International Journal of Pharmaceutics 515 (2016) 384–389

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

In vivo skin penetration of macromolecules in irritant contact dermatitis

Mona M.A. Abdel-Mottaleb[a,b,c,*], Alf Lamprecht[b,c]

[a] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt
[b] Institute of Pharmaceutical Technology, Pharmacy institutes, University of Bonn, Bonn, Germany
[c] FDE (EA4267), university of Bourgogne Franche-Comté, Besançon, France

ARTICLE INFO

Article history:
Received 23 August 2016
Received in revised form 30 September 2016
Accepted 19 October 2016
Available online 19 October 2016

Keywords:
Skin
Dermatitis
Protein
Peptide
Quantum dots
Tape stripping

ABSTRACT

Recently, a selective preferential accumulation of polymeric nanoparticles (in the size range around 100 nm) has been observed in the follicular system of dermatitis skin. The present investigation aimed at clearly investigating the effect of irritant contact dermatitis on the barrier permeability for colloidal systems below this size range, namely quantum dots and hydrophilic macromolecules. Irritant dermatitis was induced in mice and the penetrability of quantum dots (5 nm) and hydrophilic dextran molecules has been tracked in both healthy and inflamed skin using confocal laser scanning microscopy. The selective accumulation of the quantum dots was clearly observed in inflamed skin while hydrophilic dextran behaved similarly in both healthy and inflamed skin. The therapeutic potential for the transdermal delivery of peptide drugs through inflamed skin has been also tested in rats. Results revealed that the transdermal permeation of insulin and calcitonin was not significantly enhanced in dermatitis compared to healthy skin. On the other side, permeation through stripped skin was significantly higher. However, the effect was limited and shorter compared to the SC injection where t_{min} was 0.5 h and 2 h with a 70% and 46% reduction in blood glucose levels for the stripped skin and the SC injection respectively. Similarly, t_{min} was 4 h and 8 h with area under the curve of 161 ± 65% and 350 ± 97% for the stripped skin and the SC injection respectively. In conclusion, the changes in skin permeability accompanied with skin inflammation did not affect its permeability to peptide drugs. Our findings also underline that experiments with the tape stripped skin model as a surrogate for inflamed skin can risk misleading conclusions due to significant difference of skin permeability between the tape stripped skin and inflamed skin.

1. Introduction

Recently, the selective accumulation of polymeric nanoparticles in a size and charge dependent manner in the pilosebaceous units of inflamed skin has been reported (Abdel-Mottaleb et al., 2012a, 2012b). It was found that nanoparticles in the size range between 50 and 100 nm selectively accumulate in the inflamed pilosebaceous units but without any deeper penetration or transcutaneous passage. This selective accumulation in the inflamed skin was accompanied by higher drug concentration in the inflamed parts of the skin but with minimal skin permeation. Therefore, polymeric nanoparticles in such size could be considered as a potential targeting moiety for the localized treatment of skin diseases while minimizing the systemic exposure to the delivered drugs. Proteins or macromolecules having molecular sizes in the nano-range, are possible to be accumulating in a similar pattern or alternatively they may have higher penetrability and transcutaneous passage capabilities due to their smaller particle size.

Protein, Peptide and other macromolecular drugs are currently very important for the treatment of various diseases such as diabetes, osteoporosis, cancer and others especially with the significant attainments made in the recombinant DNA technology. Stability issues along with their complex structures make them difficult drug candidates for delivery. Therefore, most of the macromolecules are administrated predominately by parenteral routes, which are not favored by most of the patients especially with the short half-lives of many proteins that necessitates

* Corresponding author at: Institute of Pharmaceutical Technology, Pharmacy institutes, University of Bonn, Gerhard-Domagk str 3, 53121 Bonn, Germany.
E-mail address: mona.abdelmottaleb@pharma.asu.edu.eg (M.M.A. Abdel-Mottaleb).

http://dx.doi.org/10.1016/j.ijpharm.2016.10.042
0378-5173/

repeated administration (Kalluri and Banga, 2011). Transdermal delivery offers an appealing alternative to the parenteral route but its efficacy is limited by the limited permeability of the skin to the hydrophilic macromolecules. The excellent barrier properties of the skin mostly provided by the highly organized lipophilic outermost layer, the stratum corneum, strictly hinder the penetration of proteins and allow only small lipophilic molecules to passively penetrate the skin (Choi et al., 2012). However, the significant achievements of transdermal delivery of many short half-lived drugs and the relatively low proteolytic activity of the skin appeals research efforts to bypass the permeation barrier and achieve successful transdermal protein and macromolecules delivery (Pillai and Panchagnula, 2003). Several penetration enhancement techniques have been proposed to enhance their permeation through the skin such as electrophoresis, microneedles, iontophoresis, sonophoresis. One of the important factors affecting the transcutaneous drug delivery is disease states that can affect the barrier properties of the skin like dermatitis or even the stress to which the skin can be exposed during normal physiological processes such as walking. A correlation was found between the particle penetration and flexing movement used to simulate the barefoot walking (Tinkle et al., 2003). Evidence of increased penetration of nanoparticles in the skin flexed for 60 and 90 min compared to normal control skin has been also reported (Rouse et al., 2007). However, the exact mechanism of enhancer penetration was not yet clarified. Dermatitis or skin inflammation is one of the most common and widely spread skin disorders worldwide. Contact dermatitis describes the skin inflammation resulting from exposure to irritants or allergens, which leads to the two types; irritant contact dermatitis (ICD) or allergic contact dermatitis (ACD) respectively. The first step in the pathology of contact dermatitis is disturbance of the skin barrier by the irritant thus triggering the immune reactions leading to the dermatitis which in turn will further facilitate the penetration of the irritant leading to more irritation (Proksch and Brasch, 2012). The lamellar bilayers of the stratum corneum consist mainly of lipids rich in cholesterol, fatty acids and ceramides, removal of these lipids by several irritants is considered a major reason for skin barrier disruption in irritant contact dermatitis. In response to this irritation, a heomeostatic immune response is initiated within the nucleated epidermis for the rapid restoration of these lipids and normalization of the barrier function (Berardesca et al., 2001). The release of chemokines, vasodilation, inflammatory cells infiltration into the epidermeis and dermis, epidermal damage, spongiosis, microvesicle formation, erythema, induration and edema are considered common signs in ICD. Such factors are expected to significantly affect the skin permeation to different drugs, allergens, proteins and other macromolecules. Surprisingly, this has not been yet thoroughly explored in literature.

Therefore, present investigation aimed at clearly investigating the effect of irritant contact dermatitis on the barrier properties of the skin especially their penetrability to “smaller systems” such as proteins and macromolecules. For such a purpose, in vivo penetration of 5 nm quantum dots (QDs) nanoparticles and fluorescently labeled dextran in a dithranol-induced dermatitis model in mice ears has been performed. The two systems have been chosen as an example of smaller particulate system than the ones tested before (QDs particle size is 5 nm compared to 50 nm polymeric nanoparticles reported earlier) and dextran was a model hydrophilic macromolecule. The study was then followed by exploring the delivery through inflamed skin as an appropriate delivery route for the therapeutic administration of macromolecular drugs. Skin penetration of two widespread peptides namely insulin and calcitonin through healthy and inflamed skin and the subsequent pharmacological response has been tested in rats.

2. Materials and methods

2.1. Materials

Calcitonin salmon (97% powder, Molecular weight 3500 Da), (Lumidot™ CdSe/ZnS 590, core-shell type quantum dots, 5 mg/ml in toluene), insulin from bovine pancreas (activity ≥ 27 USP units/mg, molecular weight 5800 Da), Tetramethylrhodamine isothiocyanate–Dextran (TRITC-Dextran average molecular weight 4400 Da) were purchased from Sigma Aldrich Chemie GmbH, Steinheim, Germany. Labrafac™ CC (Medium chain triglyceride) was a kind gift from Gattefossé, France. Calcium assay kit was from Abnova GmbH (Heidelberg, Germany). All other chemicals were of analytical grade or equivalent quality.

2.2. Preparation of different formulations

The provided quantum dots are solubilized in Toluene, so for the removal of toluene, particles were diluted in Labrafac in the ratio of 1:10 followed by toluene evaporation under reduced pressure. Similarly, for the preparation of dextran dispersion (2 mg/ml), TRITC-Dextran powder was directly dispersed in PBS. Calcitonin solutions were simply prepared by direct dissolution in PBS. Insulin solutions were prepared by mixing 10 mg in 500 μl DMSO and 9.5 ml PBS. Further dilutions were performed as required.

2.3. Skin penetration experiments

2.3.1. Animals

All animal experiments were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, and National Academy of Sciences, US). Induction of irritant dermatitis was performed according to the dithranol induced irritative dermatitis in mice ears model which is well-recognised experimental model suitable for the evaluation of both topically and transdermally administered agents (Gabor, 2003). The model offers several advantages as it is quick, simple, reproducible procedure accompanied by very low possibilities of error. Male BALB/c mice (average weight 25 g, n = 3/group) were used for all experiments. Dithranol solutions in acetone (0.4%) were then applied to the surfaces of mice ears where skin inflammation was to be induced. After 24 h of dithranol application, inflammation was verified by redness observation as well as measuring ear thickness. Inflamed skin of different animals was then treated with 50 μl of either QDs suspension in Labrafac ™ (0.5 mg/ml) or the fluorescently labeled TRITC-Dextran (2 mg/ml) in PBS for three consecutive days. The same quantities of both preparations were also applied to the ears of healthy animals without dithranol application to check for the penetration in healthy skin. Animals were sacrificed 24 h after the last particle administration, and their ears were further investigated.

2.3.2. Confocal laser scanning microscopy (CLSM)

The penetration of the fluorescent quantum dots and the fluorescent dextran into different skin layers was tracked by the CLSM. Skin samples from mice ears were examined directly using the inverted confocal laser scanning microscope Nikon Eclipse Ti (Nikon Corporation Inc., Tokyo, Japan). Examination was performed using the objectives of Plan-Apochromat10x/0.45 DIC, Plan-Apo VC 20×/0.75 DIC N2.The system was equipped with an argon laser (excitation wavelength at 488 nm) and HeNe laser (excitation wavelength at 543 nm) for the observation of mouse skin auto-fluorescence and red particles or dextran fluorescence, respectively. The fluorescence signals were collected simultaneously in two different channels using bandpass filters of 525 and