Editorial

Low-frequency ultrasound may improve drug penetration in colonic mucosa

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Ulcerative colitis (UC) is a chronic relapsing inflammatory disease that is characterized by disruption of the mucosal barrier and excessive inflammatory reactions in the colon and rectum (1). The common symptoms of UC include diarrhea, hemafecia, abdominal pain, and malnutrition (2). Although immense progress has been made in developing efficient therapeutic strategies, there is still no fully effective cure for UC. Given the lack of treatment options, UC patients currently suffer from a compromised quality of life and an increased risk of developing colorectal cancer (3,4).

The need for rapid drug penetration through the colonic mucosa is a great hurdle for UC therapy due to the presence of physiologic and structural barriers, such as mucus and a wealth of digestive enzymes (5). Mucus is a viscoelastic and adhesive gel that is mainly formed by mucin fibers, which have hydrophobic main chains and highly glycosylated side segments (6). These structural features enable mucus to form a stable three-dimensional network that traps pathogens and foreign particles through hydrophobic, electrostatic, hydrogen, and/or steric interactions. These trapped materials tend to be eliminated *via* the constant turnover of mucus. For a drug to be delivered to the colonic mucosa, it must avoid degradation in the colonic lumen, diffuse through the mucus, and enter the mucosal tissue via the epithelial enhanced permeability and retention effect. Therefore, a strategy that facilitates rapid drug penetration into the colonic mucosa might hold great promise for clinical applications. Enema-based administration is one of the most common therapeutic approaches for UC therapy (7). The challenge with medicated enema is to extend its residence time in colon, and thus maximize the opportunity for drug penetration into colonic mucosa. However, the rapid turnover of mucus and diarrhea make it difficult to achieve long-term drug retention in UC.

Ultrasound (US), which is a mechanical and longitudinal wave with frequencies above the human hearing range (>20 kHz), has been widely accepted as a safe and noninvasive therapeutic modality in the clinic (8). High-frequency US (>1 MHz) is applied in a variety of medical fields (e.g., ultrasonography, tumor ablation, and lithotripsy). In contrast, low-frequency US (<100 kHz) is commonly used in transdermal drug delivery due to its unique capacity to produce a phenomenon known as transient cavitation (9). Although low-frequency US of typical intensities was associated with higher cavitational activity than that triggered by high-frequency US at the same intensities (10), low-frequency US has seldom been implemented to enhance drug delivery to the colonic mucosa.

Schoellhammer *et al*., in a paper published in *Gastroenterology*, recently hypothesized that low-frequency US could provide rapid drug delivery to the colonic mucosa for UC therapy (11). The authors first investigated whether low-frequency US could enhance the delivery of macromolecules to fresh porcine colon tissues mounted in Franz diffusion cells. The results of this *ex vivo* experiment indicated that exposure to low-frequency US (20 kHz)

could enhance the delivery of dextran (3 kDa) tagged with Texas red almost 7-fold compared to passive diffusion within the same timeframe. Further confocal imaging demonstrated that the fluorescent dextran was dispersed homogenously throughout the tissues. In contrast, non-US-exposed tissues showed negligible fluorescent dextran signals, indicating that the enhanced penetration and accumulation of macromolecules in colonic mucosa tissues could be attributed to the application of low-frequency US. On the basis of these exciting *ex vivo* results, Schoellhammer *et al*. further assessed the potential of low-frequency US to facilitate macromolecule delivery *in vivo*. They rectally administered fluorescent dextran into the colons of mice, and then placed the US probe. They found that low-frequency US could enhance the delivery of dextran by 3-fold compared to passive diffusion, and that the fluorescent signal from dextran was still markedly increased in colonic tissue at 2 hours after the administration of the drug plus low-frequency US. Moreover, US appeared to enable the fluorescent dextran to penetrate more deeply into tissues immediately after administration. To demonstrate that low-frequency US could enhance the therapeutic efficacy of drugs, the authors next used a siRNA targeting the tumor necrosis factor (*Tnf*) mRNA. Their *in vivo* findings demonstrated that mice treated with *Tnf* siRNA plus US showed significantly better fecal scores and statistically lower histology scores than any other experimental mouse group. When normalized by the total protein amount in colon tissues, the *Tnf* protein levels were 7 to 8-fold lower in mice that received *Tnf* siRNA plus US compared to the other experimental mouse groups, indicating that *Tnf* was successfully knocked down by this strategy. In addition, the authors showed that daily administration of low-frequency US was safe and well tolerated.

The lack of a siRNA carrier is a major restriction in the study presented by Schoellhammer *et al*. Although the investigators obviously increased the cellular uptake efficiency of siRNAs using low-frequency US, they found that the unencapsulated siRNAs directly entered the cytoplasm and failed to show a constant release pattern (11). A consistent release pattern is considered to be a critical prerequisite for long-term RNA interference (RNAi) (12). Owing to the inherent properties of unencapsulated siRNAs (e.g., negatively charges, high stiffness, high aqueous solubility, and easy degradation by extra- or intracellular nucleases), it has proven extremely difficult to use them as a therapeutic agent (13). Instead, the successful application of siRNAs has proven to be largely dependent on their carrier. The encapsulation of siRNAs into nanoparticles (NPs) has been shown to protect them against premature degradation, improve their transportation across the cytomembrane, and facilitate their constant release to the cytoplasm of target cells, where they can enter the RNAi pathway (14). Our previous study showed that unencapsulated siRNAs exhibited negligible cellular uptake, whereas NP-encapsulated siRNAs exhibited significant cellular uptake (15). In addition, US has been shown to increase the cellular uptake efficiency of NPs. A recent study by Mead *et al*. (16) revealed that approximately 42% of cells internalized NPs when treated with US, whereas less than 6% of cells took up NPs without US treatment. Thus, it is reasonable to speculate that the use of a carrier could increase the cellular uptake efficiency of siRNAs and provide a constant release profile following the application of US.

A great deal of research effort has focused on the development of siRNA carriers. One of the most promising carriers is bioreducible poly(amido amine)-based NP, which has a lot of unique merits, including: (I) well-defined chemical structures comprising disulfide bonds, primary amines, secondary amines, and tertiary amines; (II) an excellent siRNA-encapsulating capacity, with the positively charged groups preferentially condensing the negatively charged siRNAs into the NP; (III) a "proton sponge" effect, in which the abundant amine groups in the polymers trigger lysosomal damage and escape; and (IV) good bioreducibility and biocompatibility. The extracellular liquid is an oxidizing environment, while the intracellular compartment is full of glutathione and other molecules with free thiol groups (17). Thus, bioreducible poly(amido amine)-based NPs are relatively stable in the extracellular environment, but are liable to be degraded in the cytoplasm. Meanwhile, their cytotoxicities are very low due to polymer degradation. Recently, Xiao *et al*. (15) synthesized a bioreducible poly(amido amine) polymer, and further fabricated siRNA-loaded NPs that they called TPP-PPM/siRNA NPs. They found that treatment of lipopolysaccharide-stimulated macrophages with TPP-PPM/siRNA NP decreased in *Tnf* expression to a degree comparable to that seen in parallel experiments using cells transfected with *Tnf* siRNA and the leading commercial transfection reagent (Oligofectamine^{TM}). Thus, these NPs appeared to have an efficient anti-inflammatory effect. Another promising class of carriers for siRNA delivery are the poly(lactic-*co*-glycolic acid) (PLGA) NPs. PLGA is an FDA-approved biodegradable polyester that has been widely applied in drug delivery (18). Saltzman *et al*. showed that siRNA/spermidine complexes

encapsulated within PLGA NPs could confer efficient and sustained gene silencing (19). To further increase the therapeutic efficacy of drugs and reduce their potential adverse effects, scientists have sought to achieve active-targeting drug delivery. The interactions between targeting moieties on the surface of NPs and specific receptors over-expressed on the surfaces of target cells are expected to increase the internalization efficiency of NPs into target cells (20). The ligands used to date for colitis-targeted drug delivery include lectin (e.g., wheat germ agglutinin), antibodies (e.g., against transferrin receptor, CD98, and F4/80), monosaccharides (e.g., mannose and galactose) and polysaccharides (e.g., hyaluronic acid) (21).

The conventional strategies for UC therapy are limited to control inflammation. However, the main goal of clinical UC therapy is to not only decrease inflammation in colitis tissue but also achieve mucosal healing (22). Novel therapeutic strategies are urgently needed to achieve these two goals. Our recent *in vitro* and *in vivo* results collectively suggested that CD98 siRNA plus curcumin co-loaded into NPs exhibited a synergistic therapeutic effect against UC by preventing mucosal damage and relieving inflammation (23). These data suggested that combination therapy holds great promise against UC. In their work, Schoellhammer *et al*. (11) open the door to a broader application of US while providing a simple and robust strategy to address the barrier limiting the penetration of drugs into mucosal tissues. While their preclinical studies are promising, the clinical benefits of low-frequency US in drug delivery have yet to be investigated. The future clinical application of low-frequency US will depend on continued efforts to optimize various combinations of drugs, drug carriers, and targeting ligands.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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