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Polymer conjugated retinoids for controlled transdermal delivery

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ABSTRACT

All-trans retinoic acid (ATRA), a derivative of vitamin A, is a common component in cosmetics and commercial acne creams as well as being a first-line chemotherapeutic agent. Today, formulations for the topical application of ATRA rely on creams and emulsions to incorporate the highly hydrophobic ATRA drug. These strategies, when applied to the skin, deliver ATRA as a single bolus, which is immediately taken up into the skin and contributes to many of the known adverse side effects of ATRA treatment, including skin irritation and hair loss. Herein we present a new concept in topical delivery of retinoids by covalently bonding the drug through a hydrolytically degradable ester linkage to a common hydrophilic polymer, polyvinyl alcohol (PVA), creating an amphiphilic nanomaterial that is water-soluble. This PVA bound ATRA can then act as a pro-drug and accumulate within the skin to allow for the sustained controlled delivery of active ATRA. This approach was demonstrated to release active ATRA out to 10 days *in vitro* while significantly enhancing dermal accumulation of the ATRA in explant pig skin. *In vivo* we demonstrate that the pro-drug formulation reduces application site inflammation compared to free ATRA and retains the drug at the application site at measurable quantities for up to six days.

1. Introduction

All-trans retinoic acid (ATRA), a metabolite of Vitamin A, is a key component in the topical treatment of numerous skin disorders, including: acne, psoriasis, and UV-induced photo aging [1–8]. ATRA therapy acts by reducing abnormal follicular epithelial hyper-keratinization as well as repressing UV-induced cell signaling pathways that lead to up-regulated expression of metalloproteinases [9–14]. Use of ATRA however is limited by its serious side effects such as skin irritation and hair loss as well as its poor chemical stability [15–20]. Previous investigations have looked to control these undesirable characteristics through controlled release formulations such as creams, microparticles, and emulsions [17,21–24]. These strategies, however, rely on bolus delivery of active ATRA that, in the case of creams and emulsions, can become immediately available. This rapid increase in local concentration causes a number of adverse side effects [10,25]; while on the other hand, microparticle approaches require injection across the dermis, increasing the potential for immunologic response and infection. To date, there has been limited research into polymer-conjugated forms of ATRA, with the focus on application in cancer therapies [26–28].

Poly (vinyl alcohol) (PVA) is an excipient of choice in many pharmacologic formulations and is commonly used in the preparation of biodegradable particles [29–32]. PVA is well tolerated and has demonstrated a very good safety profile *in vivo*[33–36]. Applications for PVA in drug delivery primarily focus on it as a surfactant, providing excellent drug loading while allowing control over particle size and stability [37–40]. Recent reports have highlighted the mucoadhesive nature of PVA coated particles, suggesting that the hydrogen bonding ability of PVA promotes interaction with mucosal proteins [41–44]. This adhesive nature of PVA to mucosal components suggests a broader potential use, one that can take advantage of the hydrogen bonding to increase the residence time of drugs within tissues. Conjugating the hydrophobic ATRA to the very hydrophilic PVA through a hydrolytically degradable ester linkage, creates a new approach to formulate ATRA into a controlled release nanomaterial with potentially enhanced tissue residence.

Controlling ATRA release presents many substantial improvements over the current techniques for ATRA delivery. Currently ATRA is placed as a bolus at the sight of interest, immediately activating retinoic acid receptors and generating a strong pro-inflammatory response that can cause serious pain, irritation, and damage to the dermis. Bolus

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delivery also requires constant re-application to the sight of interest, often multiple times per day. By delivering ATRA as a controlled-release conjugate, we can avoid these side effects and promote a more healthy response from the tissue with better control over ATRA action. Using micellar delivery of ATRA using a modified PVA has previously been explored for similar reasons [45,46].

In this report, we describe the synthesis and evaluation of a PVA-ATRA polymer-drug conjugate (PATRA) for topical controlled delivery of ATRA. When hydrated, PATRA forms a nanoparticle micelle, which is soluble in water and provides protection from UV degradation of ATRA. Release of ATRA from the polymer conjugate was sustained for up to ten days *in vitro*. Delivery into skin was evaluated first using explant pig dermis which showed a near four-fold increase in ATRA accumulation within the dermis and a ten-fold reduction in ATRA that fully permeates through the tissue after 12 h compared to bolus ATRA administration. When tested *in vivo* PATRA was observed to elicit minimal inflammatory response compared to ATRA therapy, while remaining present for up to nearly five days post-application. These marked improvements in delivery profile, retention, and lowered side effects suggest that PATRA may be a promising new approach to the retinoid treatment.

2. Materials and methods

2.1. Materials and reagents

PVA (M_w ~ 31 kDa) and ATRA were purchased form Sigma-Aldrich (St. Louis, MO). Phosphate-buffered saline (PBS, 10 ×), Advanced-MEM, fetal bovine serum, antibiotic-antimycotic solution, and 100 mM L-Glutamine solution were purchased from Life Technologies (Grand Island, NY). AlexaFluor 647 NHS ester was purchased from Life Technologies (Grand Island, NY). NIH-3T3 cells were purchased from ATCC (Manassas, VA).

2.2. Fabrication of PATRA

The synthesis was performed using a previously reported procedure with modifications [47,48,53]. 0.138 g DMAP (1.12 mmol), 5.7 mmol DCC and ATRA (**2**, 0.341 g, 1.14 mmol) were added to 1.0 g PVA (**1**, ~ 31 kDa) in a mixture of 30 mL of anhydrous DMF and 70 mL of DMSO (at least 1:90 weight ratio). A 10% degree of functionalization of all hydroxyl groups from PVA was targeted for conversion. The reaction was allowed to run for 48 h or as indicated by TLC. After this reaction, the DCU precipitate was filtered out and the filtrate was concentrated *in vacuo* at low pressure and precipitated in ether.

After centrifugation of the ether suspension at 5000 rpm for 15 min, the residue was collected, dissolved in water, and dialyzed against water for 24–48 h to remove polar impurities. Lyophilization of the dialyzed product yielded yellowish PATRA conjugate (**3**). ^{1}H NMR (400 MHz, D2O): 3.98–4.02 (m, −CH−OH, PVA), 1.5–2.25 (m, −CH2, PVA); ^{13}C NMR (400 MHz, D_2O): 64.71–67.68 ($-CH_2-CH-OH$), 164.95 (CH−O−CO−).

2.3. In vitro analysis of PATRA

Release studies were carried out in a hydroalcoholic solution (50:50 ethanol: water) at two physiologically relevant temperatures, 20 °C and 37 °C. The impact of ATRA on cell proliferation was assessed by supplementing the media of sub-confluent NIH-3T3 cells with ATRA, PATRA, PVA, and PBS in 24-well plates at concentrations equivalent to 10 μM ATRA. ATRA was prepared in a concentrated hydroalcoholic solution of 10 μL. A similar dose of ethanol (5 μL) was added to each treatment group immediately after addition of the testing agents. The cell number calculated from imaging of those wells is used as the reference in the calculation of relative cell density.

Uptake, retention, and penetration of ATRA in pig skin were investigated using a Franz diffusion cell. Skin was harvested from the flanks of adult female Yorkshire pigs 1 h after sacrifice with subcutaneous fat removed. Skin was sectioned and frozen at − 80 °C for up to six months prior to use. Skin was prepared for diffusion experiments by thawing in PBS for 1 h after which time all hair on the skin was shaven off. Test samples of skin were cut to 30 mm × 30 mm square samples and placed into the diffusion cell such that the top of the dermis faced a 3 mL testing retention reservoir containing the drug and/or drug components, and the underside faced a 15 mL penetration reservoir containing no drug components. All studies were run at room temperature. The skin samples were checked for physical disruption using a hydrophilic dye, trypan blue, prior to and after completion of the study.

Samples were placed into the retention reservoir with a drug concentration of 0.1 mg/mL and followed for up to 12 h. ATRA concentration was evaluated in both the retention and penetration reservoirs every four hours during this period *via* UV absorbance measurements at 360 nm. ATRA was first solubilized in a concentrated alcohol solution (5 mg/mL) prior to being diluted into the retention well. A similar amount of ethanol (60 μL) was added to the PATRA retention well to control for ethanol concentration. For both groups, the penetration reservoir was filled with a hydroalcoholic solution so that the ATRA that could penetrate the skin would be soluble for UV absorbance measurement.

2.4. In vivo PATRA application

All animal studies were approved by the MIT Institutional Animal Care and Use Committee (IACUC). Animals were housed and cared for in the USDA-inspected MIT Animal Facility under federal, state, local, and NIH guidelines for animal care. Six-week-old Balb/CJ mice (n = 18) were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were either used for irritation assessment or for IVIS PATRA retention testing.

Mice used for the assessment of irritation were given each of the two different treatments on 1 cm^2 regions of their dorsum, these included PATRA, ATRA, PVA, and PBS. A total of twelve mice were used in this assessment. 50 μL of 10 μM ATRA solution was placed on two 1 cm^2 shaved areas on the flanks of a Balb/CJ mouse on either side of midline. PATRA (0.092 mg/mL) and PVA (0.089 mg/mL) concentrations were controlled for 10 μM ATRA dosing. These solutions were rubbed into the skin using a cotton-tipped applicator for 30 s.

Mice used for PATRA retention testing were given two applications of one material, either PATRA or the unconjugated AF-647 dye, on two 1 cm^2 regions on their dorsum on midline. The material was allowed to adsorb into the skin for 30 min and then the mice were cleaned with a wetted towel to remove excess. Mice were imaged daily for up to seven days.

2.5. Histology

Tissues were fixed in zinc fixative without formalin for 48 h. The excised tissues were cut on center and then embedded cut-face down in paraffin. Sections were taken at the wound center and at one further level of 500 μm reaching a total of 1 mm sampling length through the application site. At each level an H & E slide was stained. Unstained slides were also taken for potential IHC analysis of the tissue. All sections were 5 μm thick. Image analysis was performed using Image J.

2.6. Statistics

Statistical analysis was performed between groups using Student's *t*-test and rectified by ANOVA for comparisons between multiple groups. Values are represented as mean ± S.D. A value of $p < 0.05$ was used to indicate statistical significance.

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