J. Pharm. Pharmacol. 1979, 31: 853

COMMUNICATIONS

The stability of melphalan in the presence of chloride ion†

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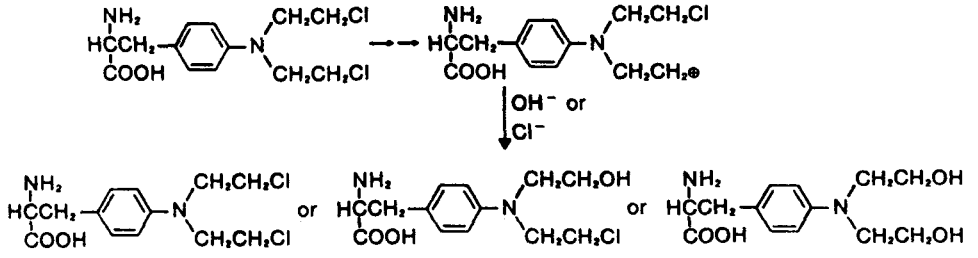
Melphalan (L-phenylalanine mustard, NSC 8806) a drug used in the treatment of several types of cancer, is known to undergo hydrolysis, the rate of which is altered by pH (Furner et al 1976; Ross 1962), temperature (Chang et al 1978a, b), bovine serum albumin, plasma proteins (Chang et al 1978b) and the type and concentration of anionic species (Gould 1959; Ross 1962) in aqueous solutions. This study shows the effect and mechanism of the chloride ion on melphalan stability.

Melphalan was analysed by h.p.l.c. using dansylproline as an internal standard (Chang et al 1978a). The h.p.l.c. apparatus consisted of two pumps (Waters 6000)‡, a solvent programmer (Waters 660)‡, a detector (Waters 440)‡, and a reverse phase column (Micro C_{18} Bondapak)‡. Various incubation mixtures containing melphalan and internal standard were injected directly onto the column.

Melphalan, 50 μg, and internal standard 100 μg, were incubated at 37 °C in 2 ml of different anionic and pH composition as follows: 0·156 M NaCl (0·9% normal saline, Travenol), 0·013 M HCl (pH = 2·4), 0·013 M H_3PO_4 (pH = 2·6), 0·06 M KH_2PO_4 (pH = 7·4), 0·06 M K_2HPO_4 (pH = 7·4), 0·156 M KBr (pH = 7·4), Burroughs-Wellcome diluent (0·047 ml HCl (36%), 1 ml ethanol, 108 mg K_2HPO_4, 5·4 ml propylene glycol, and water to 10 ml), and Burroughs-Wellcome diluent with the phosphate buffer and water replaced by 0·9% saline. At varying times aliquots were removed and analysed for total unchanged melphalan. The disappearance rate constants of the unchanged drug were calculated by $k = 2{\cdot}3 \log \frac{Co}{Ct}/t$, where Co and C_t are the concentrations of melphalan at times zero and t. The bromo-derivatives of melphalan were identified as their trimethylsilyl (TMS) derivatives by a mass spectrometer.

Melphalan disappearance in various incubation media at 37 °C is shown in Fig. 1. All experiments were in triplicate and the standard deviations of the disappearance rate constants were included. In the phosphate buffers (pH = 7·4) melphalan showed a disappearance rate constant of k = 1·02 s.d. 0·02 h^{-1}. In equimolar concentrations of HCl and H_3PO_4 the disappearance rate constants were k = 0·44 s.d. 0·1 h^{-1} and k = 0·78 s.d. 0·02 h^{-1}, respectively. Hydrogen ion concentration in both HCl and H_3PO_4 were similar (pH = 2·4)—2·6. Melphalan was very stable in 0·156 M NaCl (pH = 5·35) solution (k = 0·28 h^{-1}). Melphalan in Burroughs-Wellcome diluent gave a disappearance rate constant of k = 0·034 s.d. 0·003 h^{-1}. When the diluent was modified by replacing dipotassium phosphate and water by 0·9% saline, the disappearance rate constant was k = 0·017 s.d. 0·001 h^{-1}. An h.p.l.c. analysis of melphalan incubated in 0·156 M KBr solution at 37 °C for 1 h is shown in Fig. 2. The identity of bromo derivatives of melphalan was confirmed by mass spectrometry. The mass spectra of the trimethylsilyl derivatives of the bromo compounds were compared with trimethylsilyl derivatives of melphalan and monohydroxy melphalan. Since no standard bromo melphalan was available, bromine isotope characteristics and corresponding mass increase due to bromine



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† Supported in part by grant CA-17094 from the National Cancer Institute and by grant T32-GMO7533, National Institutes of Health, Department of Health, Education and Welfare and by a donation from Burroughs-Wellcome Co., Research Triangle Park, North Carolina. We wish to thank Dr Wayne Brenckman for his critical review of the manuscript.

‡ Waters Associates, Milford, MA.

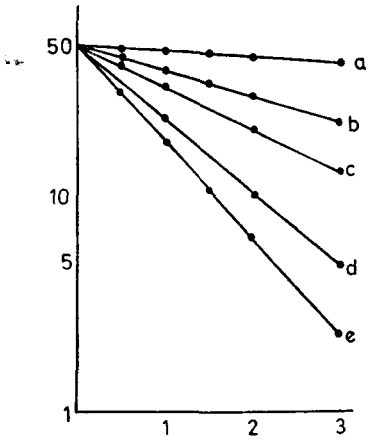

FIG. 1. A recovery of melphalan in various media at 37 °C. Melphalan (50 μg ml^{-1}) was incubated in different media: 0·156 M NaCl (0·9%), 0·156 M HCl (pH = 1·70), 0·013 M HCl, 0·013 M H_3PO_4, and 0·06 M phosphate buffers (KH_2PO_4 or K_2HPO_4 at pH = 7·4). At different times aliquots were taken and analysed for total unchanged drug.

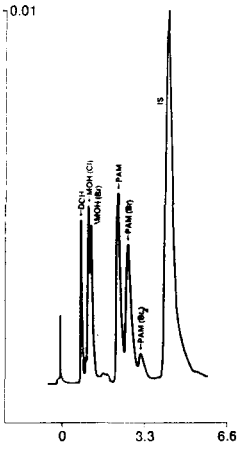

FIG. 2. H.p.l.c. chromatogram of melphalan incubated in 0·156 M KBr solution at 37 °C for 1 h. Dihydroxy melphalan (DOH), monohydroxy melphalan [MOH(Cl)], monohydroxybromo melphalan [MOH(Br)], melphalan (PAM), monobromo melphalan [PAM(Br)], dibromo melphalan [PAM(Br_2)], and internal standard (IS) are shown.

substitution served as the identification of bromo melphalan. Melphalan concentration seen in Fig. 2 is 43% of the starting concentration.

Melphalan was least stable in the presence of the phosphate ions. Although a lower pH gave some stability, a greater stability was noted in the presence of chloride ion. Since the pH of the HCl (2·4) and H_3PO_4 (2·6) solutions were similar, their differing recovery rate constants can be attributed to the presence of chloride ions. Even at a higher pH (5·4), melphalan showed its greater stability in the NaCl solution ($k = 0{\cdot}28\ h^{-1}$). In 0·156 M KBr solution, no stabilization of melphalan was observed. Instead, bromo-derivatives of melphalan and its hydrolysis products were formed, as confirmed by mass spectrometric and high pressure liquid chromatographic analysis (Fig. 2). Melphalan in the KBr solution showed similar stability as melphalan incubated in water ($k = 0{\cdot}83\ h^{-1}$) (Chang et al 1978b).

The kinetics of melphalan in an aqueous solution (Fig. 1) are characterized by monomolecular nucleophilic substitution (S_N1) (Chang et al 1978c; Livingstone & Carter 1970). Ross (1962) suggested that carbonium ion formation could be retarded and/or reversed by the presence of a 'common ion'. The stability of melphalan seen in Fig. 1 is probably not a result of the 'common ion' effect. Rather the reaction proceeds at the same rate in a forward direction regardless of anionic species or concentration. The greater melphalan stability in the NaCl solution results from its regeneration by chloride ions and not from a slowed carbonium ion formation.

The mechanism of melphalan stability in the NaCl solution appears to be different from that reported for its stability in bovine serum albumin solutions or sodium taurocholate (Chang et al 1978 b,c). Melphalan stability in these latter media is probably related to hydrophobic interaction between the chloroethyl moiety of melphalan and taurocholate or protein molecules, respectively.

Melphalan is relatively unstable in K_2HPO_4, even at low pH at 37 °C. The very good stability ($k = 0{\cdot}03\ h^{-1}$) of melphalan observed in the Burroughs-Wellcome diluent is likely to be due to both chloride ion and hydrophobic interaction of melphalan with propylene glycol. The omission of dipotassium phosphate from and addition of 0·9% saline to the diluent resulted in further stability of melphalan ($k = 0{\cdot}017$).

March 19, 1979

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