THE SYNTHESIS AND ENZYMATIC DEGRADATION OF DEXTRAN-METHOTREXATE CONJUGATES

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Several biocompatible polymers have found use in drug delivery applications as carrier molecules (Duncan et al 1988). Dextrans are naturally occurring polysaccharides, comprised essentially of linear polymers of [6(D-glucose)1]. They possess low toxicity, low pharmacological activity, high water solubility, and a range of different weight fractions are available. Dextrans have a high density of functional groups which are capable of forming covalent bonds to suitably functionalized drug molecules. We have examined the synthesis of methotrexate (MTX) conjugated to a range of dextrans.

Dextran T40 was oxidized with 17% aqueous sodium periodate solution (Malaprade reaction). Schiff base formation with diamines including 1,3-diamino-2-propanol (Shih et al 1988) and 1,3-diaminopropane, and polyamines e.g. spermine followed by sodium borohydride reduction afforded amino-modified dextrans. Biodegradable amide linkages were introduced by a carbodiimide (ECDI) catalysed reaction between the amino-modified dextran polymer and the carboxylic acid groups of the L-glutamic acid residue in MTX (in 40mM aqueous sodium hydrogen carbonate solution, pH 7.4). The incorporation of MTX was achieved most satisfactorily with 1,3-diamino-2-propanol.

The degradation of the synthetic dextran-MTX conjugates was examined over a 48h period. The dextran, amino-modified dextran, and the conjugates were incubated with dextranase (EC 3.2.1.11) (0.067M aqueous Sorensen's buffer, pH 6.0, 37°C). The conjugates were also incubated with an α-glutamyl specific protease (type XVII-S strain V8) (pH 7.8, 37°C), and with γ-glutamyl transpeptidase (0.050M aqueous tris-HCl buffer, pH 8.5, 37°C). Furthermore, the conjugates were sequentially incubated with dextranase (4h) followed by protease. Within 3h, dextran T40 was degraded to smaller oligosaccharides, mainly disaccharides e.g. maltose. The extent of degradation by dextranase of dextran, amino-modified dextrans, and the derived MTX-conjugates, decreased with increasing modification of the dextran. The highest levels of MTX release were achieved on incubation of the conjugates with dextranase followed by protease. The covalent bond between the primary amine of the spacer linkage and a carboxylic acid group of MTX may be protected from enzymatic cleavage due to steric hindrance from the bulk of the dextran polymer. This bond becomes more accessible to, and therefore more susceptible to, enzyme catalysed hydrolysis as the size of the carrier decreases. The enzymatic fragmentation of the main chains of the dextran polymer causes a significant reduction in the steric protection afforded to this bond. Dextranase is present in the liver, intestinal mucosa, colon, spleen, and kidneys of humans (Schacht et al 1988; Larsen 1989).

Duncan, R. et al (1988) Brit. J. Cancer 57: 147-156
Larsen, C. (1989) Adv. Drug Delivery Rev. 3: 103-154
Schacht, E. et al (1988) J. Bioact. Compat. Polym. 3: 72-80
Shih, L.B. et al (1988) Int. J. Cancer 41: 832-839