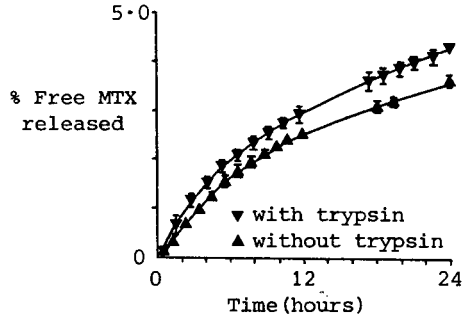
HYDROLYTIC DEGRADATION OF METHOTREXATE-ALBUMIN CONJUGATES IN THE PRESENCE AND ABSENCE OF TRYPSIN

R.J.Marriott and C.W.Pouton, School of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY

Garnett et al (1983) have shown that cell-selective cytotoxic action can be achieved *in vitro* by targeting methotrexate(MTX)-human serum albumin(HSA) conjugates to specific cells by way of monoclonal antibodies. One important facet of the design of such targeting agents is the optimisation of drug release in the presence of lysosomal enzymes (Duncan et al, 1983). We report here studies of the degradation of MTX-HSA conjugates in the presence and absence of a model enzyme, trypsin.

MTX was conjugated to HSA via a peptide bond using a water-soluble carbodiimide reagent (ECDI) in phosphate-buffered saline (pH 7.4,I=0.29M). The molar ratio of the reactants was 1:117.2:172.7 (HSA:MTX:ECDI) at a fixed concentration of HSA (10mg/ml). The reaction proceeded in the dark at 25°C for 24 hours before it was terminated by fractionation of the reaction mixture on a gel-filtration column (Sephadex G-15, pH 7.4, I=0.29M). The MTX-HSA conjugate eluted first and was collected for analysis of the molar conjugation ratio MTX/HSA(MCR). The mean MCR was determined by spectrophotometry at 376nm (E1%/1cm=162.4) and was found to be 20.3±1.4. The association of MTX with HSA to form MTX-HSA could not have been explained by physical adsorption since it was shown quantitatively that mixtures of MTX and HSA in the absence of ECDI were completely separated by one pass through a size-exclusion chromatography column. The stabilities of freshly fractionated solutions of the conjugate were examined at 37°C and pH 7.4 in the presence and absence of trypsin (the molar ratio HSA/trypsin was 10). Samples were injected hourly onto an HPLC size-exclusion column (Superose 12) which was calibrated using molecular weight standards and for MTX concentration. This allowed determination of the release of free MTX, which eluted after 50mins(0.5ml/min), and also assessment of changes in the molecular weight distribution of the degrading MTX-HSA conjugate.

Fig.1. *In vitro* release of MTX from MTX-HSA conjugates at 37°C and pH7·4.



In common with Halbert et al (1982) we found that MTX was released in the absence of enzyme, as shown by Figure 1. The presence of trypsin had little effect on the rate of release of free MTX (Figure 1) but there was substantial change in the molecular weight distribution of the conjugate over a 24 hour period. Enzymatic degradation of the conjugate was reproducible and resulted in MTX-peptide fragments of characteristic molecular weight.

Release of MTX in the absence of enzyme is an undesirable property of the conjugate which we attribute to the formation of bonds other than peptides. We advocate the use of synthetic polymer carriers which would simplify the chemistry involved. Our results show that the presence of covalently bound MTX did not prevent enzymatic degradation of albumin.

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Garnett, M.C. et al (1983) Int. J. Cancer 31: 661-670
Halbert, G.W. et al (1982) J. Pharm. Pharmacol. 34: 8OP