International Journal of Pharmaceutics, 64 (1990) R1–R4
Elsevier

IJP 10019

Rapid Communication

A study of the stability of taurolidine in plasma and protein-free serum

David S. Jones, D.F. McCafferty, A.D. Woolfson and S.P. Gorman

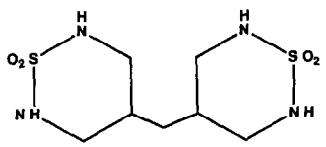
School of Pharmacy, Medical Biology Centre, The Queen's University of Belfast, Belfast (U.K.).

(Received 18 August 1990)
(Modified version received 18 September 1990)
(Accepted 22 September 1990)

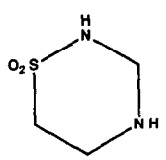
Key words: Taurolidine; Taurultam; Taurinamide; Stability; Plasma; Protein-free serum

Taurolidine (Fig. 1a) [bis(1,1-dioxoperhydro-1,2,4-thiazinyl-4)methane] is a broad-spectrum, non-antibiotic, antimicrobial agent available in Western Europe as a 2.0% w/v solution, Taurolin™. Chemically, it is derived via a condensation reaction involving two molecules of the aminosulphonic acid taurine and three molecules of formaldehyde. It is clinically advocated for the treatment and prophylaxis of Gram-negative and Gram-positive, aerobic and anaerobic infections; endotoxic shock and peritonitis, where it is administered locally (particularly intraperitoneally and in the bladder) (Taurolin™, Data Sheet Geistlich). In a previous publication (Woolfson et al., 1988), it was reported that, in aqueous solution taurolidine undergoes hydrolysis to primarily liberate the monomeric form, taurultam (Fig. 1b), and, that in an acidic environment, taurultam degrades further to liberate methylene glycol (Fig. 1c) and the antimicrobially inactive metabolite, taurinamide (Fig. 1d). Consequently, it has been suggested that the reservoir of taurolidine present accounts for the observed zero-order kinetics of the aqueous hydrolysis reaction (Woolfson et al., 1989).

As it has been reported that taurolidine is rapidly absorbed from the peritoneal cavity (Erb et al., 1983) and, in the light of the above findings



(a)



(b)

$CH_2(OH)_2$

(c)

$H_2N\,CH_2\,CH_2\,SO_2\,NH_2$

(d)

Fig. 1. Chemical structures of taurolidine (a), taurultam (b), methylene glycol (c) and taurinamide (d).

Correspondence (present address): D.S. Jones, Department of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand.

concerning the acid instability of taurolidine solutions, this study was performed to monitor the chemical behaviour of taurolidine in plasma and protein-free serum. Taurolidine, taurultam and taurinamide were supplied as the pure substances by Geistlich Sons Ltd, Chester, U.K. All other chemicals used were of AnalaR, or equivalent, quality.

Analyses of taurolidine and taurinamide were performed using pre-column derivatisation and HPLC with fluorescence detection as previously reported (Woolfson et al., 1989).

Removal of proteinaceous materials prior to injection onto the HPLC column was performed by adding 5 ml of the sample to 10 ml ice-cold acetonitrile, vortexing thoroughly for 30 s and then centrifuging at 3000 rpm for 3 min at 2°C. The addition of acetonitrile had no adverse effect on the stability of taurolidine solutions.

The concentration of formaldehyde present in the biological fluids under examination was analysed using a differential pulse polarographic method, as previously reported (Woolfson et al., 1985).

Fresh whole blood was obtained from the Northern Ireland Blood Bank, Belfast, when required. Plasma was obtained via centrifugation of whole blood (3000 rpm for 10 min) and the supernatant (plasma) removed carefully using a pipette. Protein-free serum was obtained by adding acetonitrile to plasma (in a ratio of 2 parts to 1), vortexing for 30 s, centrifuging at 3000 rpm for 5 min and removing the supernatant carefully using a pipette. Both plasma and protein-free serum were prepared prior to use in the stability studies.

Taurolidine was added to freshly prepared plasma and protein-free serum samples to produce a final concentration of 0.1% w/v. These samples were subsequently incubated at 37°C and, at 20 min intervals, samples removed and analysed for the presence of taurolidine, taurinamide and formaldehyde. In the analytical assay, plasma and protein-free serum samples (without added taurolidine) were treated similarly and used as controls. All experiments were performed in triplicate and the results expressed as mean values.

There are several reports of the instillation of taurolidine, as the commercially available solution Taurolin™, into various body cavities for the treatment and prophylaxis of clinical conditions. These include; the peritoneal cavity, in peritonitis (Buhler et al., 1978) and in the prevention of peritoneal adhesions (Leaper, 1985), and the empyemic space for the treatment of empyema thoracis (Conlan et al., 1985). Due to the rich blood supply either within, or close to, these cavities, taurolidine will encounter blood filtrates. In addition, it has been reported that taurolidine is rapidly adsorbed from the peritoneal cavity (Erb et al., 1983) after which it will encounter both plasma and serum (Rosenfelt et al., 1981). Therefore, it is appropriate that the stability of taurolidine in plasma (and protein-free serum) should be performed. With increasing time of storage in plasma, it is apparent (Fig. 2) that there is a decrease in taurolidine concentration and a subsequent liberation of the metabolite, taurinamide (Fig. 3). This is consistent with the observations of Knight et al. (1981) who were able to detect taurinamide in plasma samples after peritoneal administration of taurolidine. The observed rate

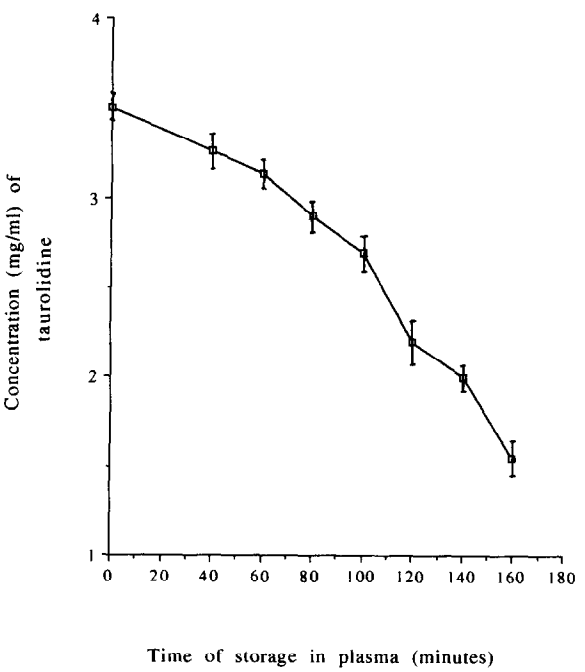


Fig. 2. The effect of storage in plasma at 37°C on the concentration of taurolidine (bars show standard deviation).

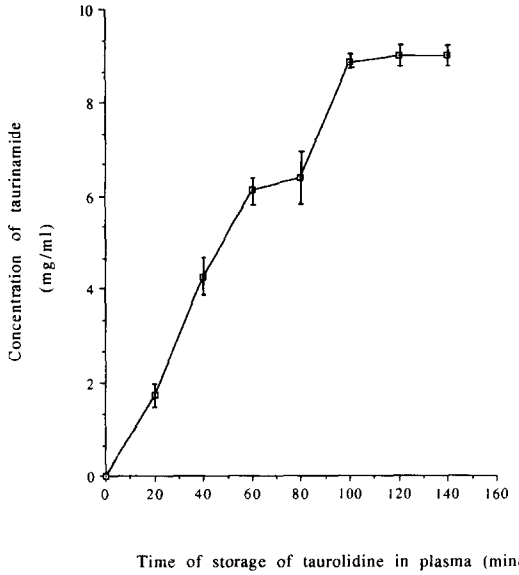


Fig. 3. The effect of storage of taurolidine in plasma at 37°C on the subsequent liberation of taurinamide (bars show standard deviation).

constants for the degradation of taurolidine and the subsequent production of taurinamide, calculated from these results, were $3.9 \pm 0.35 \times 10^{-3}$ and $4.5 \pm 0.31 \times 10^{-3}$ min^{-1}, respectively. The similarity of these values indicates that the degradation of taurolidine closely corresponds to the release of taurinamide. Analysis of the plasma samples using differential pulse polarography revealed the absence of free formaldehyde. Incubation of taurolidine (0.1% w/v) in protein-free serum for similar time periods resulted in no such degradation, i.e. > 99% taurolidine remained after 160 min storage and consequently, there was no liberation of formaldehyde and taurinamide. These findings may be explained on the basis of the known stability of taurolidine and the biochemical differences between plasma and protein-free serum. Both plasma and serum contain non-specific enzymes and it is postulated that these systems catalyse the degradation of taurultam, i.e. the monomeric form of taurolidine which exists in aqueous solution after hydrolysis of the parent dimer molecule. The enzyme catalysed degradation results in a cleavage of the taurultam ring structure, with the consequent liberation of taurinamide and methylene glycol, the latter being an isomer of formaldehyde. Therefore, with increasing times of storage in plasma, greater amounts of taurinamide may be detected, corresponding to an increased extent of taurultam degradation. However, no formaldehyde was found in the plasma samples. This observation was not totally unexpected as formaldehyde has been reported to interact with amino groups present in a variety of biochemical molecules (Grossman et al., 1961) and therefore will interact with proteinaceous materials present in plasma (Mashford and Jones, 1982). Thus, the liberated formaldehyde has effectively been protein-bound and, as a result, no formaldehyde may be detected in the plasma samples. Protein-free serum is produced after precipitation of the proteinaceous materials from plasma and is thus devoid of any enzyme systems. The removal of these enzyme systems may account for the stability of taurolidine after 160 min storage in this fluid.

Although formaldehyde has been associated with toxicity reports, limiting its clinical usage (e.g. Marzuli and Maibach, 1974), the use of taurolidine solutions (Taurolin™) has not been associated with such problems (Buhler et al., 1978). One suggested reason for this is that any liberated formaldehyde may be chemically bound to proteinaceous material and therefore remain unavailable to exhibit the toxic effects associated with free formaldehyde.

References

Buhler, H.U., Mikic, St. and Wicki, O., Neuartige chirurgische Spullosung. *Helv. Chim. Acta*, 45 (1978) 143–145.

Conlan, A.A., Abramor, E., Delikaris, P. and Hurwitz, S.S., Surgical drainage and 2% Taurolin™ instillations for empyema thoracis: a study of 50 cases. In Bruckner, W.L. and Pfirrmann, R.W. (Eds), *Ein Neues Konzept zur Antimikrobieller Chemotherapie chirurgischer Infektionen*, Urban and Schwarzeberg, Munich, 1985, pp. 281–284.

Data Sheet (Taurolin™), Geistlich Sons Ltd, Wolhusen, Switzerland.

Erb, F., Imbenotte, M., Huvenne, J.P., Vankemmel, M., Scherpereel, P. and Pfirrmann, R.W., Structural investigation of a new organic antiseptic: taurolidine. Analytical study and application to identification and quantification

in biological fluids. *Eur. J. Drug. Metab. Pharmacokinet.*, 8 (1983) 163–173.

Grossman, L., Levine, S.S. and Allison, W.S., The reaction of formaldehyde with nucleotides and T2 bacteriophage DNA. *J. Mol. Biol.*, 3 (1961) 47–60.

Knight, B.I., Skellern, G.G., Browne, M.K. and Pfirrmann, R.W., The characterisation and quantification by high performance liquid chromatography of the metabolities of Taurolin™. *Br. J. Clin. Pharmacol.*, 12 (1981) 439–440.

Leaper, D.J., Prevention of peritoneal adhesions after thermal injury using the formaldehyde carriers noxythiolin and Taurolin™. In Bruckner, W.L. and Pfirrmann, R.W. (Eds), *Ein Neues Konzept zur Antimikrobieller Chemotherapie chirurgischer Infektionen*, Urban and Schwarzeberg, Munich, 1985, pp. 115–119.

Marzuli, F.N. and Maibach, H.I., The use of graded concentrations in studying skin sensitizers: experimental contact sensitization in man. *Food Cosmet. Toxicol.*, 12 (1974) 219–227.

Mashford, P.M. and Jones, A.R., Formaldehyde metabolism by the rat: a reappraisal. *Xenobiotica*, 12 (1982) 119–124.

Rosenfelt, F.L., Glover, J.R. and Marossy, D., Systemic absorption of noxythiolin from the pleural cavity in man and in the rabbit. *Thorax*, 36 (1981) 278–281.

Woolfson, A.D., McCafferty, D.F., Gorman, S.P. and Anderson, L., Differential pulse polarographic determination of formaldehyde in stored noxythiolin solutions, *J. Clin. Hosp. Pharm.*, 10 (1985) 177–183.

Woolfson, A.D., McCafferty, D.F., Gorman, S.P. and Jones, D.S., Polarographic analysis of taurolidine, a non-antibiotic antimicrobial agent. *Int. J. Pharm.*, 48 (1988) 167–173.

Woolfson, A.D., Gorman, S.P., McCafferty, D.F. and Jones, D.S., Assay procedures for Taurolin solutions using precolumn derivatisation and high performance liquid chromatography with fluoresence detection. *Int. J. Pharm.*, 49 (1989) 135–141.