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Protein Delivery: Is Iontophoresis a Feasible Option?

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ABSTRACT

Biotechnology is now contributing almost one third of new drugs, and the trend is expected to be similar based on the number of molecules in different clinical trials. Most of these protein based pharmaceuticals are delivered by injections, which are painful and carries a risk of infection. Alternative delivery routes/techniques are being investigated and transdermal iontophoresis is one of them. This article is an attempt to evaluate recent development and the possibilities with it.

INTRODUCTION

Rapid progress in biotechnology has led to the increased use of protein therapeutics. Given their physicochemical properties and stability needs, they are usually delivered by subcutaneous or intramuscular injection and sometimes intravenously–although not ideal, they can be considered as the "traditional routes" for biopharmaceutical delivery. In addition to efficacy, which is obviously paramount, several other factors can influence the choice of delivery method and the development of a pharmaceutical product – these include product stability, systemic toxicity or other safety issues, requirement for local or systemic delivery, patient convenience/ compliance and market competition. In an effort to move away from parenteral administration, several new methods have been investigated int he last thirty years for the delivery of biopharmaceuticals [1](Table 1). However,"non-traditional"routes for protein administration encounter various challenges – biological membranes are not always designed to facilitate molecular transport, the presence of proteolytic enzymes and the potential risk of eliciting an immunological response are all factors that must be considered (Table 2).

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Table 1. Delivery options for protein therapeutics based on different target site and dose requirements [1].

DOSE	LOCAL	SYSTEMIC
High (> 2 mg/kg)		
Bolus	IV/IM/SC	IV/IM/SC (devices)
Sustained	Depots, pumps	Depots, pumps
Pulsatile	IM/SC	IM/SC (devices)
Medium (0.05-2 mg/kg)		
Bolus	IV/IM/SC, pulmonary	IV/IM/SC, pulmonary
Sustained	Depots, pumps	Depots, pumps
Pulsatile	IV/IM/SC, pulmonary	IM/SC, pulmonary
Low (< 0.05 mg/kg)		
Bolus	IV/IM/SC, pulmonary, intranasal, ocular	IV/IM/SC, pulmonary, oral, transdermal
Sustained	Depots, pumps, transdermal	Depots, pumps, transdermal
Pulsatile	IV/IM/SC, pulmonary, pumps	IV/IM/SC, pulmonary, pumps

Transdermal administration is a potential alternative for the delivery of potent therapeutic molecules. The skin is the largest organ in the body with a surface area of 1.5 to 2 m^2 and is obviously easily accessible; however,its outermost layer the stratum corneum, which is typically only 10-20 µm thick,is an extremely formidable barrier to molecular transport [2]. It is composed of 10 to 20 layers of dead cells (corneocytes) filled with keratin filaments (70%) and fat (20%), arranged within an extracellular

Table 2. Challenges to protein delivery encountered with "non-traditional" administration routes [1].	
ROUTE OF DELIVERY	**CHALLENGES**
Oral	- Epithelial cell barrier (tight junctions) - Proteases - Extreme pH conditions
Pulmonary	- Epithelial cell barrier - Proteases - Alveolar macrophages - Rapid rate of absorption - Dose limitations
Transdermal	- Stratum corneum barrier - Proteases - Macrophages - Limited rate of administration - Limited application area

lipid matrix (ceramides (40%), free fatty acids (20%) and cholesterol (~25%)) in a so-called"brick and mortar"assembly [3, 4]. Passive delivery of peptides and proteins is rendered impossibleby this thin but highly tortuous membrane. Thus, in recent years several methods have been developed in order to breach the defensive barrier of the stratum corneum [5-10] (Table 3).

Table 3. Strategies to overcome the skin barrier function [5-10].				
INTACT SKIN		**MINIMALLY INVASIVE**		
Formulation Optimization	Enhanced Passive diffusion	Active transport	Mechanical	Energy driven
Drug chemical modification	Electroporation	Iontophoresis	Microneedles	Laser
Encapsulation technologies	Sonophoresis		Velocity based technologies	Radiofrequency
Supersaturation	Photomechanical waves		Suction blister	Thermal ablation
Chemical penetration enhancers	Heat assisted drug delivery		Microscissioning	

Iontophoresis is a promising technique for the delivery of hydrosoluble charged molecules [10, 11]. It employs a low intensity electrical current to facilitate and control molecular transport through the skin. An iontophoretic device consists of a patch containing two electrodes (an anode and a cathode) which are connected to a power source (Figure 1). For small molecules, electrotransport is usually dominated by electromigration, but is also affected by electroosmosis; the relative contributions depend on the physicochemical properties of the molecule and the pH. Electromigration is a result of the charge on the molecule; electroosmosis describes the convective solvent flow generated by virtue of applying an electric field across the negatively charged skin [12-16].

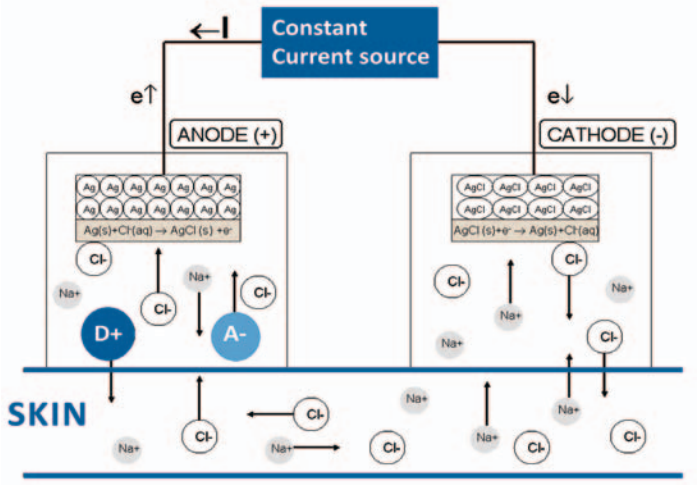

Figure1: Schematic representation of iontophoresis [10].

Besides the inherent advantages of the transdermal route, iontophoresis offers the possibility of additional control: modulation of the applied current intensity and profile enables drug administration to be adapted to the needs of each patient or to each phase of a treatment. This feature also allows complex input kinetics, e.g., pulsatile administration (e.g., for insulin, LHRH and other peptides) [17-22]), that may simulate the natural secretion patterns of endogenous molecules.It may also be important for active ingredients that produce different pharmacological effects depending on the administration profile and hence provide a more effective treatment. Until recently, it was thought that proteins were too large to be delivered by transdermal iontophoresis - this was shown not to be the case with the successful delivery of cytochrome c, a small 12.4 kDa protein [23]. Furthermore recent studies have also demonstrated that it was possible to deliver intact biologically active proteins non-invasively across the skin by using transdermal iontophoresis [24-26]. These studies have also highlighted the role of protein physicochemical properties on electrotransport.

Transdermal delivery of several biological molecules through iontophoresis offers several advantages. The classical limitations associated with parenteral and oral delivery can be eliminated using iontophoretic delivery and hence can be better targeted with little toxicity effects. The method offers simplified skin absorption system through which patient can control the drug administration and hence results in better patient compliance. However, local skin delivery through current application can result in local skin damage, pain, hypersensitivity to drugs, localized erythema and cutaneous reactions may limit the application of this technique to several patients.

Pros and Cons of using Iontophoresis for protein delivery

Advantages of Iontophoresis

- Bypasses hepatic first pass effect.
- Avoid risks and limitations associated with parenteral and oral absorption.
- Simplified delivery and hence achieves better patient compliance.
- Can prevent the chance of under dosing or over dosing.
- Permit the rapid termination of administration.
- Enzymes with short biological half life can be easily delivered via iontophoresis.
- Eliminates the toxicity problems associated with the chemical enhancers present in pharmaceutical formulations.

Limitations of Iontophoresis

- Transdermal delivery may cause potential local, irritant cutaneous reactions.
- Variability in drug delivery due to person to person variation in skin structure.
- Protection of the skin from electric shock is needed.
- Pain and burns may occur due to local changes in current density and electrolyte characteristics.
- Current induced damage may cause local vasodilatation which in turn activates nociceptive fibres terminating in the epidermis.
- Expensive technique.
- Can't be applied to patients with hyper responsiveness to drug administered and damaged or broken skin.

Several therapeutic peptide/protein/enzyme molecules have been tried in various in vitro, in vivo studies and have been proved to have better absorption characteristics, enhanced diffusion through skin and even distribution under dermis and epidermis layers of skin (Table 4). Further, the functional activity of proteins was not compromised as suggested by SDS-PAGE and MALDI-TOF spectral analysis. In view of the above reports, iontophoresis offers a much warranted therapeutic drug delivery system with greater efficiency and less adverse effect profile and hence can replace the existing model drug delivery systems in the coming future. Further combining iontophoretic delivery with novel strategies such as microneedles can able to overcome the difficulties associated with transdermal delivery of biological macromolecules and can be applied to achieve better therapeutic concentrations by transdermal delivery.

CONCLUSION

Iontophoresis offers a viable alternative to the uprising need for protein delivery. This becomes even more interesting when considering iontophoresis for dermal application of proteins, where delivery protein is particularly difficult. Although iontophoresis holds promise for future but the candidate proteins should be carefully chosen. Further understanding in this direction will be very helpful. There is a need of developing rapid and reliable screening method for selecting ideal protein candidate. With these developments a true iontophoretic device for protein/peptide could be a feasible possibility in future.

Table 4. List of peptides and enzymes being tried for iontophoretic delivery

S.No.	Therapeutic molecule	Mode of drug delivery	Method of Characterization	Reference
1.	Ribonuclease A	Transdermal iontophoretic delivery	Methylene blue assay and laser scanning confocal microscopy of rhodamine tagged ribonuclease A for skin permeation and distribution. PAGE and MALDI-TOF spectral analysis for functional activity.	[24]
2.	Ribonuclease T1	Cathodal iontophoresis	Activity was confirmed by MALDI-TOF spectral analysis. Confocal scanning laser microscopy imaging of molecule in dermis and epidermis.	[25]
3.	Human basic fibroblast growth factor (hbFGF)	Transdermal iontophoresis	Skin permeation was quantified by ELISA. Functional activity was confirmed by SDS-PAGE analysis. Confocal scanning laser microscopy proved even distribution throughout dermis and epidermis.	[26]
4.	Porcine Insulin	Electrically enhanced iontophoretic delivery	Application of depilatory lotion along with iontophoretic supply enhances transdermal delivery of insulin as evident by reduced blood glucose levels.	[27]
5.	FITC labelled phosphorothioate oligonucleotides (FITC-PS)	Topical delivery by iontophoresis and electroporation	Fluorescent microscopy and laser scanning confocal microscopy confirmed the delivery of oligonucleotides across skin.	[28]

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