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Multiply repeatable and adjustable on-demand phototriggered local anesthesia

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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.

Graphical abstract

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Keywords

liposome; near-infrared; photosensitizer; tetrodotoxin

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-administrating 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 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.

Materials and methods

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 PdCl₂ (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 H₂O. The end product was characterized by UV-Vis absorption and had an absorption peak at 734 nm in dichloromethane.

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 (1mg/mL PBS with 1M 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% H₂O 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.

TTX release studies in vitro

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

DMED release studies in vitro

The DMED release study was performed by placing 100 μ L 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/cm², 10 min) at the 0 h and 24 h time points. The concentration of DMED was determined by HPLC.

Pharmacokinetic studies

Animals were anesthetized with isoflurane-oxygen and injected with 50 μ L of 10 mg/mL sulforhodamine B PBS solution at the sciatic nerve co-injected with or without 50 μ L of 50 μ g/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 $4000 \times g$ 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 $20,000 \times g$ 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 μ L of PS liposomes loaded with sulforhodamine B (Lipo-PS-Srho) with 200 μ L 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 previous paragraph.

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 350–400g 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, consistent with the norm in the field [17].

Under brief isoflurane-oxygen anesthesia, animals were co-injected with 200 μ L of liposomes co-loaded with PS and TTX (Lipo-PS-TTX) or liposomes with PS only (Lipo-PS) and 200 μ L 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 2s 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.

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.

Cell Viability

C2C12 mouse myoblasts [American Type Culture Collection (ATCC) CRL-1772] and PC12 rat adrenal gland pheochromocytoma cells (ATCC, CRL-1721) were cultured as reported [21, 22]. C2C12 cells were cultured with DMEM (20% FBS, 1% Penicillin Streptomycin) medium and seeded into 24-well plates at 25,000 cells/well. Cells were then differentiated to myotubules in DMEM (2% horse serum, 1% Penicillin Streptomycin) medium for 10 – 14 days. PC12 cells were cultured with DMEM (12.5% horse serum, 2.5% FBS, 1% Penicillin Streptomycin) medium and seeded into 24-well plates at 2,000 cells/well. The cells were differentiated in DMEM with 1% horse serum, 1% Penicillin Streptomycin, and 50 ng/mL nerve growth factor (Life technologies) for 7 days.

The cytotoxicity of the diffusible components from the liposomes was evaluated using methods previously reported [14]. Liposomes of 100 μ L/well were exposed to cells by a 24-well Transwell® membrane (Costar 3495, pore size 0.4 μ m) and were irradiated with a 730-nm laser (100 mW/cm², 10 min). Cell viability was determined by the MTS assay 96 h after exposure to liposomes.

Statistical analysis

Histological scoring (inflammation and myotoxicity) was described with medians and quartiles due to its ordinal character, and statistical comparisons were done with the Mann-Whitney U test. All other data groups were described with means and standard deviations and compared with the Student t-test. GraphPad Prism (La Jolla, CA, USA) was used for statistical comparisons.

Results

Characterization of liposomes

TTX and the PS [1,4,8,11,15,18,22,25-octabutoxyphthalocyaninato-palladium(II) [23] (PdPC(OBu)₈)] were co-loaded into liposomes (Lipo-PS-TTX) that contained the unsaturated lipid 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC) as previously reported [14]; see Methods for liposome composition. The mean size of Lipo-PS-TTX was $4.9 \pm 3.0 \mu\text{m}$, and the loading efficiency was 25%. DMED was loaded into separate liposomes with the same lipid composition (Lipo-DMED; Fig. S1). Lipo-DMED had a mean size of $6.3 \pm 4.4 \mu\text{m}$ and a loading efficiency of 36%.

Effect of DMED and Lipo-DMED on local drug distribution

We hypothesized that DMED would induce local vasoconstriction, limiting drug redistribution from the injection site. To validate this hypothesis, the pharmacokinetics in blood of a hydrophilic fluorescent dye, sulforhodamine B (Srho), was studied after co-injection with DMED *in vivo* at the sciatic nerve (Fig. 1A). Co-injection of Srho and DMED decreased the peak Srho plasma concentration by 27.5%, and delayed the time to that peak level from 30 min to 60 min after injection.

To demonstrate that Lipo-DMED could have the same effect, it was co-injected with Srho-loaded liposomes (Lipo-Srho) at the sciatic nerve, and the pharmacokinetics of Srho was studied (Fig. 1B). Co-injection of Lipo-DMED and Lipo-Srho (1:1 vol) decreased the Srho peak plasma concentration by 79.6%, and the Srho plasma concentration was significantly lower for up to 7 h after injection compared with co-injection of blank liposomes (Lipo) and Lipo-Srho. There was no significant difference in Srho plasma concentration 10 h after injection.

These results suggest that DMED, free or encapsulated, significantly reduced the redistribution of Srho from the injection site into the systemic circulation, trapping the dye at the local site of injection.

In vitro phototriggered release

The release of TTX from a Lipo-DMED + Lipo-PS-TTX (Fig. 2A; 1:1 vol, here and in all subsequent studies) mixture was studied by determining the concentration of non-encapsulated TTX in filtrate from a centrifugal filter at predetermined intervals at 37°C (Fig. 2B). In the absence of irradiation, 3% of total TTX was released in the first 3 hours, followed by slow release. Irradiation (730 nm, 10 min, 100 mW/cm²) at the 0 h time point released 20% of TTX within the first hour, after which a return to slower release was observed. Further irradiation events at the 4 and 8 h time points released 11% and 25% within the subsequent hours respectively, followed by a return to slower release. These results demonstrated that the release of TTX from the Lipo-PS-TTX + Lipo-DMED mixture was controllable by light.

To examine whether DMED had any effect on the release of TTX from particles, release from Lipo-PS-TTX + Lipo-DMED was compared to that from Lipo-PS-TTX + blank

liposomes (Lipo), both irradiated with a 730 nm laser (10 min, 50 mW/cm²), at 37°C after the initial TTX release was completed (Fig. S2, see Methods). No significant difference was observed, suggesting that an observed biological effects would be due to drug effects in tissue rather than differences in release kinetics.

DMED release from the Lipo-DMED + Lipo-PS-TTX mixture was studied by dialyzing 100 µL of the liposomal mixture against 1.4 mL of PBS (Fig. 2C). Irradiation at the 0 h and 24 h time points showed no significant difference in release kinetics when compared with the non-irradiated group, demonstrating that DMED release was not phototriggerable.

Cytotoxicity

The cytotoxicity of diffusible components from Lipo-DMED + Lipo-PS-TTX with and without irradiation was assessed in two different cell lines relevant to local anesthetic-related tissue injury: C2C12, a myotube cell line, and PC12, a pheochromacytoma cell line frequently used for the assessment of neurotoxicity. 100 µL of liposomes (Lipo-DMED + Lipo-PS-TTX 1:1 vol mixture) were placed in the upper well of Transwell® systems, with cells in the lower well in 600 µL of media, following which irradiation (730 nm, 100 mW/cm², 10 min) was performed (Fig. S3). There was no significant decrease in cell viability in any group in either cell line.

Sciatic nerve blockade

Liposomal formulations were injected at the sciatic nerve *in vivo*, followed by neurobehavioral testing (Fig. 3). Nerve block was assessed by placing the animal's hindpaw onto a hotplate at 56°C and measuring the thermal latency, defined as the time the animal allowed its paw to remain on the hotplate. A thermal latency of 2 s indicated no nerve block (baseline), and a thermal latency of 12 s indicated deep nerve block. (Hindpaws were removed from the hotplate by the operator after 12 s to prevent thermal injury.) Successful nerve block was defined as a thermal latency above 7 s. Co-injection of Lipo-DMED and Lipo-PS-TTX caused an initial nerve block lasting 32.9 ± 7.1 h. Starting at the 41 h time point, i.e. after return to baseline latency, the injection site was irradiated (730 nm, 5 min, 75 mW/cm²). If the irradiation event resulted in nerve block, it was allowed to resolve before the next irradiation was performed. The first seven irradiation events achieved effective nerve block (thermal latency > 7 s). The 8th and the 9th induced an increase in thermal latency, but without exceeding 7 s (6.2 s and 4.5 s respectively). Co-injection of Lipo-PS-TTX and Lipo (no DMED) induced an initial nerve block of 16.8 ± 1.5 h, followed by a return to baseline latency. Irradiation (730 nm, 5 min, 75 mW/cm²) did not produce nerve block, indicating that Lipo-DMED was necessary for the induction of nerve blockade at low irradiance with short duration. Co-injection of PS-loaded liposomes (Lipo-PS) and Lipo-DMED did not result in initial or triggered nerve block, demonstrating that it was not irradiation itself and/or Lipo-DMED that resulted in nerve block. Lipo-PS-TTX was necessary for the induction of nerve block.

To demonstrate that the duration of triggered nerve block could be adjusted by controlling the duration of irradiation (Fig. 3B), Lipo-PS-TTX + Lipo-DMED were co-injected at the sciatic nerve and irradiation was performed 41 h after injection, after the thermal latency had

returned to baseline from the initial block. The duration of nerve block increased with increasing duration of irradiation (Fig. 3B). There was a linear relationship between the irradiated energy density (the product of irradiation duration and irradiance) and nerve block duration (Fig. 3C) for both the Lipo-PS-TTX only and Lipo-PS-TTX + Lipo-DMED groups. Co-injection of Lipo-PS-TTX and Lipo-DMED decreased by 94% the irradiation threshold that was necessary for nerve block compared with animals injected with Lipo-PS-TTX only. In addition, the slope of the nerve block duration with respect to energy density, which reflects the therapeutic effectiveness per unit of irradiation energy (J/cm^2), increased by 2.9 fold with the addition of Lipo-DMED.

Tissue reaction

The sciatic nerve and surrounding tissues were collected 4 days after the last irradiation event. All animals injected with PS-loaded liposomes had green particle deposits surrounding the sciatic nerve (Fig. 4A), whereas animals injected with non-PS-loaded liposomes had white particle deposits (Fig. 4B). These results showed that the liposomes were deposited at the target site and the injections were performed accurately. The tissues did not appear edematous, discolored, or with other signs of tissue injury.

The tissues were sectioned, processed for hematoxylin-eosin staining, and the inflammation and myotoxicity were scored (Table 1). All animals injected with liposomes showed mild inflammation (median inflammation score = 1) (Fig. 4), consistent with findings upon injections of other particulates [24, 25]. Foamy macrophages were observed, indicating uptake of liposomes. No significant myotoxicity (median inflammation score = 0) or other tissue injury was observed in animals injected with liposomes, whether or not irradiated for nine separate and consecutive 5-min irradiation events.

LED-triggered sciatic nerve block

The fact that repeated nerve block could be achieved with low irradiances from a 730-nm laser suggested that a LED could be effective. Lipo-PS-TTX + Lipo-DMED was injected at the sciatic nerve. 41 hours later, i.e. after the initial nerve block wore off, irradiation with a 725–755 nm LED at $50 \text{ mW}/\text{cm}^2$ for 15 min (Fig. 5) induced a nerve block of 47 ± 19 min. LED irradiation of animals with Lipo-PS-TTX + Lipo (no DMED) induced a slight increase in thermal latency that did not exceed 7 s. Irradiation with an LED was less effective than with a laser, as seen in the fact that animals injected with Lipo-PS-TTX + Lipo-DMED and irradiated with a laser ($50 \text{ mW}/\text{cm}^2$, 15 min) 41 h after injection developed a nerve block lasting 85 ± 26 min (Figure S4). The difference in the effects of laser and LED light was likely related to the fact that PdPC absorbed approximately 48% of light reaching it from the LED and 99.8% of the light from the laser (Figure S5).

Discussion

The major goal of this work was to demonstrate that the goals described above for improving photoresponsive systems (greater sensitivity, more effect from a given irradiance) can be achieved by co-encapsulating a second drug that affects the efficacy of the first. We had previously shown that Lipo-PS-TTX were effective in triggering nerve block *in vivo* but

required 330 mW/cm² over 15 min to do so [5]. With the addition of DMED, effective triggering could be achieved with 75 mW/cm² over 5 min, an energy so low that it was ineffective with Lipo-PS-TTX. The threshold for achieving nerve block was 76 J/cm² with Lipo-PS-TTX, and the addition of Lipo-DMED decreased that threshold by 94 % to 4 J/cm², i.e. much less irradiance was required to achieve therapeutic effect (Fig. 3). Moreover, the increase in therapeutic effect for a given increase in irradiance was improved by addition of DMED, as evidenced by the ~3-fold steeper slope of the relationship between irradiance and effect (Fig. 3C). That improvement in therapeutic effect was due to an effect of DMED on tissue, not on the formulation itself (Fig. S2).

Both Lipo-PS-TTX and its combination with Lipo-DMED showed a linear relationship between nerve block duration and irradiation energy density (Fig. 3C), demonstrating that therapeutic effects can be modulated by adjusting the irradiance and/or duration of irradiation. This relationship could allow patients to conveniently adjust the onset, intensity, and duration of pain relief at will, according to their changing pain conditions, providing a personalized approach towards pain relief.

Another consequence of the DMED was the enhanced therapeutic effect of released TTX – and therefore that less TTX was required to have a given effect. Consequently, more nerve block events could be triggered; nine (two of which did not achieve our criteria for nerve block) (Fig. 3) compared to two for Lipo-PS-TTX [5]. Assuming that this formulation works in humans as it did in rats (Fig. 3), one could envision the following potential use. Injection before the procedure would provide approximately one day of local/regional anesthesia, which would then wear off over the ensuing twelve hours. At any point, the patient could irradiate the site of injection to achieve nerve block to the desired degree. This could be repeated at will until a total of at least 2.5 days of analgesia had been achieved – which covers the most painful period for many procedures. Potentially, triggering less often or with less irradiance would allow more triggering events because there would be less depletion of TTX, although that would have to be balanced against loss of TTX through slow untriggered release.

DMED is known to prolong the effects of local anesthetics, both conventional [26–28] and site 1 sodium channel blockers [9]. One type of potential mechanism for this prolongation is analgesic effects mediated by α_2 -adrenergic agonism or blockade of hyperpolarization-activated cation currents [29, 30]. A second mechanism is α_2 -adrenergic receptor mediated vasoconstriction [8, 31, 32], which would restrict the redistribution of local anesthetics from the injection site [8, 32]. Data in this study (Fig. 1) support the view that vasoconstriction played a role with these formulations. Interestingly, 10 h after injection the plasma concentration of Srho from Lipo-Srho + Lipo was similar to that from Lipo-Srho + Lipo-DMED, suggesting that DMED-induced vasoconstriction was no longer in effect, even though the effect of DMED lasted on nerve block was observed > 40 h after injection of Lipo-DMED + Lipo-PS-TTX (Fig. 3). This suggests that ongoing vasoconstriction may not have been responsible to the enhancement of nerve block at later time points. Vasoconstrictive effects suggest caution regarding the use of this formulation near end arteries (fingers, eyes, etc.), or perhaps in patients with poor peripheral circulation (e.g. diabetics). However, a similar improvement of nerve blockade might be achievable by co-

delivering TTX with agents which enhance its effect without significant vasoconstriction, such as conventional local anesthetics [6, 20].

A major limitation of many phototriggerable drug delivery systems [5, 33, 34] is the high dosage of light necessary for drug release or other therapeutic effects to be activated. This problem is exacerbated by attenuation as light passes through tissues *in vivo*, and can have deleterious effects on effectiveness and safety. Such attenuation of light may make the amount of therapeutic released within the body insufficient to have an effect. Conversely, increasing the irradiance to the point where it is effective may cause burns. [5]. NIR light between 700 nm – 900 nm penetrates deeper into tissue than do UV and visible light [35], but can still be considerably attenuated [10, 11]. Consequently, it is important for NIR-responsive systems to be as sensitive to irradiation at the appropriate wavelength as possible, and to have the greatest possible therapeutic effect from a given drug release event (i.e. from a given amount of irradiation), especially when targeting deeper tissues.

LEDs have the advantage of low cost, light weight, and requiring less energy to operate compared to laser systems [36]. However, since LEDs have a broad output spectrum, they will have a lesser effect for a given irradiance (Figure S5). We demonstrated that phototriggering of nerve block from Lipo-PS-TTX+ Lipo-DMED could be achieved with an LED, while this could not be achieved with Lipo-PS-TTX (Fig. 5). Being triggerable by LEDs is likely to be an advantageous feature for clinical translation as there can be safety and cost concerns related with laser systems for outpatient treatment [37, 38].

Conclusion

We have demonstrated a liposomal formulation that could provide multiply repeated, on demand local anesthesia when triggered by low irradiances over short periods. Tissue reaction was benign. The general approach of adding a second compound that enhances the efficacy of the first may be useful in improving the performance of other phototriggered drug delivery systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Turk DC. Clinical effectiveness and cost-effectiveness of treatments for patients with chronic pain. *Clin J Pain*. 2002; 18:355–365. [PubMed: 12441829]
2. Dolin SJ, Cashman JN, Bland JM. Effectiveness of acute postoperative pain management: I. Evidence from published data. *Br J Anaesth*. 2002; 89:409–423. [PubMed: 12402719]
3. Popping DM, Zahn PK, Van Aken HK, Dasch B, Boche R, Pogatzki-Zahn EM. Effectiveness and safety of postoperative pain management: A survey of 18,925 consecutive patients between 1998 and 2006. *Br J Anaesth*. 2008; 101:832–840. [PubMed: 18945716]

4. McAlvin, JB., Kohane, DS. Prolonged duration local anesthesia. In: Domb, AJ., Khan, W., editors. *Focal Controlled Drug Delivery*. Springer US; 2014. p. 653-677.
5. Rwei AY, Wang W, Kohane DS. Photoresponsive nanoparticles for drug delivery. *Nano Today*. 2015; 10:451-467. [PubMed: 26644797]
6. Adams HJ, Blair MRJ, Takman BH. The local anesthetic activity of tetrodotoxin alone and in combination with vasoconstrictors and local anesthetics. *Anesth Analg*. 1976; 55:568-573. [PubMed: 1085112]
7. Takman BH. The chemistry of local anaesthetic agents: classification of blocking agents. *Br J Anaesth*. 1975; 47(suppl):183-190. [PubMed: 1148096]
8. Yabuki A, Higuchi H, Yoshitomi T, Tomoyasu Y, Ishii-Maruhama M, Maeda S, Miyawaki T. Locally injected dexmedetomidine induces vasoconstriction via peripheral alpha-2A adrenoceptor subtype in guinea pigs. *Reg Anesth Pain Med*. 2014; 39:133-136. [PubMed: 24448513]
9. McAlvin JB, Zhan C, Dohlman JC, Kolovou PE, Salvador-Culla B, Kohane DS. Corneal anesthesia with site 1 sodium channel blockers and dexmedetomidine. *Invest Ophthalmol Vis Sci*. 2015; 56:3820-3826. [PubMed: 26066750]
10. Henderson TA, Morries LD. Near-infrared photonic energy penetration: can infrared phototherapy effectively reach the human brain? *Neuropsychiatr Dis Treat*. 2015; 11:2191-2208. [PubMed: 26346298]
11. Jagdeo JR, Adams LE, Brody NI, Siegel DM. Transcranial red and near infrared light transmission in a cadaveric model. *PLoS ONE*. 2012; 7:e47460. [PubMed: 23077622]
12. Burkow L. The use of near infrared light emitting diodes in treating sports-related injuries: a review. *Research*. 2014
13. Dujovny M, Morency E, Ibe O, Sosa P, Cremaschi F. Contributions of near infrared light emitting diode in neurosurgery. *Insights in Neurosurgery*. 2016
14. Rwei AY, Lee JJ, Zhan C, Liu Q, Ok MT, Shankarappa SA, Langer R, Kohane DS. Repeatable and adjustable on-demand sciatic nerve block with phototriggerable liposomes. *Proc Natl Acad Sci USA*. 2015; 112:15719-15724. [PubMed: 26644576]
15. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol*. 1959; 37:911-917. [PubMed: 13671378]
16. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*. 1983; 16:109-110. [PubMed: 6877845]
17. Mogil JS, Ritchie J, Sotocinal SG, Smith SB, Croteau S, Levitin DJ, Naumova AK. Screening for pain phenotypes: Analysis of three congenic mouse strains on a battery of nine nociceptive assays. *Pain*. 2006; 126:24-34. [PubMed: 16842916]
18. McAlvin JB, Padera RF, Shankarappa SA, Reznor G, Kwon AH, Chiang HH, Yang J, Kohane DS. Multivesicular liposomal bupivacaine at the sciatic nerve. *Biomaterials*. 2014; 35:4557-4564. [PubMed: 24612918]
19. Thalhammer JG, Vladimirova M, Bershadsky B, Strichartz GR. Neurologic evaluation of the rat during sciatic nerve block with lidocaine. *Anesthesiology*. 1995; 82:1013-1025. [PubMed: 7717536]
20. Kohane DS, Yieh J, Lu NT, Langer R, Strichartz GR, Berde CB. A re-examination of tetrodotoxin for prolonged duration local anesthesia. *Anesthesiology*. 1998; 89:119-131. [PubMed: 9667302]
21. Epstein-Barash H, Shichor I, Kwon AH, Hall S, Lawlor MW, Langer R, Kohane DS. Prolonged duration local anesthesia with minimal toxicity. *Proc Natl Acad Sci USA*. 2009; 106:7125-7130. [PubMed: 19365067]
22. Zhan C, Wang W, McAlvin JB, Guo S, Timko BP, Santamaria C, Kohane DS. Phototriggered local anesthesia. *Nano Lett*. 2016; 16:177-181. [PubMed: 26654461]
23. Soldatova AV, Kim J, Rizzoli C, Kenney ME, Rodgers MAJ, Rosa A, Ricciardi G. Near-infrared-emitting phthalocyanines. A combined experimental and density functional theory study of the structural, optical, and photophysical properties of Pd(II) and Pt(II) α -butoxyphthalocyanines. *Inorg Chem*. 2010; 50:1135-1149. [PubMed: 21188985]
24. Kohane DS, Lipp M, Kinney RC, Anthony DC, Louis DN, Lotan N, Langer R. Biocompatibility of lipid-protein-sugar particles containing bupivacaine in the epineurium. *J Biomed Mater Res*. 2002; 59:450-459. [PubMed: 11774302]

25. Anderson JM. In-vivo biocompatibility of implantable delivery systems and biomaterials. *Eur J Pharm Biopharm.* 1994; 40:1–8.
26. Brummett CM, Norat MA, Palmisano JM, Lydic R. Perineural administration of dexmedetomidine in combination with bupivacaine enhances sensory and motor blockade in sciatic nerve block without inducing neurotoxicity in rat. *Anesthesiology.* 2008; 109:502–511. [PubMed: 18719449]
27. Brummett CM, Padda AK, Amodeo FS, Welch KB, Lydic R. Perineural dexmedetomidine added to ropivacaine causes a dose-dependent increase in the duration of thermal antinociception in sciatic nerve block in rat. *Anesthesiology.* 2009; 111:1111–1119. [PubMed: 19858875]
28. Yoshitomi T, Kohjitani A, Maeda S, Higuchi H, Shimada M, Miyawaki T. Dexmedetomidine enhances the local anesthetic action of lidocaine via an α -2A adrenoceptor. *Anesth Analg.* 2008; 107:96–101. [PubMed: 18635472]
29. Kosugi T, Mizuta K, Fujita T, Nakashima M, Kumamoto E. High concentrations of dexmedetomidine inhibit compound action potentials in frog sciatic nerves without α (2) adrenoceptor activation. *Br J Pharmacol.* 2010; 160:1662–1676. [PubMed: 20649570]
30. Brummett CM, Hong EK, Janda AM, Amodeo FS, Lydic R. Perineural dexmedetomidine added to ropivacaine for sciatic nerve block in rats prolongs the duration of analgesia by blocking the hyperpolarization-activated cation current. *Anesthesiology.* 2011; 115:836–843. [PubMed: 21666435]
31. Afonso J, Reis F. Dexmedetomidine: Current role in anesthesia and intensive care. *Revista Brasileira De Anesthesiologia.* 2012; 62:118–133. [PubMed: 22248773]
32. Kohane DS, Lu NT, Cairns BE, Berde CB. Effects of adrenergic agonists and antagonists on tetrodotoxin-induced nerve block. *Reg Anesth Pain Med.* 2001; 26:239–245. [PubMed: 11359223]
33. Timko BP, Dvir T, Kohane DS. Remotely triggerable drug delivery systems. *Adv Mater.* 2010; 22:4925–4943. [PubMed: 20818618]
34. Barhoumi A, Liu Q, Kohane DS. Ultraviolet light-mediated drug delivery: Principles, applications, and challenges. *J Controlled Release.* 2015; 219:31–42.
35. Steiner, R. Laser-tissue interactions. In: Raulin, C., Karsai, S., editors. *Laser and IPL Technology in Dermatology and Aesthetic Medicine.* Springer; Berlin: 2011. p. 23-36.
36. Mang TS. Lasers and light sources for PDT: Past, present and future. *Photodiagn Photodyn Ther.* 2004; 1:43–48.
37. Smalley PJ. Laser safety: Risks, hazards, and control measures. *Laser Therapy.* 2011; 20:95–106. [PubMed: 24155518]
38. Schmidt MH, Bajic DM, Reichert KW, Martin TS, Meyer GA, Whelan HT. Light-emitting diodes as a light source for intraoperative photodynamic therapy. *Neurosurgery.* 1996; 38:552–556. [PubMed: 8837808]

Fig. 1.

Pharmacokinetics of the fluorescent dye sulforhodamine B (Srho) injected at the sciatic nerve with and without DMED. Plasma concentration of Srho over time following peri-sciatic injection of (A) Free Srho in PBS solution with and without free DMED. (B) Lipo-Srho with Lipo-DMED or with blank liposomes (Lipo). Data are means $\pm$ SD, $n=4$. [‡] $P=0.01$; * $P = 8.7 \times 10^{-6}$; ** $P = 0.0014$, *** $P = 0.0014$.

Fig. 2.

Characterization of Lipo-PS-TTX and Lipo-DMED drug release *in vitro* upon NIR irradiation. (A) Schematic of the liposomes Lipo-PS-TTX and Lipo-DMED and their corresponding particle size. (B) Cumulative TTX release from Lipo-PS-TTX + Lipo-DMED with and without irradiation. (C) Cumulative DMED release from Lipo-PS-TTX + Lipo-DMED with and without irradiation. The dotted grey lines indicate irradiation events at 730 nm, 100 mW/cm², for 10 min. Data are means ± SD, n=4.

Fig. 3.

Repeatability and adjustability of phototriggered nerve block *in vivo*. (A) Phototriggerability of different liposome combinations using low power NIR light. Black arrows indicate irradiation events at 730 nm for 5 min at 75 mW/cm². (B) Effect of duration (730 nm, 75 mW/cm²) of irradiation events at 41 h after injection of Lipo-PS-TTX + Lipo-DMED. (C) Nerve block duration as a function of irradiation (730 nm) energy density, induced by Lipo-PS-TTX with and without co-injection of Lipo-DMED. Data are means $\pm$ SD, n=4.

Fig. 4. Animal tissue dissection and histology after administration. (A–B) Representative photographs of liposome residue at site of injection (4 days after the last irradiation event). (A) Lipo-PS-TTX + Lipo-DMED; (B) Lipo-TTX + Lipo-DMED. (C–D) Representative light micrographs of H&E stained sections of tissues injected with Lipo-PS-TTX + Lipo-DMED (C) without and (D) with nine separate and consecutive irradiation events at 730 nm, 75 mW/cm² for 5 min. Scale bars are 100 µm.

Fig. 5. LED-triggered nerve block *in vivo*. Animals were irradiated with a 725 nm – 755 nm LED at 50 mW/cm² for 15 min, 41 h after injection of Lipo-PS-TTX with and without Lipo-DMED. Data are means $\pm$ SD, n=4.

Table 1

Tissue reaction to Lipo-PS-TTX + Lipo-DMED

	No Light	Irradiated ^{***}
Inflammation [*]	1 (1-1)	1 (1-1)
Myotoxicity ^{**}	0 (0-1)	0 (0-0.25)

^{*} Inflammation scores (0 = normal to 4 = severe inflammation; see Methods). Data are medians $\pm$ quartiles, n=4. P = 0.32 comparing Lipo-PS-TTX + Lipo-DMED groups with and without irradiation.

^{**} Myotoxicity scores (0 = normal to 6 = severe myotoxicity; see Methods). Data are medians $\pm$ quartiles, n=4. P = 0.50 comparing Lipo-PS-TTX + Lipo-DMED groups with and without irradiation.

^{***} Rats were exposed to nine separate and consecutive 5-min irradiation events with a 730-nm laser at 75 mW/cm², 41 h after injection of formulation.