Biocompatibility and *In Vivo* Tolerability of a New Class of Photoresponsive Alkoxylphenacyl-Based Polycarbonates

DANIEL WEHRUNG,[1] SHUANGYI SUN,[2] ELAHEH A. CHAMSAZ,[2] ABRAHAM JOY,[2] MOSES O. OYEWUMI[1]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, Northeast Ohio Medical University, Rootstown, Ohio 44272

[2]Department of Polymer Science, The University of Akron, Akron, Ohio 44325

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ABSTRACT: Potential toxicities of chromophoric or polymeric units of most photoresponsive delivery systems have impacted clinical relevance. Herein, we evaluated the biocompatibility and tolerability of alkoxylphenacyl-based polycarbonates (APPs) as a new class of photoresponsive polymers. The polymers were applied as homopolymer or copolymers of polyethylene glycol (10%, w/w) or polycaprolactone (10%, w/w). APP polymers were comparable to poly(lactic-co-glycolic acid) (PLGA) based on cytotoxicity, macrophage activation, and blood compatibility. Data from biodistribution studies in BALB/c mice showed preferential accumulation in kidney and liver. Meanwhile, potential application of APP polymers as immediate or sustained (implants) drug delivery systems indicated that liver and kidney functions were not distorted. Also, plasma levels of tumor necrosis factor-alpha and interleukin-6 were comparable to PLGA-treated mice ($p > 0.05$). A histological analysis of liver and kidney sections showed no detectable damage for APP polymers. The overall data strongly supported potential consideration of APP polymers as photoresponsive delivery systems especially as implantable or tissue-mimicking photopatterned biomaterials. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:1650–1660, 2013

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INTRODUCTION

The advent of "smart" biomaterials has afforded the capability of designing and developing delivery systems that are responsive to various stimuli such as pH, temperature, light, ultrasound, redox, or chemical changes.[1–3] As such, smart delivery systems can offer great therapeutic advantages over traditional systems in which release of active/diagnostic agents can be controlled according to disease-specific (proximal) or nondisease-specific (external) stimuli.[4] In general, proximal stimuli (disease specific) such as local pH, temperature, or enzyme expression levels require physical interaction with delivery systems for activation, whereas external stimuli (nondisease specific) such as magnetic fields, ultrasound, or light can be operated remotely without the need for physical interaction.[5,6] A major concern for systems that rely on proximal stimuli (disease specific) is that the levels of these triggers can fluctuate widely from patient to patient and even within the same patient at different times or stages of the disease.[7–9] Variations in levels of proximal stimuli could translate into poor reproducibility of therapeutic effectiveness. Meanwhile, systems that utilize external triggers (stimuli) are amenable to many disease cases because stimuli responsiveness is not dependent on differences in biological processes between disease tissue and healthy tissue.[9,10] We are of the opinion that delivery systems that operate on external stimuli will ensure consistent responsiveness when applied alone or in combination with proximal responsive systems. Of special interest to this work are photoresponsive delivery systems that are able to achieve spatial and temporal release of therapeutic payload through remote activation. As such, the trigger can be precisely controlled through manipulation of wavelength, intensity, and duration of the light.[11]

To date, the vast majority of work regarding photoresponsive materials has focused on the

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Correspondence to: Moses Oyewumi (Telephone: +330-325-6669; Fax: +330-325-5936; E-mail: moyewumi@neomed.edu)

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photochemistry of different chromophores, whereas little attention has been paid to biocompatibility and biodegradability of chromophoric and polymeric units. It is noteworthy that photoresponsive polymers that are biocompatible and biodegradable are highly desirable for potential translation into clinical applications. In this regard, we recently reported, for the first time, the synthesis of alkoxylphenacyl-based polycarbonates (APPs) as a new class of photoresponsive polymers.[12] APP polymers responded efficiently to light between 270 and 320 nm, which resulted in controlled chain scission and photoresponsive release of incorporated payloads.[12] Another important earlier observation is the demonstration of thermal and mechanical stability of APP polymers, which is considered as desirable qualities in designing and developing implantable delivery systems while guarding against premature leakage of incorporated therapeutic agents. The potential biodegradable nature of these alkoxylphenacyl-chromophore-containing polycarbonates will be relevant in the context of a drug delivery platform. These aforementioned advantages prompted the current investigation of detailed biocompatibility and *in vivo* tolerability assessments of APP polymers as important aspects of consideration as photoresponsive drug delivery systems. The studies were carried out using the APP homopolymer as well as copolymers with polyethylene glycol (PEG-10%, w/w) and polycaprolactone (PCL-10%, w/w). *In vitro* assessments were based on cytotoxicity, macrophage activation, and blood compatibility. Particular attention was paid to biodistribution, blood biochemical parameters, and histopathological examination of tissue sections after intravenous (i.v.) or subcutaneous (s.c.) injection (implants) in BALB/c mice. The study included biocompatibility and tolerability assessment of APP polymers before or after photoirradiation (activation) with the understanding that the studies on APP polymer (without photoactivation) will be relevant in potential applications as implantable or photopatterned biomaterials.

MATERIALS AND METHODS

Materials

Phosphate-buffered saline (PBS), Pluronic® F-127, dimethyl sulfoxide (DMSO), and dialysis tubing [molecular weight cut off (MWCO, 12,000 Da] were obtained from Fisher Scientific (Pittsburgh, Pennsylvania). Poly(lactic-co-glycolic acid) (PLGA; 50:50) was obtained from Boehringer Ingelheim (Ingelheim, Germany). Kits to measure alanine aminotransferase (ALT) and creatinine were obtained from Teco Diagnostics (Anaheim, California) and Invitrogen (Grand Island, New York), respectively. eBioscience (San Diego, California) provided the kits for measuring cytokine levels. Thiazolyl blue tetrazolium blue (MTT) dye, lipopolysaccharides (LPS), and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) were obtained from Sigma–Aldrich (St. Louis, Missouri). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, Georgia).

Synthesis of APP Polymers

The synthesis of the APP polymer (homopolymer and copolymers) was carried out based on the method described earlier.[12] Briefly, hydroxyacetophenone was chain extended with hydroxyl terminated spacer. The product was converted to the corresponding bromide without purification and then reacted with sodium acetate to yield the acetate-protected derivative in high yield. Following deprotection, the alkoxylphenacyl diol was reacted with triphosgene to provide the corresponding polycarbonates. The homopolymer (APP-H) and copolymer with PEG (APP–PEG) or PCL (APP–PCL) were synthesized and purified accordingly.[12]

APP Nanoparticle Preparation

Nanoparticles were prepared from APP polymers (APP-NPs) using a modified nanoprecipitation method.[13] In the process, the polymers (2 mg/mL) were solubilized in 0.25 mL of DMSO, and then added dropwise into 1 mL of a 1%–5% (w/v) solution of Pluronic® F-127 (Fischer Scientific) at 40°C under vigorous stirring. In all, APP-NPs were purified by dialysis against water (MWCO = 12,000 Da). APP-NPs were characterized based on size, size distribution, and stability in biological relevant media. Fluorescent-labeled APP-NPs were prepared to contain coumarin-6 according to our earlier reported method.[14] Additional assessment on fluorescent-labeled APP-NPs included the determination of entrapment efficiency and retention of coumarin-6 within APP-NP matrix after incubation in 10% FBS in PBS (pH 7.4; 37°C). Using the same procedure, reference NPs were prepared with PLGA (PLGA-NPs).

Characterization of APP-NPs

Particle Size

Sizes of the NPs were measured using Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, United Kingdom) at 25°C. Prior to particle measurements, NP suspensions were diluted (1:5, v/v) with filtered water (0.22 μm filter; Nalgene International, Rochester, New York) to ensure that light scattering signals are within the sensitivity of the instrument.

Entrapment Efficiency of Fluorescent Dye

Microcon Ultracel YM-100 centrifugal devices (MWCO = 100 kDa; Millipore, Billerica, Massachusetts) were used to determine entrapment efficiencies. Briefly, 400 μL of NP suspensions was added