Developing circadian enzyme-controlled polymer therapeutics

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Background:

Chronotherapy, where a drug is administered at a time of day when it will be most effective, best tolerated and encounter optimal pharmacokinetics, is one approach to minimise side effects and increase efficacy and tolerability. Chronotherapy may be achieved using specialised drug delivery systems (DDS), such as polymer therapeutics, nanoparticles or liposomes, that aim to release a drug in a sustained manner, or in response to a trigger (e.g. temperature, pH, enzyme). Enzymatic triggers are attractive as they underpin physiological function and are dysregulated in many disease-associated microenvironments and aberrant cell processes. Despite this, exploitation of the biological rhythm of enzymes to trigger temporally-controlled release of a drug remains unexploited. Several studies have, however, reported circadian patterns of enzyme secretion which vary in amplitude. For example, salivary and serum amylase shows diurnal variation; activity being lowest 30 minutes after waking and peaks in the early evening. This study sought to study drug release rate from a model enzyme-responsive polymer-drug conjugate (dextrin-dexamethasone) at peak and trough circadian enzyme concentrations, as proof of concept for the design of circadian enzyme-controlled DDS.

Methods: A two-compartment static dialysis bag model was used to compare drug release rate from dextrin–dexamethasone conjugates in the absence and presence of predicted peak (100 IU/L) and trough (30 IU/L) diurnal serum amylase concentrations. The model system comprised an inner compartment containing dextrin-dexamethasone conjugate (3 mg/mL in PBS, 5 mL) +/- amylase and an outer compartment containing sterile PBS (15 mL), separated by a 10,000 g/mol dialysis membrane. The aseptically-sealed beaker was incubated at 37°C for 72 h. Samples were collected at various time-points from each compartment and stored at -20°C prior to analysis of dexamethasone content by HPLC with UV detection (at 246 nm).

Results: Dextrin (209,500 g/mol, 30 mol% succinoylation) was bound to dexamethasone to produce conjugates with a molecular weight of 285,000 g/mol and containing 14.10 µg/mg drug loading. Negligible free drug was detected in the outer compartment at 72 h in the absence of amylase, which was mirrored by minimal change to the dexamethasone concentration in the inner compartment. When amylase was added to the inner compartment, a time- and concentration-dependent increase in drug concentration in the outer compartment was detected, with a reciprocal decrease in dexamethasone concentration in the inner compartment. Diffusion of drug was significantly greater in the presence of the peak (100 IU/L) amylase concentration.

Conclusions: These studies demonstrate the feasibility of achieving enhanced drug release from enzyme-responsive nanomedicines at times of peak diurnal enzyme activity. Ongoing work is characterising the circadian rhythm of relevant enzymes *in vitro* and *in vivo* to inform the design of specialised DDS; utilising the daily variation in enzyme activity to trigger drug release to coincide with worsening symptoms.