Injectable Hydrogels with Controlled Release of Covalently Incorporated Dexamethasone

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Statement of Purpose: The anti-inflammatory and immunosuppressive drug Dexamethasone (Dex) is useful in a wide range of clinical applications, but it may have undesirable systemic side effects. A number of techniques whereby the drug has been incorporated into polymers and hydrogels, either by entrapment or covalent binding, have thus been developed to deliver the substance locally for uses ranging from vascular stants to occular inserts. Polyethylene glycol (PEG) is a well-favoured material due to its inertness, non-toxicity and ease of elimination from the body. The use of Michael-type addition reactions to form PEG gels from a simple admixture of two components have many advantages over other systems, as they occur at physiological conditions without the need for initiators or the release of by-products. Their spontaneous in situ gelation after injection is especially useful in applications such as myocardial infarct therapy, where we have shown their beneficial effect [1, 2]. The gels are also useful as potential carriers of stem cells, and the inclusion of slow-release dexamethasone in the gels has potential to reduce inflammatory and immune responses to therapeutic viral vectors.

This study shows how Dex can be covalently incorporated into degradable and non-degradable PEG gels, how controlled release of the Dex is achieved in vitro, and how the eluted Dex retains its activity. An in vivo proof of principle, in which the efficacy of adenoviruses encoding for green fluorescent protein (ADV-GFP) in the rat heart is shown to be improved by the use of Dex release from PEG gels, is also given.

Methods: PEG prepolymers (20kDa, 8-arm, 20PEG-8OH, Nektar) were derivatized with either acrylate or vinyl sulfone groups by reaction with acryloyl chloride or divinylsulfone to yield 20PEG-8Ac or 20PEG-8VS. Dexamethasone was acrylated and subsequently reacted with 2kDa linear dithiolated PEG (2PEG-2SH; Sunbio PEGSHOP) either in a 1:1 (A) or 1:5 (B) ratio to render it water soluble and capable of reaction with the derivatized PEG polymers in aqueous media. Reactions products were verified by NMR, MS and MALDI. The Dex-Ac-S-PEG-SH products were incorporated into both 20EG-8Ac and 20PEG-8VS using additional 2PEG-2SH crosslinker where required. Gels were immersed in PBS and swelling ratios were followed in vitro. Dex elution was quantified by UV spectroscopy. The activity of released Dex was determined using a human mammary gland cell line that expresses luciferase under control of an MMTV promoter that contains response elements to glucocorticoids, followed by quantification of luciferase as fold increases over controls.

ADV-GFP ($1.5x10^9$ pfu) was subsequently delivered to rat hearts (n=5 per group) by direct myocardial injection using Dex-modified and control gels for one week. The gels were similar to the PEG-VS gels used in vitro, but were crosslinked using enzymatically degradable, modified MMP substrate sequences instead of 2PEG-2SH. GFP expression, T-Lymphocytes, macrophages, and tissue damage were assessed.

Results: Swelling of PEG-Ac gels showed two phases, namely an increase to equilibrium values in 2 days, followed by steady increase in swelling ratios up to the 12-day point, indicating the decrease in crosslink density with gel degradation. Non-degradable (PEG-VS) gels showed similar initial swelling, but ratios remained constant after the initial period, confirming the non-degradability of the gels at these conditions. In both gel types, Dex containing gels swelled marginally more than controls, due to slight decrease in the crosslink density caused by the attachment of the pendent drug. For both PEG-Ac and PEG-VS gels, all of the soluble Dex-phosphate used in non-covalently incorporated control samples was essentially fully eluted after 2-3 days. The covalently incorporated Dex, on the other hand, showed controlled, near linear (R^2=0.988) release over the 12-day observation period for PEG-Ac gels, and controlled, first order release (R^2=0.991 to 0.996 for methods A and B) over the 35-day observation period for the non-degradable PEG-VS gels. The eluates from both gel types showed retained activity (15 fold induction) over their respective periods, while the activity of eluates from the Dex-phosphate controls decreased rapidly after the first 2 days. In the in vivo pilot experiment, there was a 7.7-fold greater expression of GFP in the Dex group ($p<0.05$). When normalized to GFP expression, both T-Lymphocyte and macrophage responses were also markedly reduced by the drug: 5-fold ($p<0.05$) and 20-fold ($p<0.01$), respectively. In addition, there was a near 10-fold reduction in fibrosis ($p<0.1$) when Dex was used.

Conclusions: Dexamethasone can be covalently linked to both degradable and non-degradable PEG gels in such a way as to control the release of the drug over extended periods. As the chemistry is chosen in such a way as to achieve release of the drug in its unmodified form, the drug retains its activity upon release. Dex releasing gels are also potentially useful in gene therapy applications by decreasing inflammatory responses to the delivered vectors and increasing gene expression.

References:

[1] Dobner S et al.. J Card Fail. 2009;15:629-36.
[2] Kadner K et al. Biomaterials.33:2060-6.