Investigation of drug absorption through physiological barriers

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Theses of the Ph.D. Dissertation

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Introduction

The main scope of the experimental work is the investigation of the properties of two different physiological barriers, the nasal and the dermal barrier.

Previously, our research group has successfully investigated various P-glycoprotein (P-gp) model drugs crossing the blood-brain barrier in a rat model by *in vivo* microdialysis (MD). Both young and middle-aged animals were studied, so that by comparing the results, they were able to successfully investigate the changes on the drug absorption and elimination caused by physiological aging [1]. Bors et al. developed an intranasal administration method in which the brain uptake of a P-gp substrate, quinidine (QND) could be examined in rats in the presence or absence of a P-gp inhibitor or a sympathomimetic drug [2]. Based on these previous results, we posed the question of how pathological aging (e.g. Alzheimer's disease (AD), atherosclerosis etc.) affects the absorption of different model drugs through the blood-brain barrier (BBB). The previously used intranasal administration procedure was further developed for mice, thus the alterations in the nasal

barrier function caused by pathological aging using two age-related neurodegenerative disease models, in APOB-100 and in APP-PSEN1 mice could be examined. By comparing the levels of different cerebral cytokines in the two transgenic models compared with age-matched wild type (WT) mice, we could further characterize the differences between physiological and pathological aging. We also investigated the cerebro-morphological status of the animals by magnetic resonance imaging (MRI).

Then the results of two research topics related to the dermal barrier are presented. Nowadays, there is a growing demand to develop various human skin substituents that could mimic appropriately the physiological and pathological processes that take place in the skin. However, the complex anatomical structure and the wide variety of macrostructures present in the skin make this a difficult task. The first research topic was the investigation of the permeability of human abdominal skin and a human skin substituent with a topically applied caffeine cream in a skin-on-a-chip device.

The second research topic is connected to the altered properties of the dermal barrier due to psoriasis,

which is a chronic, inflammatory skin disease of unknown origin. In the development of this disease, in addition to genetic factors, environmental triggers also play an important role. Psoriasis severely disintegrated the dermal barrier, resulting in a significant increase in skin permeability. Various therapeutic approaches are available for the treatment and relief of symptoms, which can be used either as monotherapy or in combination [3], but in most cases some topical treatments are also applied [4]. The involvement of Transient receptor potential ankyrin 1 (TRPA1) and Transient receptor potential vanilloid 1 (TRPV1) ion channels in psoriasis has been confirmed recently [5–7], therefore the examination of the effect of the genetic deletion of these cation channels on the permeability was aimed in a mouse model of Aldara-induced psoriasis-like inflammation using topically applied caffeine cream in a skin-on-a-chip device.

New scientific results

The experimental work related to the nasal barrier was divided into three main parts: 1) investigation of the alterations in the nasal barrier function, 2) the cerebral levels of different cytokines and 3) the cerebro-morphological status of the APOB-100 and APP-PSEN1 mice.

Thesis 1: *In our experiments (dual-probe in vivo microdialysis in APOB-100, APP-PSEN1 and wild type mice), it was demonstrated that nasal barrier function is unchanged in the transgenic models, and no significant role of P-gp can be seen in the process of cerebral absorption of intranasally administered quinidine. In addition, I showed that an inflammatory balance shift is present in the brain of transgenic animals, increased cerebral levels of VEGF, PDGF-BB and IL-17A were demonstrated in APOB-100 mice and upregulation of resistin, IL-17A and GM-CSF in APP-PSEN1 mice were shown. These cytokines can be relevant biomarkers of the pathological processes.*

Related publication: [J1]

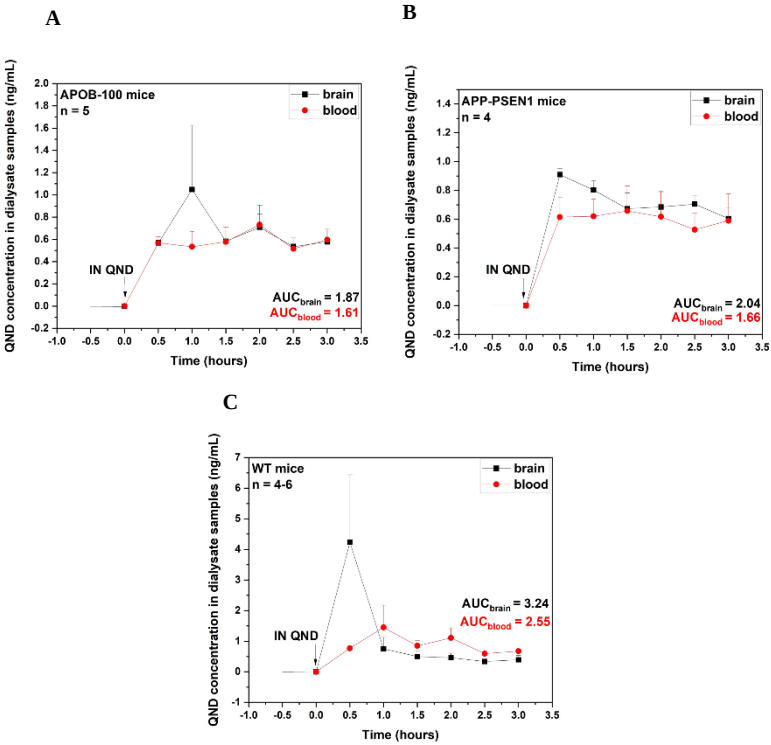


Figure 1. Investigation of the temporal characteristics of the absorption of quinidine following intranasal treatment (A) in APOB-100 and (B) in APP-PSEN1 mice compare to (C) wild type (WT) animals measured by dual-probe *in vivo* microdialysis. Intranasal administration was performed at t = 0. Black and red symbols represent the quinidine mean concentration in the brain and in the

systemic circulation (plasma). Data shown as means ± SEM. (IN QND: intranasally administered quinidine; AUC: area under the curve)

Intranasal drug delivery is a non-invasive, promising method of administration that allows each drug to bypass the blood-brain barrier and enter the brain, However, the efficiency of nasal-brain absorption may be significantly affected by several efflux transporters in the nasal mucosa, one of which is P-gp. The neurodegenerative diseases are characterized by breakdown of the blood-brain barrier and its increased permeability, moreover altered function and downregulation of P-gp has been observed in Alzheimer's disease [8], and recently demonstrated by Hoyk et al. in APOB-100 animals [9]. After the IN administration, rapid absorption peak of quinidine was observed in each group of animals, followed by a long-lasting plateau phase, where the release and the absorption of QND was continuous (Figure 1.). Based on the results of microdialysis experiments, unchanged function of the nasal barrier was observed, as the absorption pattern of

quinidine was similar in all cases. It can be concluded that P-gp does not have any significant role in drug absorption in the nasal cavity.

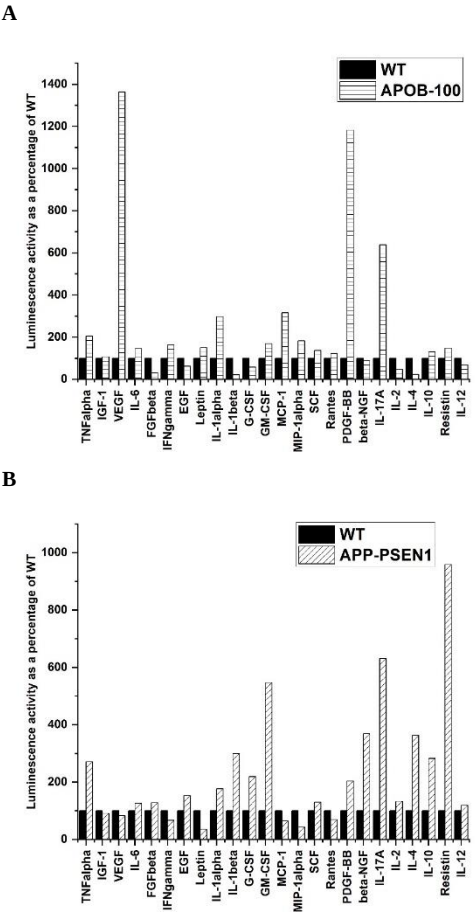


Figure 2. Comparison of LIR values of the tested cytokines compared to the wild type animals (A) APOB-100 and (B) APP-PSEN1 mice. Pooled samples were used

for both transgenic mouse strains as well as wild type animals (n = 5).

Based on the results of the ELISA cytokine assay, APOB-100 mice showed significantly elevated levels of VEGF, PDGF-BB, and IL-17A (Figure 2. A), which may even be useful in detecting cerebrovascular lesions and brain dysfunction caused by hyperlipidemia. Süle et al. showed that in this transgenic mouse strain, hyperlipidemia has a serious effect on the cerebral vascular network, primarily affecting vascular network density and capillary diameter [10]. Furthermore, it is hypothesized that hyperlipidemia may inhibit the process of angiogenesis. In addition, strongly elevated levels of VEGF may even cause breakdown of the blood-brain barrier [11], experimental results confirming this claim have been described in APOB-100 animals [9]. The double-humanized, APP-PSEN1 mice showed highly elevated levels of resistin, IL-17A and GM-CSF (Figure 2.B), which may serve as important markers of AD. Demirci et al. described elevated resistin levels in the serum of AD patients [12], suggesting that this cytokine may play an important role in the pathology of AD and be

useful as a marker. GM-CSF is involved in the inflammation processes by regulation of the number and function of macrophages. Significantly increased IL-17A levels were measured in both transgenic strains, which suggests the overproduction of cyclooxygenase-2 (COX-2) and nitric oxide (NO) [13].

In order to characterize the morphological consequences of the neurodegeneration in the two animal models, MRI was acquired on APOB-100, APP-PSEN1 and wild type male mice. Based on MRI data, several anatomical changes were detected in the brain in both transgenic mouse strains compared to the control animals, presumably due to pathological processes characteristic of vascular atherosclerosis and Alzheimer's disease. Significantly enlarged lateral and dorsal ventricles and a smaller but remarkably increased fourth ventricle volume was observed in APOB-100 mice, which is in line with previous results [9,14].

I also presented some new results of two research topics related to the dermal barrier. The first research topic was the investigation of the permeability of human

abdominal skin and a human skin substituent with a topically applied caffeine cream in a skin-on-a-chip device. It was followed by the presentation of the second research topic, which was connected to the altered properties of the dermal barrier due to psoriasis. The involvement of different TRP ion channels in this chronic immune-mediated disease has been confirmed recently, thus the genetic deletion of TRPA1 and TRPV1 cation channels on the permeability was studied in a mouse model of Aldara-induced psoriasis-like inflammation using topically applied caffeine cream in a microfluidic device.

Thesis 2: *I compared the time-course of caffeine on three different diffusion samples – human abdominal skin, cell-free electrospun PCL membrane (mesh) and HaCaT cell culture on electrospun membrane (human skin substituent) – with a topically applied 2% caffeine cream in a skin-on-a-chip-device. The time-course of caffeine concentration determined by spectrophotometric analysis confirmed the similarity of the human skin substituent and the human abdominal skin, demonstrating that the skin*

equivalent is, although greatly simplified, a sufficient model system for studying transdermal absorption.

Related publication: [J2]

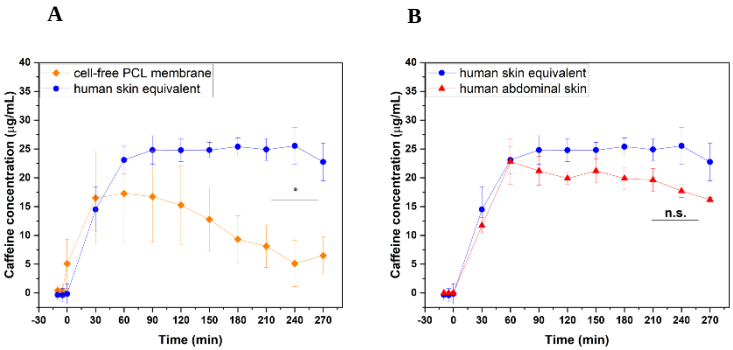


Figure 3. Concentration-time profiles of caffeine penetration of human abdominal skin, cell-free electrospun PCL membrane (mesh) and human skin equivalent. The caffeine cream was placed in the donor cell at time t = 0, then samples were taken every 30 min after caffeine exposure for all three different diffusion samples. Comparison of caffeine penetration pattern of (A) cell-free PCL membrane and human skin equivalent (B) human skin equivalent and human abdominal skin. For each sample, at least three parallel measurements (n = 3) were performed, results are expressed as mean ± SEM, p

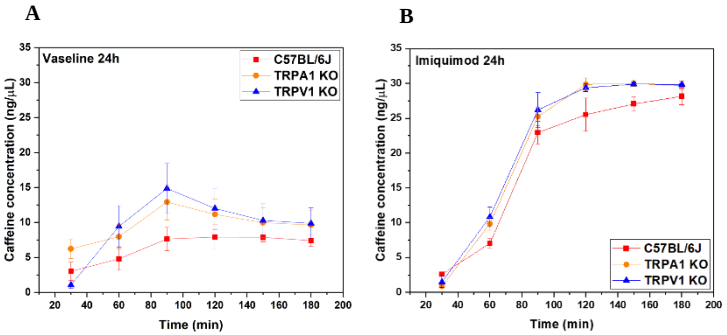
< 0.05 indicates statistically significant difference between caffeine penetration in mesh and human skin equivalent.

A similar penetration pattern was observed for all three samples, with the maximum penetration (C_{max}) measured 1 h after the onset of caffeine exposure (Figure 3.). Thereafter, by the end of the experiment, the caffeine concentration-time curves of human skin equivalent and human abdominal skin showed the same course. In contrast, a continuous decrease in caffeine concentration was observed for the cell-free PCL membrane, which can be explained by the fact that during the experiment, the close contact between the caffeine cream placed in the donor cell and the membrane was disrupted by dissolving the cream in the perfused medium. This process resulted in a gradually decreasing contact between the topically applied cream and the diffusion sample thanks to which a reduced amount of caffeine could pass through the membrane by passive diffusion. This phenomenon was not observed in the case of human skin substituents, because the cells were actively involved in the penetration of

caffeine, thus preventing the formation of gaps separating the cream from their surface.

Thesis 3: *I compared the degree and kinetics of the penetration of topically applied 2% caffeine cream during the time-progression of Aldara-induced psoriasiform inflammation in C57BL/6J (WT), TRPA1 KO and TRPV1 KO mouse skins in a skin-on-a-chip device. Based on the caffeine content of the samples of the vaseline-treated and Aldara-treated groups determined by spectrophotometric analysis, it can be concluded that psoriasiform inflammation significantly disintegrated the dermal barrier, resulting in a significant increase in the permeability of psoriasiform-inflamed skin.*

Related publication: [J3]



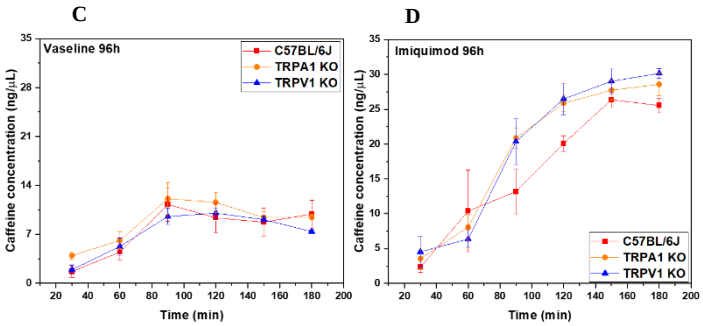


Figure 4. Concentration-time profiles of caffeine penetration using the dorsal skin samples of three different mouse strains (A) after 24 hours vaseline-treatment, (B) after 24 hours imiquimod-treatment, (C) after 96 hours vaseline-treatment and (D) after 96 hours imiquimod treatment. Red, orange and blue symbols represent the average concentration of caffeine obtained from C57BL6/J (WT), TRPA1 KO, TRPV1 KO mice. In each case, at least three independent measurements were performed (n = 3), results are expressed as means ± SEM.

Permeability of caffeine was examined in a skin-on-a-chip device using the dorsal skin of three different mouse strains (C57BL/6J, TRPV1 KO and TRPA1 KO) treated with Aldara or vaseline for 24 hours or 96 hours.

The pattern of caffeine penetration in each mouse strain was significantly different between the two treatments, since the permeability of healthy (vaselin-treated) skin was significantly lower than that of psoriatic (Aldara-treated) skin which is due to impairment of skin barrier function due to the psoriasiform inflammation (Figure 4.). Furthermore, the two observation time points allowed us to examine the time progression of the disease. No significant differences in the time-characteristics of caffeine penetration were seen for the three strains, just a moderate reduction of the caffeine absorption was observed in case of TRPA1 KO and C57BL/6J animals between the two observation points. However, no remarkable difference was seen between the Aldara treatments for 24 hours and 96 hours in TRPV1 KO animals, which is in accordance with the results published by Zhou et al. [6].

List of publications related to the thesis points

[J1] Varga-Medveczky, Zs.; Kovács, N.; Tóth, M.E.; Sántha, M.; Horváth, I.; Bors, L.A.; Fónagy, K.; Imre, T.; Szabó, P.; Máthé, D.; et al. Age-Related Inflammatory Balance Shift, Nasal Barrier Function, and Cerebro-Morphological Status in Healthy and Diseased Rodents. *Front Neurosci 2021, 15, 700729*, doi:10.3389/fnins.2021.700729

[J2] Tarnoki-Zach, J.*; Mehes, E.*; Varga-Medveczky, Z.*; Isai, D. G.; Barany, N.; Bugyik, E.; Revesz, Z.; Paku, S.; Erdo, F.; Czirok, A. Development and Evaluation of a Human Skin Equivalent in a Semiautomatic Microfluidic Diffusion Chamber. *Pharmaceutics 2021*, 13, 910. https://doi.org/10.3390/ pharmaceutics13060910
* These authors contributed equally to this work.

[J3] Kocsis, D.; Horváth, S.; Kemény, Á.; Varga-Medveczky, Z.; Pongor, C.; Molnár, R.; Mihály, A.; Farkas, D.; Naszlady, B.M.; Fülöp, A.; et al. Drug Delivery through the Psoriatic Epidermal Barrier—A

"Skin-On-A-Chip" Permeability Study and Ex Vivo Optical Imaging. *Int. J. Mol. Sci. 2022*, 23, 4237. https://doi.org/10.3390/ ijms23084237

Other publications related to the topic of the doctoral dissertation

- D. Kocsis, V. Klang, E.-M. Schweiger, Zs. Varga-Medveczky, A. Mihály, Cs. Pongor, Zs. Révész, Z. Somogyi, F. Erdő: Characterization and *ex vivo* evaluation of excised skin samples as substitutes for human dermal barrier in pharmaceutical and dermatological studies, *Skin Res Technol. 2022,* 1-13. 10.1111/srt.13165
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Other publications related to other previous research activity of the author

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- Szabó, Z.; Volk, J.; Horváth, Zs. E.; Medveczky, Zs.; Czigány, Zs.; Vad, K.; Baji, Zs.: Atomic layer deposition and annealing of Ga doped ZnO films, *Material Science In Semiconductor Processing*, *2019*, 101 pp. 95-102, 8p., https://doi.org/10.1016/j.mssp.2019.05.028

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- Zs. Varga-Medveczky: Comparative evaluation of permeability properties of a human skin equivalent

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- Zs. Varga-Medveczky: Characterization of age-related neurodegenerative disease models in transgenic animals, *PhD Proceeding Annual Issues of the Doctoral School Faculty of Information Technology and Bionics,* vol. 16:2021 pp. 115-118., 4 p (2021)
- Zs. Varga-Medveczky: Drug delivery through physiological barriers: investigation of the permeability of blood-brain barrier, nasal- and dermal-barriers, *PhD Proceeding Annual Issues of the Doctoral School Faculty of Information Technology and Bionics*, vol. 15:2020 pp. 117-119., 3 p. (2020)
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