

Can colorectal delivery technology provide a platform for enteral oligonucleotide-based therapeutics?

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Summary

Nucleic acid-based therapeutics including antisense and siRNA oligonucleotides has been expected as an innovative treatment for intractable diseases. Oral drug delivery is the most patient-friendly route of administration but developing an effective delivery system for oligonucleotides remains a major challenge. In this commentary, we discuss the potential benefits of the colorectal route as another platform for the development of oral oligonucleotide therapeutics. The importance of the targeting or the availability of oligonucleotides in targeted tissue is highlighted in contrast to systemic availability, while the liver-targeted enteral siRNA delivery technology that we recently developed is introduced.

Keywords: Oligonucleotide, drug delivery, siRNA, large intestine, liver, lymph

Oligonucleotides are promising candidates for effective therapeutic entities, not only for genetic or intractable diseases, but also for various other illnesses (1,2). Most major chronic diseases that account for the majority of deaths are refractory in nature. It is becoming clear that nucleic acid medicines are effective treatments for these diseases. Suppressing disease-specific gene expression using small interfering RNA (siRNA) or antisense oligonucleotide is quite effective and a promising treatment modality (3), but the success of this technique depends on nucleic acid molecules selectively reaching the target gene in the body. It also goes without saying that the *in vivo* stability and target specificity of the nucleic acid molecules themselves are important factors. Advances in nucleic acid synthesis techniques with chemical modifications including the introduction of phosphorothioate linkages and 2'-*O*-methylation of the ribose moieties have led to the development of nucleic acids that are resistant to nucleolytic enzymes and are extremely stable *in vivo*, improvements in the selectivity and affinity towards target genes, and significant reductions in the risk of off-target adverse effects (4-9). The cost of synthesizing nucleic acid

molecules has also continued to decline, while the cost-benefit ratio, which has been a bottleneck for oral drug development, has also been rapidly improving (10). However, many significant hurdles must be overcome to develop oral drugs based on nucleic acids. First, there is an issue with stability with respect to enzyme degradation in the alimentary canal and permeability through the intestinal epithelium by nucleic acids, which are polyanionic macromolecules. Another unresolved issue is the mechanism by which they are transported selectively to the target tissue. On the other hand, there have been a number of recent reports on the oral and enteral delivery of nucleic acid molecules, including in humans, suggesting its feasibility (11-13). However, these studies have focused on absorption through the small intestine and accordingly it is necessary to include large quantities of nucleic acids as well as absorption promoters. Therefore, these results may not provide sufficient grounds for initiating drug development based on high-cost nucleic acid molecules. We designed a delivery system aimed at the systemic liver-specific delivery of nucleic acid molecules from the large intestine, including the colon and rectum (14). This system succeeded in suppressing serum lipid levels by specifically delivering siRNA in mice from the colon to the target organ, which was the liver, where the targeted *ApoB* gene was suppressed. In this system, vitamin E was bonded to the siRNA to achieve liver-specific delivery. In order to improve

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intestinal epithelial permeability, this was prepared in the form of nanoparticles combined with long-chain unsaturated fatty acids. By post-prandial administration when chylomicron synthesis is elevated, effective and specific delivery from the alimentary canal to the liver was achieved. This system is characterized by utilizing an endogenous transport carrier without the use of an artificial carrier, such as a virus or polymer. In addition, the importance of targeting or the availability of oligonucleotides in targeted tissue should be highlighted in contrast to systemic availability, which does not directly reflect the effects of oligonucleotide. This is because a lower systemic availability can be compensated by a higher accumulation of the oligonucleotide in the target tissue.

There are differences between drug preparations targeting the small intestine and those targeting the colon and rectum. The small intestine has the advantage of high rates of synthesis and internal chylomicron secretion. However, there is a high likelihood of significant nucleic acid losses as well as reductions or fluctuations in the effectiveness of absorption promoters. This occurs by many mechanisms, including high decomposition activity owing to the secretion of digestive enzymes, significant dilution of active ingredients and adjuvants eluted from drugs by food and digestive fluids in the gut lumen, and a relatively low residence time. In addition, the small intestine has much larger surface area for digestion and lower susceptibility to the effects of most absorption enhancers compared to the large intestine (15). Therefore, it is necessary to load large amounts of nucleic acids and absorption promoters, resulting in high costs. In addition, the risk of adverse effects may also be high.

On the other hand, water absorption takes place in the colon and accordingly there is a relatively low degree of dilution and a significant reduction in enzyme activity compared to the upper gastrointestinal tract. In addition, the local physicochemical environment in the colon and rectum can be temporarily controlled to an extent using additives. In our system, the formation of complexes with chylomicrons is the key to targeted delivery to the liver, but the chylomicrons secreted in the small intestine accumulate at high concentrations in intestinal lymphatics and eventually thoracic lymph duct. Macromolecules, such as siRNA, exhibit excellent selective transfer to the lymphatic system, and molecules with hydrophobic modifications have particularly high self-associating properties as well as a high rate of bonding with proteins, potentially enabling increased targeting to the lymphatic system (16). As a result, vitamin E-modified siRNA that passes through the colonic epithelium progresses within lymph vessels and has many opportunities to form complexes with chylomicrons before entering the systemic circulation *via* the thoracic duct. Macromolecular movement towards the lymphatic system increases as the

molecular weight increases, but absorption through the gastrointestinal tract decreases with molecular weight (17). As a result, it is highly likely that an optimal molecular weight exists for the liver-specific delivery of nucleic acid molecules *via* lymph vessels from the gastrointestinal tract. However, further insight in this area will require additional research.

Our systemic delivery technique for nucleic acids *via* the large intestine not only has the potential to be developed into a drug for use as an enema or suppository, but could also be developed into an oral drug, taking advantage of a colonic drug delivery system (18-20). Thus, this colorectal delivery technique may provide a new foundation for the development of oligonucleotide-based oral therapeutics.

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