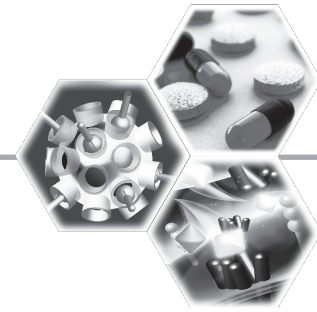

Oral delivery of nucleic acid-based therapeutics

"…today's relevant question is, can nucleic acid-based therapeutics delivered by the per os route be developed into drug products?"

Keywords: cost of therapy ■ nucleic acid-based therapeutics ■ oligonucleotide ■ oral bioavailability ■ oral delivery ■ penetration enhancers

Can nucleic acid-based therapeutics (NABTs) be delivered orally? The simple answer is 'yes,' when properly formulated. As evidenced by an increasing number of preclinical reports, the appearance of intact NABTs in the systemic circulation after per os (p.o.) dosing can lead to expression of pharmacology [1–3]. Additionally, a few clinical pharmacokinetic reports have appeared that include descriptions of measured pharmacologic effect as well as target reduction after repeated dosing [4,5].

Therefore, today's relevant question is 'can NABTs delivered by the p.o. route be developed into drug products?' While technical challenges remain to further develop p.o. delivery (as detailed below), the primary obstacle to administering NABTs by this route is the absolute and relative cost of therapy. In this regard, it is useful to examine the current economics of NABT therapy, specifically:

- Dose/potency;
- Cost of goods;
- Efficiency of delivery.

Dose/potency

Historically, NABTs that accessed their targets via the systemic circulation required doses of greater than 1 mg/kg/day. However, with the investment into more metabolically stable and higher affinity chemistries, and constructs with different modes of action, the effective potency is increasing dramatically. We now see potencies described in terms of less than 0.1 mg/kg/day [6].

Cost of goods

A fair number of single- and double-stranded NABTs are currently under development, and while the cost may vary depending upon the chemistry, they utilize similar synthesis technologies, which we can roughly speak of as a group. To date, it could be expected that the small quantities required for discovery, preclinical and clinical work, would cost more than US$1000/g. However, with recent advancements in the processing and purification of NABTs, combined with the economies of scale for both raw materials and finished goods, we can expect to see these prices fall to the $100/g range for metric ton/year quantities.

Efficiency of delivery

In considering this part of the equation, it is important to understand the interplay between a route's efficiency and the cost of convenience. For example, on the surface, with 10% absolute p.o. bioavailability, a p.o. dose would cost ten-times as much as an intravenous dose. Assuming an approximate intravenous dose cost of $100, the choice between convenience and cost seems clear. However, if economies of scale in drug production continue to drop and drug potencies continue to increase, the argument for the convenience of orally administered therapies becomes much stronger. If a therapeutic dose costs closer to $1.00, the convenience of a p.o. form with less than 100% efficiency becomes attractive. Given these trends, it seems as if this is the appropriate time to be investing in the research and development necessary to bring p.o. dosage forms of NABTs to market.

Oral bioavailability enhancement of NABTs

The p.o. absorption of hydrophilic macromolecules such as soluble NABTs, is limited due to poor permeability across the gastrointestinal membranes [7]. As an example, single-strand oligonucleotides, constructed with a variety of chemical modifications to impart presystemic

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stability and of varying lengths from 14 to 22 nucleotides, show an unaided p.o. bioavailability of much less than 1% [8,9]. However, it is well known that certain permeation enhancers can transiently open the epithelial tight junctions at the apical side of the enterocytes, thus, significantly increasing paracellular absorption of a variety of hydrophilic and charged molecules [10]. One such enhancer, the fatty acid sodium caprate (C10), has been extensively studied for this application and was found suitable for p.o. delivery of oligonucleotides in both animals and man [11]. Permeation data from *in vivo*/*in situ* rodent studies of oligonucleotides calculate tight junction opening after C10 dosing to be consistent with the cross-sectional diameter of the single-strand oligonucleotides [12]. It remains to be seen if double-stranded constructs will show the same degree of increased permeation when paracellular junctions are opened to the same degree.

Creating solid dosage forms

Based on promising bioavailability data from solution presentations of oligonucleotides with penetration enhancers, efforts to create solid dosage forms for delivery of oligonucleotides have been reported in literature [13]. Initially, these efforts showed that when solution formulations of C10 are formulated as immediate-release solid dosage forms, much, if not all, of the bioavailability seen with solution formulations was lost. Analysis of these data show that, when presented as an immediate-release dosage form to the stomach, the C10 enhancer is both diluted in the intestinal milieu and rapidly absorbed, decreasing the effect on downstream paracellular junctions, ultimately leading to a reduction in the amount of oligonucleotide absorbed. Considering the dynamics of gut absorption, it is easy to imagine that the oligonucleotide moves beyond the mucosal site made more permeable by the transient enhancer. This can be demonstrated rather easily using rodent models that contrast a static GI tract with a flowing GI tract, as well as with a controlled-release dosage form having synchronous release of C10 and drug [14].

Expanding upon this simple observation, a variety of innovative studies have been reported that provide insight into the pharmacodynamics of sodium caprate [15]. The most useful of these data come from animal models and human trials using solution presentations of formulations, typically through the use of ports or catheter-intubations, wherein the timing and the amount of enhancer released (relative to the drug and GI tract location) were directly controlled. From the understanding of sodium caprate's pharmacodynamics, criteria for engineering of an effective controlled-release solid dosage form were established. The three key criteria determined were:

- A threshold amount of Caprate is required to open the paracellular junctions;
- The effect has a limited duration;
- In oligonucleotide dose ranges >1 mg/kg, no dose dependency is noted (i.e., a first-order absorption process) [16].

Using these insights, dosage forms were prototyped and characterized *in vitro* and *in vivo* in dogs. Attention was paid to both the release characteristics of the drug and the penetration enhancer; either of which do not necessarily need to occur at the same time, place or rate. Selected dosage forms (having different release profiles) were studied clinically in normal, healthy human volunteers. p.o. absorption of the 2′-*O*-(2-methoxyethyl) modified antisense oligonucleotide, was demonstrated to have an average 9.5% plasma bioavailability across four formulations tested. The greatest average performance achieved for a single formulation was 12% bioavailability, with an individual dose and subject range of 2–28% [5]. Fortunately in the case of most NABTs, the dose-to-dose and patient-to-patient variability is acceptable because of the broad therapeutic window and the long pharmacodynamic half-lives. Nonetheless, we would expect these variations to decrease as the absolute bioavailability for advanced formulations increase.

Questions of immediate interest & where do we go next?

The question of the safety of using a penetration enhancer as a formulation adjunct is fair and appropriate as this adjuvant/excipient is intended to have pharmacological activity. One of the most advanced penetration enhancers, indeed registered in certain regions of the world, is capric acid also known as C10. Excellent review articles have been published, which collect valuable information for the safe and effective use of this enhancer [11,17]. Directly in conjunction with NABT p.o. dosing, caprate was generally well tolerated in clinical studies with up to 3 months of daily dosing [4]. Based upon this body of data, it would be prudent for anyone exploring this route of dosing a NABT to start with this enhancer.

The next question may well be, 'Is there a better formulation, one which achieves a higher oral bioavailability?' There are certainly a number of other penetration enhancing agents, which accomplish the same objective of opening the paracellular junction [18]. It is conceivable that an alternative permeation enhancer would offer greater formulatability, permeation enhancer action, improved safety profile and bioavailabilities approaching 25%.

Also unanswered at this time, is the question of the impact of a greatly decreased dose. Is there a point where alternate mechanisms become important to the p.o. uptake of a NABT? In relation to this point it has been recently reported that miRNA can be taken up into the circulation at very low levels, and in at least one example, influence upon circulating macrophages has been reported [2]. These reports are consistent with the GI tract sampling of the gut-associated lymph tissue system but, at this time, do not seem to offer a therapeutic opportunity for most NABTs.

Conclusion

Convergent increases in absolute bioavailability resulting from formulation efforts and the decreased cost of therapy arising from increased potencies and lower cost of goods would point to the ultimate success of orally delivering future NABTs. However, the most current development programs are still emerging from low-potency and high-cost-of-goods programs, so it may be a while before we see real reports of clinical development of p.o. dosage forms of NABTs.

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