Cancer Chemother Pharmacol (1984) 12: 198–200

Cancer Chemotherapy and Pharmacology

The analysis and animal pharmacokinetics of 1,2,4, triglycidyl urazol using a high-pressure liquid chromatographic technique

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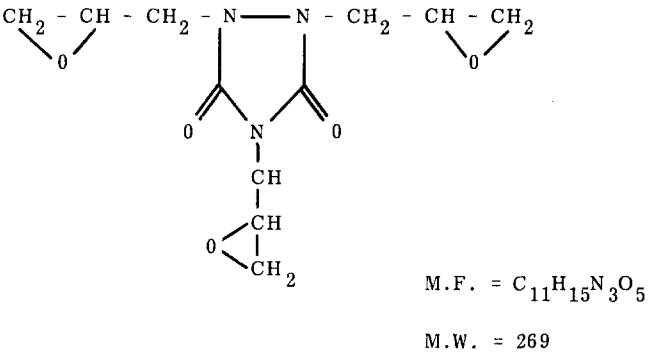
Summary. *This article details a procedure for the analysis of TGU by a simple high-pressure liquid chromatographic (HPLC) method. Linearity is maintained over the range from zero to at least 30 μg 1,2,4, triglycidyl urazol (TGU). The sensitivity of the assay is 250 ng/ml. A second peak, as yet unidentified, was detected on the chromatogram and probably represents a metabolite of TGU. The pharmacokinetic profile of TGU in Porton mice shows a first-order elimination process with a half-life ($t_{1/2\alpha}$) of 1.5 min for the distribution phase and a $t_{1/2\beta}$ of 5 min. The apparent volume of distribution is 0.75 ml and the clearance 0.10 ml/min with a elimination rate constant of 0.14 min.*

Introduction

The role of certain chemotherapeutic agents in the treatment of malignancies, such as testicular teratoma, choriocarcinoma, the haematological malignancies and reticulo-endothelial cancers, has become more clearly defined in recent years. However, there are many malignancies which remain relatively unresponsive to cytotoxic drug therapy, e.g., colon neoplasia, pancreatic tumours, and malignant melanoma. Consequently, the search for new drugs with wider spectra continues. Recently 1,3,5, triglycidyl-S-triazinetrione (α-TGT) was synthesised. This drug which is a tri-epoxide derivative, appears to act as an alkylating agent, and showed activity in animal screening. It has undergone phase-I studies but factors which may have affected the drug's evaluation were its relative instability and poor solubility [1]. To circumvent this drawback an analogue was synthesised, namely 1,2,4, triglycidyl urazol (TGU, Fig. 1). The daughter compound was designed specifically to improve solubility and stability, and this has been achieved by rational drug modification. Animal screening data has shown the anti-tumour potential of TGU to be similar to that of TGT. This paper presents a simple, accurate and rapid HPLC method for the estimation of TGU and illustrates the use of this method in establishing the pharmacokinetics of TGU in mice.

Materials and methods

Analytical. Methanol and chloroform were of HPLC grade, obtained from Fisons, Loughborough, Leicestershire, and were not redistilled. P'aminoacetophenone, the internal standard (IS) was obtained from the Aldrich Chemical Co.,



M.F. = $C_{11}H_{15}N_3O_5$

M.W. = 269

Fig. 1. Molecular structure of the synthesised analogue 1,2,4, triglycidyl urazol (TGU)

Ltd., and was 99% pure. Double-distilled deionised water from a quartz-glass still was used in the study. TGU was received from Henkel, Düsseldorf, and was dissolved in aqueous solution at room temperature immediately prior to use.

Pharmacokinetics. Female Porton mice were obtained from Bentin and Kinghorn, Hull. Thirteen groups with five mice in each group received 6 mg of TGU in 0.2 ml of water via a tail-vein injection. This procedure was repeated with a further 65 mice. The mice were killed and blood collected at the following intervals: 0, 5, 10, 15, 20, 30, 45, 60, 120, 240, 60, and 480 min. At each time point blood from five mice was collected and the blood pooled. The plasma was immediately separated by centrifugation and samples stored at (−20° C) for subsequent analysis.

The $t_{1/2}$ was estimated from a plot of log of the concentration of TGU versus time and the following pharmacokinetic parameters were calculated:

$$\beta \text{ Elimination constant } (K_{el}) = \frac{0.693}{t_{1/2\beta}\ \text{min}^{-1}}.$$

Area under the plasma concentration curve (AUC) was calculated by the trapezoidal rule and was extrapolated to infinity:

$$\text{Clearance } (Cl) = \frac{\text{dose}}{AUC}$$

$$\text{Apparent volume of distribution } (V_D) = \frac{Cl}{K_{el}\ \text{min}^{-1}}.$$

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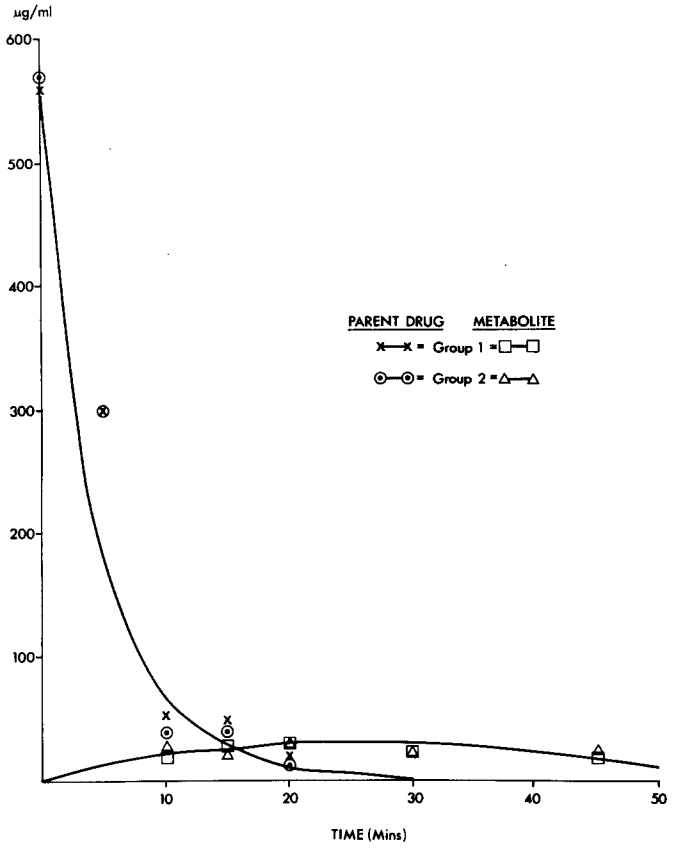

Fig. 2. Graph showing the plasma decay curve of TGU and its metabolite after tail vein administration to Porton mice. (Values shown were derived in duplicate experiments performed on different days, using five mice per time point for each experiment)

Extraction and analytical method. The extraction of TGU from mouse plasma involved the addition of 15 µl of internal standard (1 mg/ml P'aminoacetophenone). The volume of serum extracted was 50 µl for the first two time points and thereafter 200 µl. Each sample was vortexed at room temperature for 1 h. It was then centrifuged for 5 min at 3,000 rpm and the solvent layer retained. The chloroform was then removed by evaporation at ambient room temperature, using a Buchler Vortex Evaporator. The residue was redissolved in 100 µl of water. The HPLC instrument (Model Altex 100A, Altex Scientific Inc., Berkley, California, USA) contained a single constant-flow pump, which was used to deliver a flow rate of 1.5 ml/min. The stainless steel column (250 × 5 mm 1D) was packed with spherisorb 5 µm ODS (HPLC Technology Ltd., Cheshire, England). At ambient room temperature and the conditions stated above, a pressure of 2,000 psi pertained. Samples were injected onto the column via a manual injection valve containing a 200-µl loop (Altex 210). The mobile phase which afforded the best separation was methanol to water (25 : 75). The eluent was freshly prepared and degassed daily. A variable wavelength ultraviolet detector model (LC-UV Pye Unicam, England) was set at 215 nm and 0.16 aufs was employed to detect eluting compounds. The output signal was recorded by a Tekman potentiometric pen recorder (Tekman Ltd., England). All separations were performed at ambient temperatures using isocratic elution. A standard curve prepared by plotting the ratio of the peak heights of TGU to IS against the TGU concentration was used for calibration. This curve exhibited linearity to at least 30 µg/ml of TGU (correlation coefficient was 0.999). The assay was sensitive to a concentration of 250 ng/ml, i.e., 5 ng on the column, the lowest reliable peak height being taken as three times greater than the height of the baseline noise.

Table 1. Pharmacokinetics of 1,2,4, TGU and its metabolite in Porton mice

	TGU	Metabolite
$t_{1/2}\,\alpha$ (min)	1.5	
$t_{1/2}\,\beta$ (min)	5	31
$AUC^{0-\infty}$ (µg/ml · min)	57.74	20.43
Cl (ml/min)	0.10	
βK_{el} (min^{-1})	0.14	
V_D (ml)	0.75	

Results

The extraction procedure of TGU from spiked plasma was 90% efficient ± 4.8 SD. The extraction efficiency was calculated by comparing peak heights obtained with a known concentration of unextracted TGU with those obtained after extraction of the same initial concentration of TGU. The retention times for TGU and IS were 4.8 and 9.6 min respectively. Figure 2 shows the plasma concentration time profile for TGU and the unknown product in female Porton mice. Table 1 lists the calculated pharmacokinetic parameters.

Discussion

A simple, accurate and rapid HPLC assay for TGU has been described. We recommend that blood samples are immediately centrifuged and the serum separated and stored at −20° C until analysis. TGU appears to undergo metabolism in mice, as shown by the presence of a third peak on the chromatograph. This compound has not yet been identified but has a retention time of 3.2 min. This peak is similar to one of the peaks obtained after acid hydrolysis of TGU. In view of its shorter retention time on the column, the unidentified compound is presumably more polar than its parent compound and probably represents a hydrolysis product of TGU. Further studies to identify this compound are proceeding.

TGU shows first-order kinetics in Porton mice and exhibits a short $t_{1/2}\beta$ of 5 min. This value is similar to $t_{1/2}$ obtained with α TGT in mice (G. Atassi, personal communication 1982).

Stability and solubility studies have shown TGU to be more suitable from a pharmaceutical view point than TGT. The extraction and analytical method described appears to be sufficiently accurate to be useful in determining the pharmacokinetics of TGU in humans, providing the drug is handled similarly in man.

There are several examples of cytotoxic agents in clinical use which possess formulation and stability problems. Prior to phase-I clinical trials, we believe it is essential to investigate the physical characteristics of any new drug systematically and scientifically and hence to facilitate formulation, develop a reliable analytical technique and show that the technique is functionally operative in order that the pharmacokinetics of

the trial agent may be determined early after its initial clinical usage.

Acknowledgements. The study was partially funded by the Cancer Research Campaign Grant to the Department of Clinical Oncology, Grant No. SP 1429. We are grateful to the EORTC Pharmacokinetic and Metabolism Group for supplying the TGU and to Mrs. J. Ferguson and Mrs. S. Cochrane for typing the manuscript.

Reference

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Received March 23, 1983/Accepted November 28, 1983