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Stability of tauromustine (TCNU) in aqueous solutions during preparation and storage

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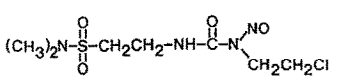
Summary

Tauromustine (TCNU, LS2667, 1-(2-chloroethyl)-3-[2-(dimethyl-aminosulfonyl)ethyl]-1-nitrosourea) stability in solution has been investigated by reversed-phase high-performance liquid chromatography. Dissolved in dimethylsulfoxide and diluted with an appropriate buffer, the drug was found to be most stable in either citrate buffer (pH 4.0) or normal saline (pH 6.25). TCNU degraded under a variety of conditions: alkaline pH, elevated temperatures, light, and in phosphate-buffered saline (pH 7.4). Long-term studies showed that the drug could be stored in 150 mM citrate buffer at −20 °C or −70 °C for 3 months with no loss of drug. No adverse affects were found when using different types of container material. Filtration with polyvinylidene difluoride filter units gave rise to a high degree of drug adsorption whilst with other filter units minimal loss was observed.

Introduction

The chloroethylnitrosoureas carmustine (BCNU) and lomustine (CCNU) have found a limited role in clinical oncology, mainly in the treatment of brain tumours (Weiss and Issel, 1982). The drugs, however, possess only modest antitumour activity and therefore further analogue development continues in the hope that this may be improved or that dose-limiting toxicities may be reduced. Of particular interest recently has been the development of chloroethylnitrosoureas with a special carrier backbone. One of these, TCNU (I, LS2667, tauromustine, 1-(2-chloroethyl)-3-2[2-(dimethylaminosulfonyl)ethyl]-1-nitrosourea) (see scheme 1) is based on the β-amino acid taurine (Hartley-Asp et al., 1988) and is currently under clinical investigation (Smyth et al., 1987).

We are using TCNU in in vitro drug sensitivity assays in an attempt to predict tumour response in vivo (Bird et al., 1988), and are therefore concerned to handle solutions of the drug in a way that leads to their greatest stability (Bosanquet, 1989). Thus, a stability-indicating high-performance liquid chromatography (HPLC) method has



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Scheme 1

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been developed for the determination of TCNU stability under conditions pertaining to the handling of the drug for in vitro assays.

Materials and Methods

Tauromustine was obtained from A.B. Leo, Sweden. Dulbecco's phosphate-buffered saline was obtained from Oxoid Ltd. (Basingstoke, Hampshire, U.K.), and sodium chloride injection B.P. 0.9% w/v in glass bottles (pH 6.25) from Phoenix Pharmaceuticals Ltd. (Gloucester, U.K.). RPMI 1640 medium (×1) and fetal bovine serum were purchased from Gibco Europe Ltd. (Uxbridge, U.K.), sodium bicarbonate and fungizone from Flow Laboratories (Irvine, Ayrshire, U.K.) and gentamycin from Roussel Laboratories Ltd. (Wembley, U.K.). Agar Noble was obtained from Difco Laboratories (Detroit, MI).

For stability in different containers, Quickfit glass test tubes were used either with or without treatment with dimethyldichlorosilane solution (2% v/v in 1,1,1-trichloroethane, BDH Ltd., Poole, U.K.). Polyethylene ampoules (2 ml) and polystyrene tubes (5 ml) were obtained from Sterilin, Teddington, Middlesex, U.K. and 1.2 ml polypropylene cryotubes (Nunc) were purchased from Gibco Europe Ltd. Polyvinylchloride (PVC) infusion bags were obtained from Travenol (Thetford, U.K.).

Solutions were held at various temperatures by a hot block (Grant Instruments Ltd., Cambridge) when above ambient. A cold room and commercial freezers were used for 2°C, −20°C, and −70°C. Methanol was HPLC grade (Rathburn Chemicals Ltd., Walkerburn, U.K), and acetonitrile (BDH Chem. Ltd., Poole, U.K.) was far UV grade. Ammonium acetate and sodium citrate were AR Grade (Fisons, Loughborough, U.K.), as was dimethyl sulphoxide (DMSO, Sigma Chem. Co., Poole, U.K.). Water was double-distilled into glass.

TCNU was dissolved at 500 mg/ml in dimethylsulphoxide (DMSO), diluted to 1 mg/ml with citrate buffer (150 mM, pH 4.0) then aliquoted and frozen. Experiments were usually carried out at 1 mg/ml or 10 μg/ml. All dilutions were carried out in the shortest time possible, usually at 4°C and with the exclusion of light.

The standard solutions of TCNU were made up at 0.5 mg/ml and 10 μg/ml by dissolving TCNU in DMSO and diluting in mobile phase. They were stored at −20°C. A 1 mg/ml standard in mobile phase initially used was found to crystallise out after 1 week at this temperature.

Samples were analysed with a previously described HPLC system (Bosanquet and McLoughlin, 1985) with the addition of a diode array detector (Hewlett Packard 1040A). A Spherisorb ODS1 5 μm 250 × 4.6 mm column was maintained at 40°C by a block heater. Two different mobile phases were used, one was 50 mM aqueous ammonium acetate in methanol (3 : 2 v/v) at a flow of 1 ml/min. The second was water : acetonitrile (7 : 3 v/v) at a flow rate of 1.3 ml/min. TCNU peak heights were measured and compared to the peak heights of the external standard solutions of TCNU. Calibration of TCNU standard solution in the mobile phase was linear ($r > 0.999$) over the range 4–1000 μg/ml. Six replicate injections of the lower and upper limits of the standard curve produced relative standard deviations of 6.0% and 2.6%, respectively.

Experiments were performed to determine that the HPLC method was stability-indicating for TCNU. The drug was degraded at extremes of pH, at elevated temperatures and in intense light. Chromatography of the solutions was performed using a diode array detector. During these initial experiments no change in the upslope, apex or downslope UV spectra (210–600 nm) of the TCNU peak was observed and all decomposition peaks were clearly resolved from the parent drug. The second mobile phase was chosen to change the chromatography conditions and lengthen the retention time of TCNU to see if any other decomposition products could be found. No further peaks were resolved under these conditions. No degradation peaks were observed in the standard solution of TCNU in mobile phase during storage at −20°C over 3 months.

The effect on solutions of TCNU of varying pH (3–9), temperature (−70–72°C), light (sunlight vs lablight), and concentration (1–100 μg/ml) were all investigated. Solutions of TCNU

were stored in the dark in glass containers covered in aluminium foil for experiments at 4°C and above or in polypropylene cryotubes at negative temperatures. Degradation was also investigated in different container materials (polystyrene, polyethylene, glass, siliconised glass and PVC). TCNU solutions were frozen and thawed repeatedly to investigate the stability of the drug under these conditions. Three long-term experiments were undertaken at each of 2°C, −20°C and −70°C using TCNU at 1 mg/ml in 150 mM citrate buffer; duplicate aliquots were analysed each week for 3 months.

In experiments on the degradation of TCNU in medium, TCNU at 1 mg/ml in 150 mM citrate, pH 4 was added to RPMI 1640 medium containing 10% sodium chloride, 24 mM sodium bicarbonate, 2.5 μg/ml fungizone, 80 μg/ml gentamicin, and 1% L-glutamine either with or without 10% fetal bovine serum. When agar was incorporated a final agar concentration of 0.3% was used. The final drug concentration was 10 μg/ml and the decay of TCNU was followed at 37°C. Aliquots were removed and the protein precipitated by addition of 2 volumes of ice-cold methanol. After microcentrifugation (Bruckard type 270a microcentrifuge, Rickmansworth, U.K.) the supernatants were analysed by HPLC.

The concentration vs time data of the degradation studies were fitted to an exponential equation assuming first order kinetics using the Statgraphics computer program (STSC, Maryland, U.S.A.).

Filter units (Acrodisc 0.2 μm, Gelman Sciences, Northampton, U.K.), with membranes made of nylon, polysulfone, polytetrafluoroethylene (PTFE), and polyvinylidene difluoride (PVDF) were tested for drug adsorption with no prewetting or washing. A solution of 10 μg/ml TCNU was filtered so that 1 ml was collected in approximately 5 s. The drug concentration in the filtered solution was compared with that of the unfiltered control.

Results and Discussion

Three representative chromatograms of TCNU and its decomposition products are reproduced in Fig. 1. The first chromatogram shows the 0.5 mg/ml TCNU standard in mobile phase. The second shows TCNU in citrate buffer after more than 3 months storage at −70°C, no decrease in TCNU concentration being seen over this period. The third shows the greatly reduced peak height and resolved degradation peaks of TCNU after 4 h on a sunny laboratory windowsill.

Generally the stability of the chloroethylnitrosoureas is pH-dependent, showing poor stability under alkaline conditions and stability maxima between pH 4 and pH 5 (Bosanquet, 1985). TCNU does not depart from this general rule having a half life of only 36 ± 4.1 min (mean ± S.D.) in sodium bicarbonate buffer (150 mM, pH 9.1, 100 μg/ml TCNU, 22°C). To further explore the effect of pH, TCNU was degraded in citrate buffer made up over the pH range 2–7 (150 mM citrate, 10 μg/ml TCNU, 22°C). As can be seen from the results shown in Fig. 2, TCNU in citrate buffer shows maximum stability at pH 4.0.

The effect of heat on TCNU solutions (150 mM citrate, pH 4.0, 1 mg/ml) is summarised in Table 1. Half-lives range from 20 min at 72°C to

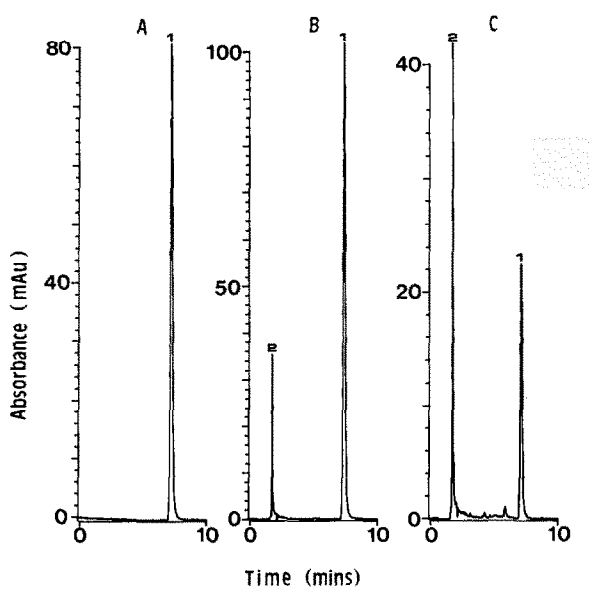

Fig. 1. Representative chromatograms of TCNU. A: TCNU standard in mobile phase. B: TCNU in citrate buffer pH 4.0 after storage at −70°C for more than 3 months. C: TCNU in citrate buffer pH 4.0 after exposure to sunlight for 4 h. 1, TCNU; 2, citrate.

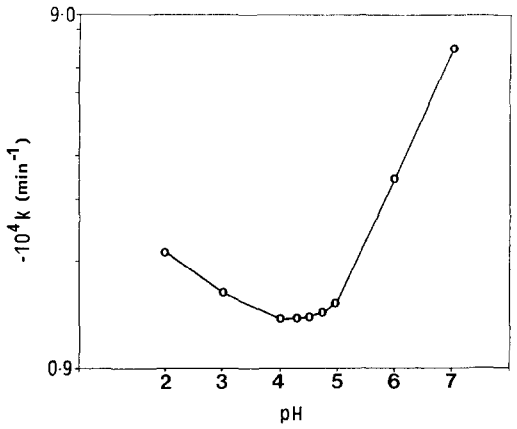

Fig. 2. pH profile of solutions of 10 μg/ml TCNU in 150 mM citrate buffer.

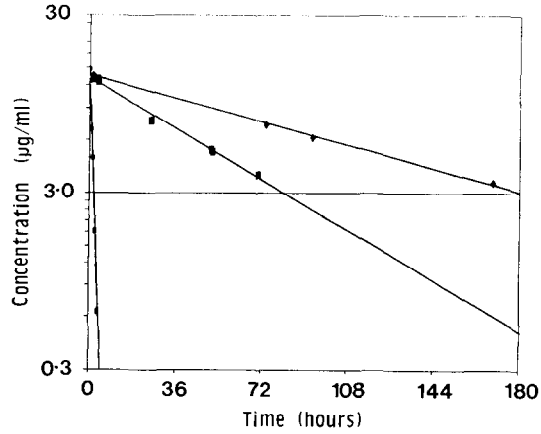

Fig. 3. Decay of TCNU solutions (10 μg/ml, 150 mM citrate buffer pH 4.0) under different lighting conditions: ◆, dark; ■, normal laboratory lighting; ●, a sunny laboratory windowsill.

46.5 days at 2°C. These results compare well with those found for carmustine (Fredriksson and Lundgren, 1986), suggesting a similar mode of degradation for both drugs. At lower temperatures (−20°C, −70°C) we have observed no loss of TCNU in citrate compared to the control in mobile phase over a period of 3 months.

TCNU solutions also show poor stability under intense lighting conditions (Fig. 3). On a sunny laboratory windowsill (150 mM citrate buffer, 10 μg/ml TCNU, 25°C) the drug degraded by 50% in only 0.9 ± 0.1 h whereas under normal lighting conditions the $t_{1/2}$ averaged 38.4 ± 2.4 h. It has only recently been reported that any other nitrosourea shows sensitivity to light (Fredriksson and Lundgren, 1986), and here again, the effect was quite marked. This suggests that in general solutions of nitrosoureas should be protected from intense light.

Drug concentration had minimal effect on the stability of TCNU (150 mM citrate, pH 4.0, RT) with a variation in half life to less than 5% over the range 1–100 μg/ml.

Using different diluents had a considerable effect on the stability of TCNU (Table 2). TCNU degradation was relatively rapid in phosphate buffered saline. In normal saline degradation of TCNU was retarded resulting in even better stability than that offered by citrate buffer at pH 4, however its limit of solubility in this buffer was much lower.

There was only a 5.1% variation in half lives when TCNU solutions were degraded in polystyrene, polyethylene, PVC, glass or siliconised glass containers. This suggests that unlike carmustine which is absorbed rapidly by PVC (Fredriksson and Lundgren, 1986), container material has no effect on TCNU stability. Presumably this is due to the more hydrophilic dimethyl-aminosulfonyl group on TCNU as com-

TABLE 1

Stability of solutions of TCNU (150 mM citrate pH 4.0, 1 mg/ml) at different temperatures

Temperature (°C)	$t_{1/2}$ (h)
2	1116.0
25	49.1
37	8.48
52	4.72
72	0.33

TABLE 2

Stability of solutions of TCNU in different diluents

Diluent	pH	$t_{1/2}$ (h)
Citrate	4.0	82.1 ± 4.0
PBS	7.4	16.4 ± 3.3
NS	6.4	105.9 ± 15.3

Values for $t_{1/2}$ are mean ± S.D.; $n = 3$.

pared to other nitrosoureas, reducing the solubility of TCNU in the PVC plasticisers.

Less than a 1% decrease in concentration was measured when a solution of TCNU (150 mM citrate, 1 mg/ml) was subjected to 3 cycles of freezing and thawing. However, if PBS was used as the diluent, losses of 3% and 14% occurred on the second and third thaws, respectively.

TCNU degraded rapidly when incubated in medium at 37°C with a $t_{1/2}$ of less than 40 min. Only an 8% variation in half life was observed on the addition of either 10% fetal bovine serum or 0.3% agar to the medium.

Four filtration units were investigated to determine whether solutions of TCNU could be filter-sterilized without drug loss. Minimal adsorption was found with PTFE and nylon filters, the filtered samples being 99.7 ± 0.6% and 97.7 ± 2.1% respectively of the unfiltered control. With polysulfone filters a little drug adsorption was noted, the filtered samples in this case being 92.7 ± 2.9% of the control. PVDF filter units, however, gave low and extremely variable recoveries (47.3 ± 21.0%) and therefore cannot be recommended for use with this drug.

Conclusion

In general we find that the stability of solutions of TCNU closely mimics that of carmustine. The drug is best stored frozen in either citrate buffer pH 4.0 or normal saline pH 6.4. Care must also be taken to exclude intense light during preparation and storage of such solutions. Unlike carmustine, which is rapidly absorbed by PVC, container material did not affect the stability of TCNU. Apart from PVDF filter units, which should be avoided, adsorption to filter units was minimal.

Acknowledgements

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