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Lipid and polymer blended polyester nanoparticles loaded with adapalene for activation of retinoid signaling in the CNS following intravenous administration

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ABSTRACT

Small molecule retinoids are potential therapeutics for a variety of neurological diseases. However, most retinoids are poorly water soluble and difficult to deliver *in vivo*, which prevents further study of their utility to treat disease. Here, we focus on adapalene, an FDA approved drug that is a specific agonist for the retinoic acid receptor β (RARβ). We sought to develop nanoparticle delivery systems that would enable effective delivery of adapalene to the CNS. We developed strategies to produce nanoparticles based on the hypothesis that incorporation of hydrophobic molecules into a polyester base would improve adapalene loading. In the first scheme, poly (lactic acid)-poly (ethylene glycol) (PLA-PEG) was blended with low molecular weight poly (lactic acid) (PLA) or poly (caprolactone) (PCL). In the second scheme, poly (lactic-*co*-glycolic acid) (PLGA) was blended with 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino (polyethylene glycol) (DSPE-PEG). Our data demonstrate that blending low molecular weight polyesters or DSPE-PEG into the primary nanoparticle base improves encapsulation of adapalene, presumably by enhancing adapalene solubility in the nanoparticle. Peripheral administration of these nanoparticles activated retinoid signaling in the brain and spinal cord of healthy mice. These studies provide new approaches for nanoparticle fabrication and establish proof of principle that systemically administered, adapalene-loaded nanoparticles activate retinoid signaling in the CNS.

1. Introduction

Retinoic acid (RA) signaling has long been studied for its role in central nervous system (CNS) development but has more recently garnered attention as being essential for maintenance of the adult CNS, as well, with functions including synaptic plasticity, neuronal repair, and modulation of neuroinflammation [24]. Members of the retinoic acid signaling pathway have been demonstrated to be altered by disease, trauma, or other stressors, including vitamin A deprivation [2,16]. Considering the multitude of ways that RA signaling supports CNS homeostasis, it is not surprising that these signaling changes have functional consequences. For example, dietary deprivation of vitamin A or mutation of RA receptors produces defects in spatial learning and memory [24]. RA signaling has also been implicated in regeneration and reinnervation after peripheral nerve damage. For example, genes encoding enzymes that are important for RA synthesis and receptor expression are increased following nerve crush, and enhanced RA receptor expression, specifically the retinoid acid receptor β (RARβ), has been demonstrated to improve neurite outgrowth and neuronal regeneration [8,40]. In addition, dysregulation of RA signaling pathway has been directly linked to neurodegeneration. Vitamin A deprivation in rats produces a motor neuron disease phenotype that resembles amyotrophic lateral sclerosis (ALS) with pathology that includes neuroinflammation and neuronal loss in the spinal cord [5]. Furthermore, gene expression and proteomic studies have found evidence that members of the RA signaling family are dysregulated in Alzheimer's, Parkinson's and ALS patients [16,18,25,26]. Studies in rodent models of neurodegeneration have also demonstrated beneficial effects of RA or retinoid supplementation in various mouse models of neurodegeneration [7,34]. This existing body of work highlights the therapeutic potential of targeting retinoid signaling for the treatment of neurological diseases.

Here, we focus on retinoid signaling via the action of the small molecule adapalene, an agonist of RARβ. Adapalene is a third-

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generation, poly-aromatic retinoid that is currently FDA approved for the treatment of acne and cervical neoplasia. Adapalene has been shown to promote cellular differentiation and have anti-inflammatory effects in multiple *in vitro* models [31]. We previously demonstrated that adapalene was neuroprotective *in vitro* against oxidative stress [18]. However, like most retinoids, adapalene is poorly water soluble and cannot be administered *in vivo* in free form, which prevents further study.

The goal of the studies described here was to develop an approach that would enable activation of retinoid signaling in the CNS after intravenous administration. To reach this goal, we focused our effort on the encapsulation of adapalene within polymeric nanoparticles. Polymeric nanoparticles have been demonstrated to improve circulation, delivery and efficacy of a range of insoluble drugs by us and others in a variety of disease models [4,13,14,42]. We previously demonstrated that peripherally circulating nanoparticles are capable of depositing lipophilic payloads in the brain even when the nanoparticles have not been designed for blood brain barrier (BBB) passage [4,29]. The well-established ability of circulating nanoparticles to improve drug bioavailability in the CNS supports our rationale for using nanoparticles to deliver adapalene to the brain. However, our initial attempts to encapsulate adapalene within polymeric nanoparticles resulted in poor loading. We hypothesized that incorporation of hydrophobic molecules into a polyester nanoparticle base would improve loading of adapalene. Two approaches were utilized to test this hypothesis: first, poly (lactic acid) − poly (ethylene glycol) (PLA-PEG) was blended with short chain poly (lactic acid) (PLA) or poly (caprolactone) (PCL) (*blended nanoparticles*), and, second, high molecular weight poly (lactic-*co*-glycolic acid) (PLGA) was blended with lipidated PEG (*lipid-polymer hybrid nanoparticles*). We focused first on developing PLA-PEG nanoparticles due to their established history of use in preclinical and clinical studies. Lipid-polymer PLGA hybrid nanoparticles were developed as a contrast to traditional PLA-PEG nanoparticles, since they harness the advantages of using a polyester base (e.g. high structural integrity, stability in storage, and biocompatibility) and liposomes (improved encapsulation and release characteristics). Lipid-polymer hybrid nanoparticles are composed of polymer core (here, PLGA) and an outer lipid layer (here, lecithin and 1,2,-distearoly-*sn*-glycero-3-phosophoethanolamine-N-carboxy (polyethylene glycol) (DSPE-PEG) [3].

The data described here provide biophysical characterization of a novel library of PLA-PEG and PLGA nanoparticles, demonstrating that blending polymers and lipids into the nanoparticle base improves encapsulation of adapalene. These studies also demonstrate that nanoparticle encapsulated adapalene (Adap-NP) retains bioactivity, as evidenced by activation of retinoid signaling in the CNS when Adap-NPs are administered intravenously to healthy mice. Importantly, the polymer and lipid blending approaches described here are not specific to adapalene, highlighting that this work is expected to be relevant to encapsulation of any number of small, hydrophobic drugs in solid polymeric nanoparticles.

2. Materials and methods

Adapalene, acetonitrile, dichloromethane (DCM), dimethyl sulfoxide (DMSO), ethanol, Dulbecco's Phosphate Buffered Saline (PBS), potassium ferricyanide, potassium ferrocyanide, sodium cholate, and sodium deoxycholate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ester terminated poly (lactic-*co*-glycolic acid) (50:50; inherent viscosity = 0.59 dL/g) (PLGA) was purchased from Lactel (Birmingham, AL, USA). MPEG-P (D,L)LA (MW 5,000:16,000 Da) (PLA-PEG) and poly (caprolactone) (MW 1,000–5,000 Da) (PCL) were purchased from Akina Inc (West Lafayette, IN, USA). Soybean Lecithin was purchased from MP Biomedicals (Solon, OH). DSPE-PEG was purchased from Avanti (Alabaster, AL). Slide-A-Lyzer Dialysis Cassettes (MWCO 5,000Da) were purchased from Thermo Fisher Scientific (USA).

2.1. Nanoparticle fabrication

Blended PLA/PLA-PEG or PCL/PLA-PEG nanoparticles were synthesized by single emulsion-solvent evaporation [4,13,29]. Briefly, 50 mg of polymer (PLA-PEG:PCL ratios of 1:0, 9:1, 8:2, 6:4) and 2 mg of adapalene were dissolved in 2 mL of dichloromethane (DCM). The dissolved polymer and drug were added dropwise into 4 mL of a 1% w/v sodium cholate aqueous solution while rapidly vortexing. Emulsions were probe sonicated on ice for three, 10 s bursts at 40% amplitude. The resulting emulsion was dispersed in a 20 mL 0.3% w/v sodium cholate and gently stirred for 3 h to evaporate solvent. Formulation steps were performed either at room temperature or with solutions held on ice water.

Lipid-polymer hybrid nanoparticles were synthesized by a nanoprecipitation technique [3] with modifications. 50 mg of PLGA and 2 mg of adapalene were dissolved in 10 mL of acetonitrile. A 10 mL aqueous solution was prepared by dissolving lecithin and DSPE-PEG (7:3 M ratio) in ethanol prior to diluting with water to a final concentration of 4% EtOH and 1 mg/mL of lipid. Dissolved PLGA was poured into the aqueous volume while stirring, and solvent was evaporated under a stream of air for 2 h.

Following solvent evaporation for both nanoparticle types, solutions were passed through a 0.22μm sterile bottle top filter to remove large drug aggregates. Particles were further washed and concentrated with Amicon Ultra-15 Centrifugal filters (MWCO 100k). All formulations were prepared in triplicate.

2.2. Adapalene loading quantification

Percent loading was quantified by comparing fluorescent intensity of samples dissolved in DMSO to control curves. Control curves were constructed by dissolving non-loaded particles in DMSO (1 mg/mL) and spiking 50 μL with 10 μL of a series of adapalene dilutions (final concentration range of 9–167 μg/mL). Samples were plated on black flat-bottom 96 well plates in triplicate for technical repeats. All control and nanoparticle samples were acidified by adding 10 μL of 10 mM HCl, which we observed selectively enhanced adapalene fluorescence above polymer background to improve detection sensitivity. Fluorescent intensity was measured at excitation/emission wavelengths of 360/420 nm, determined by excitation/emission scans resulting in the greatest fluorescent intensity on a Tecan microplate reader.

2.3. Nanoparticle characterization: yield, percent loading, encapsulation efficiency

Nanoparticle aliquots were lyophilized and weighed to determine percent drug loading (DL), batch yield, and encapsulation efficiency (EE). The control curves were used to determine the amount of adapalene per 1 mg/mL of lyophilized nanoparticles to measure drug DL [38]; $\mathrm{DL} = \frac{Adap\ (mg)}{1\ (mg)\ lyophilzed\ nanoparticle} X\ 100$. Yield was calculated as a function of polymer output relative to polymer input; $\mathrm{Yield} = \frac{Initial\ polymer\ (mg) - Polymer\ mass\ after\ fabrication\ (mg)}{Initial\ polmer\ (mg)} X\ 100$. Encapsulation efficiency was calculated by determining the amount of adapalene encapsulated in nanoparticles after fabrication relative to initial adapalene added to emulsion; $\mathrm{EE} = \frac{Initial\ adapalene\ (mg) - adapalene\ \ after\ fabrication\ (mg)}{Initial\ adapalene\ \ (mg)} X\ 100$

2.4. Size and zeta potential

Hydrodynamic radius, polydispersity index, and zeta potential was measured with a Nanobrook 90 Plus Zeta instrument (Brookhaven) on samples suspended in 1 mM KCl at a concentration of 1 mg/mL.

2.5. Controlled release

Adapalene release profiles were evaluated by dialyzing

nanoparticles against 2 L of 1X PBS at 37 °C. All nanoparticle formulations were diluted to an adapalene concentration of 0.1 mg/mL, and 400 μL of this dilution was added to Slide-A-Lyzer Dialysis Cassettes with a MWCO of 3,500 Da. At pre-determined time points (0, 1, 2, 4, 6, 24, 48, 72, 144, 168 Hr), 10 μL samples were removed from dialysis cassettes and dissolved in 190 μL of DMSO. Because we removed solutions from the source compartment, not the sink compartment, it was important to maintain concentration, and we did not replace the samples with additional PBS. Samples (50μL) were plated in triplicate on a black, flat-bottom 96-well plate. The concentration of adapalene at each time point was quantified as described above.

2.6. In vivo bioactivity

All procedures and animal care practices were performed in accordance with the Barrow Neurological Institute's Institutional Animal Care and Use Committee, in agreement with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) (OLA assurance number A3519-01). Bioactivity of adapalene-loaded nanoparticles in the central nervous system was evaluated in transgenic mice expressing a beta-galactosidase reporter gene under the control of the retinoic acid responsive element (RARE) (Jackson Laboratories stock # 008477) [35]. Adapalene loaded nanoparticles were administered intravenously via lateral tail vein injection to 4 to 6-week-old mice. Mice (n = 3 per group) received either adapalene at a dose of 3 mg/kg or drug empty nanoparticles at a matched polymer concentration. At 4 or 24 h post-administration, mice were anesthetized and perfused with heparinized saline. Brains were post-fixed in 4% PFA (48 hrs at 4oC) and cryopreserved in 30% sucrose (48 hrs at 4oC). Brains were frozen, cryo-sectioned to a thickness of 16 μm, and mounted on charge microscope glass slides. Relative β-galactosidase expression was evaluated by X-gal staining of tissue sections. Sections were washed in a 2 mM $MgCl_2$, 0.01% sodium deoxycholate staining buffer for 10 min at room temperature while rocking. Sections were transferred to a staining solution composed of staining buffer supplemented with 1 mg/mL X-Gal, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide and incubated overnight at 37 °C. Slides were then washed with 1X PBS and mounted with gelvatol and a glass cover slip. Slides were imaged on an Olympus BX40 light microscope.

2.7. Statistical analysis

All data were analyzed using GraphPad Prism 7 statistical software. The effect of temperature on drug loading was analyzed using the Student's t-test. The effects of polymer blending on biophysical characteristics of nanoparticles were analyzed using One-way ANOVAs with Tukey's post-hoc test.

3. Results

3.1. Optimizing adapalene loading

To form nanoparticles by single-emulsion solvent evaporation, drug is dissolved with polymer in the organic phase and emulsified with a water phase containing stabilizing agent [28]. Following solvent evaporation, free drug is washed from the nanoparticle solution via centrifugation or other forms of filtration. Our initial efforts to produce nanoparticles by a standard emulsion approach resulted in a relatively low adapalene loading of ~0.3% w/w. We predicted that maintaining a colder temperature during fabrication would slow the diffusion of adapalene into the water phase, enabling a higher total loading. To test this prediction, nanoparticles were formulated at all steps with cold solutions (on ice bath or in a temperature controlled centrifuge, 4 °C) or at room temperature (RT). We observed that maintaining a cold temperature (4 °C) improved loading by 80% percent over room temperature preparations, which was a statistically significant difference (Fig. 1; $p = 0.04$). Thus, moving forward, all nanoparticles were prepared under cold conditions.

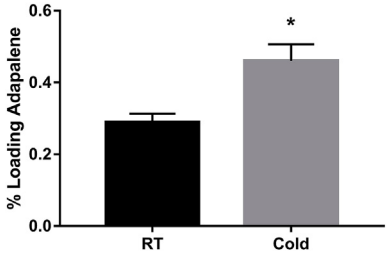

Fig. 1. Effect of emulsion temperature on adapalene loading. Loading measurement revealed that maintaining a lower temperature significantly increased adapalene loading into PLA-PEG nanoparticles. * = $p < 0.05$ Student's t-test.

We next aimed to improve encapsulation by altering the nanoparticle composition. We used two distinct approaches to achieve this. First, low molecular weight PLA or PCL was blended with the PLA-PEG base (10, 20, or 40 wt% of total polymer). PCL is more hydrophobic than PLA. By using different polymers with different hydrophobicity, it was possible to asses the effect of this characteristic on loading and yield of the resulting nanoparticles. We postulated that incorporation of short chain polyesters would facilitate adapalene loading by increasing hydrophobicity of the nanoparticle core. Second, DSPE-PEG and lecithin were blended with a PLGA base to produce lipid-polymer hybrid nanoparticles. PLGA was chosen for its ability to encapsulate hydrophobic drugs, lecithin was chosen to generate a lipid monolayer, and DSPE-PEG was chosen to insert within the monolayer and PEGylate particles to improve expected circulation properties [3]. The resulting nanoparticles were composed of an inner PLGA core with an outer lipid (DSPE-PEG and lecithin) shell.

Results for loading and yield are shown in Fig. 2, with TEM images in Fig. 3 and hydrated diameter and surface charge in Fig. 4. Blending low molecular weight PLA reduced loading at 40% PLA and reduced percent yield in direct proportion to how much PLA was added (Fig. 2). In contrast, incorporation of low molecular weight PCL significantly improved loading (0.70%–1.17% with 40 wt% PCL in PLA-PEG) without affecting yield. These improvements were observed for PCL blending while also not affecting diameter or surface charge of the resulting nanoparticles (Fig. 4). The lipid-polymer hybrid approach produced the highest encapsulation out of any method tested (1.92%), while not affecting percent yield (Fig. 2). These lipid-polymer hybrid nanoparticles were slightly larger than PLA-PEG nanoparticles, with decreased variability in diameter (130.3 ± 0.03 nm vs 115.1 ± 8.0 nm) (Figs. 3 and 4). The low variability of diameters was clearly demonstrated in TEM images of hybrid adapalene particles. Lipid-polymer hybrid nanoparticles were also characterized by a highly negative surface charge (−31.77 ± 2.31 mV) (Fig. 4), which was significantly more negative than PLA-PEG nanoparticles. The relatively negative surface charge of the lipid-polymer hybrid nanoparticles is consistent with previous zeta values reported for nanoparticles of similar composition [3].

3.2. Release profiles of blended-polymer and lipid hybrid formulations

To test whether different encapsulation strategies altered the release of adapalene from nanoparticles, each formulation was suspended in PBS, incubated at 37 °C under constant stirring motion, and sampled at regular intervals. No significant differences in adapalene release were observed for any blended formulation compared to PLA-PEG control. A slight tendency for slower release was observed for the lipid-polymer hybrid formulations, although this difference was not significant. Approximately 20% of adapalene was released after 24 h, and complete

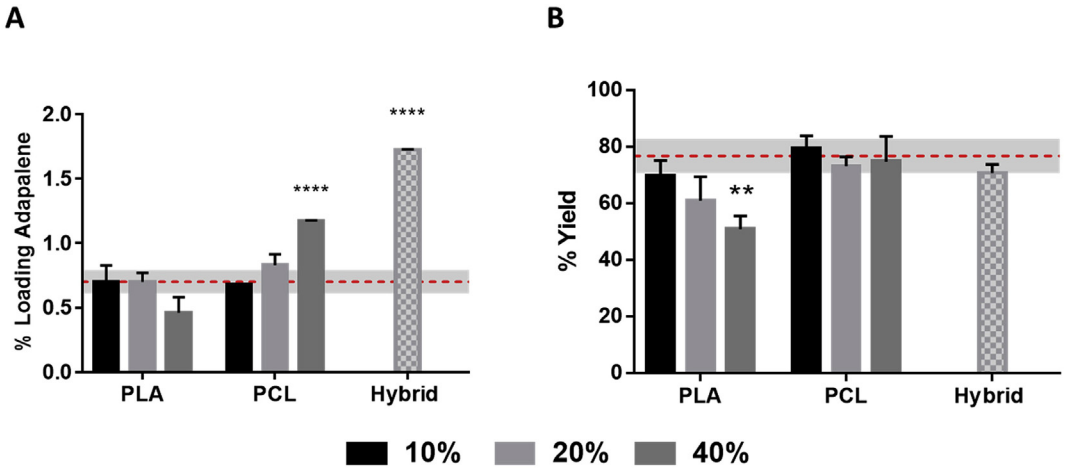

Fig. 2. Loading and yield of multiple formulations of blended nanoparticles engineered to encapsulate adapalene. Dashed lines show comparison to initial (non-optimized) formulation (dashed red line). A) The highest loading was achieved using hybrid nanoparticles. Of the blended formulations, the highest loading was achieved by blending a PLA-PEG base with 40% w/w short chain PCL. B) Effect of polymer composition on yield. Only blending with short-chain PLA caused a significant reduction in yield. Error bars indicate ± SEM for batches formulated in triplicate. ** = $p < 0.01$; ** = $p < 0.0001$ in comparison to initial (non-optimized) formulation. One-Way ANOVA with Tukey post hoc test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

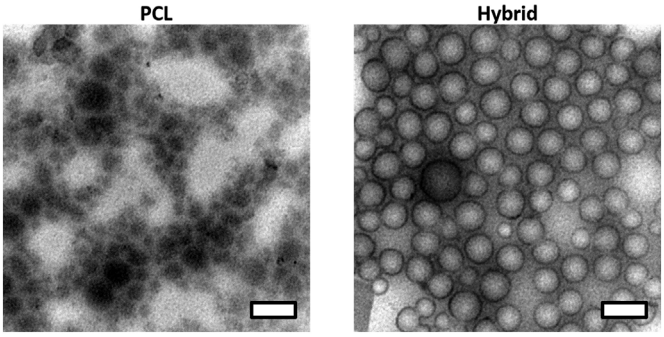

Fig. 3. TEM images of adapalene loaded nanoparticles. Left panel) PLA-PEG blended with PCL at a ratio of 60/40%, respectively. Right pane) Hybrid nanoparticles demonstrated high uniformity compared to blended formulations. Scale bar = 200 nm.

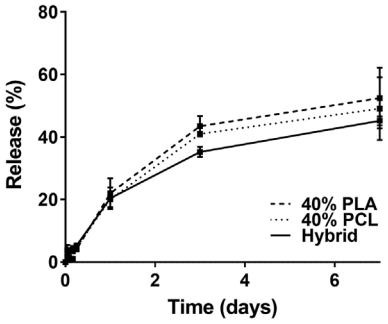

Fig. 5. Controlled release of adapalene from nanoparticle formulations: Adapalene is slowly released into buffered saline at 37 °C. Lipid hybrid nanoparticles tended to release adapalene more slowly than other formulations, although these differences were not statistically significant. Error bars indicate ± SEM.

release was not observed even after 7 days (Fig. 5). The highly hydrophobic nature of adapalene likely produces the slow and incomplete release observed in our studies. Similarly slow release with other poorly-water soluble drugs, such as paclitaxel, encapsulated within polymeric nanoparticles [1]. Given the good encapsulation efficiency of the 40% PCL-blended and lipid-polymer hybrid nanoparticles, we proceeded with these two formulations for *in vivo* testing.

3.3. Testing the bioactivity of adapalene loaded nanoparticles

As with many other retinoids, adapalene is poorly water soluble and has been shown to be cleared rapidly from circulation [6], which prevents effective administration *in vivo*. We thus sought to determine whether adapalene would be bioactive when delivered from PCL-blended or lipid-polymer hybrid nanoparticles. These studies utilized

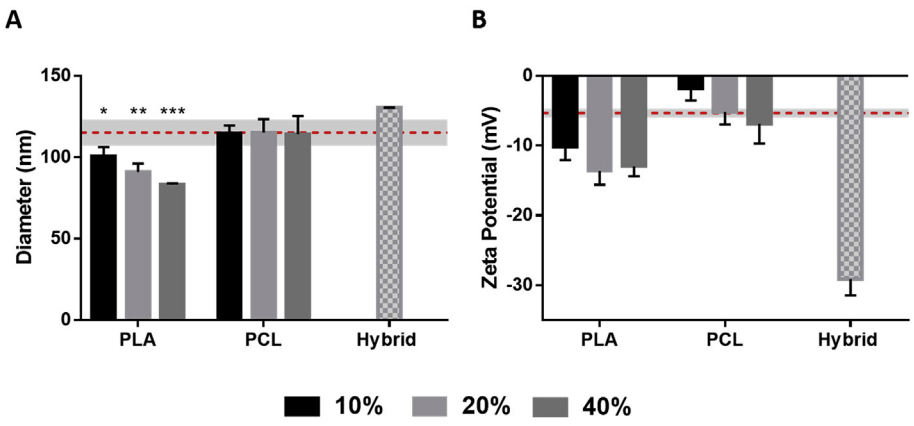

Fig. 4. Effects of blending polymers on diameter and zeta potential: Dashed lines show comparison to initial (non-optimized) formulation (dashed red line). A) Diameters derived by dynamic light scattering of nanoparticles formulations. Only blending with short-chain PLA reduces average diameter. B) Zeta potentials of different adapalene nanoparticle formulations. Error bars indicate ± SEM for batches formulated in triplicate. * = $p < 0.05$; ** = $p < 0.01$; ** = $p < 0.0001$ compared to initial (non-optimized) formulation. One-Way ANOVA with Tukey post hoc test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

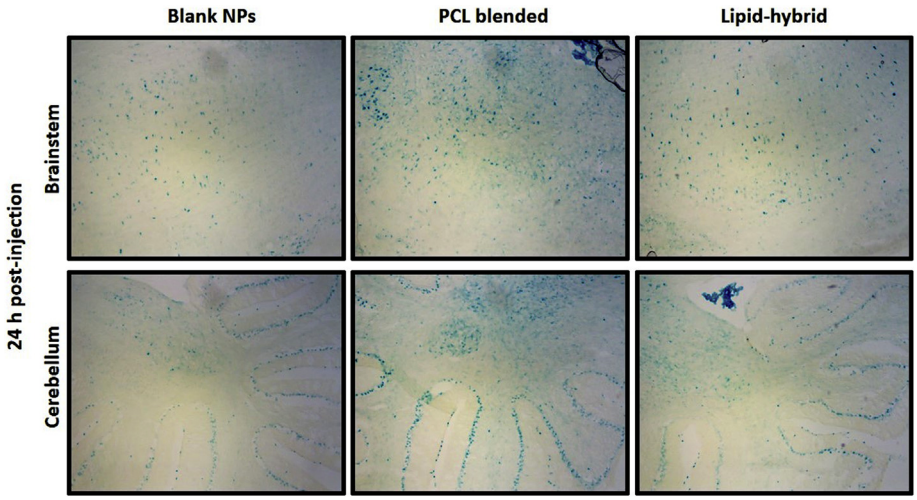

Fig. 6. *In vivo* activity of adapalene loaded particles 24 h post administration. PCL and lipid-polymer hybrid nanoparticles elicit an increase in retinoid signaling *in vivo* 24 h post administration in the brainstem (top row) and cerebellum including the purkinje cell layer (bottom row) as demonstrated by X-gal staining (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

transgenic reporter mice, which express the LacZ gene (encoding for β-galactosidase) under the control of the RA response element (RARE) [35], which allowed us to detect retinoid signaling activation. RARE mice were treated with adapalene loaded nanoparticles (3 mg/kg adapalene) via lateral tail-vein injection and sacrificed 4 and 24 h later. Treatments were well tolerated, and we did not observe any evidence of an adverse reaction at any dose of adapalene loaded nanoparticles. β-Galactosidase activity was evaluated by X-gal staining. Mice treated with control blank particles demonstrated low levels of reporter gene expression throughout the brain, including the brainstem and cerebellum. However, mice that were treated with adapalene loaded, PCL blended and lipid-polymer hybrid nanoparticles showed increased reporter gene expression, as demonstrated by increased X-gal staining (blue), after 24 h in the brainstem, thalamus and the Purkinje cell layer of the cerebellum compared to mice injected with control nanoparticles (Fig. 6). Similar results were observed when tissues were examined 4 h after injection (Fig. S1). These data establish that adapalene is bioactive in the CNS when blended or hybrid nanoparticles are administered intravenously.

4. Discussion

RA, which is the main metabolite of vitamin A, mediates its effects on gene transcription through both retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Each RAR receptor has three isotypes (α, β and γ), with multiple isoforms of each subtype generated by alternative splicing and differential promoter usage [2,19]. RARs are activated by all-*trans* retinoic acid (ATRA) or 9-*cis*-retinoic acid (9-*cis*-RA) and mediate gene expression by forming heterodimers with RXRs, whereas RXRs are activated only by 9-*cis*-RA and modulate gene expression either as homodimers or by forming heterodimers with RARs or a variety of orphan nuclear receptors [27]. In the absence of ligand, RA nuclear receptors function as transcriptional repressor [30]. RA activates RARs to form a heterodimer with RXRs which then bind to a DNA sequence called the retinoic acid-response element (RARE). Binding of RA to its receptors allows the RARs to bind to coactivators of transcription to activate gene expression regions of more than 500 genes, including those that encode for enzymes involved in neurotransmitter biosynthesis, ligand gated channels, and G protein-coupled receptors [21,32]. Adapalene demonstrates selectively high affinity (Kd 34 nM) for RARβ and, to a much lesser extent, RARγ and RARα (Kd values 130, 1000 nM respectively). Additionally, adapalene does not interact with RXRs [31]. Expression studies have demonstrated that RAR and RXRs are distributed throughout the adult CNS [2,19], with certain CNS regions such as the spinal cord, and the striatum expressing all RAR or RXR receptors, or demonstrating enrichment of specific subtypes. For example, RARβ is expressed in high abundance in the brainstem, striatum, and hypothalamus [19].

Retinoid signaling has long been understood to be essential for neurodevelopment and, more recently, for normal function of the adult CNS. Dysregulation of the RA signaling pathway has been implicated in the pathogenesis of a number of neurodegenerative diseases. A number of studies have demonstrated the beneficial effects of increasing RA signaling using in different models of neurodegeneration. However, small molecule retinoids suffer from delivery issues, including low solubility and rapid clearance, which have limited their application *in vivo*. In addition, high doses of retinoids can lead to undesirable toxicities and side effects, which means that it will be important to control treatment associated toxicity. Encapsulation within nanoparticles could provide a way to circumvent these drug delivery issues to develop new therapies for the treatment of central nervous system disease [4,13,14,42].

Methods for creating adapalene loaded nanoparticles have previously been described for topical delivery [10,12,15]. Here, we aimed at generating nanoparticles that would be suitable for the intravenous delivery of adapalene. We utilized the PLA-PEG and PLGA polymers as our base polymer because of their ability to encapsulate a multitude of hydrophobic small molecules and well-characterized biocompatibility [20]. Our initial efforts to formulate nanoparticles by standard techniques resulted in low loading of adapalene. Blending approaches have been previously reported to provide favorable nanoparticle characteristics such as improved drug loading and release [22,33,39]. We reasoned that adapalene loading could be improved by blending hydrophobic molecules into a polyester base.

Both polymer [9,23] and lipid [3] blending methods have been described by others for formation of particles via nanoprecipitation, although our work is the first that we are aware of to generate blended PLA-PEG nanoparticles via emulsion. Our data demonstrate that addition of short chain PCL increased drug loading and does not change total yield. Conversely, we find that addition of short chain PLA reduces loading of adapalene and also reduces total yield. PCL is more hydrophobic than PLA, and so its addition likely improved solubility of drug within the hydrophobic polymer core, whereas increasing the PLA content merely reduced stability of the nanoparticle, resulting in lower yield. Lipid-polymer hybrid nanoparticles have been utilized to combine favorable characteristics of polymeric nanoparticles (e.g. high structural integrity, stability in storage, and biocompatibility) and liposomes (improved encapsulation and release characteristics) [11]. Additionally, drug release can be adjusted by altering the lipid-content, thus allowing for optimization and selection of specific drug release profiles [3]. In this work, the lipid-polymer hybrid nanoparticle achieved the highest loading of all tested formulations. Release data

suggest that adapalene tended to be released more slowly from lipid-polymer hybrid nanoparticles compared to blended nanoparticles, although this difference was not significant. Secondary to achieving high loading, we also aimed to maintain a nanoparticle diameter of ~100 nm and a zeta potential around ~ -10 mV, since these parameters are known to be favorable for CNS drug delivery via intravenously administered nanoparticles [36]. The lipid-polymer hybrid formulations possessed a more negative zeta potential compared to the polymer-*blend* formulations. This relatively negative surface charge is similar to what was observed to lecithin/DSPE-PEG nanoparticles produced by others [3,41] and is presumably due to presentation of the lipids on the surface of the nanoparticle.

The data presented in Fig. 6 and in Fig. S1 demonstrate that adapalene loaded PCL blended nanoparticles and lipid-polymer hybrid nanoparticles activate retinoid signaling in the CNS following intravenous injection. Activation of retinoid signaling was seen throughout multiple regions of the brain, including the hippocampus and cerebellum. Retinoid activation was especially increased in regions such as the striatum and brainstem, regions that have been previously shown to have high expression of the RARβ receptor [2,19]. Activation of the retinoid pathway was sustained, being observed 24-h post injection.

Retinoid treatments have been utilized for a variety of indications, including cancers and dermatological treatments, but these studies are limited to topical application [10,12,15]. Recently, retinoid therapy has also been proposed for the treatment of neurological disorders such as schizophrenia, Alzheimer's, and amyotrophic lateral sclerosis [17,21,34,37], and efforts have been made to develop nanoparticles that will improve delivery and efficacy of retinoids for neurological diseases [36]. Retinoic acid (RA) loaded nanoparticles have previously been used to improve controlled delivery of RA in the brain in mouse models of Parkinson's disease [7]. These nanoparticles were delivered to the brain through intracerebellar injection and were able to promote neuroprotection in a mouse model of Parkinson's disease. To our knowledge, our data provide the first evidence that retinoids encapsulated in nanoparticles activate retinoid signaling in the brain following intravenous administration. These data demonstrate bioactivity of our nanoparticles and present novel approaches for developing retinoid-modulating therapies. These studies establish a nanoparticle-based drug delivery platform that may be useful for the treatment of CNS diseases.

5. Conclusions

We present new blended polymer and lipid-polymer hybrid nanoparticle formulations to encapsulate adapalene for delivery into the CNS. Previous micro-/nanoparticle adapalene formulations have been developed for topical applications. Here, conditions were optimized to improve loading and controlled release by blending PLA-PEG with low molecular weight PCL and PLGA with DSPE-PEG/lecithin to generate adapalene loaded nanoparticles suitable for intravenous administration. Importantly, treatment with adapalene loaded nanoparticles was able to elicit a biological response as quickly as 4 h post injection in reporter mice, and these effects were sustained for a minimum of 24 h. This is the first evidence to demonstrate that polymeric nanoparticles can deliver adapalene to the CNS. Our future goal is to test our formulations in models of neurodegeneration to evaluate the therapeutic potential of modulating the RA signaling pathway by delivering adapalene intravenously via polymeric nanoparticles.

Declaration of interests

DXM, RB, and RWS founded and own stock in a company (NP Therapeutics, Inc.) whose purpose is to advance retinoid activating nanoparticles into the clinic. The studies described in this report were performed prior to formation of the company. DXM, EPC, and RWS are named inventors on a patent related to this work (WO2018232366A1).

Acknowledgment

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2019.04.013.

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