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The effect of hyaluronan on the binding of diclofenac to human albumin using equilibrium dialysis

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Hyaluronan (HA) is a naturally occurring polysaccharide composed of D-glucuronate and N-acetyl-D-glucosamine residues. It is found extensively within the extracellular matrix of human connective tissue such as the skin. The versatile physical and chemical nature of this polymeric molecule has led to its development as a topical drug targeting/delivery vehicle. It has recently been employed as an excipient in a HA/diclofenac hydrogel formulation indicated for symptomatic relief in the treatment of osteoarthritic pain, actinic keratosis and basal cell carcinoma.

The claimed benefit of including HA in such topical preparations is that it promotes the intradermal localisation of the applied diclofenac (Brown et al 1995). Although the precise mode of action of HA is still unclear, studies across human skin have shown that HA controls and sustains the release of diclofenac in the skin by forming a resevoir of drug in the epidermis (Brown et al 1995). Furthermore, additional studies using circular dichroism have suggested that HA may modify the binding of diclofenac for albumin (Brown et al 1996 a,b). The aim of this research was to investigate further the influence of HA on the binding of diclofenac to albumin using equilibrium dialysis as an alternative technique.

All experiments were carried out using two 5-cell equilibrium dialyser units rotating at 20 rpm and employed semi-permeable cellulose membranes with a molecular weight cut-off between 12000-14000. Radiolabelled diclofenac with concentrations ranging from 54 mM to 2.1 μM and a specific activity of 746 kBq/ml, (Ciba-Geigy, Horsham, UK), was initially added in the donor compartment while human albumin at a concentration of 250 μM, (Sigma Pharmaceuticals, St Louis, USA), was included in the receptor compartment. In some experiments HA, (Hyal Pharmaceutical Corporation, Toronto, Canada), was added with the human albumin. Diclofenac concentrations in the donor and receptor compartments were measured using liquid scintillation counting and the amount of diclofenac bound to albumin determined at pH 5.5 or pH 7.4 (phosphate-buffered saline) each at a temperature of 32 or 37 °C with and without HA 1 % w/v; (n=5).

At a respective diclofenac:albumin molar concentration of 3:1 (pH 7.4; 37 °C), HA 1 % w/v was shown to significantly decrease the binding of diclofenac to human albumin by 5.02 ± 0.08 % ($p \leq 0.05$) when compared to diclofenac and albumin alone. Temperature and pH studies displayed that in the absence of HA, the greatest amount of unbound diclofenac (8 ± 0.03 %, $p \leq 0.05$), occurred at pH 7.4 and 32 °C. Overall, these results correlate well with those derived from the optical circular dichroism studies performed previously (Brown et al 1996 a,b).

Diclofenac is reported to be 99.7 % bound to human albumin (Sjoholm et al 1979). Consequently, even doubling the amount of unbound diclofenac available at the required site action could have a significant effect on its therapeutic activity. Such observations *in-vitro* may assist to further elucidate the changes or biodisposition of drugs *in-vivo* when delivered with hyaluronan.

References

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