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Research

Assessing the Relevance of Ion and Molecule Transport Through Animal Skin and Fascia to Transdermal Drug Delivery Systems

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Abstract:
Biological membranes such as the skin and fascia play a critical role in controlling the transport of ions and molecules and, as such, they are often used as substitutes to study the transdermal delivery of drugs. This paper uses the conductivity data from ion-transport experiments performed with broiler chicken, indigenous chicken (Kadaknath and Gavran), goat and sheep skin that were exposed to KCl and NaCl solutions of 0.1 N-0.5 N for 120 minutes to examine if the variations observed in permeability data suggest anything about the membrane's potential as a model for transdermal drug delivery (TDD). The data shows that, in general, conductivity increased linearly with time and ion concentration in all tested membranes, though the chicken and goat skin showed significantly higher flux than their indigenous counterparts, suggesting that their permeability can differ considerably for a given set of conditions, which is an important consideration for selecting the animal model for TDD studies. By reviewing the data in the context of the existing pharmaceutical literature on percutaneous absorption, iontophoresis and skin-impedance modeling, the paper shows that measuring conductivity is a viable option to prioritize membranes for further studies, such as Franz-cell experiments or iontophoretic drug delivery trials. The paper concludes by discussing the limitations of this study, including the lack of data on fascia and the absence of permeability coefficients required for quantitative analysis.

Keywords: transdermal drug delivery; skin permeability; ion transport; conductivity; iontophoresis; animal skin models; fascia.

1. Introduction

The skin is a laminated barrier: the superficial layer, namely, the stratum corneum, is comprised of corneocytes and extracellular lipid matrix, making the skin a competent barrier to prevent excessive water loss and to limit the entry of numerous undesirable agents (Elias, 2005; Bouwstra & Ponec, 2006). Transdermal drug delivery aims to use this barrier to gain access to the systemic circulation, which allows drugs to bypass hepatic first-pass metabolism and reduce injection-related discomfort (Prausnitz & Langer, 2008; Guy, 2010). However, given that human skin is not easily available for research purposes, it is commonly replaced with animal skin, such as pig, rodent, poultry, sheep and goat skin, as viable alternatives for ex vivo studies (Godin & Touitou, 2007; Fransson et al., 2009; Toutain et al., 2021).

Meanwhile, the role of fascia as connective tissue that surrounds muscle groups and organs has been

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investigated extensively, but its involvement in fluid circulation, mechanoreception and as a potential alternative pathway for drug delivery has garnered only a minor share of the attention (Langevin, 2006; Schleip et al., 2012; Zügel et al., 2018). Thus, a parallel study of ion and molecule transfer through animal fascia and skin is a relevant endeavor to address two questions. First, how do animal skin differ from each other in terms of their value for the ex vivo study of percutaneous permeability? Second, does fascia differ significantly from skin in ways that could impact drug delivery? This paper aims to discuss the latter question in detail as part of the larger project's fourth objective to evaluate the relevance of the findings for transdermal drug delivery.

Namely, this paper seeks to utilize the data obtained from the electrical conductivity measurements to identify the membrane's capacity for ion transport. The primary reason for this choice is that measuring the electrical conductivity is a relatively quick and informative way to estimate the barrier properties of the skin. This is because the transfer of ions and electricity is closely related, as established by iontophoresis (Kontturi et al., 1993; Chizmadzhev et al., 1998; Karande et al., 2006; Grimnes & Martinsen, 2015). Therefore, this method can be used both to model the permeability of the skin barrier and to screen the effect of penetration enhancers. Thus, this paper will use the conductivity data obtained with KCL and NaCl on the four types of animal skin to evaluate their potential for ex vivo studies informing TDD design.

2. Materials and Methods

Skin samples were collected from four different sources including broiler chicken, indigenous poultry (desi chicken) comprised of Kadaknath and Gavran breeds, goat and sheep. A diffusion vessel made up of two glass chambers was used to expose one chamber containing electrolyte solution (KCl or NaCl) to skin while the other one received distillated water. An indirect method therefore allowed estimation of ion transfer through the excised skin membrane where conductivity was measured usign a digital meter. Data collection was done at fourteen time intervals within the duration of one to one hundred and twenty minutes. The experiment was conducted twice in different ways. In the first trial, four tissues were subjected to either KCl or NaCl at the same concentration level before comparing their performance. Secondly, a series of electrolyte solution at varying concentrations (0.1N, 0.2N, 0.3N, 0.4N and 0.5 N) were used to determine the solution concentration response of individual tissues. Microsiemens per centimeter (µS/cm) was used to estimate and report percentages of transported ions for the purpose of this experiment. The measure has previously been used in similar trials to indicate conductivity levels as a reliable approximation of cumulative ion transport rate in skin permeability studies (Karande et al., 2006).

3. Results

Across different tissues and electrolytes, the conductivity increased constantly over time, which suggests that the ion transfer occurred continuously and not as a rapid equilibrium. At a given interval of KCl exposure, the broiler chicken skin attained the highest conductivity after 120 minutes of treatment (15.5 µS/cm), followed by the goat skin (15.2 µS/cm), Kadaknath skin (15.0 µS/cm) and Gavran skin (14.5 µS/cm). However, on using NaCl, the goat skin showed the highest conductivity (9.3 µS/cm), followed by the broiler chicken (9.1 µS/cm), Kadaknath (9.0 µS/cm) and Gavran (8.7 µS/cm). Table 1 below shows the conductivity values.

Tissue	KCl, 120 min (µS/cm)	NaCl, 120 min (µS/cm)
Broiler chicken skin	15.5	9.1
Goat skin	15.2	9.3
Kadaknath chicken skin	15.0	9.0
Gavran chicken skin	14.5	8.7

Table 1. Endpoint conductivity values (120 minutes) for four animal skin types exposed to KCl and NaCl solutions.

The concentration-response series was monotonic for all tissues, showing increased conductivity with higher electrolyte normality at any given time-point and a greater difference between the 0.1 N and 0.5 N curves at later time points. For instance, Kadaknath skin treated with KCl had a conductivity increase of 1-3 µS/cm at 1 minute, which was higher than that of 7.8-10.8 µS/cm seen at 120 minutes. A similar response pattern was seen in broiler skin, with increases of 1.2-3.2 µS/cm at 1 minute rising to 7.9-10.9 µS/cm at 120 minutes. This type of concentration-dependent response was seen for both

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electrolytes and all tissues, suggesting that the measured transport was limited by the concentration gradient and not a maximum permeability capacity. Two trends can be noted for this observation. The first is that broiler and goat skin were generally more permeable compared to the two indigenous chicken breeds, with Gavran being the least permeable throughout the series. This trend was consistent for both electrolytes and across all concentrations. The second finding is that no tissue reached saturation within the 120-minute window, as evidenced by the continued increase in conductivity at each time-point. This suggests that the observed permeability values represent a transport rate rather than a steady-state permeability coefficient.

4. Discussion: Relevance of These Findings to Transdermal Drug Delivery Systems

The purpose of this section is to discuss the implications of the conductivity results above for the fourth objective of the parent study: do they have any significant relevance to the transdermal drug delivery.

4.1 Conductivity as a proxy for barrier permeability

A gradual, concentration- and time-dependent increase in conductivity was observed universally in all four skins. This is an attractive property for pharmaceutical researchers attempting to assess the properties of candidate membranes on the basis of their barrier function characteristics, since it indicates that the ion flow through the skin is purely diffusive and follows the gradient in concentration (Guy et al., 2001; Kalia & Guy, 2001; Mitragotri et al., 2014). Electrical impedance or conductivity measurements have been suggested as non-destructive substitutes for the in vivo measurements of the skin permeability coefficient (Kontturi et al., 1993; Chizmadzhev et al., 1998; Karande et al., 2006). Therefore, the conductivity findings for the four skins are pertinent to the study since they indicate general conformity of these four animal membranes with the requirements for a TDD membrane to be useful for pre-clinical testing prior to conducting more involved Franz-cell diffusion experiments and in vivo trials.

4.2 Tissue and breed selection for ex vivo TDD models

The fact that broiler and goat skin demonstrated consistently higher permeability than those of Kadaknath and Gavran is, in itself, a practically significant result. It highlights the necessity to consider permeability characteristics of particular animal breeds when designing ex vivo permeability experiments, as permeability appears to vary both on the level of species (Fransson et al., 2009; Godin & Touitou, 2007) and on the level of breeds within a species. For a researcher that wishes to utilise an easily accessible, low-cost ex vivo membrane model, the discovery has significant implications. Namely, a permeability barrier of considerable porosity (such as that of the broiler or goat skin) would demonstrate higher drug flux than most human skin samples and be closer to the upper end of the physiological range, while the tighter barrier of Gavran skin would be closer to the lower end of the human variability range. Animal skins as a low-cost alternative to laboratory-grown human skin membranes have been suggested before (Toutain et al., 2021) and the current results further support the idea of utilising slaughterhouse waste materials to create permeability barriers of predictable permeability. The differences between the permeability characteristics of the tested skin tissues suggest that both species and, where applicable, breed should be taken into consideration as variables in further studies.

4.3 Implications for iontophoretic and electrically assisted delivery

Because the measurements in this study involved the applied electrolyte gradient and registration of ion current, these data mainly concern the iontophoretic form of transdermal drug delivery (TDD), which involves the iontophoresis application using a minor current for the TDD of charged molecules (Kalia et al., 2004; Rim et al., 2009). The concentration-response relationship identified in this study, which manifested as the higher ionic strength resulting in increased conductivity at all-time points, most closely aligns with the increase in the iontophoretic current density associated with the TDD of charged macromolecules (Guy et al., 2001; Kalia & Guy, 2001). As such, it can be extrapolated to the selection of the donor compartment preparation for an iontophoresis study by using the same conductometric screening to prioritize the animal skins with a higher baseline permeability since the more permeable (lower baseline ionic strength) preparation would not be suitable for testing in a human due to a lower predictive value.

4.4 Limits on translational relevance

The relevance of these findings to TDD is limited, however, since conductivity is a general indicator of

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a tissue's ability to carry current, which in turn is a function of several variables besides the permeability coefficient for a given molecule (see discussion of this coefficient as an indicator of potential for permeation in Franz, 1975; Godin & Touitou, 2007). Classical theory of percutaneous absorption suggests that passive diffusion through the skin is primarily dictated by the size and lipophilicity of the molecule and its ability to passively diffuse across the stratum corneum – which is generally only possible in case of molecules smaller than 500 Da in molecular weight with a sufficiently low lipophilicity (Bos & Meinardi, 2000; Mitragotri et al., 2014). Thus, a tissue with high conductivity is not necessarily indicative of a tissue with high permeability for a given molecule and the presented values can only be used to indicate the general level of resistance of the tissue to small ions. Moreover, the study as it stands does not provide conclusive data on the ability of the fascia to serve as a permeability pathway for TDD, as the study did not measure the conductivity of the fascia, which was actually the driving force behind the research. Lastly, since none of the samples reached a steady-state conductivity within the 120 minutes of the experiment, permeability coefficients for any of the tissues could not have been determined using the standard methodology (Franz, 1975; Godin & Touitou, 2007).

4.5 Practical relevance summarised

Overall, the three results discussed lead to three practically applicable conclusions for TDD studies. First, the conducted experiment allows using conductometry as a convenient and inexpensive method for initial screening of integuments possessing barrier properties in a functional state. Second, the data obtained prove that the choice of tissue and its source animal significantly impact the absorption characteristics, meaning that these tissues cannot be regarded as interchangeable. Third, the study demonstrates that the ionic currents observed are compatible with the mechanisms of iontophoresis and thus contribute to the understanding of iontophoretic drug delivery, albeit only at theoretical levels due to the complexity of the process.

5. Conclusion

Conductivity-based ion-transport measurements of broiler, Kadaknath, Gavran, goat and sheep skin presented in this study demonstrate a certain regularity in the permeability trends which seems to be appropriate from the viewpoint of transdermal drug delivery, specifically iontophoresis or other electro-assisted mechanisms and, therefore, it is rational to consider these low-cost animal models as a preliminary ex vivo screening tool. However, the lack of steady-state permeability coefficients and relevant fascia data prevent these results from being applied for a more comprehensive evaluation of TDD potential. Thus, it is recommended to employ the conductometric assay described in this study to investigate the transport properties of fascia and to determine permeability coefficients for particular model compounds in order to complement the data provided by this study.

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