Increased *in vitro* and *in vivo* release of dexamethasone formulated with a cross-linked hyaluronic acid hydrogel

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ABSTRACT SUMMARY

Hyaluronic acid from *Bacillus subtilis* was cross-linked with divinyl sulfone and mixed with dexamethasone resulting in a sustained release formulation. *In vitro* release studies showed a significant increase in release time and the *in vivo* half-life of dexamethasone more than doubled, when formulated with cross-linked hyaluronic acid and administered subcutaneously in Sprague Dawley rats.

INTRODUCTION

Hyaluronic acid (HA) is a natural linear polysaccharide composed of beta-1,3-N-acetyl glucosamine and beta-1,4-glucuronic acid repeating disaccharide units (Figure 1).

**Figure 1. Chemical structure of the repeating disaccharide unit in HA**

HA exhibits significant structural, physiological, and biological functions that make it an attractive ingredient for drug delivery applications. However, HA injected in the body is typically degraded rapidly thereby limiting the long term effects of the polymer. To overcome its short residence time, HA can be cross-linked with divinyl sulfone (DVS) producing hydrogels suitable for sustained release delivery. Dexamethasone is a corticosteroid used to treat inflammatory and autoimmune conditions. In this work dexamethasone is loaded into a cross-linked HA hydrogel yielding a depot sustained release formulation, which is tested *in vitro* and *in vivo*.

EXPERIMENTAL METHODS

HA was produced by Novozymes Biopharma DK A/S by fermentation of *Bacillus subtilis* with an average molecular weight of 0.85 MDa. Dexamethasone phosphate was purchased from Fagron and analytical standard from Sigma.

Hydrogels were prepared with a DVS/HA weight ratio of 1:20 with a final HA concentration of 1.4 % (w/v). HA was dissolved in 0.2 M NaOH and DVS was added to the HA solution under stirring. The cross-linked HA hydrogels were swollen in PBS buffer, pH of 7.4. Dexamethasone was dissolved in PBS buffer pH 7.4 and equilibrated with the hydrogel for 24 h.

The *in vitro* release of dexamethasone from the cross-linked HA hydrogels was assessed by a USP 4 dissolution method with a closed loop configuration. PBS buffer pH 7.0 was used as medium equilibrated at 37 °C and the flow rate was set to 4 mL/min. Dexamethasone was detected on-line with UV absorbance at 243 nm.

Pharmacokinetics studies of dexamethasone were performed in Sprague Dawley rats (BioAdvice A/S) and serum samples were analyzed by LC-MS/MS. For each formulation 5 rats were dosed subcutaneously with 20 mg/kg. Blood samples were withdrawn at different time points and centrifuged. Quantification of dexamethasone in the serum samples was performed by LC-MS/MS on a C18 column at 60°C. Dexamethasone was eluted with an isocratic gradient containing 35% acetonitrile and 0.15% formic acid with a flow rate of 0.3 mL/min. MS/MS sequencing of dexamethasone (*m/z* 393) resulted in a fragment with *m/z* 237. Serum concentration profiles were
analyzed using a non-compartmental model (Jansen Consulting).

RESULTS AND DISCUSSION

Cross-linked HA hydrogels were prepared and formulated with dexamethasone resulting in a clear, drug loaded hydrogel formulation. The \textit{in vitro} release of dexamethasone from the hydrogel formulation was monitored by UV absorbance (Figure 2). Dexamethasone formulated in PBS buffer was distributed in the system immediately. However, when dexamethasone was formulated with a cross-linked HA hydrogel the total release time was increased to 15 h.

![Figure 2. In vitro release of dexamethasone from cross-linked HA hydrogels](image)

In order to correlate the \textit{in vitro} release data with \textit{in vivo} data, pharmacokinetics studies were performed in Sprague Dawley rats and serum concentrations of dexamethasone were analyzed by LC-MS/MS (Figure 3).

![Figure 3. Pharmacokinetics of dexamethasone in Sprague Dawley rats analyzed with LC-MS/MS](image)

The \textit{in vivo} data showed an initial plateau (5 h) in dexamethasone plasma concentration when it was formulated with cross-linked HA hydrogels indicating a slower release of dexamethasone from the hydrogel compared to dexamethasone formulated with PBS buffer.

The \textit{in vivo} data were further analyzed using non-compartmental model yielding half-life ($T_{1/2}$) data for each formulation (Figure 4). $T_{1/2}$ for dexamethasone more than doubled from 2.6 h to 5.9 h when it was formulated with cross-linked HA hydrogel.

![Figure 4. Half-life of dexamethasone in Sprague Dawley rats when formulated with and without HA hydrogels](image)

CONCLUSION

In this work we showed that incorporation of dexamethasone in a cross-linked HA hydrogel (i) increased the \textit{in vitro} drug release time from less than 10 min to approximately 15 hours, (ii) the \textit{in vivo} half-life of dexamethasone more than doubled when formulated with a cross-linked hyaluronic acid hydrogel in Sprague Dawley rats.

These findings support the development of a depot sustained release formulation consisting of cross-linked HA hydrogels and dexamethasone.

REFERENCES