PHYSICAL AND BIOLOGICAL CHARACTERISATION OF METHOTREXATE/ ALBUMIN CONJUGATES

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Much attention is currently being focussed on the use of drug delivery or targetting systems as a means of reducing the toxicity involved in systemic cancer chemotherapy (Trouet 1978). Methotrexate (MTX)/Bovine Serum Albumin (BSA) conjugates initially showed promising results in vivo (Jacobs 1971). Further work indicated that improvements in this system were still possible (Chu 1977). In the present work the physical and biological properties of the MTX-BSA conjugates were studied with a view to increasing our understanding of their behaviour in vivo.

The conjugates were synthesised by coupling MTX to BSA utilising a water soluble carbodiimide in 0.05M $NaHCO_3$ buffer (pH 7.6) at room temperature, and separating the reactants by gel exclusion chromatography with final isolation of the conjugate involving dialysis and lyophilisation. Two series of conjugates were prepared and designated 'high' or 'low' strength. High strength preparations (n=11) contained an average of 12.3 mol MTX/mol BSA (range 9.5-18.1) and low strength conjugates (n=11) contained 4.9 mol MTX/mol BSA (range 2.7-8.7).

The conjugates were characterised using several physical and biological techniques. Stability experiments performed on the conjugates, using a simple dialysis method showed that over 24 hours at pH 7.4 at 37°C, less than 10% of the attached MTX was released from either the high or low strength conjugates. Similar results were obtained at a pH of 5.0, indicating that the attachment of MTX is strong and unlikely to be broken under normal biological conditions. SDS-polyacrylamide gel electrophoresis of the conjugates revealed that most of the BSA exists as the monomer, but that its molecular weight is increased to a varying degree, depending on the amount of MTX attached.

Viscosity studies showed that the attachment of MTX has little or no effect on the hydrodynamic characteristics of the BSA. The particle size of the conjugates was measured utilising photon correlation spectroscopy, although this technique is complicated by the fact that MTX absorbs at the wavelength of the laser light (442nm). However, experiments have shown that this is unlikely to affect significantly the interpretation of results, which also indicate that coupling of MTX to BSA does not markedly alter the size of the molecule.

To assess the effect that impurities in the MTX may have on the conjugates and also the effect of bound lipids on the BSA, conjugates were synthesised utilising pure MTX and lipid free BSA. These were found to possess properties very similar to the conjugates prepared from MTX and BSA without special purification.

Tissue culture experiments were performed in which L1210 murine leukamia was challenged with free MTX and conjugates at equivalent concentrations; with respect to MTX. Free MTX at 10^{-7}M inhibits cell growth completely whereas the conjugates at this concentration have no effect, at 10^{-6}M however the conjugates do inhibit growth to some extent, but at a concentration of 10^{-5}M the effect is similar to that of MTX at 10^{-7}M. The results show that the conjugates are 10 to 100x less effective than free MTX and that the high strength conjugates are slightly more active than the low strength conjugates.

The results suggest that the physical properties of the conjugates do not vary greatly from native BSA, and that as a drug targetting system improvements can still be made.

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