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Passive Delivery of Protein Drug Through Transdermal Delivery

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Abstract:

Transdermal delivery of peptides and proteins avoids the disadvantages associated with the invasive parenteral route of administration and other alternative routes such as the pulmonary and nasal routes. This is a time where modern therapeutics is slowly shifting from use of small molecule drug towards macromolecular therapeutic agents, such as protein, peptides, nucleotide in origin & skin therapeutic to evolve according to cater the delivery of agents. Since proteins have a large size and are hydrophilic in nature, they cannot permeate passively across the skin due to the stratum corneum which allows the transport of only small lipophilic drug molecules. Enhancement techniques (Active system) such as chemical enhancers, iontophoresis, microneedles, electroporation, sonophoresis, thermal ablation, laser ablation, radiofrequency ablation and non-invasive jet injectors aid in the delivery of proteins by overcoming the skin barrier in different ways. In this review, we will discuss limited success due to their complex working condition & involved certain irreversible skin damage in their way. review therefore explores the delivery strategies for transport ofmainly peptide & protein drugs that do not involve any injuries (non-invasive) to the skin termed as passive delivery techniques.

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1. Introduction:

Transdermal drug delivery renders a beneficial mode of drug administration over oral and parenteral application. They have tendency to gain steady state drug levels by-pass the hepatic pass metabolism. It also increases patient compliance & reduce git adverse effect.that the skin was identified as a route for systemic drug delivery. Therapeutic agent currently marked as transdermal drug products share the common physicochemical properties, In the past last 30 years after the first launch of recombinant human insulin, different biopharmaceutical drugs like peptides, enzyme & monoclonal antibodies, recombinant protein drug in the modern pharmaceutical industry. Specificity attributed by the complex structural orientation of proteins and potency has been the cornerstone of protein-based therapeutics as compared to small molecule drugs. complexity of their nature lends the proteins difficult for delivery.The general route of administration for protein pharmaceuticals to date is parenteral (intravenous/subcutaneous).Majority of the proteins possess short half-lives this route posses'disadvantages of the repeated drug administration & poor patience compliance.skin could be the potential alternative administration of protein drugs across the skin as it bypasses the first pass metabolism, offers prolonged release of drug and exhibits minimal proteolytic activity compared to anotherroute. The protein drugs are large in size, have higher MW (average weight of human protein being 53kDa) and hydrophilic in nature.These properties are antagonistic to Lipinski rules proposed for efficient transdermal delivery, thus suggesting negligible passive diffusion of proteins across the skin.Various transdermal technologies using physical and chemical methods have been introduced in last decade to augment delivery of proteins through the skin such as use of chemical enhancers, iontophoresis, micro-needles, sonophoresis, laser ablation, thermal ablation, radiofrequency ablation, jet injection microwaves and electroporation.Transdermal therapy is widely known for its patient compliancebut when coupled with these complex techniques involving sophistication in design and delivery methods, loses its essence of being the most convenient route. Both device & drug play safety criteria performance by FDA.

2. Basic idea on delivery of Protein Drug:

Proteins are complex molecules with large molecular weights, acid–base side chains and are polar in nature. Protein drugs are large in size with high molecular weight including hydrophilic nature. Some scientist suggesting that protein drugs have negligible passive diffusion across the skin. For transdermal delivery of protein chemical enhancers,

iontophoresis, microneedles laser ablation radiofrequency ablation electroporation is used. Low frequency ultrasound sonophoresis enhances skin permeability for transport large molecule in hydrophilic proteins. Microoperation is a technology which disrupts stratum corneum in temporary & reversible manner. Microwaves which is used high intensity electromagnetic radiation for some period of time which may cause thermal & non thermal interaction with skin. [(1)]. Protein-based drugs are now very important for the treatment of several conditions such as diabetes, osteoporosis, cancer, and so on; made possible due to the developments in recombinant DNA technology which have allowed commercially viable production of pure recombinant proteins. Stability issues, along with their complex nature, make proteins difficult drug candidates for delivery [(2)].

3. Stratum Corneum & Its Lipid architecture:

The skin, the most accessible organ of the body with a large surface area, offers an appealing alternative for delivering proteins into the systemic circulation. However, stratum corneum, the outermost barrier of skin which is made up of dead keratinocytes, acts as a rate limiting barrier. This lipophilic layer allows only small, potent and moderately lipophilic molecules to partition across it passively, into the deeper layers of the skin. It consists of flat, non-nucleated keratin enriched dead cells, corneocytes surrounded by intercellular non polar lipid domains. [(3)].This limits the delivery of proteins which have a large molecular weight and are hydrophilic in nature. So, the key for delivering proteins is to overcome this barrier after which the proteins can diffuse past the viable epidermis which is comparatively more hydrophilic in nature and into the systemic circulation via the capillaries present in the dermis. The intercellular lipid matrix forms a continuous pathway from the skin surface to the skin tissue of all chemicals,

Stratum corneum lipids consist of 1. Free Fatty acid (FFAs) 2. Ceramides (CERs) & 3. Cholesterol (CHOL) in equal ratio. Free Fatty acid consists of 36 carbon atoms chain. Within these 36-carbon atoms most important ARE C18/C24/C26.Ceramides consists of two carbon chain 1. Fatty acid (acyl) amides chain 2. Sphingoid chain. The arrangement of lipid matrix in SC can be understood by lateral organization (i.e. the molecular packing of lipids in lamellar plane generally parallel to the SC surface) and lamellar organization (i.e. the symmetry and the repeated distance perpendicular to the SC surface of lipids). Lamellar organization of SC consists of stacks of lamellae of so-called broad-narrow-broad arrangement having regular

repetitive units with a repeated distance of 13nm particularly referred to as long periodicity phase (LPP). [4]

3.1 Role of Lipid in Permeability Barrier:

LPP examined in all species until now, has suggested that the presence of this phase plays an important role in skin barrier function. In the SC of patients suffering from several diseases characterized by very high skin permeability (atopic dermatitis, lamellar ichthyosis, psoriasis, Netherton syndrome and Chanarin–Dorfman disease), the periodicity of the LPP is decreased and its general appearance is significantly altered[5] During investigations on the penetration of ethyl Para amino benzoic acid and benzoic acid across model SC membranes the fluxes of these chemicals were found to elevate in the absence of LPP which supports the positive role of LPP in permeability across the skin. The lateral lipid organization within the lamellae strongly influences the SC permeability for water. The flux of water across human skin closely correlates with the type of solid phases present in the SC: the higher the OR content, the lower the flux of water [6] [7]. The permeability through the skin therefore is the interplay of arrangement of lipids in lamellar and lateral layer. Absence of LPP favours high permeability whereas prevalence of OR arrangement in lateral layer reduces the permeation. [8]

4. Carrier supportive adjuvants (CSAs):

Carrier supportive adjuvants are auxiliary chemical moieties which are used association with Liposome, microemulsion during transdermal delivery. They are classified as Chemical penetration enhancers & Peptide chain mediated delivery.

4.1 Chemical penetration enhancers:

- It is most important auxiliary chemical moieties which are used along with main penetrate moieties
- It helps to alter the function of main chemical moieties by changing permeability capacity of skin
- Association of Chemical penetration enhancers with lipids on SC create a microenvironment under epidermis for freely diffuse the drugs

  5% terpenes are used combination with ethanol for increase passive diffusion of LHRH through porcine skin [9]. LHRH penetration was increased by 3.5 times over passive permeability using oleic acid, propylene glycol as penetration enhancer. The highest

permeation across the skin was observed with surfactants, followed by the terpene and solvents CPEs. The permeation enhancement was found to be more for Tween 80 and Cremophor RH40 with 6- and 3.7-fold enhancement compared with the IFNa solution without any CPE, which corresponds to 0.82% and 0.52% of IFNa dose, respectively [(10).] CPEswere evidenced by the fact that their permeability coefficient was 2.18 times greater by the use of ethanol. CPEs are efficacious in breaching the barrier property of skin to improve the delivery of drug molecules in therapeutic amount but their safety is a potential concern [(11).]

4.2 Peptide chain mediated delivery:

Some peptides show good penetration enhancing properties or good carrier by transdermal route. Advantages posses like easy usage, diversity & capability of targeting specified cell within the skin.[(12).] They are also interpreting barrier properties & improve delivery of therapeutic agent and cargoes across the skin. At present three pathways exist in transport of drug across the skin like intercellular, intracellular lipid pathway, Trans appendageal pathway. The intercellular lipid pathway has been widely used for delivery of cargoes in free form or conjugated form.

4.2.1 Cell Penetrating Peptide:

CPPs are amphiphilic peptides consisting up to 30amino acids which is produced from natural or unnatural protein sequence from dipper tissue. They can be internally transported by cells through energy independent mechanism with or without receptor. All CPPs are positive charge at physiological pH, maximum positive charge comes from amino acid. They make electrostatic interaction with negatively charged glycoprotein at cell surface. They have unique features like structural modification for enhancing delivery of macromolecular agents. CPPs along with their cargoes have low level toxicity.

Types of Cell penetrating Peptide:

CPPs are divided into different classes based on the criteria for division. On the basisof association with cargoes, they are divided into two types:the one requiring chemical linkage with cargo other beingthe one forming stable, non-covalent complexes. On structural basis they are divided into either polycationic or amphipathic classes.[(13)]

Mechanism of CPP across the skin:

The entry ofCPP or its cargo complex into the cell is the widely studied are compared tp transport of CPP across the skin but universal mechanism of transport across the skin applied all classes of CPP.The transport could be affected byvariety of factors, including the nature of the cargo (such assize and charge), the properties of CPP (such as moleculelength, charge delocalization, hydrophobicity and other physicochemical parameters), the cell line and the CPP concentration.Non-endocytic or energy-independent pathway and theendocytic pathway among others are the two importantpathways proposed for CPP penetration inside the cells. Mechanism involved (i) membrane interaction, (ii) membranepermeation and (iii) release of CPP into the cytosol after theentry procedure.[14]The outermost layer of the skin is composed of non-viable cells; the possibility of endocytosis canbe easily ruled out despite the fact these dead cells form ametabolically active environment. Interactions between lipidsand CPPs may also play important role in their transportacross the SC.CPPs enable to transport their cargoes acrossthe skin by destabilizing SC and increasing permeability.Micropinocytosis is anotherpossible transport mechanism, by which the CPPs passthrough the mammalian cells. It has been shown that themicropinocytosis and actin reorganization are both involvedin the cellular entry and transdermal delivery of CPP/cargo complexes.[15][16]

4.2.2Antimicrobial Peptide Magnin:

Magainin is a 23-amino-acid long antimicrobial protein isolated from skin having a net (+Ve) charge that binds with (-Ve) charged phospholipids membrane with the aid of electrostatic interaction. Its ability to form pores in thebacterial cell membranes and permeabilize the lipid bilayers,it is also called as pore-forming peptide.The ability of magainin to interact with the lipid membranes, its potential utility as a skin. Magainins alonewere incapable of enhancing the transport across the skin.They require the use of surfactants to accompany them for optimal transport. .Magainins with surfactant ethanol increased skin permeability by 47 fold [17]. They described the conditions to improve skin permeability byoptimizing the pre-treatment time and concentration ofmagainins exposure. They found that skin permeability increasedwith increasing pre-treatment time. Skin permeability also increased with increase magainin concentration up to 1 Mm [18].The positive charge on magainins wasreduced and ultimately reverted to negative charge whichcaused repulsive interaction with similarly charge fluoresceininhibiting the transport.The transport of positively chargedgranisetron increased as the positive charge ofmagaininswasdecreased by enhancing pH**.**The charge on magainins

wasreduced by enhancing pH which aided in the permeation ofpositively charged granisetron but reducing pH created thepositive charge on magainins, thus reducing the transportowing to the repulsive forces acting between the drug[19].

5. Nano Carrier:

Nanocarrier is innovative design to transform the drug across the deepest layer of the skin. Nanocarrier has greater penetration than CPEs.

5.1 **Liposome:**

Liposomes were the original vesicular nanocarriers for improved topical effect compared to conventional topical agents like cream, gel, ointment etc. It helps to increase fast absorption of progesterone, corticosterone in rats skin compared to antimicrobial effect on rabbit skin (Epidermis & Dermis). [20]. After completion of few researches it has shown that Liposome is good drug carrier than drug penetration enhancers in deep layer of skin. [21].

5.2 **Ultra deformable Liposome or Transformers:**

Observing some localized problem & delivery the drug into deeper layer of skin new innovative elastic or deformable nature liposome was prepared. The membrane of transformers is prepared with Phospholipids & edge activator which is single chain surfactant molecule. Edge activator destabilize the lipid bilayer & increasing deformation by lowering interfacial tension [22].As per study diclofenac entrapped within the transformers & it was resulted 10 times more in concentration across the skin & exhibiting its effect for prolong period of time [23]. By using transformers 8-fold increase peak plasma concentration. With special reference to protein drugs, transformers were found to be equally effective in delivering them through the skin. In a study done using insulin loaded transferosomes, it was reported to show bio efficiency of at least 50% of the SC dose both in mice and humans and was able to achieve hypoglycaemia of minimum 30% of normal blood glucose level and approachable up to 50% [24]. Some good result from protein drug from low molecular weight heparin (cationic) which is called flexosome can penetrate into deeper layer of skin than anionic low molecular weight heparin solution, it is used for potential treatment of thrombosis, subcutaneous wound infection, &burns [25]. Transformer are sufficiently capable of biomolecular drug across the skin in particular therapeutic amount. They were successful in inducing antigen-specific antibodies equivalent to subcutaneously injected vesicle suspensions which was also corroborated by the

appreciable antibody titer formation after application of transferosome tetanus toxoid formulations [26].

Transferosomes by virtue of its xerophobic nature (Tendency to avoid dry surroundings enters into deeper layer skin having higher moisture content than surface layer). The elasticity of membrane assists during the transport to breach narrow gap on the surface of skin and get transported intact. Enhanced drug transfer by transferosomes can be attributed to its distribution on the skin surface [27].

6. **Micro-Emulsion:**

Water in oil (W/O) microemulsions are thermodynamically stabilized dispersions composed of small (200nm) water droplets dispersed within a continuous oil layer stabilized by the incorporation of a high concentration of surfactant/emulsifying molecules. Lipophilic surrounding in the external phase resembles the environment found in upper layer of skin, ideal candidate application on skin surface. the ease of administration of W/O micro-emulsions to the skin without the need for a delivery device makes it ideal for passive delivery of water-soluble molecules (proteins and peptides in this case) across the skin [28]. A W/O microemulsion and found rapid penetration of the molecule into the skin immediately below the site of application,and rapid lateral movement to the distal area of the skin. The distribution studies revealed that up to 4h there was very slow drainage of protein from dermal region into the circulation other tissues of the body after which penetration into the circulation increased gradually giving a clear picture of penetration ability of microemulsions [29]. Microemulsion successfully delivered about 6 % in applied dose whereas cream transported 2% of drug at a time of 300min.Microemulsion is able to deliver the drug in dipper layer of skin compare to cream. Effect of subcutaneous & topical administration of protein drug in the form of W/O microemulsion anabolic effect on percentage of muscle mass is high. The transdermal delivery of microemulsion can be two factors a. High drug loading capacity & b. Penetration enhancing effect of constituent. Higher constituent gradient &highertransdermal flux can be observed in affinity of flowing in subcutaneous tissue & time to loading free of drug. [30].

7. **Nano-Particles:**

The purpose of nanoparticle in the range of 50 – 500 nm depending on the route of administration. The method by which a drug is delivered can have a significant effect on its efficacy. Transdermal drug delivery systems (TDDS) or patches are controlled-release devices that contain the drug either for localized treatment of tissues underlying the skin or for

systemic therapy after topical application to the skin surface. It is generally observed that the capability of nanoparticles. TDDS are available for a number of protein drugs, although the formulation matrices of these delivery systems differ [(31)]. They differ from conventional topical formulations in the following ways:

1. they have an impermeable occlusive backing film that prevents intensive water loss from the skin beneath the patch;
2. the formulation matrix of the patch maintains the drug concentration gradient within the device after application so that drug delivery to the interface between the patch and the skin is sustained; and
3. TDDS are kept in place on the skin surface by an adhesive layer ensuring drug contact with the skin and continued drug delivery

   Metal-based nanoparticles of less than 10nm in size, including quantum nanoparticles are able to move across the skin [(32)]. Intracellular macromolecular matrix within the stratum corneum abounds in keratin, which does not contribute directly to the skin diffusive barrier but supports mechanical stability and thus intactness of the stratum corneum. Transcellular diffusion is practically unimportant for transdermal drug transport. The dermato -pharmacokinetics approach for nanoparticles suggested by the Food and Drug Administration (FDA) proposes to evaluate the level of a topically applied drug in the stratum corneum during its uptake and clearance so as to calculate classic pharmacokinetic parameters When applied to diseased skin, topical drug products induce one or more therapeutic responses, where onset, duration, and magnitude depend on the relative efficiency of three sequential processes, namely:

   - The release of the drug from the dosage form
   - Penetration of the drug through the skin barrier, and
   - Generation of the desired pharmacological effect.

Nanoparticles due to their distinct biological properties owing to its non-deformable an minuscule nature as compared to lipid or polymeric counter parts aid in transdermal transport. Metallic nanoparticle perturbs the lipid phase on the membrane inducing gelled areas that enhances the lipid fluidity [(33)]. The lipid fluidizing function of nanoparticles thus alters skin permeability and which in turn facilitates the passive transport to the deepest layers of the skin SC, the stratum granulosum. [(34)]

8. Lecithin Organogels:

It is a clear thermodynamically stable including thermo reversible gel consisting of phospholipids in appropriate solvents. They are mainly soluble in polar solvents like water. Lecithin organogels is jelly like phases consisting of three-dimensional polymeric chain which immobilizes the continuous or macroscopic organic phase turning liquid into gel [(35).]

It has role as carrier for both hydrophilic & hydrophobic compound & its capability to transfer across the skin [(36)]. It is imperative for the carrier system to retain structural & functional properties of protein & peptides delivery in deeper layer of tissue. Example Ascorbic acid or hydrophilic amino acid are solubilizing micelle of the lecithin gel without their deformation. Immobilization of enzyme lipase in in the lecithin/ Cyclohexane water organogels for catalytic activity of against hydrolysis of triglyceride. LOs have arguably been efficient delivery system for topical and transdermal application of low MW drugs.Their versatility for macromolecular drugs including protein and peptide medications shows a promising avenue ahead with existing evidences.The enhanced flux of molecule is attributed to lecithin component of Los [(37)].

9. **Prodrug:**

Prodrug involves the transient or reversible modification of the physicochemical properties of compounds through chemical derivatization to augment the solubility or bioavailability profile or to provide more stability compared to the parent drug while preserving the pharmacological properties of the parent drug. There are reports suggesting the derivatization of the bioactive peptides to produce prodrugs that possess enhanced physicochemical properties compared to original compounds with regard to delivery and metabolic stability [(38).]For success of the prodrug is the reliable conversion of prodrug to the parent drug through either enzymatic or non-enzymatic catalysed reaction, once the barrier to delivery has been circumvented.To preserve the physical and chemical integrity remains additional responsibility during prodrug fabrication for proteins. Prodrug has been synthesized of proteins & study show chemical reversibility of plasma after application.The pyroglutamyl peptide and its derivatives for the prodrug approach to protect a peptide against specific enzymatic cleavage by pyro glutamyl aminopeptidase and 4-imiadazolidinones derivatives formed by condensing compounds containing an alpha amino amide moiety, abundant in most peptide groups with aldehydes or ketones, are few of them [(39).]There are Proteins also

cross biological membranes by carrier-mediated processes.Conjugation of proteins or peptides by such carriers which selectively transport them across the biological membranes might be useful in delivery across the skin. The toxic protein, ricin, is transported across the cell membrane via binding to the ricin B chain found on the surface followed by internalization. Upon entering the cell, the active component, the ricin A chain, is liberated where it exerts its toxicological effects.Therefore, ricin behaves essentially as a prodrug of ricin A chain. The use of monoclonal antibody–ricin A chain conjugates for selective delivery of this peptide toxin has also been studied

10. Skin Penetration modifier:

Numerous researches analysing transcutol as a skin penetration modifier depicted that DEGEE increased the solubility ofpermeant in the skinwithout remarkably affecting its diffusivity of skin. The presence of DEGEE resulted in enhancedskin retention although the permeability and therefore thesystemic uptake were significantly decreased.Enhancement rangesfrom minimal to dramatic, use of these solvents universallyenhances skin penetration.[40]

- **Choice of Passive delivery of proteins by transdermal route:**

Active delivery helps the transport process by providing additional force for transportation or generating permanent sites through mechanical disruption of skin for smooth passage ofdrugs. They reach the systemic circulation inshorter period of time compared to passive method of delivery.These technologies available can be manoeuvred tomeet the patient specific demands. They require sophistication in terms of design, functioning and controls. Passive approaches likeCPEs, nanocarriers or peptides are relatively easy to applyand cause minimal dermal damage. They can be applied in larger surface areas when necessitated to deliverhigher number of drugs.They do not requirestrenuous effort in terms of development and implementations. After application of passive method requires certain time to reach the systemic circulation.Thisdelayed onset of action, sometimes up to several hours, afterapplication is critical in cases where patients demand immediate care like insulin therapy in diabetic patients.[41]

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