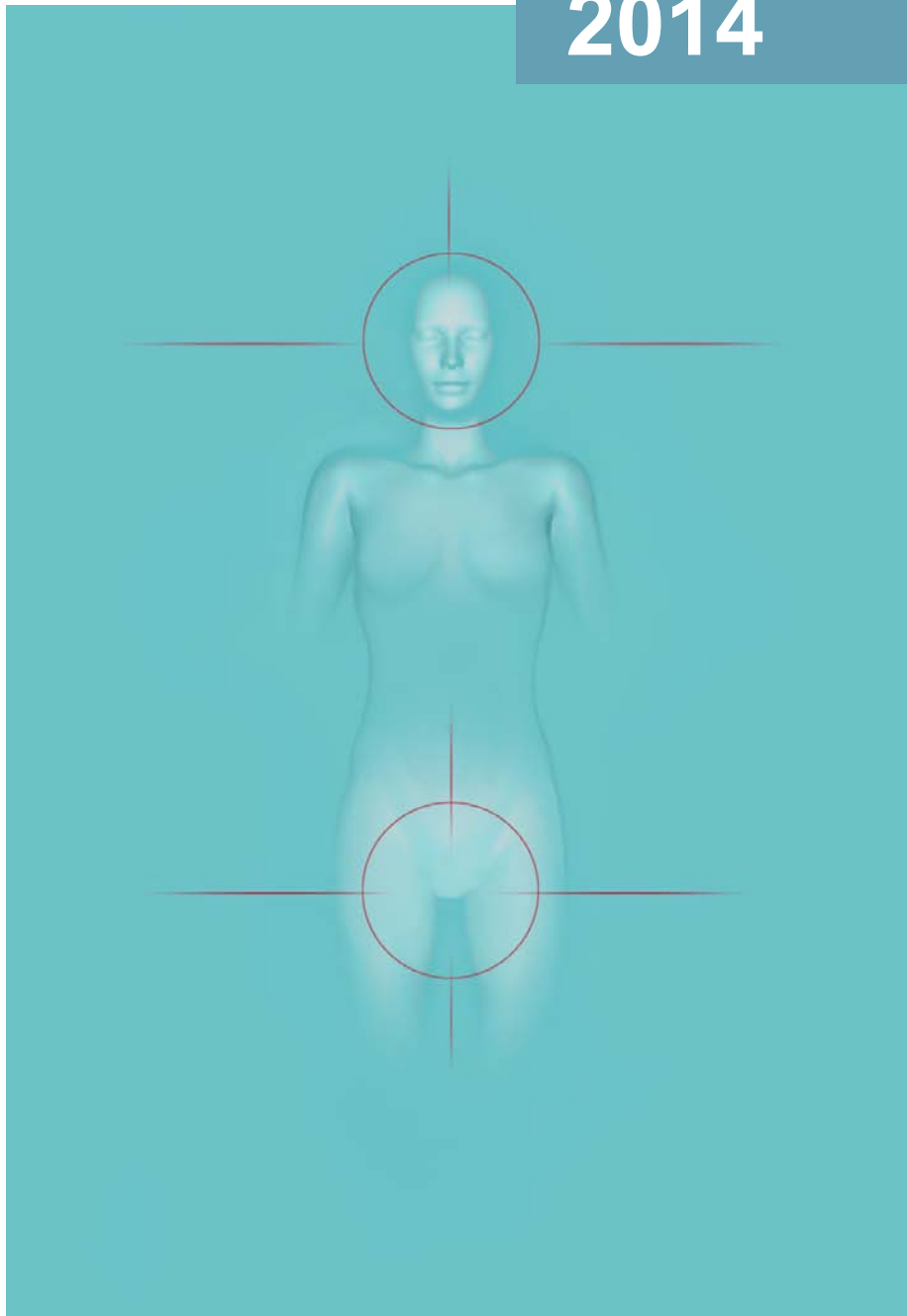# It's just mucosa, get over it...

AAPS - LIPID BASED DRUG DELIVERY AWARDS SPONSORED BY GATTEFOSSÉ

Congratulations to the 2014 winners:

- **Outstanding Awardee:**
  Soumyajit Majumdar, Ph.D.
  The University of Mississippi, USA.
  For his work on transmucosal drug delivery, notably on improving ocular delivery and disposition with lipid-based formulation strategies. Dr. Majumdar's work has demonstrated that solid lipid nanoparticles (SLN) can significantly increase ocular penetration, retention and bioavailability of lipophilic drug/drug candidates such as tetrahydrocannabinol.

- **Graduate Student Awardee:**
  Kaushik Thanki
  National Institute of Pharmaceutical Education and Research (NIPER), India.
  For his study relating to the "potentials of lipid conjugation approach in improving the oral bioavailability, therapeutic efficacy and safety profile of Amphotericin B" and "implications of lipid functionality on suitability for nanocarrier based approaches".

- **Travelship Awardees:**
  Hemlata G. Patil
  University of Mississippi, USA.
  'A novel application of Hot Melt Extrusion Technology to develop a continuous and scalable process for the production of Fenofibrate Solid Lipid Nanoparticles'.
  Vijay Kumar Nekkanti
  Western University of Health Sciences, USA.
  'Improved Oral Bioavailability of Valsartan: Proliposomes vs Self Micro-Emulsifying Drug'.

Founded and sponsored by Gattefossé, these awards aim to encourage and recognize significant research advances in the field.
We also thank all the researchers who submitted their papers for the awards.

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THE GATTEFOSSÉ FOUNDATION

The Gattefossé Foundation was established in 2008 to provide a conduit for the company's staff and family share holders to express their corporate, intellectual and philanthropic values and those of the company's founders.

The Foundation's actions are focused on three key missions; support of research and development; dissemination of scientific knowledge and involvement in community and social initiatives. To find out more please visit our website: http://www.gattefosse.com/en/gattefosse-foundation

The 'Journées Galéniques' or 'Galenic Days' is a unique scientific gathering that has been held every year since the 1960's. It was instigated by Henri-Marcel Gattefossé who believed that knowledge sharing between experts from his eclectic and international network of academics, industrialists and healthcare providers would be beneficial to addressing the challenges in modern drug development. As a result chemists, pharmacists, formulators, ingredient providers, drug developers and medical doctors would meet annually at the Gattefossé farm 'Mas Bellile' in St Rémy and discuss 'drug development' challenges during scientific sessions and a friendly social environment.

Today, the scientific challenges addressed during the 'Galenic Days' have evolved but aim and atmosphere of the meeting remain the same. Great care is taken in constructing a challenging and pertinent programme and in identifying open-minded and scientifically curious speakers and participants from around the world.

This 48th Journées Galéniques, 'It's Just Mucosa, Get Over It', was a rich gathering of key thinkers and doers in oral, nasal, ocular, rectal and vaginal drug delivery technology. The 'alternative' drug delivery route has received more interest over the past few years due to the increase in therapeutic macromolecule development, yet it is rare to be able to bring together experts from all 'alternative' fields to discuss the challenges and exchange ideas.

As President of the Gattefossé Foundation, I am delighted to share with you the content of this meeting, and I extend my sincere gratitude to the Chairmen and to all the authors for their contribution.

Lastly, whether you are a corporate, academic, or student researcher, I hope you find these articles of use, interest and possibly inspiration for your research projects.

Sophie Gattefossé - Moyrand
President of the Gattefossé Foundation

FOREWORD

IT'S JUST MUCOSA – GET OVER IT!

By Prof. Clive Wilson

The barriers to drug absorption at sites of dosage form application vary enormously around the body. The need to maintain a homeostatic environment and the vulnerability of organs to toxins necessitate isolation from the exterior world by epithelial barriers. These epithelial barriers perform many functions in addition to protection: they help regulate gas exchange, nutrient uptake, water loss and waste management. These many requirements lead to structural and physiological modification, leading to the uniqueness in absorptive properties of each route. Where the barriers are thin as in the mid-gut, the cell architecture permits entry of materials and the metabolic processes governing drug entry are more active. The squamous epithelial tissue covering many other parts of the body is usually thicker and thus we can arbitrarily divide our difficulties into getting over thin or thick barriers.

In this meeting, we gathered together experts from around the world who are at the front edge of drug delivery science. Our aim was to review what we know about the difficulties of crossing the mucosal barriers, to learn from each other and to swap ideas between disciplines for approaches and new strategies. All this in the beautiful ambience of the Provence home of Gattefosse, Mas Belille under the careful and welcoming attention of Sophie, Jacques and all the staff at 48th Journées Galéniques de St Remy.

Welcome to our celebration of science!

Jacques Moyrand (1)
Gattefossé, France

Stephen Wargacki (2)
Lohmann Therapy
Systems Corp., USA

Sophie Gattefossé-
Moyrand (3)
Gattefossé Foundation,
France

Muhammad Safadi (4)
Teva, Israel

Nabil Farah (5)
Gattefossé, France

Christopher Rupp (6)
Abbott Laboratories
GmbH, Germany

Clive Wilson (7)
University of Strathclyde,
Scotland

Olivier Midler (8)
Gattefossé, France

Johan Palm (9)
AstraZeneca, Sweden

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Lundbeck, Denmark

Mangal Nagarsenker (11)
Bombay College of
Pharmacy, India

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Pierre Fabre, France

Alan Ferrie (13)
GlaxoSmithKline,
United Kingdom

Andreas Bernkop-
Schnürch (14)
University of Innsbruck,
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Bruce Lithgow (15)
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Ferring, Switzerland

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Aptuit, Italy

Maria José Alonso (29)
University of Santiago de
Compostela, Spain

Covadonga Pañeda (30)
Sylentis - Grupo Zeltia,
Spain

Stanley Kepka (31)
MonoSol Rx, LLC, USA

Delphine Marchaud (32)
Gattefossé, France

David Brayden (33)
University College Dublin,
Ireland

*NB. Mr Vincent Jannin,
Gattefossé France, was
unfortunately parking the
car therefore not in this
years' photo.*

PRINCIPLES UNDERLYING PEPTIDE, PROTEIN AND MACROMOLECULE INTERACTIONS WITH NON-INJECTED MUCOSAE: FROM ORAL TO BUCCAL AND NASAL DELIVERY

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Abstract

While the oral route of delivery remains the preferred non-injected choice of patients, it remains a high barrier for absorption of peptides, proteins and macromolecules. This is because formulations containing such actives have to navigate past the stomach, survive overall intestinal pH values ranging from 1.5-8.0, cope with the presence and absence of food, battle against a myriad of stomach and pancreatic enzymes along with dilution and spread along the small intestine. They then face the highest hurdle of all, penetrating the single layered columnar jejunal epithelium, while surviving potential enterocyte metabolism – with still the potential impact of the liver first pass effect! With such hurdles, it is easy to see the attraction of other non-parenteral routes for peptides. The advantages of other routes of delivery including the multi-layered non-keratinized thick buccal mucosa and the single-layered thin nasal epithelium are localized access for concentrated formulations at the delivery site, somewhat increased permeability and reduced metabolism compared to the intestine, as well as avoidance of first pass and food effects. Non-oral delivery routes however, bring their own issues in terms of requirements for patient acceptability, drug-device combination research, the permeation capacity of relatively small exposure areas, and site-specific toxicology. General principles of drug flux across several epithelia (oral, buccal, and nasal) are explored with respect to structures, metabolic enzymes and role of mucus, pH differences, and epithelial turnover. With regard to oromucosal delivery, recent FDA approval of elegant buccal films of small molecules has led to re-emergence of research into creating similar constructs for peptides.

Keywords

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1. The lessons that nasal and buccal routes learned from oral delivery

For non-parenteral routes, oral delivery is much preferred by patients compared to transdermal, pulmonary, nasal and buccal delivery. Ironically, it is often stated that non-injected routes of delivery improve patient compliance, but there are still serious compliance issues even for established oral medications with excellent bioavailability [1]. There are learnings for other routes of delivery from decades of research on oral delivery of small molecules, which has been much more successful in terms of product approvals than attempts to date to orally deliver peptides, proteins and macromolecules. Many of the basic principles of drug transport across epithelia were established and confirmed using intestinal models, including study of Fick's first law of diffusion, carrier-mediated transport, the study of epithelial tight junctions, as well as peptidase categorization in different intestinal regions. For example, the advent of artificial lipid bilayers (PAMPA) to model passive diffusion of small molecules was stimulated largely by efforts to see if they could mimic passive transcellular flux across human intestinal epithelial monolayers, Caco-2 [2]. Such systems have a predictive role to play in modelling passive flux across other non-injected sites, especially when epithelial apical membrane carriers or receptors are not so relevant, as is the case for nasal and buccal delivery.

Table 1. Oral delivery factors: we have more accurate understanding about the points on the left than the right.

Intestinal peptidases	Gastric emptying times
Intestinal P-gp and P-450	GI transit times
Liver clearance and blood flow	pH changes
Molecule-related	Transporter expression and function
Log D and pKa	Role of mucus
Therapeutic index	Dilution
Formulation physicochemical aspects	Disease impact
Fick's law of diffusion	Regional metabolism
Permeability concepts	Colon residence time
	Bacterial metabolism

Table 1 divides the challenges of oral delivery between aspects that we can confirm or at least know a lot about with some degree of precision and those variables that are subject to large intra-subject variation due the vagaries of intestinal physiology. In general, we attempt to control formulation aspects of small molecules and successful products can emerge if the molecule has a high therapeutic index, which can cope with biological variability in GI transit time, dilution and regional pH [3]. It is also apparent that study of the solubility and permeability issues pertaining to the oral route led to Lipinski's 'rule-of-five' to advance small molecule design for successful absorption [4], noting that many molecules on the market do not obey them!

Furthermore, high level understanding built up over decades of research on intestinal epithelial drug transport via carriers, tight junctions and transcellular passive diffusion allowed a group of established oral drug transport investigators [5] to recently dispel spurious claims that all drug transport across lipid bilayers was carrier-mediated and the gap in knowledge was apparently that the rest of the carriers simply remain to be discovered [6]. Finally, research to deliver molecules across the nasal and buccal epithelial has benefited from the Biopharmaceutical Classification System (BCS) [7]. Although originally intended to enable biowaivers of clinical trials for immediate-release Class 1 molecules (high permeability, high solubility), the knock-on effects was to focus researchers on developing solubilizing technologies for Class 2 small molecules (high permeability, low solubility), and permeation enhancer technologies for Class 3 agents (low permeability, high solubility). Peptides and proteins could possibly be classified as Class 3 agents if the BCS could be extended to include non-small molecules (heresy in the eyes of some). It could be argued that the BCS has helped streamline formulation designs for small molecules based on their physicochemical properties, and that it subsequently helped produce candidates amenable to a formulation approach for Class II molecules for buccal delivery and for Class III molecules for nasal delivery.

In terms of oral peptide delivery, while there have been successful Phase III trials for salmon calcitonin [8] and octreotide [9], such molecules are still characterized by very low oral bioavailabilities of <1%, even in elegant enteric-coated formulations resistant to peptidases, but which in most cases contain epithelial permeation enhancers. That they may still be considered as commercial products reflects more on their high potency and relatively low cost of goods compared to 10 years ago, rather than on any great advances in formulation design. Unless the liver is the target, as is the case for insulin and glucagon-1 like peptide (GLP-1), such formulations will not preserve the peptide from liver enzyme attack. The issues of the first pass effect and food interactions in particular for oral delivery, have encouraged more effort in nasal and buccal peptide and protein delivery, which has led to several products in the case of the former, and new attempts to do the same for the latter.

2. Nasal delivery: from small molecules to first generation approved peptides

Nasal delivery of up to 10 peptides including sCT, octreotide, LHRH agonists and nafarelin has already been achieved in man over the past 20 years, but bioavailabilities are still <1% [10]. Current efforts in the clinic use permeation enhancers such as CriticalSorb™ (based on the excipient, Solutol® HS15) to improve such values and this may bring other payloads including human growth hormone into potential commercial reality [11]. Without doubt, molecules with molecular weights <1000 Da will require such formulation assistance. There are at least 5 permeation enhancers in clinical trials for oral peptides and over 100 that have been in preclinical assessment [12]. Of these enhancers/solubilizers, the ones with most clinical data are chitosan, cyclodextrins and alkyl maltosides [12].

One of the issues holding back investment in nasal delivery of peptides with permeation enhancers is the regulatory risk and costs involved in pursuing novel permeation enhancers that are not listed in National Formularies or Pharmacopoeia as excipients, or ones that do not have GRAS status.

Table 2. Nasal epithelial drug transport pros (left) and cons (right).

Accessible	Thickness of 0.5mm
Avoids GIT and food effects	Taste/foreign body
Low [enzyme]	Salivation
Systemic delivery: blood and lymph access	Challenges in adhesion and retention
Highly vascularised	Permeability issues remain
Local delivery: oral cavity diseases	Limited to passively absorbed agents (mainly)
Design for IR or CR products	Drugs must be stable at buccal pH of 6.0-6.6
Quick onset and ease of termination possible	Limited to low doses
	Low surface area versus GI

Table 2 shows the pros and cons of nasal delivery systems for peptide and protein delivery. The most important advantages are the rapid onset, lower first pass effect, zero food effect and potential to use lower doses compared to oral. The disadvantages include, patient reticence to keep adhering, local irritation, and a requirement for device technology. The thin mucosa absorbs in small surface area respiratory turbinate tissue and this can be dose limiting.

Moreover, absorption can be offset by mucociliary clearance, effects of rhinitis and colds, as well as variable pH in secretions. The opportunity for molecules to reach the CNS via nerves in the olfactory bulb remains a controversial subject [13]. Apparently, it requires precision in the device to access the olfactory region and capacity may be an issue. That said, in the case of small molecules, an intranasal naloxone spray has received fast track designation at the FDA in order to treat opioid overdose [14] and presumably it has central actions. There is also an approved nasal spray of sumatriptan to treat acute migraine attacks [15].

3. Buccal delivery: sub-lingual to sprays to oromucosal thin films

The buccal mucosa in man is a non-keratinized multilayered structure of 500-600 µm in thickness with a surface area of 50 cm^2. For drug delivery, the sub-lingual tissue is better exploited and is also non-keratinized, 100-200 µm in thickness, and has a surface area of 25cm^2 [16-18]. The buccal and sub-lingual epithelia seem to have far better passive permeability compared to the related keratinized mucosae, the gingival and palatal tissues. The pros and cons for buccal drug delivery are not unlike those of the nasal route and are presented in Table 3.

Table 3. Buccal epithelial drug transport pros (left) and cons (right).

Needle-free and safer than injection	Absorbing area is small and precise
Accessible	Requires device advances
Local and systemic delivery	Compliances issues
Rapid onset	Marketed peptides have bioavailability <1%
Lower doses/lower side effects than other routes	Molecules >1000 Da require enhancers
Avoids first pass effect	Irritation potential limits enhancer selection
T_{max} and C_{max} can compare to s.c.	Limited by colds, rhinitis
Access to CNS possible	Committed once administered

Notable differences to nasal are that the surface area is larger, and the device can be removed to facilitate termination. Though closely related to skin, basal permeability to water and horse-radish peroxidase is a lot higher across the buccal epithelium [16]. On the other hand, the subject has to cope with a foreign body and the drug molecule can be adversely impacted by saliva with a typically acidic pH value of 6.0-6.5. The buccal epithelium is better known for local delivery of agents to treat mucositis and for local anti-bacterial action, but recent advances have led to fast dissolving oral films of quite lipophilic small molecules, several of which have been approved in this format (e.g. odansetron, and buprenorphine/naloxone [19]. There is now renewed interest in delivering insulin via the buccal route for diabetes as, unlike oral delivery, it is not subject to a food effect. In terms of the buccal epithelium, it has more in common with skin than the intestine, as passive flux according to Fickian principles appears to the dominant route. It has no tight junctions and, uniquely, has unusual membrane coating granules in the paracellular space, which act as a barrier to transport. Many polar lipids are found in intercellular spaces and they may contribute to an unorthodox paracellular pathway not involving tight junctions, noting that the passive route comprises both transcellular and paracellular pathways.

In vitro buccal models remain controversial as regards prediction of drug permeability in man. In the 1990s, the human TR146 epithelial cell line was compared for transport of a range of FITC dextran- and mannitol polar passive flux markers and a reasonably good correlation was found with isolated porcine buccal mucosae within molecular weight constraints [20], but it offers a reduced barrier compared to tissue models. Since then however, the TR146 line fell out of favour, as the buccal delivery field declined in popularity. In a recent comparison of flux of metoprolol across human and porcine buccal tissue, the conclusion was that porcine tissue was indeed a reasonable model for human [21]. Nowadays, following recent small molecule buccal film and sub-lingual tablet successes, Matek Corporation's EpiOral® human epidermal keratinocytes as a component of a multi-layered differentiated model is being tested for its potential for predictive buccal peptide transport [22], but the model has been better characterized to date as a useful toxicology assessment tool for the buccal epithelium.

Finally, Generex (Canada) has an advanced buccal spray for insulin (Oral-lyn™), which completed Phase III clinical testing outside the US in 2013 [23]. It contains mixed micelles and bile salts as permeation enhancers and the spray was able to reduce plasma glycated haemoglobin to levels on a par with regular sub-cutaneous insulin injections (note that the Phase III clinical trial data has not yet been peer-reviewed). One of the issues for Oral-lyn™ however, is the requirement for up to 12 puffs [24], which may render it impractical in dosing regimes. The Generex Rapidmist™ device technology has stimulated greater interest in using film-dissolving constructs containing permeation enhancers and nanoparticles to deliver peptides systemically, and some of these systems are in preclinical testing [25].

4. Conclusions

Our thesis is that basic research on epithelial drug transport carried out in intestinal tissue models led to advances in delivery technologies for both nasal and buccal delivery of small molecules, peptides and proteins. Permeation enhancers seem to be a pre-requisite for non-injected peptide formulations that are in the clinic for oral, as well as recent improved constructs for nasal delivery, however there are challenges to maximize the therapeutic window for these agents, while at the same time keeping the required dose of payload commercially viable. Nose-to-brain delivery is being actively pursued for several CNS-targeted agents against a background of skepticism that the olfactory pathway has relevant capacity. Buccal delivery research is enjoying a resurgence due to recent approvals of film-based systems for small molecules and there are renewed attempts to adapt such systems for peptides that are normally injected. Many of the questions raised in this opening lecture of the 48th Journées Galéniques de St Rémy de Provence (*'It's Just Mucosa Get Over It'*) were then discussed in more detail in subsequent presentations.

5. Acknowledgments

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MUCUS PENETRATING NANOPARTICLES FOR DRUG AND GENE DELIVERY TO MUCOSAL TISSUES

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Abstract

The controlled delivery of bioactive molecules to target tissues can significantly improve drug effectiveness while reducing side effects by concentrating medicine at selected sites in the body. Mucus layers coat and protect nearly all entry points into the body that are not coated by skin. Until recently, human mucus was thought to be nearly impenetrable to drug delivery particles even as small as 59 nm in diameter. Particles that become trapped in mucus are typically rapidly cleared from the organ of interest, usually within minutes to a few hours. Thus, while the barrier properties of mucus provide outstanding protection against infection and other potentially toxic substances, they have also thwarted efforts to achieve uniform and sustained drug and gene delivery to mucosal surfaces. This talk will focus on our work to understand the length-scale dependent and adhesion-mediated barrier properties of mucosal fluids, and how this knowledge has guided the development of polymeric nanoparticulate carriers capable of improved drug and gene delivery to the respiratory tract, female reproductive tract, gastrointestinal tract, surface of the eye, and other mucosal tissues.

Full article not supplied.

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KEY DRIVERS IN ALTERNATIVE ROUTE DELIVERY

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Abstract

To establish a testing strategy and justify the need for alternate route delivery of a new drug candidate, it's important to gather information around following aspects: is the drug intended for chronic or non-chronic treatment and what is the desired drug plasma profile (e.g. immediate or sustained release)? How does the patient population look like (e.g. geriatric or pediatric) and will the disease influence delivery efficiency? What is the predicted therapeutic plasma concentration and can the predicted dose be delivered through the alternative mucosal route. Sub-potent drugs are generally ill-suited for alternative route delivery. What are the physicochemical characteristics of the candidate drug like molecular weight, charge, solubility, membrane permeability (e.g. Caco-2) and solid/solution-state stability? Are there any limitation or liabilities associated with the primary (oral or injectable) route of administration that need to be overcome such as extensive first pass metabolism. Due to the nature of the alternative mucosal barriers with rich vascular supply, nasal, buccal/sublingual and pulmonary delivery tends to favor treatments that need rapid systemic absorption. Although alternate route delivery have shown potential for poor permeability molecules, BCS-I and II compounds have the best probability of achieving significant systemic absorption.

Keywords

Bioavailability, buccal delivery, nasal delivery, peptide–protein drugs, transmucosal drug delivery, zolmitriptan.

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1. Introduction

Oral (gastro-intestinal) administration is the preferred and most common route for delivery of conventional small molecular dugs intended for systemic action while parenteral administration is used for delivery biomacromolecular drugs. However, for certain drugs it may also be possible or even advantageous the use an alternative route for administration in order to obtain the desired plasmas concentration profile and therapeutic effect. From our experience working with drug substances in various stages of drug development, the key drivers for investigating alternative route delivery are:

- poor absorption by conventional GI administration
- avoid extensive first pass metabolism (intestine, liver)
- improved therapeutic effect, e.g. more rapid onset of action
- convenience of dosing as compared to injection
- line extensions, offering options to suit different patient needs

The alternative routes of administration for systemic drug action can be divided into the transdermal and the transmucosal routes where the latter include the pulmonary, ocular, rectal vaginal, nasal and oral (buccal/sublingual) delivery routes. The rectal, vaginal and ocular routes of delivery have comparatively poor patient acceptability and are not commonly used for systemic delivery of "conventional drugs". This summary article focuses on transmucosal delivery via the nasal and buccal routes. Selected examples from explorative studies on compounds in early development and on new delivery options for marketed products are presented to illustrate key drivers for alternative route delivery:

- exploring nasal delivery of a large protein drug
- nasal spray of Zolmitriptan for improved migraine therapy
- exploring buccal delivery of a peptide drug

2. Advantages and limitations of nasal and oral administration

The nasal cavity is an attractive site for the delivery of drugs owing to the well perfused mucosa with a highly permeable epithelial lining and relatively low metabolic activity [1, 2]. Intranasal absorption bypass first-pass metabolism and this route afford simple and convenient drug administration for patients. This makes this a possible route for delivery of small hydrophilic molecules and peptides. Limitations are the dose and dose volume that can be administered. Irritation potential of the drug and formulation on the nasal mucosa, particularly for chronic treatment, needs to be considered [1, 2].

Oral (buccal/sublingual) administration offers several advantages including relatively large surface area and rich blood supply, easy access and convenient dosing, low metabolic activity and a robust mucosa amenable for prolonged retention systems [1, 3, 4]. Oral mucosal delivery is an intestinal alternative for:

- patients with nausea and vomiting
- patients with swallowing difficulties
- drugs that cause gastric irritation
- drugs that are unstable in the gastro-intestinal fluids
- drugs with first-pass metabolism in the gut wall or liver

Disadvantages of oral mucosal delivery are the dose limitation, taste/sensory liability, mucus and salivary clearance, patient acceptance and commercial difficulty.

There are two main mucosal sites of delivery for oral administration, sublingual and buccal, which differ in their properties [3]. The sublingual route is relatively permeable and capable of giving rapid and appreciable absorption of low molecular weight lipophilic compounds. Typical formulations are sublingual sprays and fast dissolving tablets. It is unsuitable for retentive systems due to high salivary flow, excessive movement and patient discomfort. The buccal route is relatively less permeable than the sublingual route and does not generally give the rapid onset of absorption seen with sublingual delivery. The buccal mucosa is robust and well suited to retentive systems such as adhesive tablets and patches.

In summary, compounds with BCS-I properties (high permeability and solubility) have the best potential of achieving significant systemic absorption but BCS-III compounds may also be candidates for nasal delivery and BCS-II compounds for sublingual/buccal delivery. Another key parameter is the dose limitation for nasal and buccal administration which means that the drug compound needs to be sufficiently potent.

3. Exploring nasal delivery of a large protein drug

AZ-X1 is a protein drug with a large molecular weight of approx. 4000. It is degraded by enzymes in the gastro-intestinal tract and has very poor membrane permeability, and is therefore not bioavailable following oral dosage. A feasibility study was conducted in rat to investigate if nasal administration could offer an alternative route to subcutaneous administration of AZ-X1.

Four different concentrations of AZ-X1 in a water solution (1.9 mg/ml, 7.3 mg/ml, 21.0 mg/ml, and 32.0 mg/ml) were given intranasally (IN) to rats and compared to both an intravenous bolus dose (IV) (0.18 mg/ml) and a subcutaneous (SC) (0.25 mg/ml) administration. The nasal administration gave a fast initial peak at 5-10 min with high plasma concentrations of AZ-X1 (Figure 1). A dose dependency in extent

of bioavailability was observed, bioavailabilities compared to IV administration were 1.1-4.2% at dosing concentrations 1.9-32 mg/ml, and in comparison to SC administration the bioavailabilities were 7.3- 27.2% (Table 1). Morphological studies showed that the epithelium was not damaged by the administration procedure.
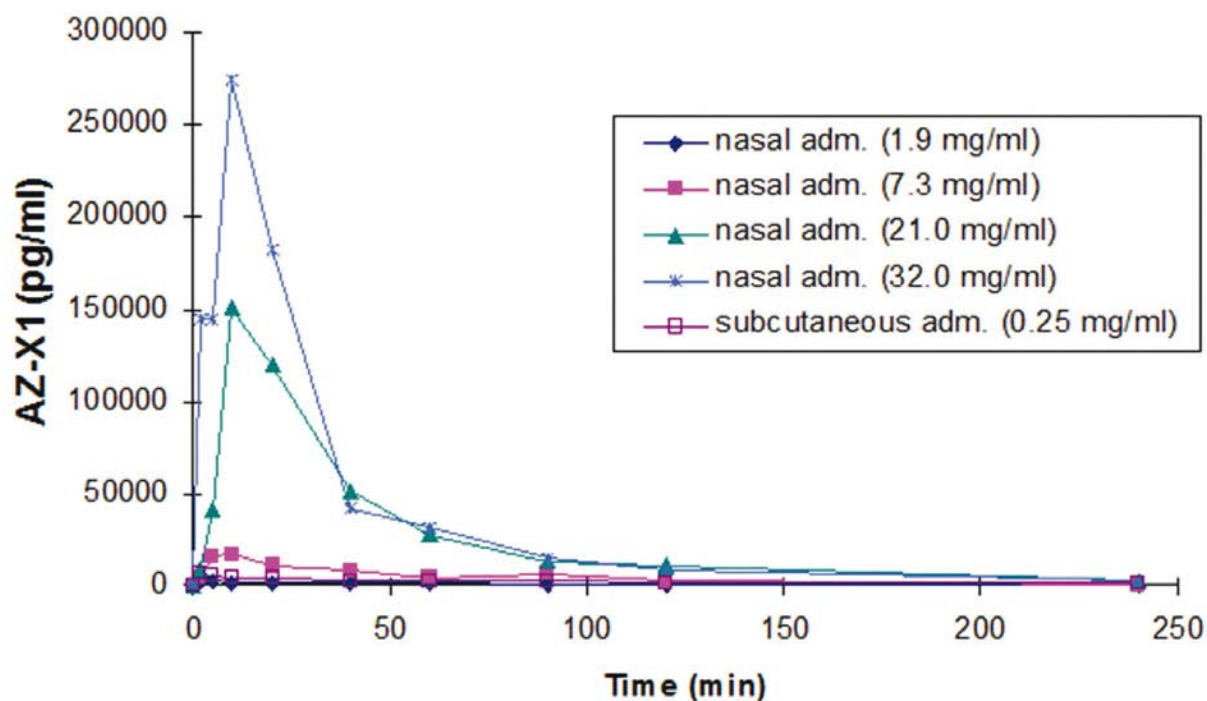

Figure 1. Average plasma concentration profiles for subcutaneous and nasal administration of AZ-X1 in rats.

Table 1. Pharmacokinetic data (mean values) for subcutaneous and intranasal administration of AZ-X1 in rat.

Parameter	SC (0.26 mg/ml)	IN (1.9 mg/ml)	IN (7.3 mg/ml)	IN (21 mg/ml)	IN (32 mg/ml)
AUC (mg L/min)	556	115	1080	5866	8029
NEA (µg L/min)	5881	432	1060	1535	1586
T_{max} (min)	2	5	10	10	10
C_{max} (pg/ml)	6774	2326	16950	150894	274081
F (%)	20.8	1.1	2.8	4.1	4.2
F_{rel} (%)		7.3	18.0	26.1	27.2

In conclusion, nasal administration of AZ-X1 in rats provided promising results by means of the obtained plasma concentration profile and systemic bioavailability. Nasal delivery is therefore a prospective alternative to subcutaneous injections of AZ-X1. A similar absolute bioavailability was later obtained in man and it was also shown that the absorption could be significantly increased with permeation enhancers.

4. Nasal delivery to obtain rapid onset of therapeutic effect

A key driver for using nasal administration is the opportunity to achieve rapid onset of drug action as illustrated by the development of the nasal spray formulation of zolmitriptan (Zomig). Basic characteristics of this compound are summarized in Table 2 [5, 6].

Table 2. Summary of characteristics for Zolmitriptan

Pharmacology	• Selective serotonin 5-HT receptor agonist (triptan) indicated for acute treatment of migraine
Chemistry	• MW=287.4, pKa=9.6 (base), logD(pH7.4)=-1.00, Solubility >1 mg/ml at pH 7.4.
Pharmacokinetics	• Mean bioavailability approx. 40%. • Well absorbed (F_a estimated to 60%, moderate Caco-2 permeability of 0.6 x10^{-6} cm/s) • Converted to an active N-desmethyl metabolite • T_{max}=2-4 h, $t_{1/2}$=2.5-3-0 h • BCS classification: BCS III

Intake of a conventional tablet is not ideal for all migraine patients since it can exacerbate migraine-associated nausea and vomiting and migraine-associated GI disturbances has been shown to be affecting oral absorption. Taking triptan therapy as early as possible in an migraine headache attack is advantageous. Therefore, administering zolmitriptan by nasal spray offers several advantages:

- convenient
- no localized pain and site reactions
- rapid onset of action because of potential for nasopharyngeal absorption
- high consistent efficacy because a good proportion of absorbed drug bypasses first-pass metabolism
- ability to be used by patients who experience nausea and vomiting

In clinical studies, it was shown that absorption begins at a high rate almost immediately after intranasal administration of zolmitriptan. Detectable levels of zolmitriptan were found in plasma 5 min after dosing and 38% of C_{max} was achieved in 10 minutes [6]. The rapid systemic absorption was translated into a rapid response in relieve of headache in migraine patients. The headache response rates at 15, 30 and 60 minutes after dosing with zolmitriptan 5 mg nasal spray was found to be 11%, 32% and 56%, respectively [6].

5. Exploring buccal delivery of a peptide drug

As described in the introduction, oral transmucosal delivery offers several advantages over injectable and enteral delivery. In this example, buccal delivery was investigated for AZ-X2, a tetrapeptide with negligible bioavailability in rat and dog following oral administration due to extensive metabolism and poor membrane permeability (ADME data summarized below).

ADME summary:

- C_{max} and AUC (rat, dog) increase proportionally with dose, no accumulation.
- F_{SC}= 50% in rats, 100% in minipigs
- F_{oral} is negligible
- Clearance from plasma= 107 ml/min/kg (rat), 33 ml/min/kg (dog)
- V_{SS}= 700 ml/kg in rats, 290 ml/kg in dogs
- $t_{1/2}$= 4-26 min in rats, 8-104 min in dogs
- *In vitro* plasma protein binding: fu=43% in rats, 28% in dogs, 25% in man
- Extensive metabolism, no unchanged drug excreted in urine

An evaluation of alternative administration routes for AZ-X2 was performed and buccal delivery came out as the first choice (Table 3).

Table 3. Evaluation of alternative administration routes for AZ-X2.

Route	Comment
Buccal	- Acceptable permeability of the buccal mucosa (Ussing) - Devices can stay in place for a few hours, new ones can be added
Transdermal	- Usually one order of magnitude lower transport - Much slower onset
Subcutaneous depot	- F_{SC}=50% in rats; F_{SC}=100% in minipigs - Short half-life: high loading required
Rectal	- Very low permeability of the rectal mucosa (Ussing) - Together with short half-life: undesirable administration route
Nasal	- Exploratory, screening in rats, possibly followed by sheep - Aimed at high and fast absorption, try for somewhat longer duration
Pulmonary	- Possibly too high doses needed - Will give pulsatile delivery

The permeability of AZ-X2 *in vitro* across pig buccal mucosa was relatively low but acceptable. The measured permeability coefficient was comparable to that of atenolol, a compound that has an enteral fraction absorbed in humans of about 50%. Studies on buccal delivery of AZ-X2 in dogs and minipigs showed reasonable absorption with Cmax occurring around 1 hour after administration. Finally, a clinical study on buccal administration of AZ-X2 to healthy volunteers was conducted. Oral transmucosal tablets were administered as single or multiple tablets. Attractive plasma profiles of AZ-X2 were seen after multiple buccal dosing. The buccal bioavailability in humans was rather low (few percent), but this could be acceptable for a potent peptide, if cost is not an issue (Table 4). However, data indicated that the desired effect of AZ-X2 disappeared before the plasma concentration declined (tolerance) and this compound was not pursued further.

Table 4. Individual, median and mean bioavailability (F) estimates following single buccal administration of AZ-X2 oral transmucosal tablets to healthy volunteers.

Subj. No.	F (%)	Subj. No.	F (%)	Subj. No.	F (%)	Subj. No.	F (%)	Subj. No.	F (%)	Subj. No.	F (%)
	3 mg		10 mg		30 mg		3x30 mg		3x10 mg		6x10 mg
101	2.8	201	1.1	101	0.4	201	0.1	101	3.5	201	1.1
102	3.0	202	1.4	102	0.9	202	0.6	102	3.1	202	1.6
103	1.8	203	2.5	103	0.3	203	0.5	103	2.1	203	1.7
104	4.0	204	2.6	104	1.1	204	0.8	104	4.4	204	2.9
Median	2.9		2.0		0.6		0.5		3.3		1.7
Mean	2.9		1.9		0.7		0.5		3.3		1.8
SD	0.9		0.8		0.4		0.3		1.0		0.7

6. Conclusions

Our feasibility studies on alternate routes of administration show that nasal and buccal administration enable systemic delivery of large peptide/protein drug molecules that have negligible bioavailability by gastrointestinal administration. However, it has proved difficult to achieve bioavailabilities greater that 5% for this type of molecules in human. Choosing an alternative administration route can be a way of salvage a new drug compound but it can also be utilized to obtain preferable exposure profiles and pharmacokinetic-pharmacodynamic relationship. Nasal administration is an attractive route of administration in this regard for potent drugs with good membrane permeability, where a rapid absorption and onset of therapeutic action is desired.

7. Acknowledgements

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CURRENT STRATEGIES FOR ANTI-RETROVIRAL DRUG TRANSPORT ACROSS VAGINAL AND RECTAL EPITHELIUM

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Abstract

Current development of microbicides against HIV-1 is largely focused on anti-retroviral drugs (ARVs). Microbicides are topically applied products that inhibit early events in HIV transmission at mucosal surfaces and thereby may prevent infection. The CAPRISA 004 human efficacy trial reported (in 2010) an overall reduction in HIV infection of approximately 40% following administration of a vaginal gel containing the nucleotide reverse transcriptase inhibitor Tenofovir as the active pharmaceutical ingredient. Separately, studies of early events in transmission of HIV at mucosal surfaces indicate that sub-mucosal CD4+ T cells form the primary foci of infection and that the number of such foci is very low with infection being established by a single virus isolate in many cases. Together these findings point to the requirement for ARV-based microbicides to penetrate mucosal epithelium and to be taken up by sub-mucosal T cells that are primary targets for infection. In two collaborative projects funded by the European Union, we are investigating factors that may influence distribution of ARVs at mucosal surfaces including the role of drug transporters. In view of the relatively low level of protection reported in the CAPRISA trial, we are also aiming to develop microbicide formulations with two or more ARVs. Both drug-drug interactions and approaches to co-formulate ARVs with incompatible physicochemical properties are also under investigation.

Keywords

Anti-retroviral drugs, drug permeability, drug transporters, HVV-1, microbicides, mucosal epithelium.

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1. Introduction

The potential for topically applied anti-retroviral drugs (ARVs) to prevent infection with HIV-1 was demonstrated in a phase II clinical trial (the CAPRISA 004 trial) where vaginal application of a gel formulation of the nucleotide reverse transcriptase inhibitor, Tenofovir, conferred 39% infection against infection compared with a placebo gel [1]. Following on from the CAPRISA 004 trial, results of a phase III (FACTS 001) trial of vaginally applied Tenofovir gel are expected during 20015 while development of a Tenofovir gel for rectal application is in progress [2,3].

Development of topically applied products, termed microbicides, to prevent HIV infection now includes other anti-retroviral drugs including non-nucleoside reverse transcriptase inhibitors such as Dapivirine which is currently being investigated in two linked phase III trials using intra-vaginal ring formulations [4,5]. Development of ARV-based microbicides represents a change of focus from earlier clinical trials that showed no protective effect of microbicides based on compounds such as surfactants [6,7] or polyanions [8,9] that possessed more broad spectrum activity and aimed to prevent fusion and entry of HIV. ARV-based microbicides inhibit post-entry events in the HIV life cycle and therefore, to be effective, must access the cells that form the primary foci of HIV infection. *In vivo* studies using the SIV models of vaginal and rectal infection [10,11] together with *ex vivo* studies of HIV infection in human cervico-vaginal tissue explants [12] indicate that sub-mucosal CD4 T cells form the founder populations of infected cells at the portal of entry. The efficacy of ARV-based microbicides therefore requires that the API (active pharmaceutical ingredient) be delivered across vaginal or rectal epithelium and enter submucosal T cells.

In this article we discuss studies, carried out in two collaborative projects (CHAARM and MOTIF) funded by the European Commission as well as those of other researchers, that aim to develop microbicide formulations that provide optimal drug distribution in mucosal tissue for the prevention of HIV infection. In particular, these studies focus on the role of solute carriers/drug transporters in the tissue distribution of ARVs as well as approaches to formulating microbicides comprising two or more ARVs.

2. HIV-1 Lifecycle: targets for microbicides

ARVs that are used in therapy of patients infected with HIV-1 target specific events in the HIV lifecycle (Fig. 1). Several of these act as inhibitors of the viral reverse transcriptase (RT) enzyme. Nucleotide analogues such as Tenofovir (analogue of adenosine monophosphate) undergo intra-cellular phosphorylation to form the active compound which competes with ATP for incorporation in the growing cDNA strand and blocks further strand elongation. Non-nucleoside RT inhibitors such as Dapivirine have a different mode of action as non-competitive inhibitors that bind to an induced allosteric hydrophobic pocket approximately 15Å from the reverse transcriptase catalytic site [13].
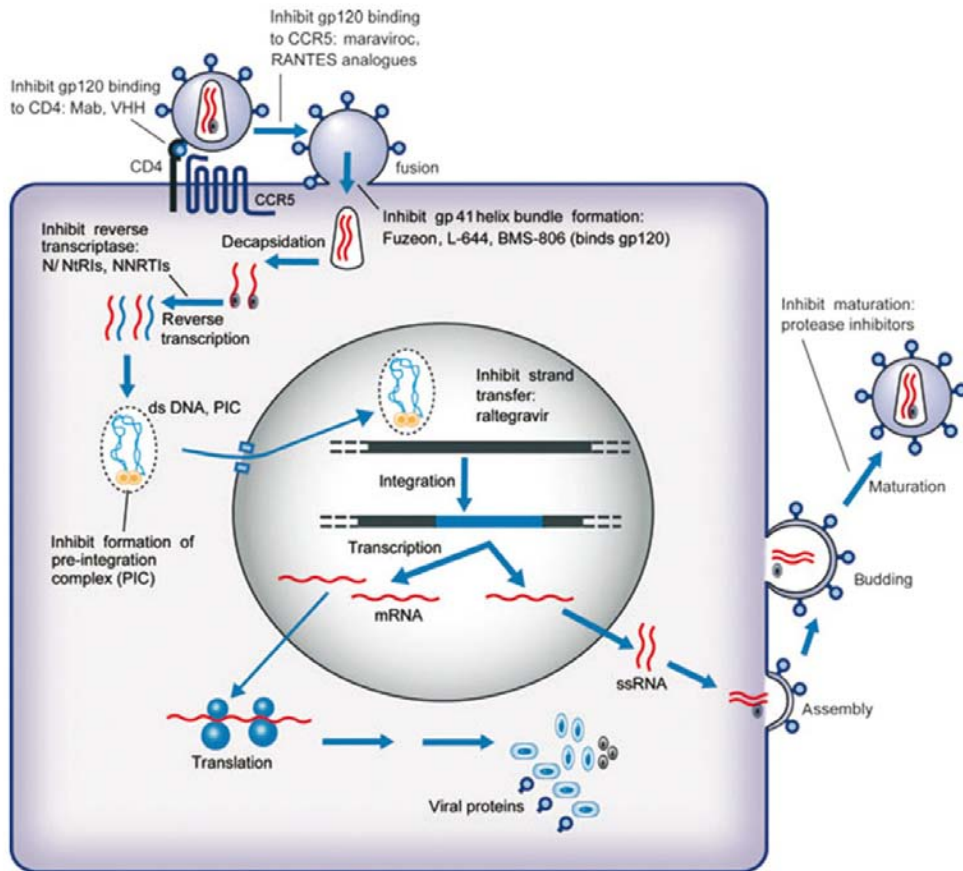
Figure 1. HIV-1 lifecycle and targets for intervention with microbicides. HIV binds to the CD4 receptor and CCR5 co-receptor triggering a conformational change in gp41 that leads to membrane fusion and release of the viral capsid into host cell cytoplasm. Decapsidation is followed by formation of the viral reverse transcription complex to produce double-stranded HIV-derived cDNA (proviral ds DNA) which in turn leads to the formation of the preintegration complex (PIC) of proviral ds DNA with HIV integrase and cellular cofactors. The PIC enters the nucleus through the nuclear pore complex, and HIV integrase mediates proviral dsDNA integration into the host cell genome. Activation of host cell transcription factors that bind the long terminal repeat of the inserted DNA (not shown in diagram) initiates viral replication by production of viral proteins and viral RNA. Assembly of viral proteins together with two copies of HIV RNA produces an immature virus particle that buds off from the host cell. HIV protease activity is required for the formation of the mature infectious virus particle. Stages in the lifecycle that have been targeted by microbicides currently under development are indicated. (Reproduced from Kelly CG and Shattock RJ, J. Intern. Med. 270 (2011) 509 -519, with permission).

Several ARVs are inhibitors of the HIV-1 protease. This enzyme is essential for the conversion of non-infectious immature virus particles to mature infectious virus – a process initiated by cleavage of gag and gag-pol polyproteins [14]. The successful use of protease inhibitors as a component in highly active antiretroviral therapy (HAART) together with their high potency and high barrier to resistance have stimulated interest in developing them as microbicides.

3. Drug Transporter Expression in Colorectal and Cervicovaginal Epithelium

Drug transporters that may affect the tissue distribution of ARV-based microbicides have been described in colonic epithelium [15,16] and more recently in cervico-vaginal epithelium [17]. Data are summarised in Fig. 2. In colonic epithelium, organic cation transporter 3 (OCT3/SLC22A3) and organic anion transporting polypeptide B (OATP2B1/SLCO 2B1) may mediate uptake of some ARVs at the apical (luminal) surface while efflux transporters (P glycoprotein/ABCB1 and BCRP/ABCG2) may reduce uptake. Efflux (MRP3/ABCC3) and uptake (MCT1/SLC16A1, OCT1/SLC22A1, OCTN2/SLC22A5) transporters on the baso-lateral surface may also influence drug distribution. Several efflux and uptake transporters are expressed in cervicovaginal epithelium although their distribution on apical or baso-lateral surfaces remains to be defined.

Using quantitative polymerase chain reaction technology, we have compared drug transporter expression in human cervical and vaginal epithelium with three human epithelial cell lines of cervical or vaginal origin. These data together with those above, confirm that expression of efflux transporters in the HEC-1A cell line correlates well with expression in cervical tissue although P-glycoprotein is likely expressed at a lower level and BCRP is not expressed in the cell line. In contrast, expression of uptake transporters in HEC-1A cells correlates poorly with tissue expression with only OATP-E (SLCO4A1) and ENT1 (SLC29A1) being expressed of those shown in Fig. 2.
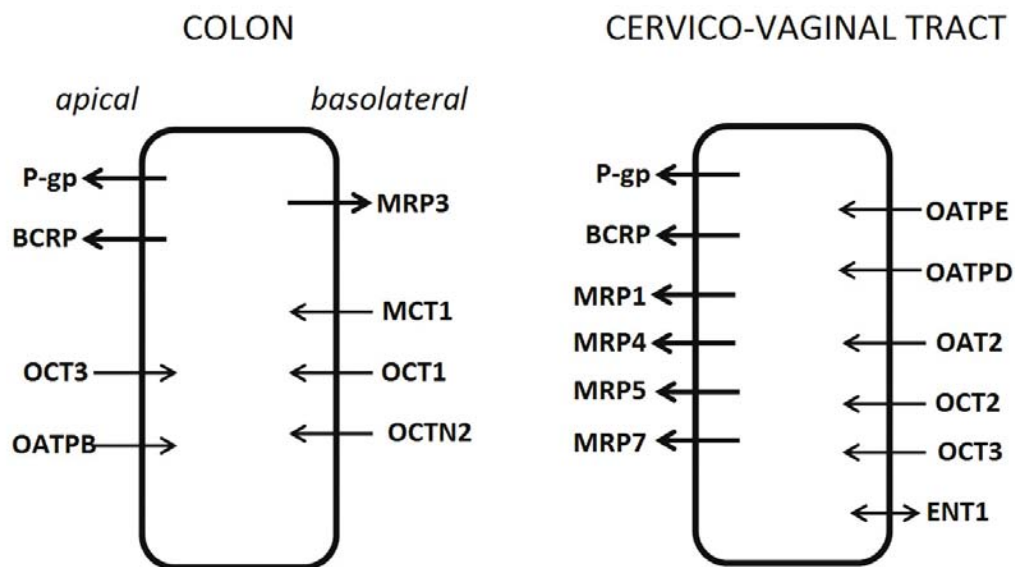

Figure 2. Transporter expression in colo-rectal and cervico-vaginal tissue. Efflux and uptake transporters have been described in the colon and cervicovaginal tissue. The apical and basolateral distribution of transporters in cervicovaginal epithelium has not been determined.

4. Drug Transport Studies

We have used *in vitro* models of colorectal and cervicovaginal barrier epithelium based on CaCo-2 and HEC-1A cell lines, respectively, in a Transwell® diffusion cell system to investigate permeability of three ARVs namely, Dapivirine, Tenofovir (provided by Gilead) and the protease inhibitor Darunavir (provided by Janssen) (Fig.3). Both cell lines form tight junctions as shown by immunostaining (Fig 4b,d). The lower trans-epithelial electrical resistance (TEER) of the HEC-1A model epithelium compared with that of CaCo-2 is reflected in the higher permeability of mannitol (paracellular marker) compared with CaCo-2 (Fig. 4c,e). In the HEC-1A model, permeability of all three ARVs was transporter-independent with similar absorptive and secretory flux. The apparent permeability coefficient (P_{app}) values for Tenofovir were similar to those for mannitol, consistent with paracellular permeability. In the Caco-2 model, permeability of Dapivirine and Tenofovir was again transporter-independent. In contrast, secretory flux of Darunavir was higher than absorptive flux.

When transport was measured with dual and triple combinations of drugs, no drug-drug interactions were evident and transport of individual ARVs was not affected.
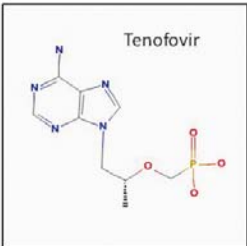
	Tenofovir	Darunavir	Dapivirine
MW	287	548	329
Log P	-1.6	1.8	5.3
pK_a	7.91	14.23	
aqueous solubility	13.4 mg/ml (25⁰C)	0.15 mg/ml (20⁰C)	insoluble

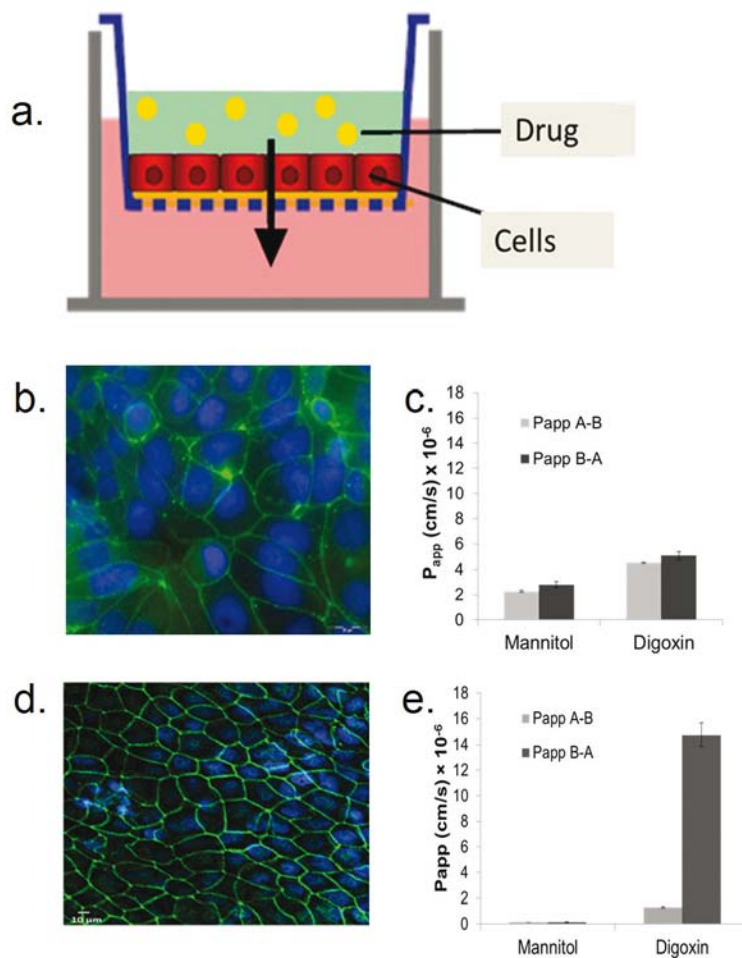*Figure 3. Antiretroviral drugs used in this study. Properties that influence formulation are shown.*

Figure 4. Models of colorectal and vaginal barrier epithelium for in vitro drug permeability studies. Panel a shows the Transwell® diffusion cell system with drug represented in medium on the apical side of the epithelial barrier. Panels b and d: immunofluorescent staining of zona occludens protein -1 (ZO-1) in HEC-1A and CaCo-2 cells, respectively. Staining indicates formation of tight junctions. Panels c and d show permeability values for mannitol (paracellular marker) and digoxin (P-glycoprotein substrate) in HEC-1A and Caco-2 Transwell® systems, respectively. The higher TEER values for the CaCo-2 barrier (approximately 2000 Ω/cm^2) compared with that of HEC-1A (approximately 250 Ω/cm^2) are reflected in the P_{app} values for mannitol.

5. Pharmocokinetic Studies

Microbicides that include 2 or more ARVs may show increased efficacy compared with a single drug. *In vitro* neutralisation studies using both cell and tissue explant systems demonstrated that the inhibitory concentrations of double or triple ARV combinations were decreased compared to single drugs [18]. Quadruple combinations conferred little advantage over triple combinations. Microbicides that include combinations of ARVs may also be more effective against viruses that are resistant to one component and present a higher barrier to possible development of resistant strains of virus.

As part of the project to develop combination microbicides, Dapivirine and Darunavir were co-formulated in a carbopol gel. Pharmacokinetic studies of this formulation together with Darunavir alone in an identical gel were performed in cynomolgous macaques – a non-human primate model that can be used for virus challenge studies. Following vaginal application of either gel, vaginal fluid and blood were sampled over a period of 72 hours and drug concentrations in both were determined by liquid chromatography linked to mass spectroscopy (LC-MS). As shown in Fig. 5, kinetics of release of Darunavir into vaginal fluid and transfer to serum were identical for both gels indicating that co-formulation with Dapivirine has no effect on release. Drug concentrations of both Darunavir and Dapivirine in vaginal fluids remained at levels over 5 logs greater than IC_{50} values reported for each drug [18,19] for 24h following application of the gel.
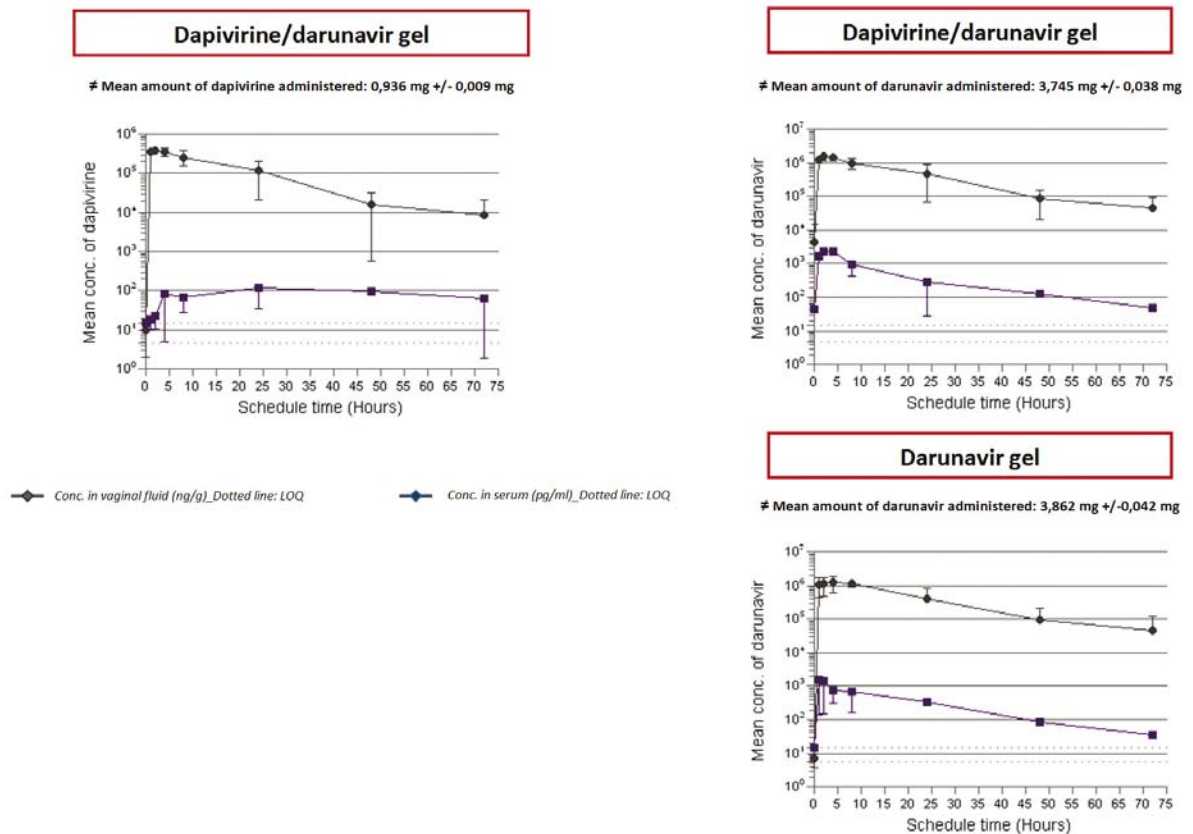
Figure 5. Tissue levels of Darunavir and Dapvirine following single application of vaginal gel in the macaque model. The graphs show the levels of drug in serum and vaginal fluid sampled at intervals over 72 hours following single vaginal application of gel containing Dapivirine + Darunavir or Darunavir alone. No effect of Dapivirine on Draunavir release is evident. (Data provided by Roger Le Grand and colleagues from Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), Fontenay-aux-Roses, France).

6. Phase I Human Trial

Further along the development pipeline within the CHAARM project, the Dapivirine and Darunavir gel co-formulation is currently being tested in a phase I clinical trial. The primary objective is to assess the safety, pharmacokinetics and pharmacodynamics of Darunavir and Darunavir + Dapivirine vaginal microbicides after a single dose and after a multiple dose regimen. Results of the trial are not yet available.

7. Conclusions and further work

Studies described above highlight the importance of formulation for microbicide delivery. While microbicides that include combinations of ARVs may be more effective than those with a single drug, co-formulation, in a single vehicle, of drugs with widely differing physicochemical properties is not a simple procedure. Increased degradation of Darunavir when combined with Dapivirine was noted when developing the gel formulation for the clinical trial. This effect was mitigated by inclusion of selected excipients. Optimising release rates of each ARV in a combination may not be possible if the drugs are simply dispersed in a homogeneous formulation. Studies are therefore in progress to develop generic approaches to encapsulate ARVs so that segregated and optimally formulated individual ARVs may then be combined in a single vehicle.

The *in vitro* epithelial cell models indicate that only Darunavir of the drugs investigated here is affected by efflux transporters at the apical surface of CaCo-2 cells. The low level of expression of P glycoprotein and some MRP efflux transporters (that have been identified in cervicovaginal tissue [18]) may represent a limitation in the HEC-1A model. Inflammation of the genital tract is associated with increased susceptibility to infection with HIV-1 [20, 21] and inflammation at mucosal surfaces may alter expression of drug transporters [22, 23]. Stimulation of inflammation in the epithelial cell models provides a convenient system to investigate effects on drug disposition.

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BUCCAL AND SUBLINGUAL DELIVERY OF SMALL AND LARGE MOLECULES: CURRENT STATUS AND CHALLENGES

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Abstract

There have been several advances in the delivery of drugs through the oral mucosa (buccal and sublingual) in recent years, which have resulted in a number of new buccal and sublingual delivery products appearing on the market. Wide variety of dosage forms, both conventional and advanced, have been designed and investigated *in vivo*, however those who are on the market are mostly the conventional ones (tablets, films, wafers etc). Delivery of the small molecules has been demonstrated to be efficient and stable whilst the delivery of the macromolecules has still obstacles to overcome. Recently, sublingual route has been extensively investigated for delivery of vaccines and allergens, hence good knowledge of the immune cells distributed within the oral mucosal tissue became essential as well.

The future potential of buccal and sublingual systems looks favorable platforms for the successful delivery of drugs, vaccines and allergens. Here, the current status of buccal/ sublingual drug delivery will be summarized, and the possibilities and limitations will be discussed in regard to quality, safety and efficacy issues.

Keywords

Buccal mucosa, drug delivery, macromolecules, sublingual mucosa.

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1. Introduction

There are many reasons that make the buccal mucosa an attractive site for the systemic delivery of therapeutic agents [1, 2]. The oral mucosa is readily accessible to the patients as well as their carers. The delivery system can easily be applied on the mucosa with high patient compliance; in other words, the delivery system can be attached on the mucosa without any pain and discomfort, while permitting easy removal in case of any adverse reactions. It is a well-vascularized tissue, therefore, drugs penetrating the mucosal epithelium are delivered directly into the systemic circulation, thus avoiding the hepatic first pass effect. The degradation by enzymes or hydrolysis is also avoided as the oral mucosa provides an environment almost free from the acidity and protease activity encountered elsewhere in the gastrointestinal tract. The cellular turnover time in the buccal mucosa is estimated to be 4 to 14 days, which would allow the attached delivery system retain on the application site for improved bioavailability.

Yet, the oral mucosa still represents a challenging area to develop an effective drug delivery technology. This arises mainly due to the various inherent functions of the oral cavity such as eating, swallowing, speaking, chewing, as well as the presence of saliva that is involved in all these activities. Furthermore, the low permeability and a smaller absorptive surface area is another limitation.

Buccal mucosa has a surface lining consisting of a non-keratinized, squamous epithelium supported by connective tissue lamina propria. The upper third to quarter of the buccal epithelium, where membrane coating granules extrude their contents (neutral lipids and glycolipids) into the intercellular space, represents the primary barrier to the entry of substances from the exterior [3]. Two major routes of absorption are involved in oral mucosal drug permeation: the transcellular or intracellular route (where drugs permeate directly through the cells) and the paracellular or intercellular route (where drugs permeate by passive diffusion through the spaces between the cells). The paracellular route is favored especially by hydrophilic drugs such as peptides/proteins which dissolve more readily in the aqueous fluids filling the intercellular spaces [4].

The permeation of drugs across the buccal mucosa is not only affected by structure and physiology of the mucosa but also properties of the drug such as aqueous solubility, lipophilicity (partition coefficient), ionisation (pH), molecular weight, required dose, which have to be taken into consideration in selection of the drug candidates for buccal delivery. An example of a drug known to penetrate via the transcellular pathway is fentanyl, which is a highly lipophilic and low molecular weight drug, while an example of a drug absorbed via the paracellular route is insulin, which is a water-soluble and high- molecular weight drug.

2. Design of drug delivery

The effectiveness of a delivery system designed for buccal/sublingual use depends on the retention of the delivery system in contact with the mucosa and permeation rate of the drug across the mucosa.

Chemical modification of the drug, incorporation of penetration enhancers and enzyme inhibitors into formulations as well as benefiting from mucoadhesive polymers have been the key approaches to improve the efficacy of the drugs via buccal/sublingual route. Physical means of enhancing drug uptake (e.g., sonophoresis, iontophoresis and electroporation) have also been investigated for buccal delivery, which would expand the current drug candidate list for this area [2].

Penetration enhancers

In order to deliver broader classes of drugs across the buccal mucosa, penetration enhancers such as bile salts, fatty acids chelating agents, surfactants, cyclodextrins, Azone, chitosan and etc. have been used to to overcome the barriers in this tissue. These penetration enhancers show their action by different mechanisms such as cell membrane fluidity, extraction of the structural intercellular and/or intracellular lipids from the membrane, the alteration of cellular proteins, or changing the mucus structure or rheology [5]. In regard to safety, the effect of the penetration enhancers should be reversible, non-toxic and should cause no side effects. Furthermore, the penetration should have no taste.

Mucoadhesives

Mucoadhesive systems have been widely used to maintain an intimate and prolonged contact of the drug with the oral mucosa [6]. Different classes of polymers have been investigated for this purpose. New generation mucoadhesive polymers which are either the modified forms of existing mucoadhesive polymers or new materials have been developed.

The most widely investigated group of mucoadhesives are hydrophilic macromolecules containing numerous hydrogen bond forming groups which are called "first generation mucoadhesives". The presence of hydroxyl, carboxyl or amine groups on the molecules favours adhesion. These include anionic polymers (e.g. polyacrylic acids-carbomers, sodium alginate); cationic polymers (e.g.chitosan) and non-ionic polymers (e.g. cellulose derivatives such as carboxymethyl cellulose, hyroxypropyl methylcellulose,etc.). In last decade, so-called "second generation polymers" which are less susceptible to mucus turnover rates, with some functional groups binding directly to mucosal surfaces have been investigated for buccal delivery as well. Amongst these polymers are lectin and the thiolated polymers derived from hydrophilic polymers such as chitosan, polyacrylates or alginates.

Some mucoadhesive polymers such as chitosan and some polyacrylic acids have been shown to exert penetration enhancing effect as well. Correspondingly, chitosan became a promising candidate as a mucosal penetration enhancer due to its favorable properties such as mucoadhesivity, nontoxicity, biocompatibility and biodegradability [7]. The effect of chitosan as a penetration enhancer was determined by measuring the flux of a peptide TGF-β across porcine buccal mucosa [8]. A six- to seven-fold enhancement of permeability was obtained in presence of chitosan. Tissue sectioning technique permitted quantitation of the compound in different strata (Fig.1). The hydrophilicity of the compound also seems to have an effect, for there was relatively greater penetration of TGF-β into the deeper tissue layers as compared to that obtained for a water insoluble small compound hydrocortisone [5].
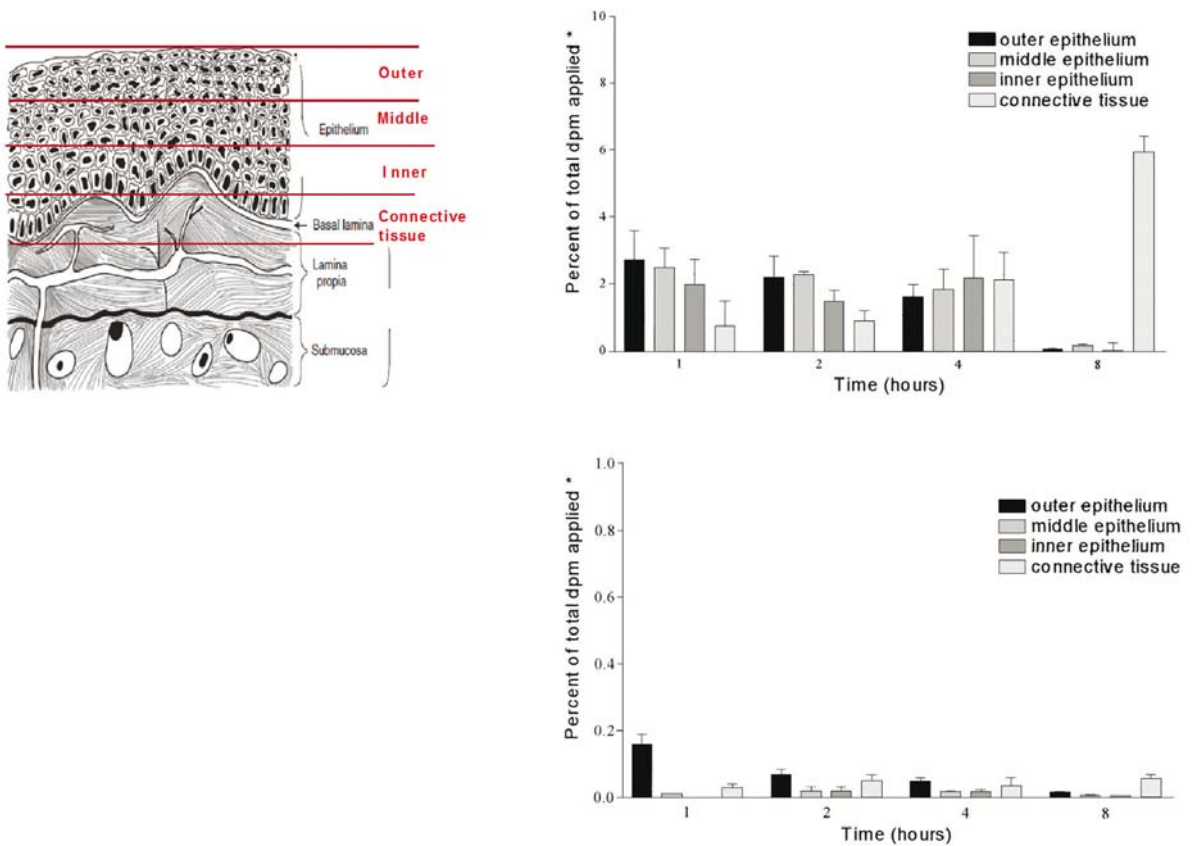
Figure 1. Distribution of TGF-β in different layers of porcine buccal tissue in presence of chitosan (a) and in PBS (b) [8].

The enhanced penetration in presence of chitosan is attributed to the bioadhesive nature of chitosan, which increases the retention of the drug at its application site. This could be mediated through the positive charge on chitosan, which could contribute to its continued effect on epithelial permeability after physical removal from the surface. This effect can be combined with the depot (reservoir) ability of the buccal epithelium. The permeabilizing effect of chitosan can also be due to its interference with the lipid organization in the buccal epithelium, which constitutes the barrier.

Testing the formulations

Permeation, dissolution test, mucoadhesion and residence time are the main *in vitro* tests performed on the buccal/sublingual formulations. In general, side-by-side (e.g. Ussing) or vertical (e.g. Franz) diffusion chambers are used to study the permeation of drugs, using artificial membranes, excised buccal/ sublingual tissue (porcine, bovine) or cell culture (EpioralTM, TR146 cell line human, SkinEtic, etc).

For dissolution tests, different USP apparatus have been used with different volume and rotation rate. In Table 1, the dissolution parameters for some FDA-approved buccal products are listed.

Table 1. Dissolution parameters for some commercially available products (FDA).

	Apparatus	Volume (mL)	Rotation Rate (rpm)	Medium
Miconazole Buccal tablet	I (Basket)	1000	60	0.5% SDS in water. pH adjusted to 6.5 ± 0.5
Testosterone Extended release buccal tablet	II (Paddle, may use sinker)	1000	60	1% sodium dodecyl sulfate in double distilled water
	I (Basket) 100 mL dissolution vessel	60 100	100	Phosphate buffer pH 6.4
	II (Paddle)	500	175	Phosphate buffer pH 4.5

Recommended sampling intervals (h):
Miconazole: 1, 2, 4, 6, 8, 10 and 12. **Testosterone:** 1, 2, 4, 6, 10, 12 and 24

Buccal / Sublingual Products

Currently, the number of the drugs that have successfully reached the market as buccal/sublingual delivery systems is still not very high. There is indeed a disparity between the intense research activity over the last two decades and the products for oral mucosal drug delivery actually reaching the market. A number of oral mucosal drug delivery systems such as tablets, lozenges, sprays, wafers, strips, films etc. have been developed for various drugs [9]. Disease conditions for which buccal/ sublingual products have been developed are summarized below:

- Breakthrough cancer pain
- Nausea and vomiting
- Status epilepticus and serious tonic–clonic seizures
- Diabetes mellitus (Type-1 and Type-2)
- Obesity
- Middle-of-the-night insomnia
- Oral chronic graft-versus-host disease (GVHD)
- Muscle spasticity
- Hypogonadism
- Opioid dependence
- Smoking cessation
- Cardiovascular
- Erectile dysfunction

Although wide variety of dosage forms including particulate systems, devices etc. has been investigated, currently tablets, lozenges and films are the leading delivery systems available on the market for buccal/sublingual drug delivery. In Table 2, examples to these products are given.

Table 2. Examples for buccal/sublingual products available on the market.

API	Product	Technology	Effect
Buprenorphine and naloxone	Suboxone® Sublingual tablet *Reckitt Benckiser Pharmaceuticals Inc.*		Treatment of opioid dependence.
	Suboxone® Sublingual film	PharmFilm® technology *(Monosol Rx)*	
	Bunavail® buccal film *BioDelivery Sciences*	BEMA (BioErodible MucoAdhesive)	
Ondansetron	Zuplenz® oral soluble film *Vestiq Holdings*	PharmFilm® technology *(Monosol Rx)*	Prevention of chemo- and radiotherapy induced and post-operative nausea and vomiting.
Prochlorperazine maleate	Buccastem M buccal tablet *Alliance Pharmaceuticals*	Mucoadhesives: PVP, Xanthan gum and locust bean gum	Symptomatic treatment of vertigo due to Ménières disease.
Testosterone	Striant® buccal system *Auxillium Pharmaceuticals*	Mucoadhesives: Carbopol 974, polycarbophil, hypromellose	Testosterone replacement therapy in males (primary or secondary hypogonadism).

In the following sections, only two examples for buccal delivery, fentanyl which is a small molecule and insulin, which is a large molecule, will be discussed in more detail due to the brevity of the current paper.

Buccal / sublingual delivery of fentanyl

Fentanyl citrate is a fast-acting opioid molecule, with a molecular weight of 528.59. It is used to treat breakthrough pain in adults with cancer who are already using opioids to control long-term cancer pain. Being a small and lipophilic compound (about 80% nonionized), fentanyl is particularly suitable for mucosal formulations. Oral mucosal formulation of fentanyl has been shown to produce more rapid and effective pain relief than oral morphine. Buccal/sublingual products appear to be more promising than oral products, and have started to attract more attention than the transdermal route (Fig. 2).

Fentanyl: Whole Drug Set (Marketed and Clinical Stage)

Products & Pipeline Count vs Route

- **53** products & pipeline in total
- **63** product programs in total
- 96 companies (This count includes all active and not active companies)
- 1 molecule types
- 4 different mechanisms are used
- at 8 different routes
- at 5 different DD Categories
- 6 dosage forms

Pharmacircle

Route	Number of Product and Pipeline
Transdermal	23 (21 marketed)
Buccal-Sublingual	23 (10 marketed)
Nasal	2
Inhalation	2
Injection	1
Oral	1

Figure 2. Products for fentanyl delivery (provided by Pharmacircle, September 2014).

The fentanyl Oralet was the first FDA-approved (1996) formulation developed for oral mucosal administration of an opioid in a formulation acceptable to children. The first product with a label claim for breakthrough cancer pain was fentanyl lollipop (Actiq, Cephalon, Inc.), which received approval in 1998. Consequently, newer oral mucosal formulations of fentanyl have been developed to further improve the management of breakthrough pain. The products that have been approved recently in Europe and/or the USA are summarized in Table 3.

Table 3. Fentanyl products available on the market.

Product	Dosage Form	Technology
Effentora/Fentora (*Teva Pharmaceuticals, Cephalon unit*)	Buccal and sublingual tablet	Oravescent technology (*Cima Lab*)
Abstral (*ProStrakan Ltd.*)	Sublingual tablet	Rapidly dissolving tablet (*Orexo*)
Breakyl / Onsolis (*MedaPharm*)	Buccal film	BEMA (BioErodible MucoAdhesive) (*BioDelivery Sciences International Inc.*)
Actiq Oral (*Cephalon*)	Lozenge	Lollipop shape with an applicator
Recivit (*Grünenthal Ltd.*)	Sublingual tablet	Unique three-layer structure (*Ethypharm*)

The dissolution parameters for these products (obtained from FDA's page) are summarised in Table 4, and pharmacokinetic results for the fentanyl buccal products are given in Table 5 [10-14]. When carefully examined, one can observe the differences between the dissolution test parameters chosen.

Table 4. Dissolution parameters for some commercially available fentanyl products (FDA).

	Apparatus	Volume (mL)	Rotation Rate (rpm)	Medium
TABLET				
Buccal	II (Paddle) Small volume dissolution apparatus	100 200	100	Phosphate buffered saline solution, pH 7.0
Sublingual	II (Paddle)	500	50	Phosphate buffer pH 6.8
Film	I (Basket) 100 mL dissolution vessel	60 100	100	Phosphate buffer pH 6.4
Lozenge	II (Paddle)	500	175	Phosphate buffer pH 4.5

Recommended sampling intervals (min):
Buccal tablet: 3, 5, 7.5, 10, 15, 20. **Sublingual tablet:** 1, 3, 5, 7, 15, 20.
Film: 5, 10, 20, 30, 45. **Lozenge:** 5, 10, 20, 30, 40

Similarly, in a study where the existing trials assessing the buccal products was examined, significant differences in many study parameters were observed, and it was concluded that it was difficult to evaluate the relative efficacies of these products on the basis of the available trials [15].

Table 5. Pharmacokinetic results for fentanyl oral mucosal products.

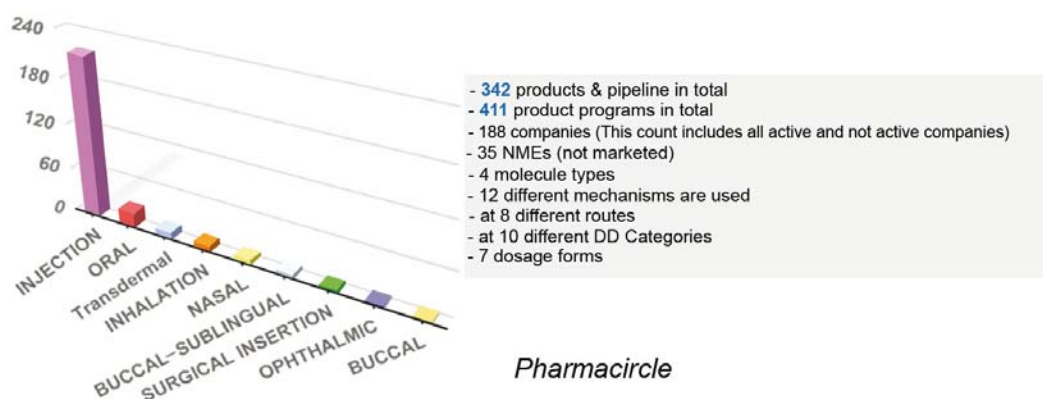| Product	Absolute Bioavailability (%)	t_{max} (minutes)	C_{max} (ng/mL)
Fentora (Cephalon)	65	46.8	1.02
Abstral (ProStrakan Ltd.)	54	30 (22.5-240)	0.2 to 1.3
Onsolis (Meda Pharm)	71	90 (45-240)	1.33 ± 0.31
Actiq Oral (Cephalon)	50	20	1.6 ± 0.5
Recivit (Grünenthal Ltd.)	70	50-90	0.4 to 2.07

Buccal delivery of insulin

Insulin is a key player in the control of hyperglycemia for type 1 diabetes patients and selective individuals in patients of type 2 diabetes. Currently available insulin delivery systems are administered subcutaneously or intravenously. Such therapy regimen involving multiple daily injections brings a heavy burden of compliance on patients and has prompted interest in developing alternative, less invasive routes of delivery such as transdermal, oral, buccal and pulmonary (Fig. 3). However, the main obstacle to these delivery routes is the difficulty of moving a large molecule like insulin across the membranes. The molecule size (5807.57), charge and hydrophilicity of insulin plays indeed an important role in its delivery across the membranes.

In the last decade, a liquid formulation (Oral-Lyn™) of regular recombinant human insulin for buccal delivery using the RapidMistTM device has been developed by a Canadian company, Generex Biotechnology [16]. This technology uses combination of insulin and specific absorption enhancers that encapsulate and protect the insulin molecules. It has the combination of a surfactant, a solubilizer, a micelle creating agent and emulsifying agents, all "GRAS" - generally regarded as safe - excipients prepared in a fashion that allows insulin to permeate across the buccal mucosa. It is reported that the micelles that are formed, containing the insulin, are smaller than 7 microns, therefore insulin does not enter the lungs. The product has received regulatory approval in Ecuador and subject to marketing approval in India.

Insulin: Whole Drug Set (Marketed and Clinical Stage)

Products & Pipeline Count vs Route

- **342** products & pipeline in total
- **411** product programs in total
- 188 companies (This count includes all active and not active companies)
- 35 NMEs (not marketed)
- 4 molecule types
- 12 different mechanisms are used
- at 8 different routes
- at 10 different DD Categories
- 7 dosage forms

Pharmacircle

Route	Number of Product and Pipeline
Injection	213 (68 marketed)
Oral	21
Transdermal	9
Inhalation	8
Nasal	6
Buccal-Sublingual	5 (1 marketed)
Ophthalmic	2

Figure 3. Products for insulin delivery (provided by Pharmacircle, September 2014).

Oral-Lyn™ is indicated for the treatment of type 1 and 2 diabetes. The combined data, completed in July-2011, from the 084 Trial in Type 1 patients and the Prevoral Trial in patients with impaired glucose tolerance provided key insights into both the short-term pharmacokinetic, and glucodynamic effectiveness of the product in reducing post-prandial increases in blood sugar as well as the one year safety and positive effect on metabolic control. The clinical trial 084, conducted in 463 patients with type 1 diabetes, demonstrated that Oral-lyn™ was effective in maintaining the hemoglobin A1c concentrations comparable to injected insulin during the 6-month head-to-head clinical trial [17]. In the Prevoral study, the safety and efficacy of Oral-Lyn™ on postprandial plasma glucose excursions and insulin levels in 31 obese subjects with impaired glucose tolerance (IGT) was demonstrated [18]. The results of a Phase 3 clinical trial, conducted in type 2 diabetes patients by Generex's Indian licensee, Shreya Life Sciences Pvt. Ltd., was announced in July, 2013 [19]. It was demonstrated that Oral-Lyn™ reduced hemoglobin A1c more rapidly and was as effective as subcutaneously injected regular insulin at the trial's conclusion establishing non-inferiority. Adverse events were rare and comparable between groups. It is reported that Phase III clinical trials on Oral-Lyn™ are continuing in USA and Canada [20].

Recently a self-dissolving strip containing nanoparticle insulin film form for insulin referred to as Midaform™ Insulin PharmFilm® has been developed for buccal delivery, combining the gold-nanoparticle technology of Midatech Ltd with PharmFilm technology of MonoSol Rx LLC. The transbuccal insulin film product delivers monomeric insulin, bound to a gold nanoparticle, through the use of a rapidly dissolving mucoadhesive film that is placed on the buccal mucosa [21]. A Phase I clinical study was conducted in 27 healthy volunteers, and positive bioavailability and pharmacokinetic results were obtained [22]. More studies are needed to show the efficacy of this product.

3. Conclusion

To date, several commercially successful delivery technologies have been developed for buccal and sublingual administration of drugs, and more are expected to appear on the market in the near future. The physicochemical properties of drugs play an important role in selecting the candidates for buccal delivery, small and lipophilic drugs being the most suitable candidates. Currently the biggest challenge for formulators is to deliver large molecules across the mucosa. Innovative formulation approaches that manipulate the barrier properties of the mucosa and that increase the retention of the delivery system on the application site will provide efficient delivery of large molecules, provided that safety is maintained. Furthermore, for high patient compliance, the size and taste of the system, ease of application, non-irritancy are the important factors that should also be taken into consideration.

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THE CHALLENGES OF THIN MUCOSA: THE NASAL MUCOSA

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Abstract

The nasal mucosa has been recognized as a place for the entry of active substances to the systemic circulation for a very long time. In spite of this, it has only been in the last decades that a number of drugs of systemic effect have reached the market. The initial formulation developments were rather simple, however, recently, efforts have been oriented towards the use of penetration enhancers and mucoadhesive biomaterials in the form of liquids (mainly sprays), gels and micro and nanoparticles, in order to facilitate bioactive compounds overcoming the barriers associated to the nasal mucosa. Within this frame our group has particularly focused on the design and use of nanocarriers for helping large molecules (peptide drugs, antigens and nucleic acids) to cross these barriers and reach their target. The criteria for their design have been: (i) stability upon contact with mucosal fluids (ii) muco-penetration and/or (iii) favored interaction with the epithelial barrier. A major out-put of this effort has been, first, the discovery of the positive effect of PEGylation on the ability of PLA-PEG nanocapsules to overcome the nasal mucosa and, second, the pioneering development of chitosan nanoparticles and the evidence of their potential for enhancing the transport of both, drugs, i.e. insulin and antigens (tetanus and diphtheria toxoids). More recently, our activity has been oriented towards the nasal vaccination field. Using advanced nanocarriers consisting of chitosan, protamine, and lipids, we have defined a series of nanostructures, which are promising vehicles for vaccination against hepatitis B and HIV. Overall, although general statements about the potential of nanocarriers for nasal drug/antigen delivery cannot be made, the rational design of nanoparticles specifically tailored for nasal administration opens optimistic prospects in this area.

Keywords

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1. Introduction: Nasal drug delivery: the pros and cons

Intranasal drug delivery is commonly associated with the treatment of local diseases of the upper respiratory tract such as nasal infections, congestion and allergy related conditions (e.g. rhinitis). However, because of the easy access, high vascularity, high permeability and limited pre-systemic metabolism of the nasal mucosa, this modality of administration has been considered an alternative route for the systemic drug delivery. Until now, low molecular weight drugs for which a rapid effect is required and with a high first-pass hepatic metabolism have been the main targeted molecules. However, there is an increasing interest in using this route for the systemic delivery of peptide drugs and vaccines as well.

Despite these advantages, the nasal route of administration has specific limitation that are mainly associated with the potential local intolerance or discomfort, the presence of mucus and the mucociliary clearance mechanism, the enzymatic activity (although much less important than in the gastrointestinal tract), the small absorption surface and the generally limited epithelial permeability for molecules larger than 1000 Daltons [1]. An additional critical limitation is the important variability in drug absorption, which is associated to the frequent alterations of the mucosa in pathological conditions.

Besides the intrinsic characteristics of the drug (chemical form, molecular weight, solubility and pKa) and the aforementioned anatomical and physiological aspects, there are other factors associated to the formulation that significantly affect nasal drug absorption. These factors include the drug concentration, pH and osmolality, presence of gelling substances, mucoadhesive agents and solubilizers. The application device has also a significant impact in drug absorption [2]. In this regard, nasal drops, the simplest form, lacks of drug dosing precision and has a limited retention in the absorbing respiratory epithelium. Nasal gels are able to reduce leakage and swallowing due to high viscosity but they also present a limited dose precision (excluding *in situ* gels, which are administered in solution for latter gelation). Alternatively, nasal powders, which provides better formulation stability without the necessity of preservatives, however, they are known to cause dryness and nasal irritancy. Finally, sprays, adequate for both solutions and suspensions, represent the most attractive form of administration. They are able to deliver exact doses and provide the drug distribution in the nasal cavity and subsequent interaction with the absorptive epithelium.

Bellow we provide an update of the nasal products on the market as well as the research efforts intended to achieve systemic drug delivery of a variety of drugs with emphasis on systemic delivery of peptides and vaccines.

2. Nasal marketed products intended to achieve systemic effects

The number of intranasal marketed medicines with a systemic effect has increased in the last years (Table 1) [3-5]. Examples of such marketed products are drugs to relieve severe pain, such as the opioid fentanyl (PecFent® or Lazanda®, Instanyl®) and ketorolac tromethamine (Sprix®); drugs for treatment of migraine attacks, such as triptans (Migranal®, Imigran®, Zomig®) and butorphanol (Stadol® NS); estradiol for hormone replacement therapy (Aerodiol®); anti-emetic drugs such as metoclopramide (Pramidin®); vitamin B12 (Nascobal®, Calomist®); and nicotine replacement therapies for smoking cessation (Nicotrol® NS). These drugs could be delivered intranasally without the need of absorption enhancers. This is because all of them have a low molecular weight and a lipophilic character, properties that enable them to reach the systemic circulation at therapeutic levels.

Table 1. Market authorized intranasal medicines with systemic effect.

Company	API	Brand name	Indications	Dosage Form	Observations
Novartis AG	Dihydroergotamin	Migranal®	Migraine	Spray solution	
GlaxoSmithKline	Sumatriptan	Imigran® Imitrex®			
AstraZeneca	Zolmitriptan	Ascotop® Zomig®			
Bristol Myers Squibb	Butorphanol	Stadol®	Migraine Post-operative pain	Spray solution	No longer on the market Generic alternatives
Novartis AG	Calcitonin	Karyl® Calsynar® Miacalcin®	Postmenopausal osteoporisis	Spray solution	
Apotex and Sandoz		Generic of Novartis (2008)			
Upsher Smith Lab		Fortical®(2005)			Peptelligence™ technology from Unigene
Ferring	Desmopressin	Minirin®	Polydipsia, polyurea and dehydration (diabetic patients)	Spray solution	
Medimmune (AstraZeneca)	Live attenuated influenza vaccine	FluMist® (2003)	Influenza vaccine	Mono-dose spray	Latest version is FluMist® Quadrivalent (2012)
Pfizer	Nicotine	Nicotrol® NS	Smoking cessation	Spray solution	
Depomed Inc (Archimedes)	Fentanyl (opioid)	Lazanda® (2013) Pecfent® (2011)	Breakthrough pain (patients with cancer)	Spray solution, in situ gelling	PecSys® Technology (pectin)
Nycomed	Fentanyl (opioid)	Instanyl®(2011)		Spray solution	

Table 2. Market authorized intranasal medicines with systemic effect.

Company	API	Brand name	Indications	Dosage Form	Observations
Pharmacia Ltd	Nafarelin	Synarel® Synarela®	Endometriosis: Fertility programs	Spray solution	
Sanofi Aventis	LHRH analogue or buserelin	Suprefact® Profact®	Endometriosis Prostate cancer	Spray solution	
	Gonadorelin	Kryptocur®	Undescended testicle		(Germany, Greece, Austria)
	TRH or protirelin	Antepan® Relefact® TRH	Thyroid diagnostics		Currently outside USA
Servier	Estradiol	Aerodiol®	Hormone replacement	Spray solution	
Novartis AG	Oxytocin	Syntocinon®	Lactation induction	Spray solution	Withdrawn in 1997 for commercial reasons. Soon remarketed by Retrophin Inc.
Regency Therapeutics	Ketorolac tromethamine	Spririx® (2011)	Severe pain	Spray solution	Short term treatment
Sirton Medicare	Metoclopramide	Pramidin®	Antiemesis	Spray solution	Only marketed in Italy
Par Pharma	Vitamin B12	Nascobal® (2005)	Vitamin B12 deficiency	Spray solution	Developed by Nastech
Flemming & Co.		Calomist®			

It is accepted that the systemic absorption of large hydrosoluble compounds, such as peptides, generally requires the use of absorption enhancers. However, there are a few exceptions of peptide products commercialized without the need of such ingredients. That is the case of, for example, desmopressin (Minirin®), calcitonin (Miacalcin®, Fortical®), nafarelin (Synarel®), buserelin (Suprefact®), gonadorelin (Kryptocur®) and protirelin (Relefact® TRH), which are efficacious at very low systemic levels. The nasal delivery of other peptides and proteins, such as insulin or PTH, still remains challenging.

Additionally, a nasal influenza vaccine spray (Flumist®) is present on the market from 2003, being its last version, Flumist® quadrivalent, commercially available from 2012.

3. Promotion/modulation of nasal absorption: Pharma players

Several companies are involved in the development of different approaches aimed at promoting and/or modulating the nasal absorption of diverse active molecules (see examples on Figure 1). For example, Archimedes Pharma (currently ProStrakan) has two proprietary drug delivery systems, PecSys® and ChiSys®, based on the use of the natural polymers pectin and chitosan, respectively. The first one was introduced on the market for the delivery of fentanyl (PecFent® in Europe and Lazanda® in the US). With regard to the second one based on chitosan, there is a morphine (Rylomine®) formulation that reached clinical Phase III in 2007, however, the current status of this product is unknown. On the other hand, there is a nasal Norovirus vaccine based on the Chisys® technology, which according to Takeda Pharmaceutical, is currently in advanced preclinical studies.

On the other hand, the Japanese company SNBL has developed a nasal delivery technology, consisting of a muco-adhesive powder prepared with GRAS excipients, named as μco™ carrier. This carrier has shown a good safety profile in clinical trials as well as the ability to enhance and prolong the absorption of several drugs. Their most advanced developments are an intranasal anti-emetic (ganisetron, Phase II completed in the US), an intranasal anti-migraine (zolmitriptan, Phase I completed in the US) and an intranasal flu vaccine (preclinical studies in Japan).
Marina Biotech Inc. proposed the use of tight junction-modulating lipid and peptide molecules to enhance nasal absorption of peptides [6]. Although the company reported the development of nasal insulin and teriparatide up to Phase II clinical trials in 2008 and 2009, respectively, no further advances of these developments have been identified.

Other delivery technologies intended to enhance nasal absorption have been reported by companies such as Critical Pharmaceuticals (CriticalSorb™ technology with Macrogol 15 hydroxiestearate; hGH in Phase I), CPEX Pharmaceuticals (CPE-215® with cyclopenta decalactone; failed Phase IIa with their Nasulin® insulin in 2010) and Nanotherapeutics (GelSite® with sodium polygalacturonate from Aloe vera; GelVac™ influenza vaccine in Phase I).
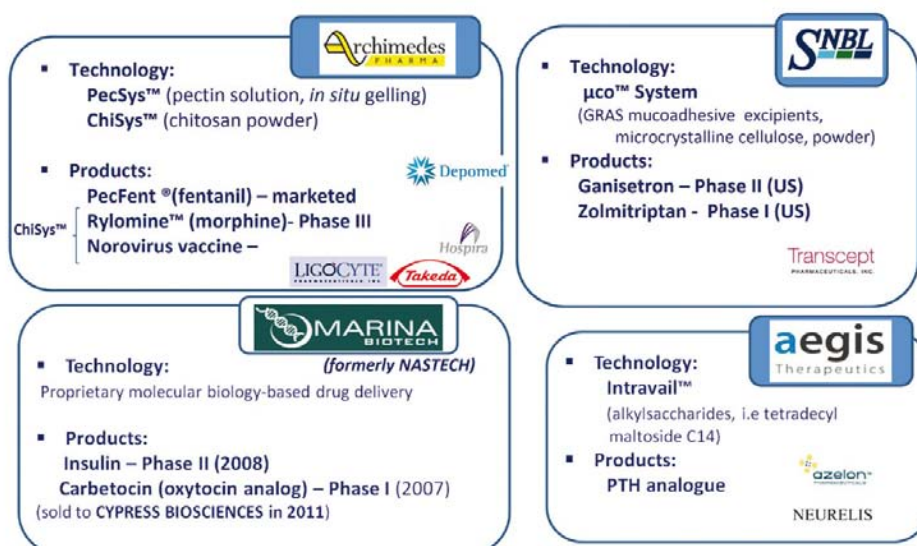

Figure 1. Promotion / modulation of Nasal Absorption: pharma players.

4. New indications of nasal medicines containing old drugs

As an alternative to accelerate the drug development process with reduced risk of failure and relatively lower costs, numerous pharmaceutical companies have adopted the strategy of drug repositioning. Just as an example, Retrophin Inc. acquired in 2013 Syntocinon® (nasal oxytocin) license from NOVARTIS AG, not only for remarketing it as lactation inducer (currently Phase III clinical trials) but also to initiate clinical trials for new indications of this old-known drug. More specifically, this drug is being investigated in Phase II clinical trials, for the treatment of schizophrenia and autism.

Another intranasal drug repositioning example is the development of a TRH (protirelin) nasal spray intended to combat suicidal thoughts that is being developed by the University of Indiana.

5. Research approaches in nasal nano-drug delivery

Over the last couple of decades, the use of nanotechnology has led to the development of a number of delivery carriers, which hold promise on their use for the delivery of complex molecules, i.e. peptide and protein drugs and antigens. Tables 3 to 6 show a number of nanocompositions, including nano-emulsions, liposomes, nanoparticles and microspheres that have been reported in the last five years with the goal of improving the nasal delivery of a variety of drugs. Efforts have been mainly oriented to deliver peptide molecules and antigens. Overall, the biomaterials used to develop such compositions are lipids and polymers, in particular chitosan and poly(lactic/glycolic) (PLGA).

Our group was the first one to report the potential of chitosan nanoparticles for improving the nasal delivery of insulin [7]. Overall, using the rabbit model, we could show that chitosan nanoparticles were able to enhance the nasal absorption of insulin in a much greater extent than chitosan used as a simple solution [8, 9]. The same composition was later used to deliver antigens such as tetanus toxoid [10] or hepatitis B surface antigen (rHBs Ag) [11]. In both cases, we could show that the association of the antigen to the nanoparticles led to a significant response in comparison to that observed after the administration of the antigen in solution. In some instances, the response was comparable to the one attained upon injection of the antigen in association to Alum [11]. More recently, we developed a nanocapsular system consisting of an oily core surrounded by a chitosan corona that contained rHBs Ag together with the TLR agonist imiquimod. The *in vivo* assessment of this prototype led us to conclude that it is possible to optimize the efficacy of nasal vaccines by the co-encapsulation of antigens in association with immunomodulatory molecules within a nano-antigen delivery vehicle [12].

On the other hand, in the mid 80's our group made a pioneering discovery with regard to the potential of nanoparticles for nasal drug delivery. We observed that hydrophobic PLGA nanoparticles aggregated upon contact with mucosal fluids and, hence, we proposed the idea of coating them with polyethyleneglycol (PEG) in order

to prevent their interaction with components of the mucus. In agreement with our hypothesis, we could verify that PLA-PEG nanoparticles were much more efficient that PLA nanoparticles in terms of enhancing the transport of the associated proteins across the nasal mucosa [13]. Moreover, we could demonstrate that both, the size of the nanoparticles and the density of the PEG coating had an impact on their ability to overcome the nasal mucosa [14, 15]. Finally, we could also show the potential of these nanoparticles as carriers for nasal immunization against tetanus toxoid [16].

Overall, taking together the results reported in the literature and those from our experience we conclude that polymer-based nanoparticles have shown in a number of animal models (mice, rats and rabbits) the possibility to enhance and prolong the delivery of peptides and proteins [17]. The success of the clinical implementation of the delivery approaches will largely depend on the safety issues and also on the pharmacological profile of the drug candidates. Probably, highly active peptide drugs with a broad therapeutic interval may represent the next generation of nasal nanomedicines. Protein and peptide antigens are also expected to potentially benefit from these delivery technologies.

Table 3. Nano-drug delivery products (described in the literature).

Components	Delivery system	Drug	Dosage Form	Ref.
DPPC, Cholesterol PVP	Liposomes	Acyclovir	Gel	[18]
Sodium deoxycholate, DC-cholesterol, soybean phosphatidylcholin		Calcitonin	Liquid	[19]
DPPC, DPPG, colesterol, Chitosan		Fexofenadine	Liquid	[20]
Tween 80, Capmul PG8, ethyl oleate	Nanoemulsions	Artemether	Liquid	[21]
Tween 80, Transcutol P, Solutol HS-15		Nitrendipine		[22]
Thiolated chitosan	Microparticles/ Microspheres	Insulin		[23]
Chitosan		Zolmitriptan	Dry powder	[24]
Chitosan + ethylcellulose		Loratadine		[25]
Chitosan		Metoprolol		[26]
C. pulcherrima galactomannan		Ondansetron		[27]
Chitosan		Verapamil	Powder	[28]
Concanavalin A-PEG-PLA	Nanoparticles	(-)		[29]
Starch (potato)		Insulin		[30]
Chitosan, anionic cyclodextrins		Insulin	Liquid	[9]
Chitosan		Olanzapine		[31]
Chitosan		Rizatriptan	Powder	[32]

Table 4. Nano-vaccine delivery products (described in the literature).

Components	Delivery system	Drug	Dosage form	REF.
N-trimethyl chitosan, PLGA, CpG, Hyaluronic acid	Nano-conjugates	Ovalbumin	Liquid	[33] [34] [35] [36] [37]
Tween 80, CPC, ethanol, highly purified soybean oil	Nanoemulsion	Hepatitis B antigen; influenza; ovalbumin; anthrax antigen	Liquid	[38] [39] [40] [41] [42]
Mygliol, phosphatidylcholine, chitosan	Nanocapsules	Hepatitis B antigen	Liquid	[12]
Cholesteryl-group-bearing pullulan	Nanogel	Clostridium botulinum type-A neurotoxin	Liquid	[43]
Chitosan	Complexes	pDNA (pcDNA3.1-pHSP65pep)		[44]

Table 5. Nano-vaccine delivery products (described in the literature).

Components	Delivery system	Drug	Dosage form	REF.
Mannosylated chitosan; Chitosan; Dextran	Microspheres	Bordetella bronchiseptica; Tetanus toxoid		[45] [46] [47]
Chitosan; Mono-N-carboxymethyl chitosan, N-trimethyl chitosan;	Nanoparticles	Tetanus toxoid; pDNA (Esat-6/3e-FL, M2e-HSP70c); influenza; Hepatitis B antigen;	Liquid	[10] [48] [49] [50] [51] [52]
PLGA; PLA-PEG ; Chitosan; γ-PGA; Polypropylene sulfide; Co-methyl vinyl ether and maleic anhydride;	Nanoparticles	Tetanus toxoid; Ovalbumin; Shigella flexneri; Bovine parainfluenza type 3 virus antigen	Liquid	[53] [54] [55] [56] [57] [58]
PLGA, PVA, chitosan	Nano- and microparticles	pDNA (foot and mouth disease virus antigen)	Powder	[59]

Table 6. Nano-vaccine delivery products (described in the literature).

Components	Delivery system	Drug	Dosage form	REF.
PC, cholesterol; dextran	Liposomes	Tetanus toxoid	Liquid	[60]
PC; cholesterol; dicetyl phosphate; Dioleoylphosphatidylethanolamine; 1, 2-Dioleoylphosphatidylglycerol; chitosan;	Liposomes	Herpes simplex virus 2 antigen; pDNA (pGJA-P/VAX)	Liquid	[61] [62]

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OCULAR DELIVERY: A PROBLEM TOUGH ENOUGH TO MAKE YOU CRY?

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Abstract

Ocular drug delivery is employed solely as a method of direct treatment of the eye, rather than a method of accessing the systemic circulation. The conditions treated topically can loosely be described as mild to chronic periocular conditions including allergic diseases, infections, and loss of tear structure. The barrier functions of lacrimal drainage, vascular clearance and the nature of the epithelial barriers in man prevent direct retinal delivery and more invasive strategies including direct intravitreal injection, must be employed for the treatment of progressive, blinding disease associated with ageing and co-morbidities.

With regard to drug access to internal structures, the eye is often treated mechanistically as a variant of 'wet' skin, with emphasis on free drug concentration and pKa and pH-partition. The tissue is however unusual in that contaminant removal processes are very active, the system works under a slight positive hydraulic pressure of between 14 and 22 mmHg, and the target structures are mostly inaccessible. The sense of vision is precious and well-protected. Moreover, the cornea represents only 17% of the available surface area and the lens divides posterior from anterior globe.

Delivery across these barriers requires technological ingenuity and an understanding of how convective barriers dominate distribution and flux. Within the last ten years, the interest in macromolecular constructs has increased greatly. It has been shown that blindness can be partially reversed in age-related macular degeneration, but the duration of treatment is too short and the patient must endure multiple and expensive reinjection. Increasing exposure and sustaining delivery are therefore key medical targets in order to exploit the next generation of potential blockbuster drugs.

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Contrary to the scary headlines, the rates of impaired eyesight and blindness have plummeted over the last 20 years in the developed world. Cataract, a leading cause of poor sight is treatable effectively and safely as with removal of the lens and replacement with a prosthetic device as a routine surgical procedure. This cause of visual loss has been replaced by blindness caused by macular degeneration and diabetic retinopathy in populations which survive longer into old age allowing expression of genetic predisposition to these diseases. Progressive diseases such as glaucoma had been routinely treated with carbonic anhydrase inhibitors, beta-blockers, alpha-2 agonists and prostaglandins as formulations applied as eyedrops or occasionally gels and device. Initially formulation scientists hoped to achieve therapy for these 'deeper' conditions by similar topical approaches. The problem is the eye is a vulnerable and important organ and evolution has protected this delicate, sensitive tissue with formidable barriers to prevent retinal function from being disturbed. Most organs in the body have a recognisable shape, but soft tissues stretch and distort to allow changes in volume by relaxation, shorten to develop tension or pack spaces to provide insulation and energy stores. The eye must preserve dimensions in order to focus on image onto the retina and therefore is inflated by production of liquid to between 13 and 23 mm Hg giving rise to convection within the organ. The retina forms a cup shaped surface and on its outer side is bathed by vessels forming an extensive capillary network to provide blood to service the extensive metabolic needs of the tissue. Internal reflection in an organ design for vision would degrade the quality of the image and therefore must be absorbed. Protection of the eye is provided by inset of the organ into the skull with the strong orbital plates partially protecting from side injury. The response to frontal stimuli is head-turning and reflex eyelid closure, accompanied by reflex tearing on chemical stimulation and blinking to recorrect refraction as the eyes well up with tears.

Particulate material is agglomerated by mucus and ejected at the corner of the eye and smaller material by hyperaemia of the conjunctivae and venous and lymphatic removal. Structurally, physiologically and metabolically, the barriers to delivery are significant. The frontal transparent cornea is often regarded as the principal barrier for the absorption of drugs following topical instruction. The cornea is a thin tough membrane composed of an outer epithelial layer joining acellular collagen arranged in a lattice with regularly spaced fibrils allowing transparency (stroma). The innermost endothelium layer is not a barrier but is metabolically important in maintaining the thickness of the stromal layer. The tear film above the epithelium provides a physiological barrier since it is partially removed and replenished on each blink. Disturbance of the normal refractive properties of the cornea results in excessive blinking and squeezing of the eyelids. In terms of assessment of the corneal route of entry, the cornea and tear film form are often considered as a functional unit but this ignores the conjunctiva which lines the eyelids and joins the sclera beyond the pars plana to form a surface five times bigger than that of the cornea.
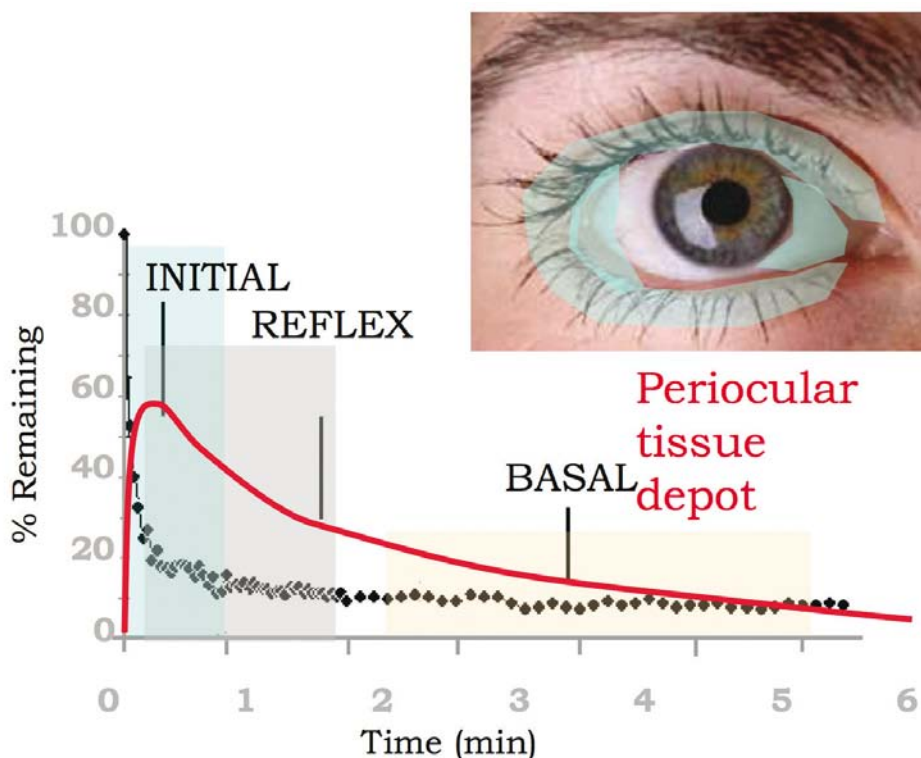
Figure 1. Diagram showing the phases of tear secretion. Inset: the conjunctiva lining the lids and over the sclera retains applied drug for a short time after topical administration.

It is this tissue which forms the real reservoir. A formulation applied as a drop will partition into this surface and subsequent redistribution of that drug, not removed by conjunctival veins, feeds the tear film concentration following classical rules of flux dictated by drug concentration and pKa/ pH-partition.

Precorneal clearance.

The human eye clears non-viscous solutions effectively by blinking, with 90% clearance from the eye in 1 minute. Gamma scintigraphy is a useful tool to monitor clearance with minimal disturbance to normal physiology [1]. A small amount of a sterile technetium-labelled non-absorbed marker, typically [99mTc]-labelled diethylenetriamine pentaacetic acid [99mTc-DTPA] is added to the formulation to facilitate imaging. This allows the flow of the formulation through the lacrimal duct into the nose. Addition of polymeric excipients will increase the surface residence time, but the effect is not dramatic. Typically viscous solutions have a residence time of 3-5 minutes in man. The response to the applied drop shows three phases as indicated- an initial increase in tear volume, reflex tearing caused by temperature and formulation related factors and finally structuring of the tear film (the basal phase). Above a certain viscosity, the mixing action of the upward sweep of the lid is insufficient to break the cohesive forces and the material then forms a reservoir in the lower fornix. Ointments spread slowly and because they blur vision are physically spread mechanically by the hand.

Peptide absorption and the sclera

As a therapeutic class, peptides and proteins have attracted much interest in the treatment of retinal disease, particularly in modulating the VEGF-cascade. These molecules show remarkable activity when injected into the vitreous and compounds such as ranibizumab (Lucentis) and bevacizumab (Avastin) have changed the scenario in treatment of macular degeneration. Many of the screens are based on small animals and in the rat and the mouse models of neovascularisation, polar compounds may be shown to be active even when applied topically. The dimensions of such small eye are such that convective forces are unimportant, and the applied drug has easy access to the back of the eye.

Compared to the cornea, the sclera allows the permeation of polar compounds, with a similar permeability for moderate lipophiles. The permeation is controlled by molecular radius rather than molecular weight as shown below.
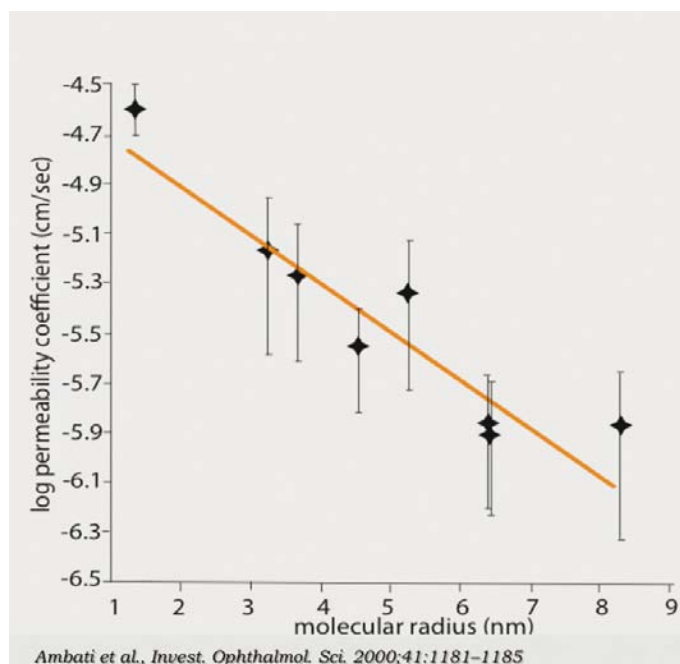

Ambati et al., Invest. Ophthalmol. Sci. 2000;41:1181–1185

Figure 2. Sclera acts as a sieving matrix and is more hydrophilic than the cornea. It therefore permits the absorption of more polar materials [2].

The sclera is composed of connective tissue. Three pathways through the sclera have been identified: along the scleral bundles, through aqueous-filled spaces between bundles and along perivascular space.
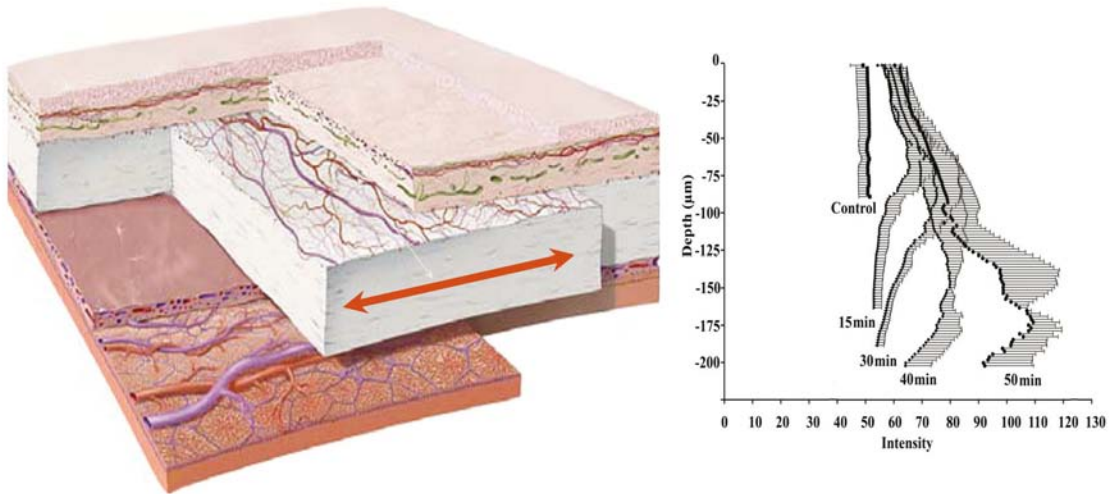
Figure 3. Distribution is aided by uveoscleral flow which distributes drugs laterally as shown.

The secretion of aqueous humour generates lateral spread of applied material at a depth of 150 to 200 µm along the scleral bundles, which as they adopt different directions in the anterior and posterior segments and crisscross at different depths, provides a complex pathway for distribution [3]. The uveoscleral flow causes material spreads faster laterally than through the tissue, a point that must be remembered when measurements of drug flux are made in Franz cell assemblies.

Blood flow

The eye is circled by the choroidal flow under the sclera. This is to support the retina, which has a high oxygen demand to support a significant glycolysis and responds quickly to anoxic stimuli. Flow processes are of immense significance in a closed structure such as the eye, whose anatomy and physiology presents such unusual geometries and matrices for drug delivery.

The extensive flow in the choriocapilliaris restricts the access of drug to the retina by vascular clearance, rather like a fast flowing river. When a drug is administered by subconjunctival injection, the transfer to retina is greatly increased by the application of gentle pressure at the site of injection [4].

Melanin binding

For drugs containing a strong primary amine function and a planar heterocyclic group, melanin binding may be significant [5]. The human eye produces melanin from two different types of pigment cells: those in the ocular pigmented epithelium and those in the uveal tract. Melanin colours the iris to different extent in different races but does not differ in the pigment epithelium. The melanin exists in two forms: Eumelanin (black to brown coloured) in darker irides and pheomelanin (yellow to red) more prominent in blue, hazel and lighter coloured irides. The effect of melanin binding is illustrated in Figure 4, which shows transfer of a melanin-bound drug from the dosed, across to the non-dosed eye through the nose and ciliary artery.
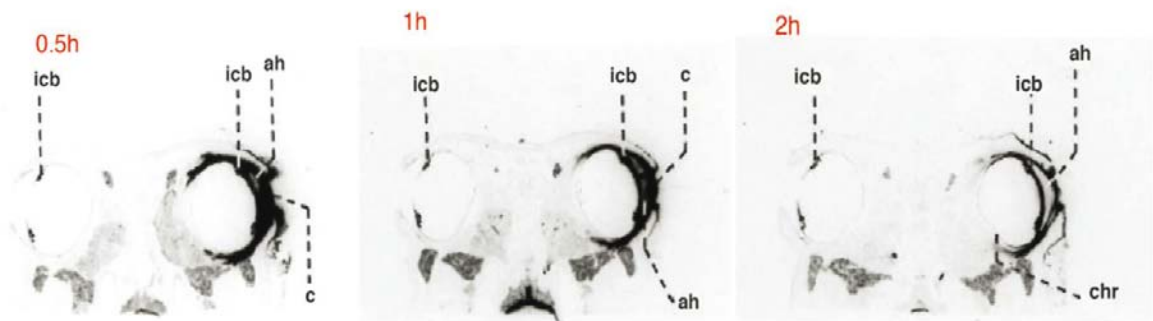
Figure 4. Microradiographic images showing transfer of radiolabelled memantine from the dosed eye of a rabbit [from reference 6].

The ageing eye

As the vitreous humour ages, it starts to collapse, with increased areas of liquefaction. This is caused by the loss of Type IX collagen which covers the sticky underlying fibrils of Type II collagen. This causes the formation of coarser fibrils which contract as they thicken. The posterior inner limiting pulls away from the back of the eye, with the remnants remaining attached to the back of the lens. The space behind the lens is no longer filled with gel and increased clearance of larger molecular constructs is likely to occur by convection [7], especially if the patient has also had cataract surgery.

6. Concluding remarks

Delivery across these barriers requires technological ingenuity and an understanding of how convective barriers dominate distribution and flux. Within the last ten years, the interest in macromolecular constructs has increased greatly. It has been shown that blindness can be partially reversed in age-related macular degeneration, but the duration of treatment is too short and the patient must endure multiple and expensive reinjection. Increasing exposure and sustaining delivery are therefore key medical targets in order to exploit the next generation of potential blockbuster drugs.

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DRUG DELIVERY ISSUES FOR ALTERNATIVE ADMINISTRATION ROUTES: FUNCTIONAL POLYMERIC EXCIPIENTS

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Abstract

The potential of functional polymeric excipients to improve the efficacy of drugs being administered by alternative routes such as intraoral, nasal, ocular or vaginal has already been shown in numerous *in vitro* and *in vivo* studies. From the drug delivery point of view in particular mucus permeating, permeation enhancing, *in situ* gelling and mucoadhesive properties are of relevance. Particulate delivery systems are in most cases more efficient when they can permeate the mucus gel layer covering mucosal epithelia. On the one hand a prolonged residence time on the mucosa can be achieved and on the other hand the drug can be released in a more concentrated manner right on the absorption membrane. Polymeric excipients that can improve the mucus permeation properties of particulate delivery systems are PEGs, mucolytic enzymes such as papain and excipient combinations leading to a high density of positive and negative charges on the surface of mirco- and nanoparticles. Permeation enhancing polymers such as polyacrylates, chitosans and thiomers are able to open tight junctions on mucosal epithelia. *In situ* gelling polymeric excipients can prolong the mucosal residence time by increasing their viscosity once having reached the mucosa. Xanthan gum, gellan gum, poloxamers and thiomers exhibit this property. Furthermore, mucoadhesive polymers such as thiomers, carbomers and chitosans can also prolong the mucosal residence time.

Keywords

Microparticles, mucoahesive polymers, mucus permeation, multifunctional polymeric excipients, nanoparticles, thiomers.

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1. Introduction

The potential of functional polymeric excipients to improve the efficacy of drugs being administered by alternative routes such as intraoral, nasal, ocular or vaginal has already been shown in numerous *in vitro* and *in vivo* studies. From the delivery point of view in particular mucoadhesive, cohesive, mucus permeating, release controlling and permeation enhancing properties are of relevance in order to prolong the residence time of drugs on mucosal membranes and to improve drug uptake from there.

2. Mucoadhesive/cohesive polymers

Generally, mucoadhesive and cohesive properties go hand in hand as high mucoadhesive properties lose their potential when the adhesive bond fails within the mucoadhesive polymer itself. Among mucoadhesive polymers such as polyacrylates, chitosans and cellulose derivatives especially thiolated versions of these polymers – designated thiomers – are known for comparatively high mucoadhesive properties [1]. By forming disulphide bonds with cysteine-rich subdomains of mucus glycoproteins as illustrated in Fig. 1 they are in contrast to all other mucoadhesive polymers covalently linked to the mucus.
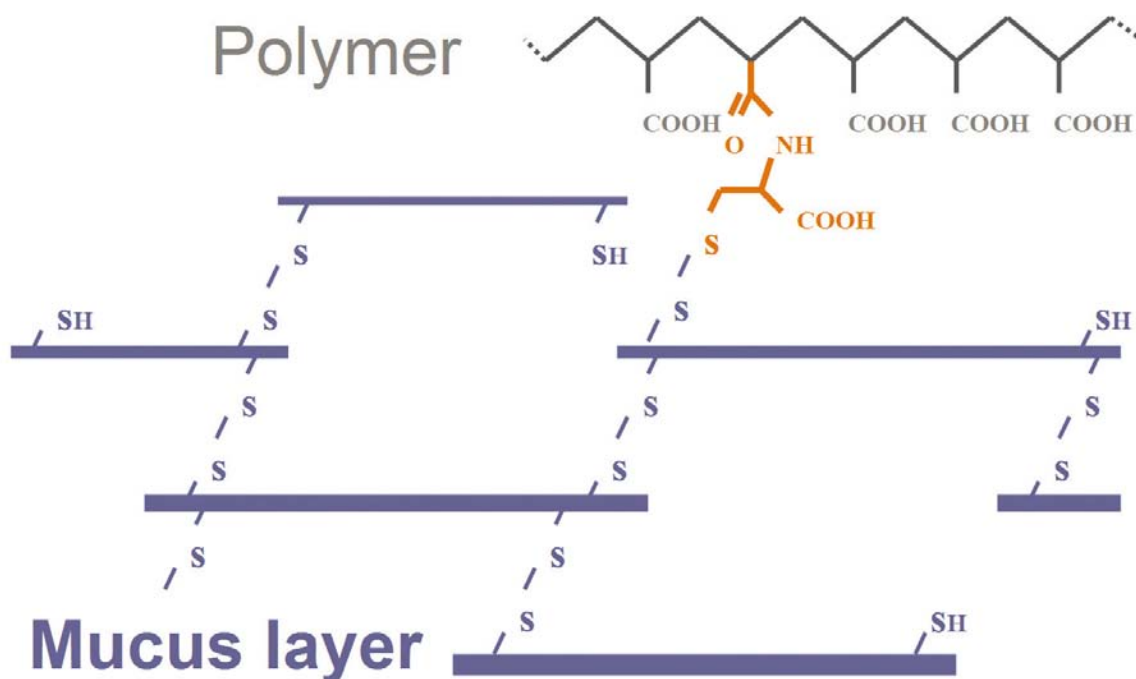

Figure 1. Schematic presentation of disulphide bond formation between a thiomer and mucus glycoproteins.

Furthermore, as they can crosslink via disulphide bond formation just within their polymeric network, they exhibit also comparatively high cohesive properties. Sakloetsakun et al., for instance, could demonstrate a more than 10,000-fold increase in viscosity within 5 minutes for a 1% (m/v) thiomer gel due to oxidative disulphide bond formation [2].

Such *in situ* gelling properties are in particular of interest for alternatives routes of administration, as an unpleasant outflow of low viscous gel formulations from nasal or vaginal mucosa can be avoided. Evidence for the potential of thiomers for alternatives routs of administration has been provided by numerous *in vivo* studies. For example Barthelmes et al. showed that after one hour less than 5% of a fluorescence marker are present on the intravesical mucosa of rats when being administered in aqueous solution, whereas there are still more than 50% of the marker present even after 6 hours when it is incorporated in thiomer nanoparticles [3]. Results of this study are highlighted in Fig. 2. Furthermore, evidence for these high mucoadhesive and cohesive properties could be provided by various studies on human ocular mucosa leading subsequently to the first product development based on thiomers for treatment of dry eye syndrome entering the market early 2015 [4]. Further products are in development.
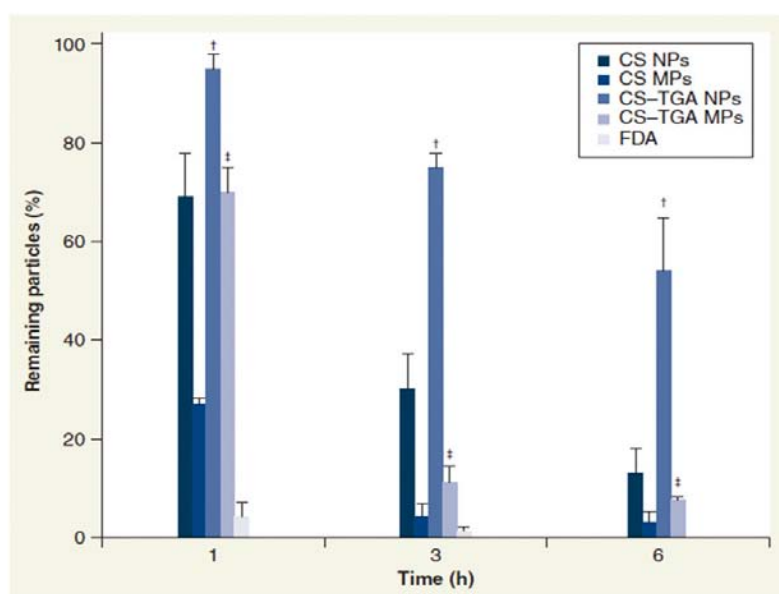

Figure 2. Fluorescein diacetate (FDA) loaded chitosan (CS) and chitosan-thioglycolic acid (CS-TGA) micro- (MPs) and nanoparticles (NPs) remaining on intravesical mucosa of rats (means ±SD; n=3); adopted from Barthelmes et al. [3]

By the covalent attachment of mercaptonicotinamide substructures via disulphide bonds to thiolated polymers as illustrated in Fig. 3 mucoadhesive properties are even substantially further improved and stability towards oxidation in aqueous media can be provided.
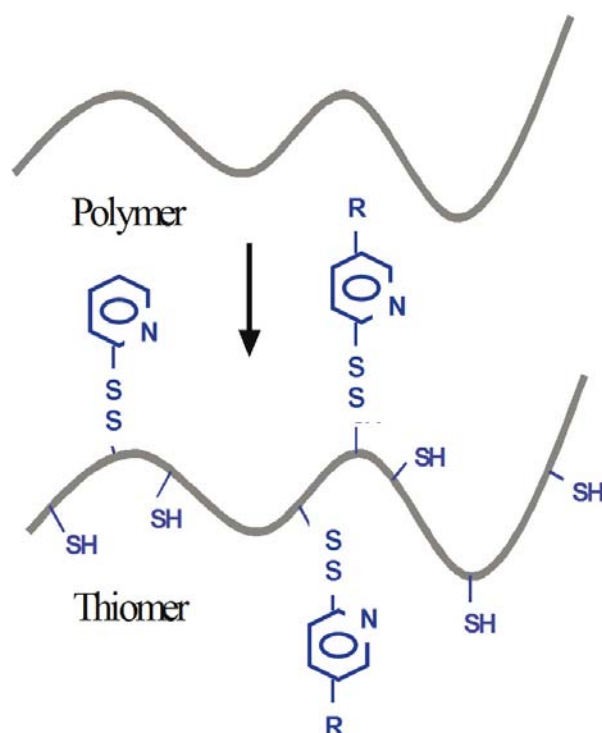
Figure 3. Schematic presentation of preactivated thiomers; R= COOH or CONH$_2$.

3. Mucus permeating polymers

When functional polymers are formulated to micro- and nanoparticles it is in many cases advantageous to render them mucus permeating so that they are less rapidly eliminated from the mucosa by the mucus turnover and that they can get closer or even directly to the epithelium. In addition, an improved spreading over the target mucosa can be achieved. Functional polymers providing such properties can generally be divided in passive and active systems. On the one hand among passive systems especially PEG-ylated polymers and Pluronics seem promising. Particles containing polymer-mucolytic enzyme conjugates such as polyacrylate-papain conjugates as illustrated in Fig. 4, on the other hand, are the currently most promising active systems cleaving mucus glycoproteins in front of particles moving in the mucus towards the epithelium.
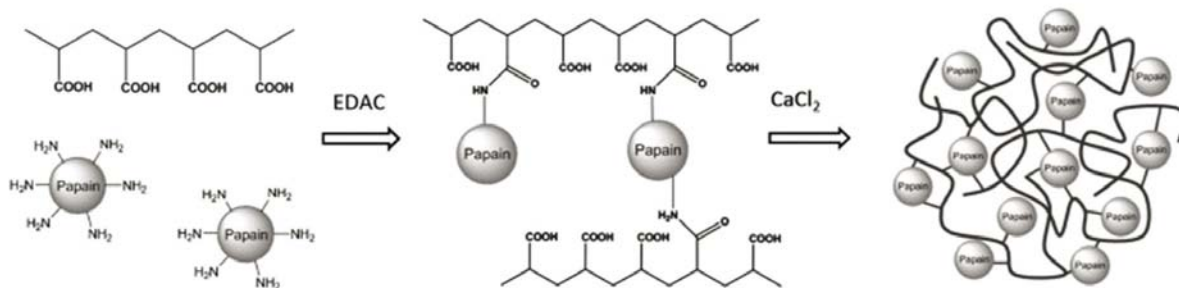

Figure 4. Schematic diagram of papain immobilization on polyacrylic acid mediated by carbodiimide (EDAC) chemistry and particle formation by ionic gelation with CaCl$_2$. Adopted from Müller et al. [5].

Evidence for their potential could already be provided by *in vivo* studies demonstrating a significantly prolonged mucosal residence time of such particles as illustrated in Fig. 5 [5]. Newer concepts regarding active systems are focusing on zeta potential changing polymers allowing the formulation of nanocarriers exhibiting a negative zeta potential when being applied to the mucus. As the mucus has a negative net charge, they can more easily move through the mucus than positively charged ones. When reaching the epithelium, however, they change their zeta potential to positive, for instance, due to the cleavage of strongly negatively charged phosphate groups from their surface by epithelial membrane bound phosphatases. The positive zeta potential on the epithelium is subsequently advantageous for effects such as endocytosis or in order to avoid a back diffusion out of the mucus.
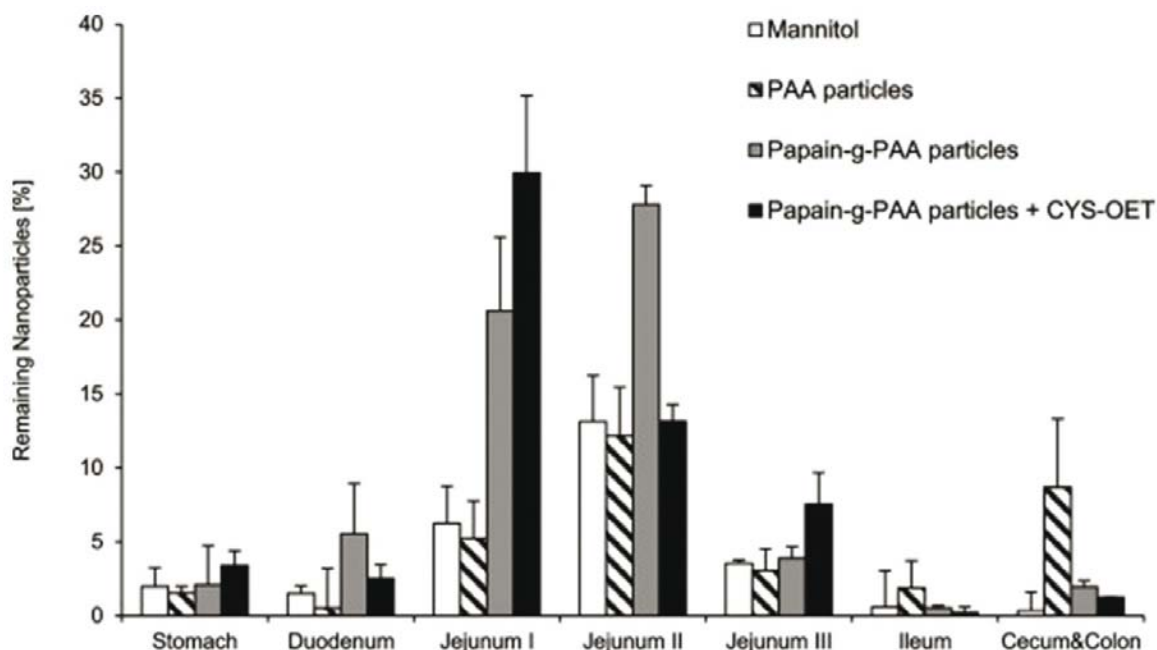

Figure 5. GI-residence time of polyacrylic acid (PAA) particles, papain-g-polyacrylic acid (papain-g-PAA) particles and papain-g-polyacrylic acid (papain-g-PAA) particles mixed with cysteine ethyl ester hydrochloride (papain-g-PAA + CYS-OET) in rats (means ±SD; n=6); adopted from Müller et al. [5]

4. Release controlling polymers

Apart from a simple release controlling via diffusion process or drug dissolution process in particular in case of micro- and nanocarrier systems drug release can be controlled by ionic interactions. The release of positively charged drugs can be strongly sustained out of negatively charged polymers such as polyacrylates and the release of negatively charged drugs can be sustained in the same way by utilizing positively charged polymers such as chitosans. Furthermore, a controlled release can be provided by making use of hydrophobic interactions of hydrophobic drugs and polymers exhibiting hydrophobic substructures [e.g. 6]. In case of non-viral gene delivery systems thiomers turned out to be a useful tool in order to guarantee a targeted release of DNA/RNA-based drugs in the cytosol.

Due to a crosslinking of the polymeric carrier matrix via disulphide bonds DNA/RNA-based drugs remain in the particles during the delivery process. Once these particles have reached their target i.e. the cytoplasma exhibiting a reducing environment, their payload is released due to reduction and subsequent cleavage of disulphide bonds within the carrier matrix [7]. In particular the sustained innovations of biomimetic nano-sized constructs with bioreducible poly(disulfide amine)s demonstrate a viable clinical tool for the treatment of cardiovascular disease, anemia, diabetes, and cancer [8].

5. Permeation enhancing polymers

Various polymeric excipients such as polyacrylates and chitosans are known for their permeation enhancing properties on mucosal membranes. In contrast to many low molecular mass permeation enhancers they are simply too big to be absorbed from the mucosa. As a consequence they remain at the absorption site providing a sustained permeation enhancing effect. A synchronized release of permeation enhancer and the drug is therefore not needed. Moreover, systemic toxic side effects of not biodegradable polymeric permeation enhancers can be excluded. Due to thiolation permeation enhancing properties of these polymers can be even further improved. Evidence for this effect could be provided by numerous *in vivo* studies in valid animal models for various alternative routes of administration. Langoth et al. for instance, could show a 1% bioavailability for pituitary adenylate cyclase-activating polypeptide (PACAP) in pigs, when being administered in a thiolated chitosan buccal patch formulation, whereas control formulations comprising the drug and unmodified chitosan did not lead to any systemic uptake at all [9].

6. Conclusions

Because of mucoadhesive, cohesive, mucus permeating, controlled release and pemeation enhancing properties functional polymeric excipients can strongly improve the therapeutic efficacy of numerous drugs by providing a prolonged drug residence time on the mucosa and an improved drug uptake. Moreover, some functional polymeric excipients are active pharmaceutical ingredients *per se*.

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PREDICTION OF THE IMPACT OF TRANSPORT MECHANISM ON INTESTINAL DRUG ABSORPTION

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Abstract

Qualitative biopharmaceutical classification schemes have been introduced for predicting the interplay between passive and transporter-mediated drug absorption. Still, important issues remain to be quantitatively resolved, including: under which conditions do transporters influence intestinal drug absorption? What is the impact of transporter inhibition under different combinations of doses, solubility, passive permeability and transporter kinetics? Attempts to answer such questions are typically at either end of a simplicity–sophistication continuum. Here, we present an intermediate approach, using a simple mechanistic model that takes advantage of data available in the early drug development.

Keywords

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1. Introduction

It is commonly assumed that passive diffusion is the major route of absorption for orally administered drugs, especially for highly dosed compounds with adequate intestinal solubility and for lipophilic ones for which passive intestinal permeability is generally high [1]. However, many modern drugs are administered in low doses, which in turn results in low intestinal concentrations. Moreover, the exploration of new target classes while preventing unwanted metabolism and off-target effects has pushed some development programs into a more hydrophilic chemical space, which can result in low passive diffusion rates. The combination of these two circumstances may under some circumstances reveal significant transporter effects in the intestine, including both enhanced absorption via uptake transporters and reduced absorption through efflux transporters. Models that give better overview of these issues are therefore warranted.

Interestingly, recent publications expressed very different points of view on the importance of active transport processes as compared to the passive route [1-2]. While some claim that "carrier-mediated" active uptake is the major pathway for drugs into cells and across cellular barriers [2], others, including the author of this chapter, support the view that passive diffusion and active transport processes operate in parallel during drug transport [3]. Misunderstanding of the relative importance of passive and active transport mechanisms can mislead drug researchers and result in missed opportunities to discover much needed drugs. We therefore set out to define the transporter landscape in the human intestine [4].

For this purpose we developed a transparent mechanistic model of intestinal drug permeation, suitable for use in drug discovery/lead optimization. As the foundation for our simulations of intestinal drug permeation, we collected >1,800 *in vitro* measurements of kinetic parameters for intestinal transporters, and introduced a method for inferring *in vivo* transporter capacities from intestinal perfusion data. Finally, we visualize our results in quantitative 'transporter-impact landscapes'.

2. Methods

The major site of absorption is the human small intestine, in particular the jejunum. In this segment, most transport proteins known to participate in the active uptake transport have their highest expression. Transporters effluxing drugs back into the intestinal lumen, such as P-glycoprotein, also have a high expression in the small intestine. In contrast to most uptake transporters, the efflux transporters are also expressed in the large intestine [4], albeit at lower levels, fig 1.
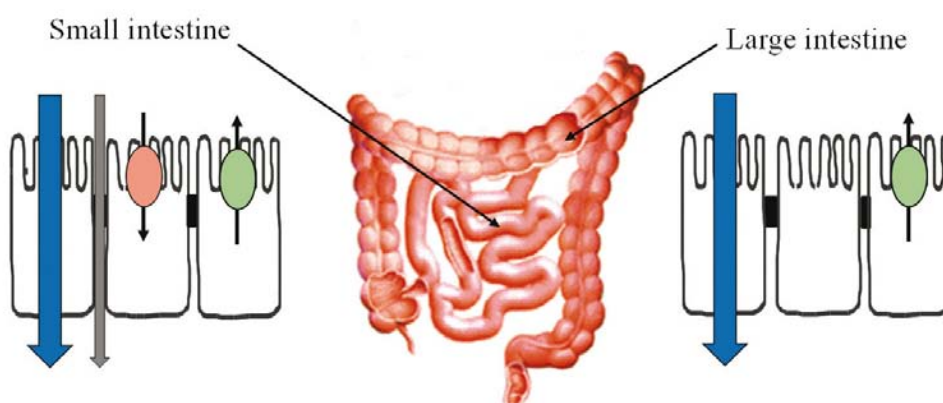
Figure 1: Dominating transport mechanisms for small-molecule drugs in different regions of the human intestine.

Left: in the small intestine, permeability is determined by passive diffusion across the epithelial cell layer (transcellular diffusion; blue arrow) or in the pores between cells (paracellular diffusion; gray arrow). In addition, transport proteins can mediate cellular drug uptake (orange) or efflux (green).

Right: in the large intestine, paracellular pores are smaller than in the small intestine, and transcellular diffusion therefore dominates the passive absorption. The expression of most apical uptake transporters is also significantly lower than in the small intestine, whereas efflux transporters are expressed (although at relatively low levels).

We therefore developed a mechanistic model of a small intestinal segment. The model consisted of three compartments (intestinal, intracellular and blood), with model parameters set to reflect the standard 'Loc-I-Gut' human intestinal perfusion experiment, a validated method for studies of human intestinal permeability in healthy subjects [5]. Compound transport across the apical membrane was incorporated as a combination of transporter-mediated flux (assumed to follow regular Michaelis-Menten kinetics) and bidirectional passive Fick's diffusion; flux across the basolateral membrane was assumed to be purely passive. Mixing in the three compartments was assumed to be instantaneous. The model was developed in R version 2.15 (http://www.r-project.org), using the *deSolve* package for differential equation solving. The permeation process was simulated over 270 min, approximating the average drug residence time in the human jejunum. All simulations included two models run in parallel: one model that incorporated both transporter-mediated and passive diffusive compound flux, and one that only incorporated the passive component.

Intestinal perfusion experiments to assess the permeability of transport substrates were collected from the literature. All selected reports were composed of one experiment each in the presence and absence of intestinal active transport, where absence was induced either through the use of chemical inhibition or knock-out animal models. We focused primarily on intestinal transporters indicated in the recent International Transporter Consortium whitepapers as the ones particularly relevant for drug development. Perfusions were included only when complicating factors such as intestinal degradation or metabolism were controlled for.

First, the mechanistic model was used to reproduce the reported passively absorbed fractions. This was done by incorporating the permeability coefficient determined in animals for which transporter function had been knocked down. Second, *in vivo* V_{max} values were estimated by adding the respective transporter components to the simulation, using K_m values from our literature database. The V_{max} was optimized to reproduce the experimental fraction absorbed in the presence of transporter-mediated flux. Parameter optimizations were performed separately for each compound, as well as collectively for all substrates of a given transporter.

3. Results

To perform a quantitative assessment of the impact of transporters, we collected more than 1,800 data points from public domain databases and from manually curated literature in the drug transport field. We excluded duplicate entries, drugs that are not administered orally, and interactions with transporters that are not expressed in the intestinal epithelium. Reported K_m values displayed a large variation, depending on the transporter and substrate. However, in many cases only single outliers deviate from quite tight K_m distributions. For such substrates, it was possible to make good approximations of the K_m value, in particular for uptake transporters. In contrast, we observed a pronounced variability in *in vitro* V_{max} estimates. Further, we wanted to predict passive and active drug transport in man, but no such clinical data were available. To circumvent these two major hurdles, we used rodent *in situ* intestinal perfusion data to validate our model. We first confirmed that the model accurately reproduced the passive diffusion component by using perfusion data from animals where the transporter of interest had been genetically or chemically knocked out (i.e., with transepithelial permeability resulting from passive diffusion alone). Our model reproduced the experimental fraction absorbed with an r^2 of 0.997. The same substrates were then modeled under non-inhibited conditions (i.e., permeability influenced by both passive diffusion and transporter-mediated flux). The passive component in the model was therefore fixed to be equal to that in the strictly passive scenario, and K_m values from our literature review were used to describe substrate affinities for the respective transporters. V_{max} was then optimized to reproduce the fraction absorbed under non-inhibited experimental conditions. When simultaneously fitting all data points for a specific transporter, the mechanistic model recapitulated the experimental fraction absorbed with an r^2 of 0.82 for the whole dataset. Thus, we conclude that our model was able to reproduce both the passive and active components of the *in vivo* permeation process. The ranges of *in vivo* V_{max} estimates confirmed those observed *in vitro*, and we therefore used these parameter ranges to further explore the impact of transporters on oral drug absorption.

To illustrate the application of our model in drug discovery, we selected a series of novel HIV-1 protease inhibitors (PIs) with low-nanomolar potency as a reference case [6]. To assess the potential for significant transporter effects we determined transporter-impact landscapes for each compound, across the ranges of K_m and V_{max} observed from the literature and modeling exercises outlined above, fig 2A.
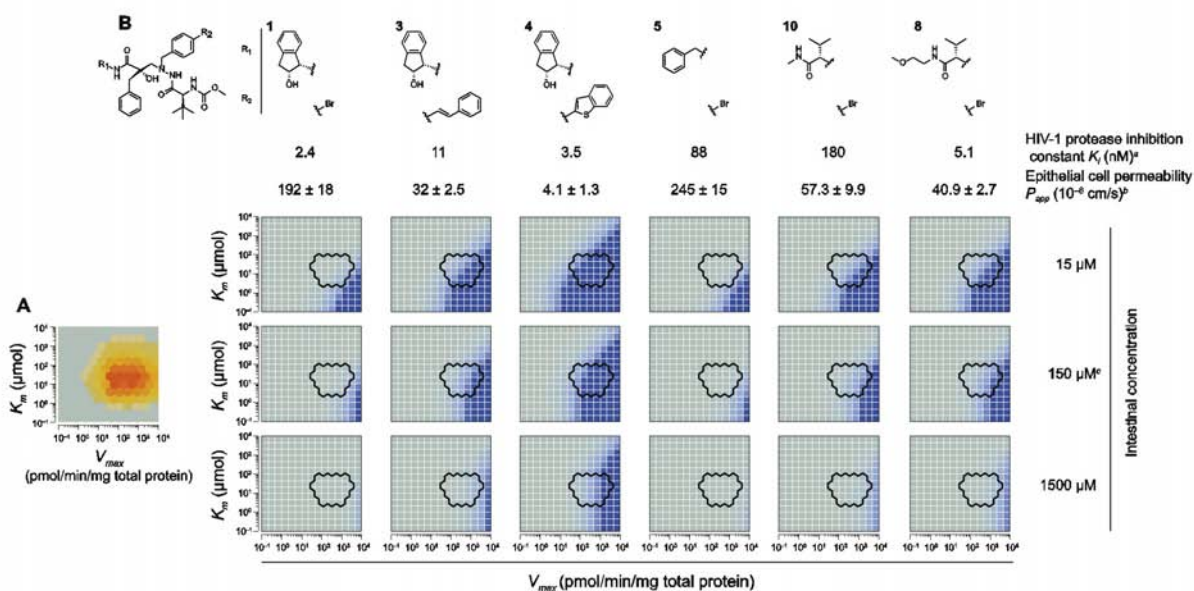
Figure 2. Prediction of transporter impact on the oral absorption of novel HIV-1 protease inhibitors. (A) The distributions of in vitro K_m and V_{max} values for intestinal efflux transporters were extracted from literature. V_{max} values obtained from the modelling are all within this space. Darker colors indicate a higher likelihood of a certain K_m–V_{max} combination, calculated based on the distributions of the individual parameters. (B) Predicted transporter impact on the absorption of HIV-1 protease inhibitors with varying passive membrane permeability. Dose-dependent transporter effects were explored by varying the initial intestinal drug concentration between 15 and 1,500 µM.

Simulations were performed at an intestinal concentration of 150 µM, based on the solubility of the structurally related PI indinavir in human fed-state intestinal fluids, fig 2B. Dose-dependent transporter effects were assessed by repeating the simulations using ten-fold lower and higher intestinal drug concentrations. By comparing the K_m–V_{max} distribution with each PI's transporter-impact landscape, we could judge the likelihood of observing a significant transporter effect, fig 2B. For instance, our simulations show that if the intestinal concentration is increased 10-fold or to 1,500 µM – corresponding to a dose of approximately 250 mg – efflux transporters will be saturated and will not affect the absorption of any of the PIs in this investigation.

4. Conclusions

In conclusion, we have used a mechanistic model to derive 'transporter-impact landscapes' that visualize the influence of active drug transport on the intestinal drug permeation process. We have demonstrated the strength of the modeling approach in exploring the impact of transporters under different scenarios that could be expected *in vivo*. These include solubility enhancement from specialized formulations or concomitant food intake, inhibition of transporters by co-administered drugs, and variable transporter expression. Our model can hopefully restrict extensive profiling of transporter interactions to only those compounds susceptible to significant transporter effects.

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