

A Renaissance in Peptide Therapeutics is Underway

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Introduction

It has long been recognised that peptide and protein drugs are among the most useful and effective therapeutics yet discovered. However, the practical use of most peptide drugs has been confined to treatment of diseases having severe or life threatening consequences as a direct result of the requirement for administration by injection. Recent advances in transmucosal drug delivery have created a rebirth in interest in peptide drugs. This was perhaps brought home most dramatically by the simultaneous approval of Exubera®, the first non-injectable insulin product to come along since the discovery of insulin, by both the European Commission and the FDA. Punctuating the point is the fact that it was not one of the more recent biopharma entries that took this innovative step, but rather **Pfizer**, the largest, and one of the most successful, pharmaceutical companies in the world.

Peptides often demonstrate high potency and high selectivity while exhibiting essentially no chemical toxicity. Because they are metabolised to naturally occurring amino acids, peptides and proteins do not invoke xenobiotic metabolic processes, nor do they accumulate significantly in organs – principle sources of small molecule drug toxicity. In spite of the many attractive aspects of peptides and proteins as potential therapeutic agents, their susceptibility to denaturation, hydrolysis and poor absorption in the gastrointestinal tract makes them unsuitable for oral administration, typically requiring administration by injection. This remains their major shortcoming. In spite of this, more than 140 peptide and protein drugs are in use today.

From a commercial perspective, the opportunity for systemically-acting peptide and protein drugs is potentially quite large. In 2003, sales of approved peptide therapeutics in the US alone totalled more than US\$9 billion (MedAd News, 2003) and sales of therapeutic proteins grew to US\$37 billion, with 2010 sales predicted to exceed US\$90 billion (Parmar, 2004). Among transmucosally-delivered drugs, the current global market for nasally-delivered medications is valued at greater than US\$6 billion (Devillers, 2003). While the growth rate for topically-acting intranasal drugs, such as those used to treat allergic

rhinitis, is about 10%, the growth rate for intranasal delivery of systemically-acting drugs is 30% (Bommer, 2002). This dramatically outpaces the growth of the overall worldwide pharmaceutical market, which is projected to grow at 6-7% during 2006 (Aitken, 2005).

Strong patient desire to avoid repeated injections, both for peptides and non-peptides alike, has spurred growing interest in researching and developing alternate administration routes. While Exubera relies upon transmucosal absorption across the pulmonary mucosa of the lungs, a number of alternative transmucosal delivery routes for macromolecular drugs, that similarly circumvent the need for injection but offer greater patient convenience as well as certain cost and other commercial advantages, are in clinical and preclinical development (*Table 1*). Each of these alternate routes has both strong and weak points and the selection of the most propitious route of administration is determined in large part by the properties of the individual drug in question (e.g. solubility, total mass required per dose, localised biological action upon mucosal tissue, and so on).

A New Class of Transmucosal Absorption Enhancement Agents

Over the past two decades, a large number of molecules, encompassing at least a dozen chemical and biological approaches, have been screened for the ability to enhance

Nasal	Aqueous spray/metered pump
Pulmonary	Dry powder inhalation
Sublingual	Oral cavity spray or 'flash-dissolve' formats
Buccal	
Intestinal	Enteric coating or targeted osmotic release
Vaginal	Suppositories
Rectal	

Table 1 – Transmucosal (non-injection) administration routes for peptide drugs.

Approaches to Transmucosal Absorption Enhancement

Aggregation inhibitory agents
 Charge-modifying agents
 pH control agents
 Degradative enzyme inhibitors
 Mucolytic or mucus clearing agents
 Ciliostatic agents
 Membrane penetration-enhancing agents
 Vasodilators
 Vasoconstrictors
 Selective transport-enhancing agents
 Stabilising delivery vehicles
 Protein complex-forming species

Examples of molecules studied as transmucosal absorption enhancers

Aprotinin	Lysophosphatidylcholine
Benzalkonium chloride	Menthol
Capric acid, sodium salt	Methoxysalicylate
Cetylpyridinium chloride	Methyloleate
Chitosan	1-O-Octadecyl-2-O-methyl-sn-glycero-3-phosphocholine
Chitosan-4-thiobutylamidine	Oleic acid
Cyclodextrins	Palmitoylcarnitine
Deoxycholic acid, sodium salt	1-Palmitoyl-2-(5'-oxo-Valeroyl)-sn-glycero-3-phosphocholine
Dextran sulfate	1-Palmitoyl-2-glutaroyl-sn-glycero-3-phosphocholine
Dodecyl azacycloheptyl-2-ketone	Poloxamer 407
EDTA	Polyacrylic acid
Ether lipids (plasmologens)	Polycarbophil cysteine
Glucosyl sphingosine	Poly-L-arginine
Glycerol	Polyoxyethylene
Glycocholic acid, sodium salt	Polyoxyethylene-9-lauryl ether
Glycodeoxycholic acid, sodium salt	Polyoxyethylene-23-lauryl ether salt
Glycofurol	Polysorbate 80
Glycosylated sphingosines	Propylene glycol
Glycyrrhetic acid	Quillaja saponin
Hyaluronic acid, sodium salt	Salicylic acid, sodium salt
2-Hydroxypropyl- β -cyclodextrin	Saponin
Lactosyl sphingosine	β -Sitosterol- β -D-glucoside
Laureth-9	Soybean derived stearylglucoside
Lauric acid	Taurocholic acid, sodium salt
Lauroyl carnitine	Taurodeoxycholic acid, sodium salt
Lauryl sulfate, sodium salt	Taurodihydrofusidic acid, sodium salt

Table 2 – Some examples of molecules studied as transmucosal absorption enhancers.

Drug	Approx. Molecular weight	Intranasal bioavailability at 0.125% TDM	Intranasal bioavailability at 0.250% TDM
Calcitonin	4kDa	55%*	96%*
Insulin	6kDa	54%	62%
leptin	16kDa	58%	74%
Human growth hormone	22kDa	30%	50%
Erythropoietin	30kDa	12%	28%

*Compared to *iv* injection; all other proteins compared to *sc* injection.

Table 3 – Intranasal bioavailability of a sampling of peptide drugs in the presence of an Intravail™ excipient (Rat model).

Drug	Approximate Molecular Weight	Bioavailability at 0.125% TDM	References
Intestinal LMWH	5kDa	6%	Yang <i>et al.</i> , 2005
Pulmonary insulin	6kDa	22%-24%	Hussain and Ahsan, 2005
Pulmonary LMWH	5kDa	80%	Yang <i>et al.</i> , 2004; 2005
Antisense polynucleotide	7kDa	Up to 18%	Aegis unpublished observations

Table 4 – Effect of Intravail™ on bioavailability via non-nasal transmucosal routes.

transmucosal delivery of peptides (Textbox; Table 2). For the most part, these agents provide only limited bioavailability or have been shown to be irritating or toxic to nasal mucosa. Facilitating the acceptance of these alternative transmucosal delivery routes, a new class of patented alkylsaccharide transmucosal delivery enhancement agents exhibiting certain well-defined and highly specific structural characteristics was discovered by two researchers (Professors Dennis Pillion and Eli Meezan) and their colleagues working in the Department of Pharmacology and Toxicology at the University of Alabama, Birmingham (Pillion *et al.*, 1994; 2006; Ahsan *et al.*, 2001). This well-defined group of molecules has been collectively designated as Intravail™ absorption enhancers to distinguish them from the many thousands of potential alkyl saccharides accessible from a combinatorial chemistry perspective. One of the most well-published members of this group is tetradecyl maltoside (TDM).

Intravail™ absorption enhancers provide unsurpassed transmucosal bioavailability, comparable to that achieved by injection, for protein, peptide, and other macromolecular therapeutics. This has been well described in a growing number of publications (Ahsan *et al.*, 2003; Arnold *et al.*, 2002; 2004; Maggio, 2005; Pillion *et al.*, 1995; 2002; 2005). Some specific results for TDM in a variety of animal models are summarised in Tables 3 and 4. From a mechanistic perspective, it has been demonstrated that these agents function to increase both paracellular and transcellular absorption. A recent study contrasts the unique ability of Intravail™ alkyl saccharides to open tight junctions compared to non-Intravail™ alkyl saccharides which are essentially inert (Chen *et al.*, 2005; Figure 1).

Intravail™ agents are non-toxic, synthetic single chemical entities that are metabolised to CO₂ and H₂O (Weber and Benning, 1984) and allow controlled transient mucosal

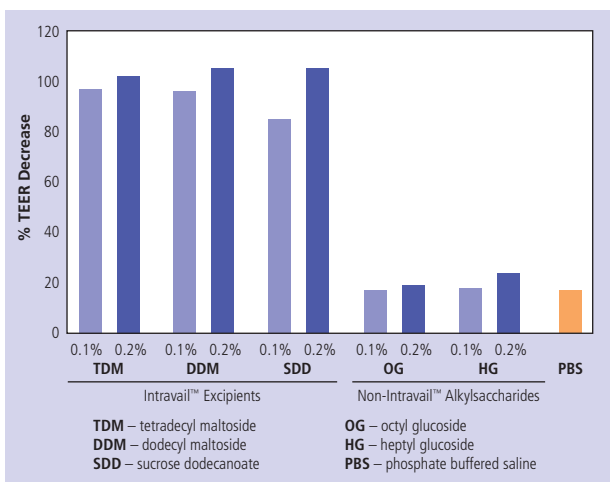


Figure 1 – Alkylsaccharides open tight junctions in normal human tracheal/bronchial epithelial cell-derived mucociliary tissue (adapted from Chen et al., 2005).

permeation by both paracellular (tight-junction) and transcellular routes. They allow for homogeneous aqueous solutions to be delivered through simple inexpensive metered nasal spray pumps – no particles, powders, or complicated pulmonary pumps. Intravaiil™ agents are compatible with routine formulation and dispensing processes for ease of scale-up and production.

They have been shown to be non-irritating when tested at 25% in the rabbit eye (Draize test). Bronchoalveolar lavage fluid analysis of animals receiving pulmonary insulin in conjunction with TDM, a highly effective absorption enhancer for insulin, shows no increase whatsoever in any of the well-accepted cell injury markers including lactate dehydrogenase, alkaline phosphatases, and N-acetylglucosaminidase (Hussain and Ahsan, 2005). The oral No Observable Effect Level (NOEL) for some Intravaiil™ compounds is approximately 20,000 to 30,000 mg per kg of body weight, which extrapolates to approximately 1.2 to 1.8 kg for a 60 kg person. The World Health Organization (WHO) specified oral Allowable Daily Intake (ADI) is approximately 15,000 times the amount that would be administered intranasally on a daily basis. Controlled intranasal studies are presently underway and, while it is obviously not appropriate to equate oral safety with nasal safety, the essential lack of oral toxicity of these agents in relatively high amounts is certainly very encouraging in view of the extremely small amounts required to promote transmucosal drug absorption.

Recently Chen et al. (2005) examined cell viability of human tracheal/bronchial epithelial cell derived mucociliary tissue upon prolonged exposure to static concentrations of various alkylsaccharides. These studies showed essentially 100% cell viability at an exposure level that is roughly 3-times the integrated exposure expected for the nasal cavity, assuming a T1/2 for mucocilliary clearance of 15 minutes (Figure 2). This lends further support to the mild nature of Intravaiil™ agents especially in light of the fact that *in vitro* test have been shown repeatedly in human trials to overstate actual *in vivo* intranasal toxicity. For example, in an extensive review of the scientific publications on

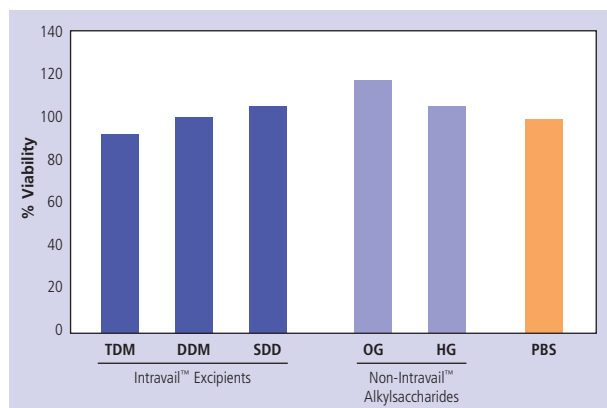


Figure 2 – Cell viability of normal human derived tracheal/bronchial epithelial cells upon prolonged exposure to alkylsaccharides in cell culture (adapted from Chen et al., 2005).

this subject, Marple et al. (2004) analysed data taken from 14 *in vivo* studies of one of the most widely used nasal excipients, benzalkonium chloride, in which *in vitro* predictions of toxicity were compared to actual *in vivo* experience in human volunteers. In every study examined, and in direct contrast to the accompanying *in vitro* results within each study, analysis of the *in vivo* data demonstrated that even prolonged use of topical formulations containing BAC caused no significant damage to the nasal mucosa. An explanation of this disparity is presented in a recent study examining biochemical indicators of proinflammatory effects, namely myeloperoxidase, IL-6, and Substance P, upon *in vivo* exposure to BAC (Riechelmann et al., 2004). The authors conclude that lack of proinflammatory effects *in vivo* is probably the result of neutralisation of BAC by components of normal nasal secretions (the predominant components of which are albumin and lysozyme). This should not be too surprising since this is essentially a basic function of the nasal secretions in the mucociliary clearance process. A more extensive review and bibliography describing the lack of correlation of *in vitro* and *in vivo* results may be found in Maggio (in press).

Extending the Range of Practical Transmucosal Drug Administration Routes

The effectiveness of Intravaiil™ agents for transmucosal delivery in tissues other than nasal mucosa has been demonstrated by a number of investigators. For example, TDM has been shown to increase pulmonary absorption of insulin by a factor of about threefold (Hussain et al., 2003; Figure 3). A similar two- to threefold increase in pulmonary absorption of low molecular weight heparin using TDM has also been reported (Yang et al., 2004; Yang, Arnold and Ahsan, 2005; Figure 4). This same group demonstrated up to a fourfold increase in intestinal absorption of low molecular weight heparin achieving maximum bioavailability of roughly 8% (Yang et al., 2004).

Conclusions

Improvements across the board in transmucosal drug delivery technology, comprising new routes of administration, new delivery devices, and breakthroughs in formulation technology are spurring a rebirth in interest in the broader use of peptide drugs. The need and the opportunity are clear since peptide drugs have potential applications across the full spectrum of human disease. Driven by strong patient desire to avoid repeated injections, the commercial opportunities for transmucosally-administered, systemically acting peptide drugs is potentially quite large. Presently, revenues for new peptide therapeutics are predicted to exceed US\$90 billion by 2010. As further improvements continue to be made, this trend is likely to accelerate.

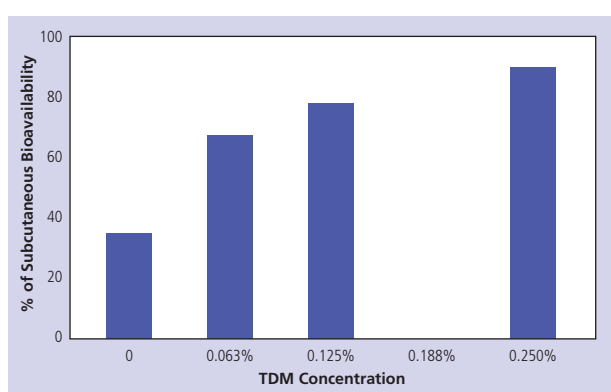


Figure 3 – TDM enhancement of pulmonary absorption – low MW heparin (adapted from Yang et al., 2004).

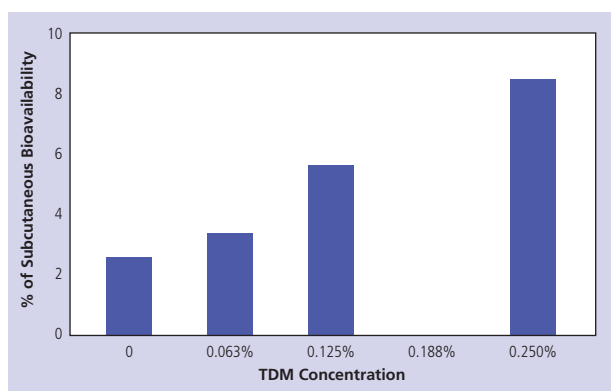


Figure 4 – TDM enhancement of oral (intestinal) absorption of low molecular weight heparins (enoxaparin) (adapted from Yang et al., 2005).

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