DDS Institute

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General Summary

We are now investigating a new drug delivery system (DDS) using nanotechnology. We have developed fabrication methods for 1) (poly)ethyleneglycol (PEG)-poly (D, L-lactic acid) (PLA)/PLA nanoparticles for targeting and sustained release of steroids, 2) $CaCO_3$ nanoparticles with insulin, and 3) intelligent antigen-responsive nanoparticles.
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Research Activities

Nanoparticle preparations of a steroid for targeting and sustained release

We have examined the therapeutic activity of betamethasone phosphate (BP) encapsulated in biocompatible and biodegradable nanopaticles consisting of PLA homopolymers and PEG-block-PLA copolymers (stealth nanosteroid), which are targeted to inflamed joints and have shown slow release and prolonged blood circulation after intravenous administration, in experimental arthritis models, including rats with adjuvant arthritis (AA rats) and mice with arthritis induced by anti-type II collagen antibodies (AbIA mice).

First, we determined the characteristics of nanoparticles, such as diameter, PEG density, BP encapsulation efficiency, BP release rate, and cellular uptake, because various types of nanoparticle can be prepared depending on different compositions and molecular weights of polymers and by various blend ratios using an oil-in-solvent diffusion method. The pharmacokinetic and biodistribution profiles were examined in normal rats, AA rats, normal mice, and AbIA mice. Furthermore the biodistribution of nanoparticles with Cy7 was determined with an *in vivo* imaging system (Optix) in CIA mice. Uptake in the RAW and LYM1 cell lines was lower and slower with stealth nanosteroid (24 hours) than with non-stealth nanosteroid without PEG (3 hours). With stealth nanosteroid nanoparticles blood circulation time was markedly higher (24 hours) and liver uptake was lower (20%) than with non-stealth nanosteroid nanoparticles (5 minutes and 70%, respectively). Furthermore, stealth nanoparticles specifically accumulated in inflamed joints in AbIA mice and remained for at least 1 week. In AA rats, the highest anti-inflammatory activity was exhibited by stealth nanosteroid nanoparticles composed of 80% PLA (molecular weight, 6,200) and 20% PEG-PLA (68: 32; molecular weight, 10,000) with a diameter of 115 nm, encapsulation efficiency of 7.1%, modest PEG density, slower release rate, and lower cellular uptake; a 35% decrease in paw inflammation was obtained in 1 day and maintained for 2 weeks with a single injection of 30 μg of this stealth nanosteroid. In AbIA mice, a single injection of 3 μg

of this stealth nanosteroid resulted in complete resolution of the inflammatory response after 1 week. In contrast, non-stealth nanosteroid and free BP did not reduce the severity of inflammation with the same dose in both models. The observed strong therapeutic benefit obtained with the stealth nanosteroid may be due to the prolonged blood circulation, to the targeting of the inflamed joint, and to its slow release *in situ*.

$CaCO_3$ nanoparticles with insulin

This study evaluated the pharmacokinetic and pharmacodynamic effects of a transdermally delivered insulin using novel $CaCO_3$-nanoparticles in normal and diabetic mice. The $CaCO_3$-nanoparticle encapsulating insulin (nanoinsulin) was transdermally applied to the back skin of normal ddY mice and diabetic dB/dB and kkAy mice after 1 hour of fasting. Serum insulin levels in ddY mice were analyzed with enzyme immunoassay, and blood glucose levels in normal and diabetic mice were monitored with a transdermal sensor (Diasensor, Diasense, Inc., Midland, TX, USA). Maximum serum insulin was 67.1 ± 25.9 μIU/ml at 4 hours with 200 μg of transdermal nanoinsulin in ddY mice, whereas that after subcutaneous injection of 3 μg of monomer insulin was 462 ± 20.9 μIU/m at 20 minutes. Transdermal nanoinsulin decreased glucose levels in a dose-dependent manner. Maximum decreases in blood glucose with 200 μg of transdermal nanoinsulin observed after 6 hours were 48.3 ± 3.9% (ddY), 32.5 ± 9.8% (dB/dB), and 26.2 ± 7.6% (kkAy), whereas maximum decreases observed after 1 hour with 3 μg of subcutaneous monomer insulin were 64.1 ± 1.0% (ddY), 57.9 ± 3.4% (dB/dB), and 24.1 ± 6.7% (kkAy). Insulin bioavailability until 6 hours with transdermal nanoinsulin in ddY mice was 0.9% based on serum insulin levels and 2.0% based on pharmacodynamic blood-glucose-lowering effects. This $CaCO_3$-nanoparticle system successfully delivered insulin transdermally, as evidenced by a significant sustained decrease in blood glucose in normal and diabetic rats. These results support the feasibility of developing transdermal nanoinsulin for human applications.

In collaboration with other institutions, we developed an immunosensor using a quartz crystal microbalance to detect dioxin, after preparing monoclonal antibody and single-chain variable fragments against tetrachlorodibenzodioxin.

Publications

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