combination showed no apparant cumulative toxic effects on liver function or the GI tract, it may prove to be especially suitable for clinical application. Our study was restricted to a 40-mg/kg dose of TGU given in combination with HD-BU. It might thus be speculated that the maximum tolerated dose of 50 mg/kg TGU would further enhance its immunosuppressive efficacy. Autopsy findings

in animals that died of GI toxicity demonstrated complete atrophy of lymphatic follicles in the lymph nodes, spleen, and gut walls, further supporting the substantial toxicity of TGU to the lymphatic system.

As has previously been described for different species, including humans [2, 11], the pharmacokinetic profile of TGU in the present study was characterized by rapid plasma clearance, with $t_{1/2}$ values in the range of 2.7 and 3.5 min being obtained. Short plasma and systemic clearance rates of cytotoxic agents used in regimens preparatory to BMT are of special importance for the avoidance of cytotoxic effects on the transplanted marrow cells. In this regard, it might have been a matter of concern that significant concentrations of TGU could be extracted from organs for up to 7 days after administration of the compound, which indicates extensive binding of the drug to tissues. However the kinetic of hematopoietic recovery in animals receiving marrow grafts after preparation with TGU-containing regimens apparently were not delayed as compared with those observed in historic controls prepared with TBI or HD-BU in combination with cyclosphospamide. Persistent binding of TGU to tissues thus appeared to have minor clinical importance with regard to marrow graft function. Our results do not enable us to draw valid conclusions as to whether there was a relationship between the binding of drug to tissues and the toxicities observed. The comparatively high concentrations of TGU extracted from upper GI-tract tissues, however, may be suggestive of such a relationship.

In conclusion, the present study in a preclinical animal model confirms that TGU may be a useful agent in regimens preparatory to BMT. The pharmacologic, myelotoxic and immunosuppressive properties of this drug warrant clinical studies for the further definition of its potential role in the framework of BMT.

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References

1. Bruntsch U, Dodion P, Ten Bokkel Huinink WW, Hansen HH, Pinedo HM, Hansen M, Renard J, Van Glabbeke M (1986) Primary resistance of renal adenocarcinoma to 1,2,4-triglycidylurazol (TGU, NSC 332488), a new triepoxide cytostatic agent – a phase II study of the EORTC Early Clinical Trials Group. Eur J Cancer Clin Oncol 22: 697
2. Cunningham D, Soukop M, Stuart JFB, Setanoians A, Gilchrist NL, Forrest GJ, Kaye SB (1986) A clinical and pharmacokinetic phase I study of 1,2,4-triglycidylurazol (TGU, NSC 332488). Eur J Cancer Clin Oncol 22: 1325
3. Cunningham D, Banham SW, Soukop M (1986) Small-cell lung cancer: results of a phase II study of 1,2,4-triglycidylurazol. Cancer Chemother Pharmacol 17: 85
4. George M, Scotto V, Carnino F, Dodion P, Ten Bokkel Huinink WW, Rotmensz N, Vermorken JB (1987) Phase II trial of anaxirone (1,2,4-triglycidylurazol, TGU) in patients with advanced ovarian carcinoma: an EORTC Gynecological Cancer Cooperative Group study. Eur J Cancer Clin Oncol 23: 867
5. Hansen SW, Bach F, Hansen HH, Kaplan S, Cavalli F (1985) Phase I trial of 1,2,4-triglycidylurazol (TGU, NSC 332488): a new triepoxide cytostatic agent. Eur J Cancer Clin Oncol 21: 301
6. Hilgard P, Peukert M, Pohl J (1984) α/β-Triglycidyl-urazol (TGU, NSC 332488, I. N. N.: Anaxirone): a new chemotherapeutic agent. Cancer Treat Rev 11: 115
7. Lund B, Hansen F, Hansen M, Hansen HH (1987) Phase II study of 1,2,4-triglycidylurazol (TGU) in previously untreated and treated patients with small cell lung cancer. Eur J Cancer Clin Oncol 23: 1031
8. Miller AB, Hoogstraten B, Staquet M, Winkler A (1981) Reporting results of cancer treatment. Cancer 47: 207
9. Nicaise C, Rozencweig M, Crespeigne N, Dodion P, Gerard B, Lambert M, Decoster G, Kenis Y (1986) Phase I study of triglycidylurazol given on a 5-day iv schedule. Cancer Treat Rep 70: 599
10. Schilcher RB, Young JD, Nowrousian MR, Hoffmann B, Schmidt CG (1986) Reversed-phase high-performance liquid chromatography determination of anaxirone in biological specimens. J Chromatogr 378: 248
11. Schilcher RB, Beelen DW, Schmidt-Weinmar AC, Schaefer UW, Nowrousian MR, Hengst B, Schmidt CG (1988) Pharmacokinetic evaluation of high dose anaxirone (TGU, NSC 332488). J Cancer Res Clin Oncol 114 [Suppl]: 151