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Multiply repeatable and adjustable on-demand phototriggered local anesthesia

Alina Y. Rwei [a,b], Changyou Zhan [a], Bruce Wang [a], Daniel S. Kohane [a,*]

[a] Laboratory for Biomaterials and Drug Delivery, Department of Anesthesiology, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA
[b] Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

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ABSTRACT

A phototriggerable system whereby patients could repeatedly and non-invasively control the timing and dosage of local anesthesia according to their needs would be beneficial for perioperative pain and perhaps obviate the need for oral narcotics. However, clinical application of phototriggerable systems have been limited by concerns over phototoxicity of lasers and limited tissue penetration of light. To address these limitations, we increased the devices' effective sensitivity to light by co-delivering a second compound, dexmedetomidine, that potentiates the effect of delivered local anesthetics. The concurrent release of dexmedetomidine enhanced the efficacy of released local anesthetics, greatly increasing the number of triggerable nerve blocks (up to nine triggerable events upon a single injection) and reducing the irradiance needed to induce nerve block by 94%. The intensity and duration of on-demand analgesia could be adjusted by varying the intensity and duration of irradiance, which could not only be delivered by lasers, but also by light-emitting diodes, which are less expensive, safer, and more portable.

1. Introduction

Current treatment for many pain states – including localized pain – often involves systemic medications such as opioids. Such treatments are sometimes ineffective and often have side effects, can be addictive, and can be diverted to illicit ends [1–3]. Locally injected anesthetics are effective, but they do not last long, and their application requires skilled personnel. Consequently, many investigators have developed drug delivery systems to provide prolonged duration local anesthesia lasting days to approximately 1 week from a single injection [4]. One limitation of such formulations is that once established, the nerve block cannot be modulated according to the patient's changing conditions. A long-lasting, safe local anesthetic formulation that could be adjusted to conform to changing patient needs would be very beneficial.

Phototriggerable drug delivery systems provide spatiotemporal control over drug release and are therefore potentially useful in many clinical areas, including pain management. However, despite the development of a number of phototriggerable drug delivery systems [5], their clinical translational potential has been limited by the strong scattering and absorption of light in tissue. We recently used a liposomal system to release the potent local anesthetic tetrodotoxin (TTX) in response to irradiation with near-infrared (NIR; 730 nm) light, resulting in on-demand adjustable local anesthesia (10). The liposomes contained a NIR-absorbing photosensitizer (PS) (11), irradiation of which led to singlet oxygen release that peroxidized liposomal lipid bilayers, leading to TTX release. Although this formulation was effective, the maximum number of phototriggerable nerve block events from a single dose was low and the irradiance required to trigger TTX release could prove limiting for reaching targets deeper inside tissues due to potential thermal injury (12–14); the same could be true if irradiation was simply prolonged. Moreover, shorter irradiation times would be more convenient for patient care, but might require higher irradiances to be effective. These potential limitations could be addressed by making the delivery vehicle more sensitive to light, or by making each amount of released TTX more effective.

Here, a pharmacological approach was used to achieve higher photosensitivity and triggering repeatability, such that lower and safer light doses were required to trigger therapeutic effects. Such an approach would involve co-administering a second drug but would not require modification of the original drug delivery system. The efficacy of TTX can be greatly enhanced by co-administration with a variety of compounds [6,7]; the α_2-adrenergic agonist dexmedetomidine (DMED) is one such compound [8]. Its enhancement of the activity of TTX may be due to local vasoconstriction, which would maintain a high local concentration of drug, but other mechanisms are possible [9]. Here we have hypothesized that if TTX could be released against a background of release of DMED, each quantity of released TTX would be more effective, allowing lower irradiances – releasing less TTX – to achieve a given therapeutic effect. The lower amount of TTX required for a given neurobehavioral effect would allow triggering at greater depths of tissue, and more triggered events at lower irradiance or

* Corresponding author.
E-mail address: daniel.kohane@childrens.harvard.edu (D.S. Kohane).

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shorter irradiation, minimizing potential thermal injury [5,10,11] and enhancing convenience for patients. This approach could also possibly allow triggering with light-emitting diodes (LEDs) which are more cost-effective, safer, and portable [12,13] than lasers.

Here we tested the hypothesis that the pharmacological approach described above would enhance the effective light-sensitivity of a phototriggerable drug delivery system (*i.e.* reduce the irradiance required to have a given biological effect), and allow more triggerable events of local anesthesia. Both of those goals would enhance clinical translation of phototriggerable drug delivery systems.

2. Materials and methods

2.1. Preparation of $PdPC(OBu)_8$

1,4,8,11,15,18,22,25-Octabutoxyphthalocyaninato-palladium(II) ($PdPC(OBu)_8$) [PS] was synthesized as reported [14]. 1,4,8,11,15,18,22,25-Octabutoxy-29H,31H-phthalocyanine (Aldrich) was dissolved in anhydrous dimethylformamide and mixed with $PdCl_2$ (Aldrich) in a 1:3 molar ratio. The solution was purged with nitrogen for 30 min, followed by stirring at 120 °C for 24 h. Purification was achieved with the addition of excess H_2O. The end product was characterized by UV–Vis absorption and had an absorption peak at 734 nm in dichloromethane.

2.2. Preparation of liposomes

Liposomes were prepared following a thin film hydration method [14]. 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC, Avanti Polar Lipids), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC, Avanti Polar Lipids), 1,2-distearoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (DSPG, Genzyme), and cholesterol (Sigma) in the molar ratio 3:3:2:3 were dissolved in a chloroform:methanol 9:1 solution. PS was added at 0.45 mol% (of total lipid) for the preparation of PS-loaded liposomes. The solvent was evaporated to form a lipid thin film, which was redissolved in *t*-butanol and further lyophilized. The lipid cake was hydrated with PBS, TTX solution (0.3 mg/mL PBS; Abcam), DMED solution (1 mg/mL PBS with 1 M HCl 18 μL/mL; Sigma), or sulforhodamine B solution (10 mg/mL PBS; Aldrich). The suspension was homogenized with a 3/8-in MiniMicro workhead (Silverson) on an L5M-A Laboratory Mixer (Silverson) at 10,000 ×*g* for 5 min. The suspension was then dialyzed against PBS for 48 h in a 1000 kDa MWCO dialysis tube (Spectrum Laboratories). Liposomes were imaged by cryo-TEM for morphology evaluation. The size of the liposomes was characterized by a Beckman Coulter Multisizer 3. TTX liposomal content was measured by ELISA (Reagen) after lipid removal using the Bligh and Dyer method [15]. DMED content was measured by HPLC with a mobile phase of 70% H_2O with 0.1% trifluoroacetic acid and 30% acetonitrile with 0.1% trifluoroacetic acid. Sulforhodamine B content was determined by UV–Vis absorption at 565 nm.

2.3. TTX release studies in vitro

TTX release was studied by incubating liposome samples at 37 °C and placing 200 μL samples into Amicon Ultra-0.5 mL centrifugal filters (100 kDa MWCO) at predetermined time points, and centrifuging them at 5000 ×*g* for 15 min. The filtrate was collected and analyzed by ELISA.

2.4. DMED release studies in vitro

The DMED release study was performed by placing 100 μL of liposome sample into a Slide-A-Lyzer MINI dialysis device (Thermo Fisher) with a 20 kDa MWCO and dialyzing it against 1.4 mL PBS at 37 °C. The dialysis solution was replaced with fresh, pre-warmed PBS at predetermined time intervals. Irradiation was performed with a 730-nm laser (100 mW/cm^2, 10 min) at the 0 h and 24 h time points. The concentration of DMED was determined by HPLC.

2.5. Pharmacokinetic studies

Animals were anesthetized with isoflurane-oxygen and injected with 50 μL of 10 mg/mL sulforhodamine B PBS solution at the sciatic nerve co-injected with or without 50 μL of 50 μg/mL DMED. At predetermined time points (15 min, 30 min, 1 h, 2 h, 4 h, 6 h), blood was harvested by tail-bleeding and centrifuged at 4000 ×*g* for 15 min. The supernatant was collected and methanol was added at a 1:1 vol ratio. Samples were left at 4 °C overnight then centrifuged at 20,000 ×*g* for 15 min, and the supernatant was collected. The concentration of sulforhodamine B was analyzed by fluorescence (excitation/emission: 560/580 nm).

Liposomal pharmacokinetics studies were performed by co-injecting 200 μL of PS liposomes loaded with sulforhodamine B (Lipo-PS-Srho) with 200 μL of blank liposomes (Lipo) or DMED-loaded liposomes (Lipo-DMED) at the sciatic nerve. At predetermined time points (30 min, 3 h, 7 h and 10 h), blood was harvested by tail-bleeding. The steps that followed were described in the preceeding paragraph.

2.6. Phototriggered nerve block in vivo

Animal studies were performed according to protocols approved by the Boston Children's Hospital Animal Care and Use Committee, which were in accordance with the guidelines of the International Association for the Study of Pain. Adult male Sprague-Dawley rats of 350–400 g were housed in groups under a 12-h/12-h light/dark cycle with lights on at 7:00 AM [16]. Animals were randomly assigned to experimental groups. All experimental groups had a sample size of 4.

Under brief isoflurane-oxygen anesthesia, animals were co-injected with 200 μL of liposomes co-loaded with PS and TTX (Lipo-PS-TTX) or liposomes with PS only (Lipo-PS) and 200 μL of Lipo-DMED or Lipo at the sciatic nerve using a 23-G needle. Sciatic nerve injection followed procedures that were previously reported [18]. Animals were irradiated using a 730-nm laser or a 725–755 nm LED at the timing, irradiance and duration indicated in the Results section.

Nerve block was examined by a modified hotplate test as previously reported [19,20]. In brief, the plantar surface of the animal's hindpaw was placed onto a 56 °C hotplate, and the thermal latency was measured: the time (s) at which the animal withdrew its hindpaw. Animals that did not withdraw their paw after 12 s were removed from the hotplate. The average of three measurements was used. Successful nerve block was defined as blocks achieving a thermal latency above 7 s (half-way between a baseline of 2 s and a maximum latency of 12 s). Duration of nerve block was calculated as the time required for thermal latency to return to 7 s.

2.7. Histology

Animals were euthanized by carbon dioxide 4 d after the last irradiation event. The sciatic nerve and surrounding tissue were harvested and H&E staining was performed. The samples were scored for inflammation and myotoxicity. The observer (A.Y.R.) was blinded to the nature of the individual samples. The inflammation score was scaled from 0 to 4, where 0 was normal and 4 was severe inflammation. The myotoxicity score was scaled from 0 to 6 as previously reported [14,18]: 0 = normal; 1 = perifascicular internalization; 2 = deep internalization (more than five cell layers); 3 = perifascicular regeneration; 4 = deep tissue regeneration (more than five cell layers); 5 = hemifascicular regeneration; 6 = holofascicular regeneration.

2.8. Cell viability

C2C12 mouse myoblasts [American Type Culture Collection (ATCC) CRL-1772] and PC12 rat adrenal gland pheochromocytoma cells