Local Drug Delivery System: Inhibition of Inflammatory Angiogenesis in a Murine Sponge Model by Dexamethasone-Loaded Polyurethane Implants

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ABSTRACT: Implants are defined as controlled sustained release delivery systems of therapeutic agents incorporated or dispersed into a polymeric carrier. These systems can be implanted in specific organs and delivered by the therapeutic agents at the target site to treat various pathological processes. In the present study, the effects of dexamethasone-loaded polyurethane implants [PU ACT (dexamethasone acetate) implants] on inflammatory angiogenesis in a murine sponge model were investigated. PU ACT implants were inserted into nonbiocompatible sponges, used as a framework for fibrovascular tissue growth, and implanted into subcutaneous tissue located on the back of mice. After 7 days of implantation, the implant system was collected and processed for the assessment of hemoglobin (Hb; vascular index), myeloperoxidase (MPO), and *N*-acetyl-β- D-glucosaminidase (NAG; inflammatory enzymes activities) and collagen content. ACT released from the polymeric implants provided a significant decrease in the neovascularization in the sponge (Hb content). PU ACT implants provided no effects on neutrophil infiltration (MPO activity) but macrophage recruitment was affected by the glucocorticoid delivered by implants (NAG activity). ACT released from implants was able to reduce the collagen deposition. The qualitative histological findings corroborated with the measured biochemical parameters. These local drug delivery systems derived from polyurethane efficiently modulated the key components of inflammation, angiogenesis, and fibrosis induced by sponge discs in an experimental animal model. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:2886–2895, 2011

Keywords: polyurethane; implants; inflammatory angiogenesis; murine sponge model; local drug delivery system; targeted drug delivery; polymeric drug delivery system; controlled release/delivery; biodegradable polymers; biomaterials

INTRODUCTION

Glucocorticoids are anti-inflammatory drugs extensively used in the treatment of severe inflammatory diseases. In addition to their anti-inflammatory action, these steroids are also immunosuppressive and antiangiogenic drugs.[1] Their therapeutic effects are considered to be mediated by different mechanisms of action, including the genomic and nongenomic mechanisms caused by the cytosolic and membrane-bound glucocorticoid receptors, respectively.[2–4]

Despite the therapeutic efficacy of steroidal treatment, the long-term systemic administration of these drugs leads to a number of well-characterized clinical side effects, such as brittle skin,[5] muscle weaknesses, osteoporosis,[6] fat redistribution,[7] diabetes,[8]

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and neurodegeneration or suppressed neurogenesis in the brain.[9] The development of targeted delivery systems of these drugs represents a therapeutic alternative, with fewer side effects, to effectively control inflammation and immunosuppressive responses, as well as angiogenesis.[10]

Implants are controlled sustained-release delivery systems of therapeutic agents incorporated or dispersed into a biocompatible carrier, usually a nonbiodegradable or biodegradable polymer. These implantable biomedical devices present several advantages, including (1) the overcoming of the natural membrane barriers, allowing drugs at therapeutic levels to be directly delivered to the targeted site; (2) prolonged drug delivery; and (3) reduction of side effects frequently observed with systemic administration.[11–13] A number of studies have demonstrated the efficacy of these drug delivery systems in suppressing inflammation and angiogenesis in experimental animal models. For example, implants derived from natural or synthetic polymers incorporated with glucocorticoids, antimetabolites, or bioactive molecules were developed and proved to be therapeutically effective at suppressing/minimizing the experimental chronic uveitis and rheumatoid arthritis induced in animals.[14–19]

Recently, implants were developed based on dexamethasone, a potent glucocorticoid, and biocompatible and biodegradable polyurethane, derived from polycaprolactone and/or polyethylene glycol (PEG), as soft segments, and isophorone diisocyanate (IPDI) and hydrazine (HZ), as hard segments.[20] This polyurethane was developed by producing a water dispersion of the polymer, followed by a drying step. This procedure of synthesis avoided the use of organic and toxic solvents usually employed in the formation of polylactic acid (PLA) and polyglycolic acid (PLGA) films. The polyurethane was also produced using low-cost raw materials and mild processing conditions that can result in a biomaterial less expensive than the PLA family of polymers for biomedical applications. The raw materials used as precursors of the polyurethane were carefully selected, so that the hydrolytic degradation process was favorable, and the probable degradation products were noncytotoxic and soluble in water.[21] Besides the intrinsic advantages of the synthesized polyurethane, there are some advantages of the implants based on this polymer. Dexamethasone-loaded polyurethane implants [PU ACT (dexamethasone acetate) implants] led to a controlled *in vitro* and *in vivo* release of the drug over a prolonged period. The mechanism of drug release involved the simultaneous erosion of the polyurethane and the diffusion of the dexamethasone through the polymeric matrix. During the release study, liberation burst of the dexamethasone from the polyurethane implants was not observed. In general, the release profile of drugs incorporated into biodegradable PLA or PLGA implants have a triphasic release pattern: an initial burst, a second stage that is derived from diffusional release before the onset of polymer erosion and swelling, and a sudden burst resulting from swelling and disintegration of the polymeric matrices. The liberation burst of the drug from theses biodegradable polymers may cause an adverse effect due to the release of an overdose of the drug.[22,23] Finally, the obtained implants, consisting of biodegradable polyurethane, did not have to be removed as they are degraded and absorbed or eliminated from the body. This reduces the need for a new surgery for implant removal after complete drug release, which can increase the patient's compliance with the treatment. But, contrary to biodegradable implants, nonbiodegradable devices must be removed after complete drug release, which can represent risks to the patient as well as a disadvantage of these systems.[11,12]

In the present work, the efficacy of these PU ACT implants in minimizing the inflammatory response and angiogenesis in a murine sponge model was evaluated. The sponge-induced inflammatory angiogenesis consists of the implantation of a nonbiocompatible synthetic polymer in the subcutaneous or intraperitoneal sites in mice.[24] This model elicits a spatial and temporal circumscribed tissue response within the matrix, allowing for the definition of the sequence of histological changes in the granulation tissue formation, the assessment of cellular proliferation kinetics, and the quantitation of various components in the inflammatory angiogenic process.[25–27] Therefore, the therapeutic efficacy of this local drug delivery system was demonstrated by monitoring local inflammation changes, an angiogenesis index, and fibrogenic responses, by implementing markers to represent these activities in the group of animals that received these drug delivery systems.

Experimental

Synthesis of the Aqueous Polyurethane Dispersion

Aqueous polyurethane dispersion (PUD) was prepared by a prepolymer mixing process, using a 250 mL three-neck glass flask equipped with a heating mantel, a mechanical stirrer, and a thermometer. The macrodiol components of polycaprolactone diol (PCL 1000; Tone Polyol 2221, $\overline{M_n}$ = 1000 g·mol^{-1}; Dow Chemical Company, Midland, Michigan, USA), PCL 2000 (Tone Polyol 0249, $\overline{M_n}$ = 2000 g·mol^{-1}; Dow Chemical Company, Midland, Michigan, USA), PEG 1500 ($\overline{M_n}$ = 1500 g·mol^{-1}; Sigma–Aldrich, Seelze, Germany), IPDI (Bayer, Barmen, Germany; NCO/OH ratio of 2.3), and 2, 2-*bis*(hydroxymethyl) propionic acid (98.3%, Fluka Chemical Corp, Milwaukee, Wisconsin, USA) were added to the reactor in the