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Intravail™: highly effective intranasal delivery of peptide and protein drugs

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Recent development of a new class of patented alkylsaccharide transmucosal delivery enhancement agents, collectively designated as Intravail™ (Aegis Therapeutics) absorption enhancers, has created opportunities for new therapeutic options across a broad spectrum of human diseases. Intravail absorption enhancers provide unsurpassed intranasal bioavailabilities, comparable to those that are achieved by injection for protein, peptide and other macromolecular therapeutics. These novel, highly effective and non-irritating excipients circumvent the two primary limitations of intranasal drug delivery, namely mucosal irritation and poor bioavailability, and offer the promise of more convenient, more effective and safer therapeutics for patients and physicians alike. For pharmaceutical companies, Intravail provides a means to capitalise on two important industry dynamics: rapidly growing industry interest in commercialising peptide and protein drugs, and increasing interest in, and use of, the intranasal route for systemic drug delivery.

Keywords: absorption enhancers, intranasal delivery, Intravail™, nasal insulin, non-invasive drug delivery, peptide drugs, protein drugs, systemic drug delivery, therapeutic peptides, therapeutic proteins, transmucosal delivery

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1. The growing importance of peptide and protein drugs

Peptide and protein drugs are among the most useful and effective drugs that have as yet been discovered. In total, > 140 peptide and protein drugs are currently in use today, and the chemical and biological diversity available through peptides is breathtaking. Genomic efforts that are aimed at revealing the complete spectrum of expressed proteins in innumerable species, from man to invertebrates to single cell organisms, promise an unending supply of new investigational candidates. If that should ever prove insufficient, the combinatorial possibilities for peptide structures of molecular weight ≤ 10,000 Da, employing the 20 natural amino acids, exceeds the number of atoms in the universe (i.e., ~ 10^{80} atoms). Many peptides demonstrate high potency and high selectivity whilst exhibiting essentially no chemical toxicity. Because they are metabolised to naturally occurring amino acids, peptides and proteins do not invoke xenobiotic metabolic processes, the source of small molecule drug toxicity. Counterbalancing the inherent lack of chemical toxicity, high biological potency can make some otherwise beneficial peptides lethal at excessive or imprudent concentrations.

Many naturally occurring peptides have direct therapeutic applications (e.g., insulin, interferon, erythropoietin, growth hormone and parathyroid hormone among others). Other peptides could provide the initial biological activity from which new peptide and protein therapeutics may be designed. Important examples include a growing number of glucagon-like-1-related peptides such as exendin-4 [1], peptide YY-related peptides [2], or leptin-derived peptides such as OB-3 [3], which promise to provide new classes of highly effective treatments for Type 2 diabetes and diabetes-associated obesity.

Table 1. Examples of current peptide and protein therapeutics now amenable to intranasal delivery.

Peptide or protein	Clinical indications	Peptide or protein	Clinical indications
Arginine vasopressin*	Primary nocturnal enuresis, haemophilia A	Insulin	Diabetes
Bivalirudin	Anticoagulant	IFN-α	Chronic hepatitis C, malignant melanoma
Buserelin (LH-RH analogue)	Prostate cancer and endometriosis	IFN-β	Multiple sclerosis
Calcitonin*	Osteoporosis	Leuprolide	Prostate and breast cancer
Cetrorelix	Premature ovulation	LH-RH	Control of ovulation
Enfuvirtide	Antiviral (HIV-fusion inhibitor)	Melatonin	Sleep regulation
Eptifibatide	Coronary thrombosis	Nafarelin acetate*	Endometriosis
Erythropoietin	Anaemia	Nesiritide	Congestive heart failure
Exendin-4/GLP-1-related peptides	Diabetes	Octreotide	Growth hormone replacement
FSH	Fertility	Oxytocin	Labour induction, milk secretion
Ganirelix acetate	Infertility	Pramlintide acetate	Diabetes
GM-CSF	Neutropenia	IL-11	Anaemia (platelets)
Glial-derived neurotrophic factor	Parkinson's disease	Somatostatin	Antisecretory in GI disorders
Glucagon	Severe hypoglycaemia	Teriparatide (1-34)	Osteoporosis
Goserelin acetate	Prostate cancer	Triptorelin	Prostate cancer
Human growth hormone	AIDS wasting, dwarfism	Zafirlukast	Asthma
Human parathyroid hormone (1-84)	Osteoporosis		

*Currently available in intranasal formulation.
FSH: Follicle-stimulating hormone; GI: Gastrointestinal; GLP: Glucagon-like peptide; LHRH: Luteinising-hormone-releasing hormone.

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 a major shortcoming. Compared with small-molecule drugs, peptides are considerably less stable. Careful attention must be paid to formulation and storage to avoid unwanted degradation. Small peptides often exhibit rapid clearance even when administered parenterally, although this particular limitation is increasingly being addressed by careful and intelligent modification of the peptide structure. Some proteins, particularly proteins with substantially non-naturally occurring amino acid sequences can be immunogenic. Other possible problems that are associated with peptides as therapeutics include the potentially high cost of synthesis and solubility challenges, although both these limitations tend to be the result of idiosyncratic properties of individual peptides. Therefore, although the range of clinical indications for therapeutic proteins and peptides is quite broad (see Table 1), the actual number of such therapeutics in general use today is quite small compared with the number of chemically synthesised and orally active pharmaceuticals that are currently on the market. Recent developments in intranasal and other forms of transmucosal delivery for proteins and peptides are creating new and expanded opportunities for practical clinical uses of peptides, proteins and other macromolecular therapeutics.

2. Non-invasive peptide drug delivery: opportunities and challenges

Most of the peptide therapeutics that are listed in Table 1 are administered by injection (the few exceptions are footnoted). Injection is an inconvenient and expensive mode of administration. For situations where the medical consequences may not be immediate or life-threatening, and in cases where the administration must be frequent and chronic, patient noncompliance naturally becomes a serious health issue. Extended half-life derivatives (i.e., via pegylation) and depot formulations of peptide and protein therapeutics, both still requiring injection, are partial but imperfect solutions, and bring with them their own set of pharmacological problems and limitations.

Strong patient preference for intranasal drug delivery over injection, both for peptides and non-peptides alike, has spurred growing interest in researching and developing alternative administration routes. The opportunity for systemically acting peptide and protein drugs is potentially quite large. In 2003, sales of approved peptide therapeutics in the

Table 2. Some investigational peptide and protein therapeutics that are amenable to intranasal delivery.

Peptide or protein	Putative indications	Peptide or protein	Putative indications
N-Acetyl oxyntomodulin (30-37)	Obesity, diabetes	Hematide®*	Anaemia
Angiostatin	Cancer	Insulin C-peptide analogue	Diabetes complications
Bombesin	Cancer	Insulin-like growth factor-1	Amyotropic lateral sclerosis
Bradykinin antagonists	Pain, cancer	IL-6 antagonist	Chronic lymphocytic leukaemia
Corticotropin-releasing factor	Brain swelling	Keratinocyte growth factor-2 (FGF-10)	Duodenal ulcer
Cyclic peptide cRGDfV	Prostate cancer	Leptin	Obesity, satiety
CZEN 002	Anti-inflammatory/ anti-infective	rNAPc2	Neonatal respiratory distress
Endostatin	Cancer	Mammary-derived growth inhibitor	Breast cancer
Daptomycin	Antibiotic	Nerve growth factor	Alzheimer's disease
FGF-2	Parkinson's disease	Neuropeptide pituitary adenylyl cyclase-activating polypeptide	Diabetes
FGF-21	Diabetes	OB-3 peptide (7-mer)	Obesity, diabetes
GAP486	Cardiac arrhythmia	Obestatin	Obesity
Ghrelin	Appetite enhancer	Oxyntomodulin	Obesity, diabetes
Glucose-dependent insulinotropic polypeptide	Diabetes	Tissue factor pathway inhibitor	Anticoagulant
Glial-derived neurotrophic factor	Parkinson's disease	Urokinase receptor inhibitor	Cancer
GM-1 ganglioside	Alzheimer's disease	VEGF antagonist peptide	Cancer

*Registered trademark of Affymax Corp.

US alone totalled > $9 billion [4], and sales of therapeutic proteins grew to $37 billion, with 2010 sales predicted to be > $90 billion [5]. The current global market for nasally delivered medications is valued to be > $6 billion [6]. Whereas the growth rate for topically acting intranasal drugs such as those used to treat allergic rhinitis is ~ 10%, the growth rate for intranasal delivery of systemically acting drugs is 30% [7], dramatically outpacing the growth of the overall worldwide pharmaceutical market, which is projected to grow from 6 to 7% in 2006.

3. Development of new approaches

Although intranasal (more broadly transmucosal) drug delivery has proven satisfactory for many small molecules, intranasal delivery of peptide and protein drugs has proven to be much more difficult. Generally speaking, bioavailability decreases with increasing molecular weight. For example, intranasal butorphanol (molecular weight 478 Da) exhibits essentially 100% bioavailability compared with subcutaneous injection [8]. More typically, other small molecules exhibit somewhat poorer bioavailability. The slightly larger dihydroergotamine mesilate molecule (molecular weight 680 Da), for example, exhibits only 33% intranasal bioavailability compared with subcutaneous injection [9]. The much larger peptide salmon calcitonin (~ 4000 Da) exhibits an average bioavailability of only 3% [10]. A need clearly exists to increase intranasal bioavailability for larger drugs.

The advantages of intranasal administration in terms of greater patient comfort and convenience, as well as the elimination of needle-stick injuries, potential transmission of blood-borne infections and syringe disposal concerns associated with daily injections, are substantial and well accepted. This is clearly evidenced by the fact that sales of nasally delivered therapeutics have demonstrated 5- to 12-fold increases over the corresponding original injectable formulations. Creation of intranasal formulations of existing injectable products can provide patent-life extension and access to new and expanded markets, and affords a means to maximise value extraction from an existing protein therapeutics franchise through the classic marketing strategies of 'product proliferation and multilevel pricing'. Moreover, at a time when many drug companies are facing expiration of key patents, intranasal formulations of some of the estimated 700 peptide drugs in preclinical and clinical development [11] can provide new therapies across a broad spectrum of human diseases and provide new sources of revenue growth. Some examples of new peptide therapeutics that are currently in preclinical development, along with their associated putative clinical uses, are listed in Table 2.

Figure 1. Various metered nasal spray pump systems. Photo provided courtesy of Ing. Erich Pfeiffer GmbH.

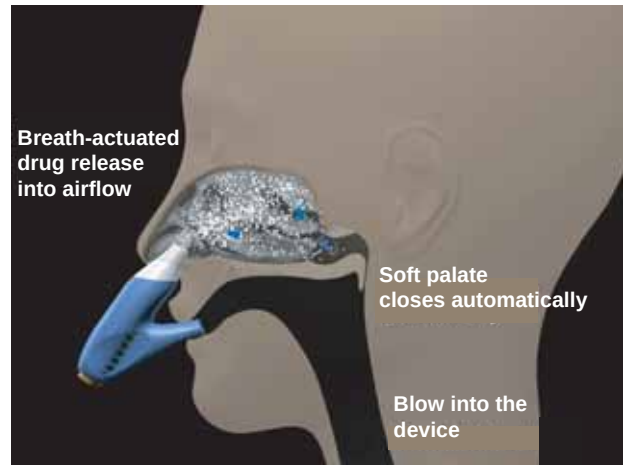

Figure 2. Breath-actuated metered spray device closes the soft palate when the user blows into the device resulting in substantially increased surface area exposure within the nasal cavity. Courtesy of Optinose A/S.

The principal limitations that are associated with intranasal delivery of peptides and proteins have included poor bioavailability and nasal irritation. The advent of highly effective and non-irritating absorption enhancement agents, such as certain alkyl saccharides [12], affords a practical opportunity to reconsider the broad use of peptides as commercially and clinically viable human therapeutics. However, issues remain, and the applicability of intranasal delivery must be assessed on a case-by-case basis. Molecules as large as erythropoietin, at ~ 30,000 Da, have been successfully administered intranasally in rats with a bioavailability of 14 – 28%, although in some epithelial cells morphological changes were observed [13]. Clearly, transmucosal delivery of monoclonal antibodies, a very important class of human therapeutics, is far beyond current capabilities and is likely to remain so. Second, the drug mass that can be administered intranasally is estimated to be no more than ~ 25 mg [14]. Some therapeutic agents may be susceptible to partial degradation in the nasal mucosa or may cause irritation to the mucosa.

4. Nasal physiology, formulation and absorption enhancement

Nasal physiology has been described in detail in a number of excellent reviews [14-16]. For the purposes of this review, a more concise description will suffice. Briefly, the human nose is divided into two nasal cavities, each with a volume of ~ 7.5 ml. The surface area of each cavity is ~ 75 cm^2 [15]. The dimensions and geometry of the nasal cavity restrict the estimated upper limit of practical intranasal drug absorption to ~ 25 mg and a spray volume of no more than 150 µl [17]. Metered spray devices such as those manufactured by Pfeiffer and Valois (Figure 1) provide a convenient, effective and relatively inexpensive means of administering intranasal drug formulations. Such devices deliver drugs to the anterior portion of the nasal cavity comprising about one-third of the mucosal surface area.

Recently, a number of delivery devices have been developed that permit the administration of drugs to larger portions of the nasal cavity. For example, a device being manufactured and tested by Optinose A/S (Figure 2), provides a means of administering drugs to an expanded portion of the nasal cavity and prevents pulmonary deposition [18]. Kurve Technology, Inc. has developed a device that applies drug formulations to the entire nasal mucosa and also delivers drugs into the paranasal sinuses. The technology has the potential to offer deeper penetration of topical drugs and greater drug absorption of systemic drugs (Figure 3) [19]. Although these devices are more expensive to manufacture than simple multiuse metered spray pumps, they provide a means of maximising systemic drug delivery through intranasal administration. They will not only find clinically important niche applications where they excel, but will undoubtedly greatly extend the range of practical applications of intranasally administered protein and peptide drugs across many disease categories.

The respiratory epithelium consists of basal, mucus-containing goblet, ciliated columnar, and non-columnar cell types [16,20]. The cilia move in a wavelike beating fashion, transporting foreign material from the nose to the pharynx for ingestion [16]. The nasal epithelium is covered by a mucus layer that is renewed every 10 – 15 min [21]. The pH of mucosal secretion ranges from 5.5 to 6.5 in adults and from 5.0 to 6.5 in children [22]. The mucus layer entraps particles and other foreign material, which is then cleared from the nasal cavity by cilial movement. This process, called mucociliary clearance, is

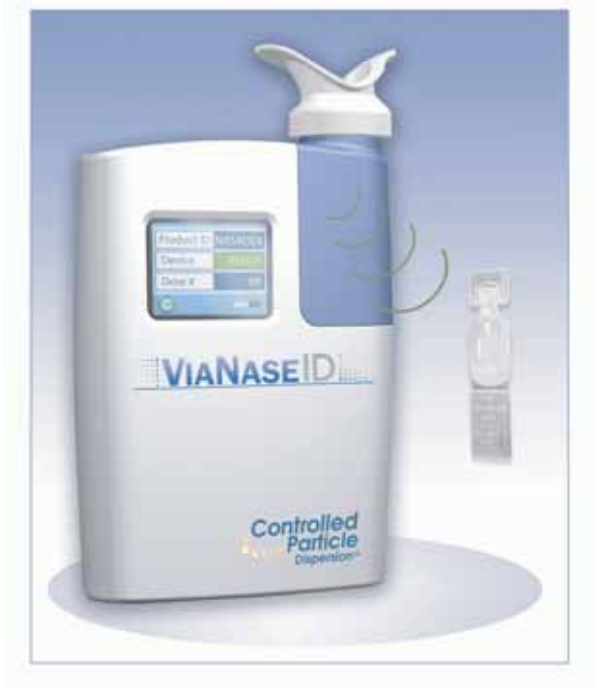

Figure 3. Kurve Technology, Inc.'s ViaNase™ device applies drug formulations to the entire nasal mucosa and the paranasal sinuses. Courtesy of Kurve Technology, Inc.

a normal mechanism for the entrapment and removal of inhaled unwanted noxious materials [23]. The rate of mucus flow through the nose is ~ 5 – 6 mm/min, resulting in a clearance half-life of ~ 15 – 20 min [15,24].

Human nasal secretions are comprised substantially of lysozyme and albumin as their main protein components [25]. Lysozyme is an antimicrobial enzyme, produced by nasal serous cells, that hydrolyses peptidoglycan bonds in bacterial cell walls and has specific activity against Gram-positive bacteria [26]. Lysozyme serves as an important antibacterial defence. Because it is active at the slightly acidic pH values found in nasal secretions (i.e., pH 5 – 6.5), it is desirable that nasal drug formulations be in this pH range. Albumin is obtained primarily from increased nasal vasculature permeability. The nasal cavity also contains numerous other enzymes [24,27,28]. In humans, CYP enzyme isoforms that have been identified are CYP1A, CYP2A and CYP2E [29]. Other enzymes detected in the human nose include carboxylesterases, glutathione *S*-transferases [30-32] and endo- and exo-peptidases [33].

Typically, nasally administered drugs are removed via mucociliary clearance. The average clearance half-life of ~ 15 – 20 min in humans can vary from person to person. Absorption can be increased to a certain extent by the addition of mucoadhesive agents to the formulation [34-39]. Certain preservatives, such as benzalkonium chloride (BAC) or chlorobutanol, shut down ciliary beating, thus extending a drug's residence time in the nasal cavity, which results in somewhat increased systemic absorption.

Box 1. Approaches to transmucosal absorption enhancement.

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

The physiological and biological properties of the nasal cavity, such as intrinsic pH, enzymatic activity, mucociliary clearance and the variety of cell types of greatly differing function, provide, in aggregate, a formidable natural barrier to the absorption of exogenous substances. At the same time, they create a significant challenge to achieving effective intranasal delivery of peptides by imposing a complicated set of restrictions on the allowable formulation compositions. Therefore, in spite of the many attractive aspects of peptides and proteins as potential therapeutics, the actual number of such therapeutics in general use today is quite small compared with the number of chemically synthesised and orally active pharmaceuticals that are currently on the market. Within the range of options defined by these practical restrictions, many approaches for enhancing drug absorption, and particularly peptide and protein drug absorption, have been researched (see Box 1).

5. Identification of transmucosal enhancement agents for peptide and protein drugs

A large number of molecules have been screened for the ability to enhance transmucosal delivery of peptides with limited success. Some of these are listed in Box 2. For the most part, these agents provide only a small percentage bioavailability for peptide or protein drugs. Some agents have multiple modes of action and the effects seem to be peptide specific. By systematically optimising combinations of a few such enhancement agents on a therapeutic-specific basis, the bioavailability can be increased somewhat; however, the percentage bioavailability compared with injection typically remains in the single digit or low double digit percentages. Of greater concern is the fact that many of these agents are irritating and toxic to the nasal mucosa.

In recent years, development of a large class of alkylsaccharide delivery enhancement agents (molecules that provide

Box 2. Examples of molecules studied as intranasal absorption enhancers.

- Benzalkonium chloride
- Capric acid, sodium salt
- Ceramides
- Cetylpyridinium chloride
- Chitosan
- Chitosan-4-thiobutylamidine
- Cyclodextrins
- Deoxycholic acid, sodium salt
- Dextran sulfate
- Dodecyl azacycloheptyl-2-ketone
- EDTA
- Glycerol
- Glycocholic acid, sodium salt
- Glycodeoxycholic acid, sodium salt
- Glycofurol
- Glycosylated sphingosines
- Glycyrrhetinic acid
- Hyaluronic acid, sodium salt
- 2-Hydroxypropyl-β-cyclodextrin
- Laureth-9
- Lauric acid
- Lauroyl carnitine
- Lauryl sulfate, sodium salt
- Lysophosphatidylcholine
- Menthol
- Methoxysalicylate
- Methyloleate
- Oleic acid
- Palmitoylcarnitine
- 1-Palmitoyl-2-glutaroyl-sn-glycero-3-phosphocholine
- Phosphatidyl choline
- Plasmologens
- Poloxamer 407
- Polyacrylic acid
- Polycarbophil cysteine
- Poly-L-arginine
- Polyoxyethylene
- Polyoxyethylene-9-lauryl ether
- Polyoxyethylene-23-lauryl ether
- Polysorbate 80
- Propylene glycol
- Quillaja saponin
- Salicylic acid, sodium salt
- Saponin
- β-Sitosterol-β-D-glucoside
- Soybean derived sterylglucoside
- Taurocholic acid, sodium salt
- Taurodeoxycholic acid, sodium salt
- Taurodihydrofusidic acid, sodium salt

unsurpassed intranasal bioavailabilities, comparable to those achieved by injection) was pioneered by E Meezan and D Pillion at the University of Alabama at Birmingham [40,41]. At present, these agents are in preclinical and early-stage clinical development for a growing number of protein and peptide drugs. The resulting families of molecules are now patented and collectively designated as Intravail™ (Aegis Therapeutics) absorption enhancement agents. There are a large number of molecules that are included in this structural class. The most studied, in terms of scientific publications, is tetradecyl maltoside; however, the scope and properties of molecules in the broad class are quite diverse. Intravail agents allow intranasal delivery or, more broadly, transmucosal delivery [42,43] of peptide, protein and non-protein macromolecular therapeutics [44] having molecular weights of up to and in excess of 20,000 Da, with bioavailabilities > 50%, compared with subcutaneous injection. They are chemically synthesised molecules that are metabolised to CO_2 and H_2O [45] that provide controlled transient permeation of the nasal mucosal barrier. The permeation effectiveness is largely a function of molecular weight of the drug and works with molecules as diverse as small-molecule drugs, peptides, proteins, polynucleotides and other charged polymers, such as heparin [44].

Very importantly, lack of toxicity or irritation of mucosal tissues has been demonstrated for a number of these molecules. For example, Intravail agents, typically employed at 0.1 – 0.2% in intranasal formulations, have been shown to be non-irritating when tested at 25% in the rabbit eye model. The oral 'no observable effect level' for this compound is ~ 20,000 – 30,000 mg/kg of body weight, which extrapolates to roughly 1.2 – 1.8 kg for a 60-kg person. The WHO-specified oral allowable daily intake is ~ 15,000 times the amount that would be administered intranasally on a daily basis. No similar intranasal data has yet been reported, and although it is not possible to equate oral safety with nasal safety, the essential lack of oral toxicity of these agents in relatively high amounts is certainly very encouraging.

Figure 4 shows the intranasal bioavailability of protein and peptide therapeutics having molecular weights in the range of ~ 4 – 30 kDa [13]. For peptides and proteins up to ~ 20 kDa, intranasal bioavailabilities > 50%, compared with subcutaneous injection, can be attained. For smaller peptides, such as calcitonin, bioavailabilities > 95% are observed. A number of clinically and commercially interesting peptide therapeutics in addition to calcitonin, such as exendin-4 and similar glucagon-like-1-related peptides, peptide YY, teriparitide-parathyroid hormone 1-34 and leuprolide, among others, fall into this category. The Intravail agents are inherently nondenaturing and are pharmaceutically compatible with virtually any peptide or drug and in some cases, such as insulin, offer dramatically extended protein stability [46].

Figure 5 shows the effectiveness of Intravail in the intranasal administration of insulin in a primate model of diabetes [13]. In the absence of Intravail, essentially no insulin is observed to be absorbed systemically. After 60 min, a second administration

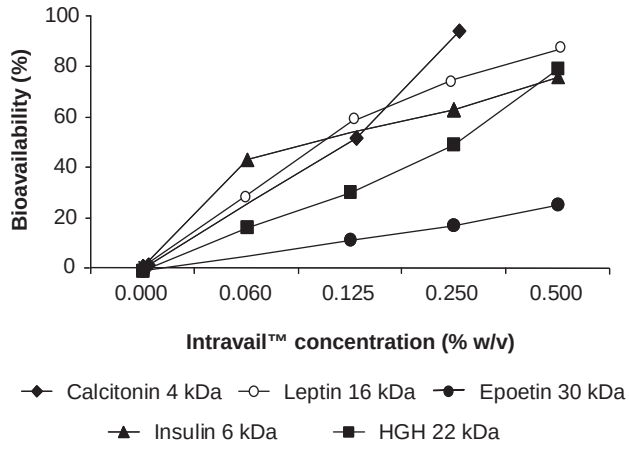

Figure 4. Intranasal bioavailability compared with injection (intravenous for calcitonin, all others subcutaneous) of equal amounts of protein and peptide therapeutics of different molecular weights of up to 30 kDa as a function of Intravail™ enhancement agent concentration [13,47]**.**
HGH: Human growth hormone.

of insulin in the presence of Intravail results in elevated systemic insulin levels and a reduction in blood glucose to euglycaemic levels. These agents have been shown to dramatically increase transmucosal delivery, and applications extend well beyond the intranasal route to also include the ocular, oral, oral cavity, pulmonary, rectal, transdermal and vaginal routes of administration.

The essentially complete lack of intranasal absorption of insulin in the absence of Intravail has been confirmed in fluorescence microscopy studies. Figure 6, for example, shows the absorption of fluorescein isothiocyanate (FITC)-labelled insulin following intranasal administration to the rat in the presence and absence of an Intravail absorption enhancer on subsequently sectioned rat nasal mucosa. Without the Intravail absorption enhancer, virtually no green colour arising from the FITC-labelled insulin could be seen (Figure 6A), whereas substantial absorption occurs when the Intravail enhancer is present (Figure 6B). Paracellular interstices do not seem to be labelled in this experiment; however, this may be an artifact of the wash procedure. This, and other data [13], suggest that absorption enhancement may proceed in part by transcellular means (i.e., increased endocytosis). A study of reduction of transepithelial electrical resistance following the treatment of normal human tracheal/bronchial epithelial cell-derived mucociliary tissue following extended exposure to a number of alkyl saccharides indicates that absorption enhancement may also proceed in part by a paracellular route [48]. Both routes seem to be rapidly reversible as the rapid reversibility and transient nature of the absorption enhancement effect of Intravail in the rat model has been demonstrated and studied in detail by Pillion, Meezan and colleagues [13,44]. Reversibility is key to lack of irritation and patient tolerance. The intranasal absorption enhancement window for the 4-kDa peptide calcitonin is nearly completely closed beyond 120 min after administration of Intravail enhancer. For larger proteins such as somatropin at 22 kDa, the rapid reversibility is even more clearly evident in that virtually no somatropin enters systemic circulation if administered 60 min after administration of the Intravail enhancement agent (J Arnold, D Pillion and E Meezan, unpublished observations).

6. New directions and challenges

Much confusion and misinformation concerning the relative tolerability or toxicity of intranasal excipients exists. This is largely the result of the currently unwarranted and uncritical reliance following *in vitro* testing methods. Recent studies directly comparing *in vitro* and *in vivo* results have clearly demonstrated a lack of correlation between *in vitro* and *in vivo* tests in predicting nasal irritation or toxicity. The best studied example is BAC. This drug has been used in nasal and ophthalmic products since 1935 at concentrations of up to 0.1%. However, over the past few years there have been conflicting reports of damage to human epithelia and exacerbation of rhinitis associated with products incorporating BAC.

In an extensive review and thorough analysis of the scientific publications on this subject, Marple *et al.* concluded that the current data indicate that any concerns raised were limited to results from *in vitro* experiments [49]. In direct contrast, analysis of the *in vivo* data suggested that even prolonged use of topical formulations containing BAC caused no significant damage to the nasal mucosa. The data analysed were taken from 14 *in vivo* studies in which changes in the function and ultrastructure of nasal cilia were determined by various types of microscopy, including light, transmission electron, scanning electron and inverted phase microscopy [50-56]. Direct mucociliary clearance was evaluated via measurement of indigo carmine saccharine transport time or saccharine clearance time, and exacerbation of rhinitis was determined by changes in nasal epithelia thickness.

In a well-controlled, double-blind, nasal biopsy study, 22 patients with perennial allergic rhinitis receiving fluticasone propionate aqueous nasal spray containing either BAC, BAC plus placebo, or BAC alone for a 6-week period were studied [54]. There were no statistical differences between indigocarmine saccharine transport time and the number of ciliated cells present for each group, and scanning and transmission electron microscopy examination of the biopsied tissues showed no effects of BAC.

In another recent study examining nasal irritation caused by BAC at 0.02%, saccharine transport time, anterior rhinomanometry, determination of nasal secretions, orienting smell test and anterior rhinoscopy showed no discernible negative effects whatsoever [57].

In a similar study by McMahon *et al.*, conducted with 65 normal volunteers over a 2-week period, no significant difference was found between subjects receiving nasal spray with or without BAC at 0.02% b.i.d. on a double-blind basis [53].

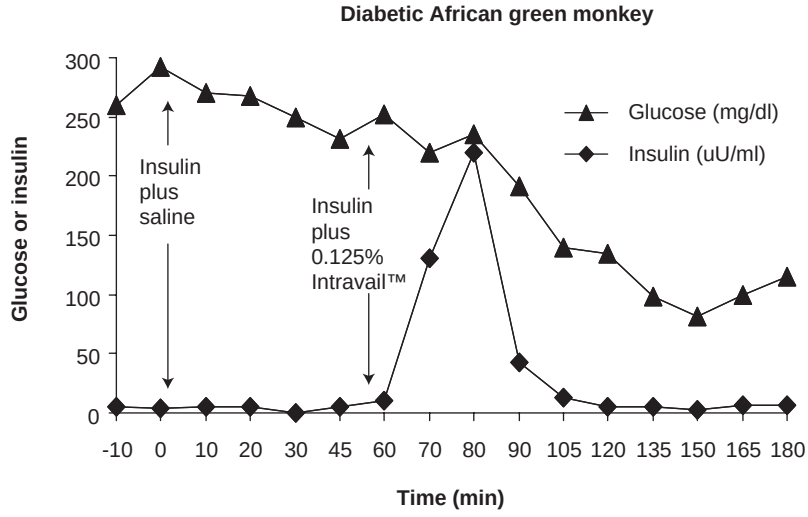

Figure 5. Intranasal administration of insulin to a diabetic green monkey. Administration of insulin in the absence of Intravail™ at 0 min provides an internal control. In the presence of Intravail™, systemic insulin levels rise and blood glucose levels fall into the normal range [47].

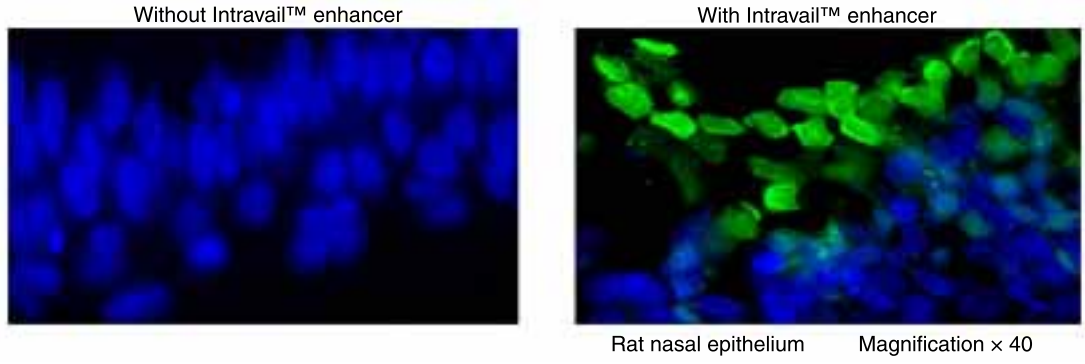

Figure 6. Effect of an Intravail™ enhancer on insulin permeation (fluorescence light microscopy). The virtually complete lack of insulin absorption upon intranasal administration in the diabetic primate model as described in Section 5 is confirmed by the lack of absorption of FITC-labelled insulin in the absence of Intravail™, as seen in these fluorescence light micrographs of vertical cross-sections of nasal septa excised from rats treated with FITC-insulin [13].
FITC: Fluorescein isothiocyanate.

Symptoms scored included acoustic rhinometry, saccharine clearance time and ciliary beat frequency. BAC caused a slight prolongation of mucosal ciliary clearance after application but had no detectable effect on the nasal mucosal function after 2 weeks of continual regular use.

And finally, in yet another study that highlights the lack of correlation of *in vitro* testing with *in vivo* experience in humans [58], and one that also offers a simple and plausible explanation of the lack of correlation, the effect of the BAC on isolated nasal cilia taken from 15 human donors was examined. In *in vitro* testing, BAC was seen to be ciliotoxic. However, once again, in *in vivo* tests BAC did not alter saccharine transport time or indicators of pro-inflammatory effects, namely myeloperoxidase, and secretion of IL-6 and substance P. The authors conclude that as no BAC-related pro-inflammatory effects are observed, any ciliotoxic effect of BAC is probably neutralised by components of secretions. This should not be too surprising as this is essentially the function of the nasal secretions in the mucociliary clearance process.

7. Conclusions

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 peptide drugs has been confined to the treatment of diseases having severe or life-threatening consequences as a direct result of the requirement for administration by injection. The recent advent of highly efficient and non-irritating Intravail transmucosal absorption enhancement agents for protein and peptide therapeutics that circumvent the two primary limitations of intranasal drug delivery in the past, namely mucosal irritation and poor bioavailability, offers the promise of more convenient, more effective and safer therapeutics for patients and physicians alike. It also promises access to new and expanded markets for pharmaceutical companies that are interested in capitalising on two important industry dynamics: rapidly growing industry interest in peptide and protein drugs and increasing interest in, and use of, intranasal delivery for systemically acting drugs.

Finally, as clinical data generated in creating an injectable therapeutic can be directly applied in seeking regulatory approval of a new (i.e., intranasal) route of administration, the creation of intranasal formulations provides a rapid path to regulatory approval and near term increased revenues with minimal risk of technical or clinical failure.

8. Expert opinion

Peptide and protein drugs are among the most useful and effective drugs yet discovered, exhibiting potent biological activity, high binding specificity for specific biological targets and low toxicity with few or no drug interactions. There are > 140 peptide and protein drugs that are in current use and their number and importance are rapidly increasing. Advances in intranasal delivery of peptides and small proteins are creating new therapeutic options across an entire spectrum of human disease. In particular, the recent advent of highly efficient transmucosal delivery enhancement agents, which circumvent the two primary limitations of intranasal drug delivery in the past, namely mucosal irritation and poor bioavailability, offers many practical opportunities for drug companies to begin to embrace the broad use of peptides as commercially and clinically viable human therapeutics.

From a commercial standpoint, companies with existing franchises in injectable protein or peptide therapeutics can realise a rapid path to regulatory approval and near-term increased revenues through the creation of corresponding intranasal formulations of existing government-approved injectables and through the introduction of new peptide therapeutics. According to IMS data, revenues for intranasal forms of previously injectable-only therapeutics have expanded by 5- to 12-fold over the corresponding injectable products, presumably as a result of increased patient convenience and acceptance, as well as expansion of the range of practical clinical use. As key patents expire in the next few years, and as companies experience the simultaneously increasing costs of drug development, regulatory approval and product liability lawsuits, novel intranasal formulations of existing and new therapeutics can provide an attractive and low-risk path that can benefit all of the affected stakeholders, patients, physicians, company employees and shareholders, and should be an integral part of planned strategic revenue growth and product life cycle management in the pharmaceutical industry.

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