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Improved intervention of atherosclerosis and cardiac hypertrophy through biodegradable polymer-encapsulated delivery of glycosphingolipid inhibitor

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ABSTRACT

D-Threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP), a glycosphingolipid synthesis inhibitor, holds promise for the treatment of atherosclerosis and cardiac hypertrophy but rapid *in vivo* clearance has severely hindered translation to the clinic. To overcome this impediment, we used a materials-based delivery strategy wherein D-PDMP was encapsulated within a biodegradable polymer composed of poly ethylene glycol (PEG) and sebacic acid (SA). PEG-SA was formulated into nanoparticles that were doped with ^{125}I-labeled PEG to allow *in vivo* bio-distribution and release kinetics of D-PDMP to be determined by using γ-scintigraphy and subsequently, by mass spectrometry. Polymer-encapsulation increased the residence time of D-PDMP in the body of a treated mouse from less than one hour to at least four hours (and up to 48 h or longer). This substantially increased *in vivo* longevity provided by polymer encapsulation resulted in an order of magnitude gain in efficacy for interfering with atherosclerosis and cardiac hypertrophy in apoE−/− mice fed a high fat and high cholesterol (HFHC) diet. These results establish that D-PDMP encapsulated in a biodegradable polymer provides a superior mode of delivery compared to unconjugated D-PDMP by way of increased gastrointestinal absorption and increased residence time thus providing this otherwise rapidly cleared compound with therapeutic relevance in interfering with atherosclerosis, cardiac hypertrophy, and probably other diseases associated with the deleterious effects of abnormally high glycosphingolipid biosynthesis or deficient catabolism.

1. Introduction

Atherosclerosis and cardiac hypertrophy are the leading causes of death worldwide. To date, methods to treat these diseases – such targeting cholesterol using statins and cholesterol absorption inhibitors (ezetimibe) – have not substantially improved outcomes. Lowering glycosphingolipid (GSL) load offers an attractive alternative method for preventing and interfering with atherosclerosis and cardiac hypertrophy, since accumulation of cholesterol, triglycerides and GSLs contribute to the plaque formation that leads to these conditions [1–3]. The promise of the widely used inhibitor of GSL synthesis, D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP), is evident in our recent study were we showed that this compound could ***slow*** and in some cases actually ***prevent*** the progression of atherosclerosis in a rodent model [4]. Unfortunately, D-PDMP is rapidly metabolized by the cytochrome P450 system and cleared from the body in less than an hour severely compromising the *in vivo* effectiveness of D-PDMP. As a result, the efficacy of this potential drug to the more stringent task of ***reversing*** already present atherosclerotic tissue or extending this treatment approach to additional endpoints such as cardiac hypertrophy is severely hindered. The current report describes a polymer-based approach that overcomes the poor pharmacological

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properties of D-PDMP and thereby improves this drug candidate's *in vivo* activity.

Although the concept of encapsulating drugs in biomaterials to improve their *in vivo* delivery is a decades-old concept [5], many challenges inherent in implementing this strategy have slowed its widespread adoption. For example, non-immunogenic materials that could be reproducibly molded into particles or wafers of defined sizes and shapes – while at the same time accommodating a robust "payload" of drug – had to be developed. Over the past decade, many pertinent technical issues have been overcome (as reviewed elsewhere, [6,7]), setting the stage for a materials-based strategies for treating prevalent human ailments such as cardiovascular disease [8,9]. In particular, in this study we extended biodegradable and biocompatible sebacic acid – poly(ethylene glycol) (SA-PEG) copolymers as drug delivery vehicles [10–12] by (1) successfully encapsulating D-PDMP for controlled release, (2) controlling the size of biomaterial particles to approximately 100 nm for release over a multi-hour time scale, and (3) incorporating radioactive tracers into the material for *in vivo* tracking. The success of this strategy in increasing the *in vivo* longevity of encapsulated D-PDMP and thereby dramatically enhancing this compound's efficacy in reversing atherosclerosis and cardiac hypertrophy was demonstrated using an animal model consisting of apoE−/− mice fed a high fat and high cholesterol (HFHC) diet [13] and an extensive set of biomarkers indicative of cardiovascular disease.

2. Materials and methods

D-PDMP was purchased from Matreya LLC (Pleasant Gap, PA). All other chemicals were purchased from Sigma–Aldrich (St. Louis, MO) unless mentioned otherwise.

2.1. Preparation of polymer-encapsulated D-PDMP

Poly(ethylene glycol) sebacic acid co-polymer (PEG-SA) was prepared following the procedure published by Fu and coworkers [10]. Briefly, sebacic acid prepolymer was made by refluxing sebacic acid (SA) in acetic anhydride followed by drying under high vacuum (evaporation), crystallized from dry toluene, washed with 1:1 anhydrous ethyl ether-petroleum ether and finally air dried. PEG prepolymer was made by refluxing of polyoxyethylene dicarboxylic acid in acetic anhydride, volatile solvents were removed under vacuum. The solid mass was extracted with anhydrous ether and air dried. The poly(PEG-SA) co-block polymer was then synthesized by the melt polycondensation method and characterized by proton NMR [10]. Note that this copolymer was used before in our laboratory and have been extensively characterized for the composition and structural identity [14].

Encapsulation of D-PDMP in poly(PEG-SA) (to prepare polymer-encapsulated drug subsequently referred to as BP-D-PDMP, with the "BP" standing for biodegradable polymer) followed by the melt polycondensation method described above for SA and PEG prepolymers but with the inclusion of D-PDMP at starting ratios of poly(PEG-SA) to D-PDMP of 70:30 by weight. Subsequently, microparticles were prepared using a single emulsion solvent evaporation method [10]. Briefly, D-PDMP and poly(PEG-SA) were dissolved in chloroform (50 mg/mL) and emulsified into a 1.0% w/w poly(vinyl alcohol) aqueous solution under sonication condition keeping the temperature below 25 °C. Particles were hardened by allowing chloroform to evaporate at room temperature while stirring for 12 h. Particles were collected and washed three times with double distilled water via centrifugation at 2600× g (30 min) and lyophilized for 48 h before use.

2.2. Preparation of [^{125}I]-BP-D-PDMP and imaging and metabolic experiments

2.2.1. Radiolabeling the SA-PEG co-polymer

L-Tyrosine (20 mg, 0.14 mmol) (Sigma Aldrich, St. Louis, MO) was introduced to 45 mCi (810 kBq) of [^{125}I]NaTyrosine in 100 mL of PBS in a glass vial containing plated Iodogen (Pierce, Rockford IL USA). The radioiodinated reaction proceeded at room temperature for 12 min before withdrawing the supernatant. The supernatant was then added to 100 mg of *O,O′*bis[2-(*N*-succinimidal-succinylamino) ethyl]polyethylene glycol (Sigma Aldrich, St. Louis MO USA) and this mixture was allowed to sit at room temperature for one hour. After one hour, the reaction mixture was then loaded onto a PBS-conditioned G25 Sephadex size-exclusion column (Pierce, Rockford, USA) to remove any unreacted iodide and free tyrosine. The absence of free radioiodine and tyrosine in the elute was confirmed using ITLC (Gelman strips, Vernon Hills IL USA) in ACD buffer (Sigma–Aldrich, St. Louis MO USA). Finally, the labeled PEG was incorporated into poly (SA-PEG) polymer.

2.2.2. In vivo γ-scintigraphy of ^{125}I labeled polymer

Drug-loaded polymer [45 mCi (810 kBq) of the ^{125}I-labeled samples] was orally administered by gavage to each of three wild type, C57bl/6 adult male mice. The mice were anesthesized using 2.5% isoflurane gas in oxygen delivered via tent. The mice were lined up side-by-side directly over a high-resolution parallel hole collimator in an X-SPECT SPECT-CT scanner (GammaMedica Ideas, North Ridge CA USA). Scans consisted of several 10 min acquisitions through two hours post-tracer administration with a CT scan followed by additional acquisitions as indicated with accompanying CT scans (512 slice, 50 keV beam). The data were reconstructed using the manufacturer's software and co-registered using AMIDE (http://www.sourceforge.net). All scintigraphy images are displayed on the same scale.

2.3. Animals and treatments

Apolipoprotein E-deficient ApoE-/-, male mice aged 12 weeks (Jackson Labs, Bar Harbor, ME) were used. At the age of 12 weeks, the ApoE-/- mice were placed on a high fat and high cholesterol diet (HFHC) of 4.5 kcal/g, 20% fat and 1.25% cholesterol (D12108C, Research Diet Inc., New Brunswick, NJ) until 20 weeks of age. This time point was chosen so as to allow for adequate atherogenesis prior to therapeutic intervention. A control group of mice on normal diet consisting only of chow food was used for comparison.

Starting at 20 weeks, mice on the HFHC diet were treated with unconjugated D-PDMP at 10 mg/kg (mpk) and with 1 and 10 mpk of polymer-encapsulation drug and compared to controls fed normal chow diet or placebo fed with HFHC + Vehicle (100 μL of 5% Tween 80 in PBS) for another 16 weeks. A sample size of n = 5–6 subjects per group was designated.

During D-PDMP treatment, vehicle control, unconjugated, and polymer-encapsulated drug was delivered daily by oral gavage. During treatment, the mice were supplied with a known quantity of food on a weekly basis allowing food intake to be estimated; the growth rate of the animals was also monitored on a weekly basis. Physiological studies and tissue harvest were performed around 12 and 36 weeks for molecular and histopathological studies. The 20 week time point was designed for the start of treatment because we have found that mice show significant plaque accumulation, significant increase in ascending aortic intimae media thickness (IMT_AsAo) and vascular stiffness using histopathology, ultrasound and pulse wave velocity (PWV), respectively at this time point (unpublished studies).