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Quantitative assessment of tissue retention, lipophilicity, ionic valence and convective transport of permeant as factors affecting iontophoretic enhancement

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Iontophoresis of the dipeptide tyrosine-phenylalanine (TyrPhe), the protected amino acid tyrosine-β-naphthylamide (Tyr-β-NA) and the glucose derivative benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside (BAd-α-Glc) used as model compounds was investigated in human epidermis in vitro *at pH 3 and pH 4.5 under constant voltage application, the purpose being to delineate the contribution of tissue retention, lipophilicity, ionic valence and convective transport of these compounds to iontophoretic enhancement in a quantitative fashion and ultimately gain an improved mechanistic understanding of iontophoresis. Retention of BAd-α-Glc in epidermal tissue during permeation was considerable and was reduced upon iontophoresis in the presence of extraneous tyrosine (Tyr) and phenylalanine (Phe) added to the donor solution, this evoking an increase of the apparent iontophoretic permeation. This suggests firstly, the occurrence of an interaction between BAd-α-Glc, Tyr and Phe impacting iontophoretic enhancement and secondly, the potential of using amino acids as adjuvants to modulate iontophoresis of selected compounds. This influence of tissue retention on iontophoretic permeation in the presence of Tyr and Phe was not observed for TyrPhe or Tyr-β-NA or when BAd-α-Glc, TyrPhe and Tyr-β-NA were used concomitantly rather than individually in the absence of Tyr and Phe. The effect of lipophilicity, ionic valence in the aqueous permeation pathway and convective transport by electroosmosis of the three permeants on iontophoresis was assessed simultaneously for all permeants by analyzing the experimental data using a theoretically derived model for iontophoretic enhancement that encompassed these factors. This model was based on an extension of the modified Nernst-Planck equation, whereas the difference of ionic valence between the aqueous domain of tissue and the bulk solution was evaluated on the basis of a pH shift due to the electrical double layer at the lipid/aqueous interface in the epidermis using the Poisson-Boltzmann equation. Using deduced parameter values characterizing the involved factors, a very good agreement between model calculated and experimental enhancement data was obtained. At pH 4.5, a very weak convective transport due to electroosmosis from cathode to anode was evident, indicating an isoelectric point of the epidermis slightly above 4.5. At pH 3, electroosmotic transport was approximately 10-fold stronger than at pH 4.5 which reflected a high positive surface charge density of the epidermis at pH 3 and a low one at pH 4.5. The pH in the aqueous permeation pathway, estimated to differ from that of the bulk in accordance with these charge densities, produced an ionic valence in this pathway dependent on pK_a that accounted consistently for the flux due to the direct effect of the electric field on the ionic permeants. The ratio of lipid to aqueous pathway passive permeability coefficient was << 1, indicating a marginal role of the lipophilicity of these compounds for iontophoretic enhancement. Hence, the applied evaluation afforded a quantitative assessment of the effect of factors relevant for iontophoresis and provided a congruent understanding of the process.*

Key words: Iontophoresis – Transdermal permeation – Physicochemical model – Amino acids – Peptide – Tissue retention – Lipophilicity – Electroosmosis – Iontophoretic enhancement.

Iontophoresis is an extensively investigated technology for enhancing and modulating topical and systemic transdermal delivery of drugs by an electric field. Using the electric field as an external physical stimulus whose strength and temporal pattern of application can easily be regulated, it was possible to control the onset and the rate of the delivery of drugs to the body as well as of the extraction of substances from the blood stream for the purpose of monitoring [1-8].

Mechanistically, next to the direct effect of the electric field driving ionic permeants through the epidermis, additional phenomena such as convective solvent flow due to electroosmosis and membrane alterations may influence the iontophoretic flux of charged and uncharged permeants. The direction of the electroosmotic flow may coincide with that of the current flow or be opposite to it, depending on the fixed charge carried by the membrane. The epidermis is a permselective membrane with an isoelectric point (pI) reportedly somewhat above 4 [9-11]. At physiological pH, epidermis is negatively charged and electroosmotic flow contributes to the current flow of an anodal delivery while cathodal delivery is retarded [12, 13]. At a pH close to the pI, a neutral membrane comes about so that no electroosmotic flow occurs. At a pH lower than the pI, the epidermis carries a positive charge and the electroosmotic flow is against the current flow of an anodal iontophoretic delivery [9, 18, 19]. Compounds may permeate the epidermal membrane by an aqueous and a lipid pathway [20]. The relative importance of each pathway depends on the lipophilicity and the ionic state of the compound. The aqueous pathway alone is responsible for the conductance of electric current and for electroosmosis. Therefore, iontophoretic permeation of a compound and permeation enhancement depend on its lipophilicity and ionization. The latter also affects the magnitude of migration that takes place as a result of the direct effect of the electric field on an ion. Furthermore, ionization of weak electrolytes depends on the pH of the environment which for a charged membrane may be affected by the distribution of charges in the electrical double layer at interfaces within the membrane.

In order to establish a formalized relationship between iontophoretic permeation enhancement and the above listed factors and make possible the evaluation of the effect of each of these factors in a quantitative fashion, a theoretical model was introduced [14, 21]. This model was based on the modified Nernst-Planck equation that included electroosmotic flow and was extended to take into account the ratio of lipid to aqueous pathway passive permeability of permeant and the ionization of the permeant in the aqueous pathway of the epidermis. The latter was considered to differ from that in bulk because of the electrical double layer at the aqueous/non-aqueous domain interface of the epidermis eliciting a pH shift in the aqueous domain that was estimated

using the Poisson-Boltzmann equation. The relationship between the iontophoretic enhancement under constant voltage application and all factors conceivably affecting enhancement that was yielded by the model was used earlier to analyze experimental iontophoresis data of a peptide and an amphoteric weak electrolyte drug in order to verify the validity of the underlying assumptions and delineate the contribution of the different factors acting simultaneously in the process [14, 21]. Those analyses provided quantitatively consistent results about the effect of the involved factors and a good agreement between calculation and experiment.

In a previous manuscript [15], anodal iontophoresis of the glucose derivative benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside (BAd-α-Glc) used as an electroosmosis marker at pH 3 and pH 4.5 caused a slight flux retardation of this compound indicating electroosmotic flow occurring in the cathode-to-anode direction in accordance with the accepted view of positive fixed epidermis charges prevailing in this pH range. Significant iontophoretic enhancement of BAd-α-Glc at the same pH values was found in the presence of the amino acids tyrosine (Tyr) and phenylalanine (Phe) produced by cutaneous metabolism of the model dipeptide tyrosine-phenylalanine (TyrPhe) that was used concomitantly in the studies. This correlated with a decrease upon iontophoresis of the amount of BAd-α-Glc retained in the epidermis. Therefore, an interaction between BAd-α-Glc and the metabolic products of TyrPhe impacting tissue retention and apparent permeation of BAd-α-Glc upon iontophoresis was suggested [15].

In the present work, the effect of *a priori* addition of Tyr and Phe to the drug solution on epidermis retention and iontophoretic permeation of three model permeants, i.e., the glucose derivative benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside (BAd-α-Glc), the dipeptide tyrosine-phenylalanine (TyrPhe) and the protected amino acid tyrosine-β-naphthylamide (Tyr-β-NA) is investigated, the purpose being to test the universality of the described effect [15] and understand its origin. TyrPhe was studied at 4°C in order to abolish its enzymatic degradation in the tissue [15] and thus establish controlled experimental conditions. Furthermore, the physicochemical model of iontophoretic enhancement developed previously [14, 21] is used for simultaneously analyzing experimental data of all three model permeants in order to quantitatively delineate the effect of permeant lipophilicity, ionic valence in the aqueous membrane domain and convective transport by electroosmosis to the measured enhancement. The goal of applying the model to the iontophoresis of more than one compounds simultaneously is to obtain results about the role of the involved phenomena and the influence of permeant properties with rather general validity. The model permeants were chosen such as to span a wide range of lipophilicity (70-fold) and ionic valence but had all approximately the same molecular size at this stage of the work to ensure comparable diffusivity and convective transport in the tortuous aqueous membrane domain. Permeation experiments were performed with heat separated human epidermis at pH 3 and pH 4.5. At these pH values the permeants carried according to their pK_a in part positive (or zero) charge allowing the use of anodal iontophoresis. Also, this pH range contained the isoelectric point of human epidermis, providing the possibility to apply the theoretical model when opposite directions of the electroosmotic flow took hold. This represents the typical situation encountered in the iontophoresis of peptides and drugs that are organic bases. Finally, constant voltage iontophoresis was used because under these conditions an analytical solution of the Nernst-Planck equation can be obtained and iontophoretic transport of concomitant ionic spieces including metabolic products and buffer salts can be treated independently of each other. The Nernst-Planck equation in combination with the Poisson-Boltzmann equation provided the theoretical framework for this analysis. It is recognized that constant voltage conditions are appropriate only for a fundamental study of the process whereas constant current application is the method of choice for controlling delivery [24].

I. MATERIALS AND METHODS

1. Materials

Benzyl-2-acetamido-2-deoxy-α-D-glucopyranoside (BAd-α-Glc), a glucose derivative with a molecular weight of 311.3, was purchased from Toronto Research Chemicals, North York, Canada. BAd-α-Glc is unionized at pH 3 and pH 4.5 and was used at a concentration of 13 mM in the donor solution. Tyrosine-β-naphthylamide (Tyr-β-NA), an amino acid protected at the C-terminus with a molecular weight of 306.4, and tyrosine-phenylalanine (TyrPhe), a dipeptide with a molecular weight of 328.4, were purchased from Bachem AG (Bubendorf, Switzerland). A pK_a of 3.5 for TyrPhe and 4.78 for Tyr-β-NA was determined by potentiometric titration in the range that was of interest considering the acidic pH values of the permeation experiments. No other pK_a was found < 7. The concentration of TyrPhe and of Tyr-β-NA in the donor solution was 3 mM at pH 3 and 2 mM at pH 4.5. The amino acids tyrosine (Tyr) with a molecular weight of 181.2 (pK_a 2.2, 9.1 and 10.1) and phenylalanine (Phe) with a molecular weight of 165.2 (pK_a 2.2 and 9.2) were purchased from Fluka BioChemika (Buchs, Switzerland) and Sigma Chemical Co. (St. Louis, MO, USA), respectively. The concentration of each amino acid that was added to the donor solution was 3 mM at pH 3 and 2 mM at pH 4.5.

Universal buffer was used at pH 3 and pH 4.5. It was composed of citric and phosphoric acid (each 6.67 mM) and boric acid (11.5 mM), which were dissolved in double distilled water and titrated to the desired pH with sodium hydroxide. The osmolarity in the donor and the receiver solutions was set to 300 mOsmol with sodium chloride. All chemicals were of analytical reagent grade.

Human cadaver skin was obtained from the Department of Pathology, University Hospital, Basel. Heat-separated epidermis with an average thickness of 43 μm (SD 5 μm, n = 16) was used in the permeation experiments. Heat separation, mounting in the diffusion cells, a gravitational leaking test and an electrical resistance test of the barrier properties of the epidermis were carried out as described in [14].

Permeation experiments were carried out in custom made two-chamber, side-by-side glass diffusion cells with a diffusion area of approximately 2 cm^2 that were connected to a current source with two working Ag/AgCl electrodes and two reference Ag/AgCl electrodes, the latter reaching the membrane by buffer-filled glass capillaries. The anode was always placed in the donor and the cathode in the receiver compartment. Universal buffer was used in both chambers of the cells. The current source (built in the Department of Physics, University of Basel) supplied direct current and was operated at constant voltage for iontophoresis. The flow of electric current was measured and recorded continually on a disk with a Digital Chart Recorder (DCR 520, W + W Instruments AG, Basel, Switzerland). Further details of the experimental set up can be found elsewhere [14, 21].

2. Protocol of permeation experiments

In a first stage, the baseline passive permeability was measured for 44 h to allow accurate determination of the flux of these slowly permeating compounds. This was followed by the iontophoretic stage during which a constant voltage of 250 mV was applied for 3 h across the epidermal membrane. This was shown previously to be appropriate for determining the steady state iontophoretic permeability [14, 21]. Subsequently, the post-iontophoretic passive permeability was measured. Samples of 0.2 ml were withdrawn from the receiver compartment every 90 min in the passive stages and every 20 min in the iontophoretic stage and replaced with fresh buffer. Samples were analyzed with no previous treatment by HPLC-MS. At the beginning of each stage, 0.05-ml samples were taken from the donor compartment and diluted 400-fold before analysis by HPLC-MS. At the end of the passive stages, a potential difference of 250 mV was applied for 5 min to record the electrical resistance and check the integrity of the epidermal membrane barrier. At the end of each experiment, the

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