Journal of Controlled Release 268 (2017) 113–119

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Defining optimal permeant characteristics for ultrasound-mediated gastrointestinal delivery

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ARTICLE INFO

Keywords:
Gastrointestinal drug delivery
Low-frequency ultrasound
Microparticle delivery
Oral delivery
Ultrasound-mediated drug delivery

ABSTRACT

Ultrasound-mediated drug delivery in the gastrointestinal (GI) tract is a bourgeoning area of study. Localized, low-frequency ultrasound has recently been shown to enable significant enhancement in delivery of a broad set of active pharmaceutical ingredients including small molecules, proteins, and nucleic acids without any formulation or encapsulation of the therapeutic. Traditional chemical formulations are typically required to protect, stabilize, and enable the successful delivery of a given therapeutic. The use of ultrasound, however, may make delivery insensitive to the chemical formulation. This might open the door to chemical formulations being developed to address other properties besides the deliverability of a therapeutic. Instead, chemical formulations could potentially be developed to achieve novel pharmacokinetics, without consideration of that particular formulation's ability to penetrate the mucus barrier passively. Here we investigated the effect of permeant size, charge, and the presence of chemical penetration enhancers on delivery to GI tissue using ultrasound. Short ultrasound treatments enabled delivery of large permeants, including microparticles, deep into colonic tissue *ex vivo*. Delivery was relatively independent of size and charge but did depend on conformation, with regular, spherical particles being delivered to a greater extent than long-chain polymers. The subsequent residence time of model permeants in tissue after ultrasound-mediated delivery was found to depend on size, with large microparticles demonstrating negligible clearance from the local tissue 24 h after delivery *ex vivo*. The dependence of clearance time on permeant size was further confirmed *in vivo* in mice using fluorescently labeled 3 kDa and 70 kDa dextran. The use of low-frequency ultrasound in the GI tract represents a novel tool for the delivery of a wide-range of therapeutics independent of formulation, potentially allowing for the tailoring of formulations to impart novel pharmacokinetic profiles once delivered into tissue.

1. Introduction

Gastrointestinal (GI)-based drug delivery is an often preferred means for delivering drugs due to the ease and convenience typically associated with this route of administration [1]. The oral delivery of a broad set of therapeutics remains an area of intense research owing to the challenges presented by the physiology of the GI tract [2]–[4]. Specific challenges around drug delivery to the GI tract include poor drug stability and low solubility of drugs in the gastric environment, low permeability owing to the mucus barrier, and extreme susceptibility to degradation by pH extremes, bacteria and degradative enzymes [5]–[7]. These challenges are amplified further when targeted delivery to a specific location in the GI tract is required, necessitating sophisticated formulation approaches [8]–[10]. Effective oral delivery for the treatment of colonic diseases, like inflammatory bowel disease, for example, not only requires the delivery of an efficacious therapeutic

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http://dx.doi.org/10.1016/j.jconrel.2017.10.023
Received 29 March 2017; Received in revised form 1 October 2017; Accepted 13 October 2017
Available online 16 October 2017

to the affected site but also protection of the molecule from the harsh, proximal GI environment. Moreover, targeting to the site of disease can be a complex formulation challenge, utilizing pH-responsive nanoparticles [11] or surface modifications to enable high selectivity [12].

These hurdles largely stem from the need for the formulation to play two roles: i) encapsulation and protection of the therapeutic from the gastric environment, and ii) high target-specificity to minimize off-target effects [8,13]. These two roles often require disparate formulation strategies, leading to therapeutics with low bioavailability and inadequate target specificity [14,15]. The ability to decouple these two requirements such that the formulation no longer has to satisfy both requirements may help expedite translation of successful therapeutics to the clinic [16]. Physical enhancers are one such method that may enable formulation-independent delivery of material and interest in their use in the GI tract has recently increased [17,18]. Ultrasound, one type of physical enhancer, has recently begun to be investigated in the GI tract with potentially broad utility, allowing the successful delivery of small molecules, biologics, and nucleic acids in an enema format [17,19].

Ultrasound is a sound wave characterized as having a frequency above the audible range of humans ($>$ 20 kHz) [20]. Ultrasound has seen broad clinical use for a myriad of applications, including imaging, lithotripsy, and lysis of fat during liposuction [21]. With respect to drug delivery, ultrasound has been investigated for decades for transdermal drug delivery [22]. The enhancement in drug uptake using ultrasound relies on a phenomenon known as acoustic cavitation [23]. When an ultrasound wave is propagating through a fluid, the oscillating pressure field spontaneously nucleates bubbles in the solution [20]. Using low-frequency ultrasound, ($\leq$ 100 kHz) these bubbles grow through rectified diffusion, and eventually become unstable [24]. They then implode, causing a microjet [25]. These microjets can physically propel drug into tissue and reversibly permeabilize tissue to allow enhanced drug uptake [17,26].

Clinically, ultrasound might lend itself to drug delivery applications in the GI tract, in addition to the skin. One such embodiment of the technology in the GI tract could be the administration of medicated enemas for targeted delivery to the rectum in the setting of diseases such as ulcerative colitis [27]. More broadly, ultrasound is also readily miniaturizable, which may enable fully ingestible capsules for the oral delivery of therapeutics currently limited to injection [28,29]. Given the potentially broad use ultrasound might have for drug delivery and the fact that its previously been shown to enable formulation-independent delivery of proteins and nucleic acids [17,19], this study sought to identify and characterize permeant properties that can modulate delivery and retention in GI tissue.

2. Materials and methods

2.1. Chemicals

Phosphate buffered saline (PBS), dextran labeled with Texas red (3 kDa and 70 kDa), dextran labeled with tetramethylrhodamine (2000 kDa), and carboxylate-modified and amine-modified polystyrene microspheres were obtained from Thermo Fisher Scientific (Waltham, MA). Sodium hydroxide was obtained from Amresco (Solon, OH). Sodium lauryl sulfate (SLS) and formalin were obtained from Sigma-Aldrich (Saint Louise, MO). All chemicals were used as received.

2.2. Tissue procurement

This research was approved by the Massachusetts Institute of Technology (MIT) Committee on Animal Care. Fresh GI tissue from Yorkshire pigs was procured within an hour of sacrifice. The tissue was washed thoroughly using PBS and excess fat dissected away. The tissue was sectioned into pieces approximately 2 cm × 2 cm for subsequent mounting in Franz diffusion cells with an exposed area for delivery of 15-mm (PermeGear, Hellertown, PA). First the receiver chamber was filled with PBS and the tissue placed on top of the receiver chamber with the muscularis layer in contact with the receiver chamber. A donor chamber was then placed on top of the tissue and the setup clamped together. PBS was added to the donor chamber to keep the tissue hydrated before treatment. Care was taken to ensure there were no air bubbles in the receiver chamber. Experiments were conducted at room temperature.

2.3. Ultrasound treatment

Ultrasound was generated with a 20 kHz, VCX500 probe from Sonics & Materials (Newtown, CT). Ultrasound was applied with the transducer positioned 3 mm above the tissue surface at an intensity of 5 W/cm^2 calibrated by calorimetry [30]. A 50% duty cycle was utilized to reduce thermal effects [31]. Immediately before treatment, the PBS was removed from the donor chamber and the coupling fluid containing the permeant of interest was added. Fluorescently labeled probes were used as the model permeants and used at a concentration of 0.2 mg/mL unless otherwise stated.

2.4. Delivery quantification in tissue

Permeant content in the tissue after delivery was quantified using an *In Vivo* Imaging System (IVIS) Fluorescent Imager (PerkinElmer, Waltham, MA). Immediately after ultrasound treatment, the donor chamber solution was discarded and the tissue washed. Tissue samples were then imaged with the IVIS Fluorescent Imager. Unless otherwise noted, imaging was performed using a binning factor of 8, f-stop of 8, and a field of view of 21.6 cm. Exposure time was varied to ensure a total photon count of $\geq$ 6000, per the manufacturer's guidelines.

2.5. Tissue clearance tests

Permeant clearance from tissue samples was also investigated *ex vivo*. Tissue samples were treated in Franz diffusion cells as described. After treatment, the treated tissue samples were thoroughly washed and placed in individual 500 mL beakers filled with 300 mL PBS to mimic an infinite-sink condition. All beakers were stirred on a magnetic stir plate at 400 rpm. 24 h after treatment, tissue samples were removed from the beakers, thoroughly washed, and imaged using an IVIS Fluorescent Imager.

2.6. Scanning electron microscopy (SEM)

In order to image microparticles within tissue after delivery, samples were imaged by scanning electron microscopy using a JEOL JSM-5000 Scanning Electron Microscope and Environmental Scanning Electron Microscope. Samples were prepared for imaging by dehydration in 200 proof ethanol at serial concentrations of 50%, 75%, 90%, 95%, and 100% ethanol. Dehydration in each concentration lasted 20 min. Ethanol-dehydrated samples were finally dried using a critical point drying instrument. Dried samples were mounted on aluminum stages using carbon black stickers and coated with gold nanoparticles by spattering. Samples were imaged using an acceleration voltage of 5 kV, working distance of 20 mm and a spot size of 20 at various magnifications.

2.7. Confocal microscopy

Fluorescently labeled permeants were also imaged for their distribution within tissue by confocal microscopy. After ultrasound treatment, the tissue was thoroughly washed and removed from the Franz diffusion cells. Tissue was fixed with 10% formalin overnight. After fixation, tissue samples were stained with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) nuclear stain for 30 min.