chemistry proceedings

MDPI

Proceeding Paper

Molecular Modelling and In Vitro Research of New Substances for the Targeted Stimulation of AQP3 in Skin †

Viktor Filatov [1,2,*], Andrey Varava [2] and Egor Ilin [3]

[1] Department of Pharmaceutical Chemistry and Organization of Pharmaceutical Business, Faculty of Basic Medicine, Lomonosov Moscow State University, 119991 Moscow, Russia
[2] Science Center, SkyLab AG, 1066 Lausanne, Switzerland; rnd.innovation@skylaboratory.ch
[3] Department of Chemistry, Lomonosov Moscow State University, 119991 Moscow, Russia; ilin.eg.2000@gmail.com
* Correspondence: filatovviktor097@gmail.com
† Presented at the 27th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-27), 15–30 November 2023; Available online: https://ecsoc-27.sciforum.net/.

Abstract: Skin dryness and xerosis are the most common clinical manifestations of different dermatological diseases. At the same time, it was established that the expression of aquaporin 3 (AQP3) is related to the pathogenesis of atopic dermatitis, psoriasis, eczema, and vitiligo. Thus, our study was focused on the search for new molecules and the investigation of their biological activity to accelerate the expression of AQP3 in the skin's epidermis. Aloin from an *Aloe barbadensis* leaf extract and trimethylglycine were chosen as new potential candidates using DiffDock computational modelling. These natural molecules demonstrated a good affinity towards the active site of AQP3 with an estimated docking score of −6.2 kcal/mol to −7.7 kcal/mol. Phyto4Health modelling predicted the anti-psoriatic, anti-inflammatory, and immunosuppressant activities that are useful in the treatment of atopic skin diseases. Furthermore, it was shown that the combination of the *Aloe barbadensis* leaf extract and trimethylglycine in a mass ratio of 1:1 revealed a clear synergetic effect to increase the AQP3 amount up to two times. Thus, the combination of the *Aloe barbadensis* extract standardized for aloin and trimethylglycine has a promising potential in drug development and the treatment of dryness.

Keywords: aloe vera; trimethylglycine; aquaporins; skin hydration; synergy; docking

Citation: Filatov, V.; Varava, A.; Ilin, E. Molecular Modelling and In Vitro Research of New Substances for the Targeted Stimulation of AQP3 in Skin. *Chem. Proc.* **2023**, *14*, 51. https://doi.org/10.3390/ecsoc-27-16088

Academic Editor: Julio A. Seijas

Published: 15 November 2023

1. Introduction

Skin performs its functions only if its barrier layers are not damaged, and sufficient transepidermal water flow is provided. It was established that skin xerosis is the most common clinical manifestation of different dermatological diseases, such as atopic dermatitis, eczema, psoriasis, and vitiligo [1]. The prevalence of dry skin by age group in Europe was up to 56% among elderly people and up to 75% of the entire population [1]. Research results show that clinically recommended emollients targeted at skin hydration and the recovery of epidermal lipid barrier are not sufficient in the treatment of atopic skin diseases and may make skin dryer [2].

Skin performs its functions only if its barrier layers are not damaged, and sufficient transepidermal water flow is provided. Water is vital for the natural functioning and healthy appearance of the skin, as skin is the largest external organ of the body with three separate layers: the epidermis, dermis, and hypodermis. Skin hydration is the result of the interaction of three basic mechanisms: the corneal layer and its barrier role in relation to water loss, natural moisturizing factor (NMF), including several hygroscopic molecules to maintain corneocyte hydration, and water transportation channels, such as aquaporin type 3 (AQP3). AQP3 provides the transport of water, glycerol, and natural moisturizing

factor molecules increasing skin hydration, the proliferation of keratinocytes, and wound healing [3]. AQP3 is an essential element for sufficient skin hydration [4].

It was found out that skin aging is related to a decrease in the moisture of the skin, increased TEWL parameters, and a decreased amount of aquaporin type 3 (AQP3) in epidermal cells [5]. Meanwhile, it was established that the expression of aquaporin 3 (AQP3) is related to the pathogenesis of atopic dermatitis [6], psoriasis [7], chronic skin irritation [8], and vitiligo [9]. In the pathogenesis of psoriasis, a decrease in the amount of AQP3 due to an excessive immune reaction is indicated [10].

The stimulation of AQP3 gene expression and protein translocation can be a potential mechanism to prevent and treat these dermatological diseases. Due to the important role of AQP3 in the skin and the need to regulate the AQP3 amount in epidermal skin, the search for an innovative plant-based combination for long-term skin moisturizing, keratinocyte proliferation, and skin barrier function maintenance remains urgent [11].

Plant-based substances, such as essential oils or plant extracts, have comprehensive compositions, multiple properties, different activities, and a low irritancy potential [12]. Extracts of various plants are irreplaceable components in dermatological products due to their anti-inflammatory, antioxidating, moisturizing, and protective effects [13]. It was discovered by the authors that the most promising substance for the treatment of skin xerosis is *Aloe barbadensis* leaf extract due to the richness of biologically active molecules [14] and [15].

2. Materials and Methods

2.1. Chemicals and Materials

Aloe barbadensis leaf extract (CAS 85507-69-3) standardized in aloin content and trimethylglycine (CAS 107-43-7), were purchased from Sigma-Aldrich (Sigma Chemical Co., Ltd., St. Louis, MO, USA). Glyceryl glucoside Hydagen® Aquaporin was purchased from BASF (BASF Personal Care and Nutrition GmbH, Monheim, Germany). All chemicals used in the in vitro research were of analytical grade. Keratinocytes HaCaT and the Aquaporin 3 ELISA kit were purchased from MatTek (MatTek Europe, Bratislava, Slovakia).

2.2. Ligand and Target Preparation

The AQP3 protein (PDB ID: 3LLQ) (Figure 1) was used to fit the three-dimensional structure of the AQP3. The protein was obtained in the.pdb format from the Protein Data Bank [16]. Using AutoDock version 4.2, the protein model was prepared by eliminating water molecules, cutting out superfluous chains, and adding polar hydrogen and charges. After processing, the protein structure was saved in the .pdb and .pdbqt formats for additional in silico study.

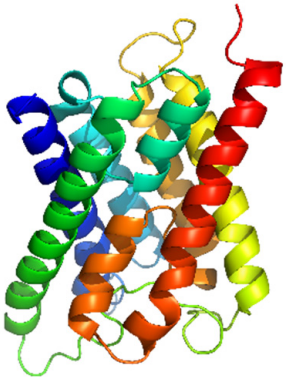


Figure 1. 3D structure of AQP3 from Protein Data Bank.

2.3. Molecular Docking of Phytochemicals with AQP3

A personal computer (PC) with an Intel Core i7-12700U CPU running at 2.3 GHz and 16 GB of RAM was the tool used for this purpose. Windows 11, 64-bit OS, was the operating system that was used. First, the native protein ligand was used in the molecular docking process to confirm that the procedure was consistent and the root mean square deviation (RMSD) was less than 2 Å. The coordinates of the grid were (X, Y, Z) 28.73, 58.834, 63.068 and the grid box was 40 × 40 × 40. The ligand was flexible, and the macromolecule remained rigid during the docking process. AQP3 (PDB ID: 3LLQ) was docked with 10 molecules and explored using AutoDock version 4.2. The molecular docking was carried out by modifying the parameter of the genetic algorithm (GA), using ten runs of the Lamarckian GA criterion.

2.4. Drug-Likeness Activity

The drug-likeness analysis was carried out with Phyto4Health (https://www.way2drug.com/p4h/ (accessed on 15 October 2023)) to predict the biological activities of natural molecules of plant origin [17]. This database contains information about more than 9000 phytoconstituents from different medical plants and herbs. All phytocompound structures are presented in InChI, InChi Key Canonical SMILES formats. This in silico program is able to predict the affinity of ligands to targets, biological activity with approximate PASS effects, and compare the physicochemical properties of molecules, such as hydrogen bond donors (HBD), the number of hydrogen bond acceptors (HBA), the number of rotatable bonds (RTB), polar surface area (PSA), and the octanol-water partition coefficient (AlogP).

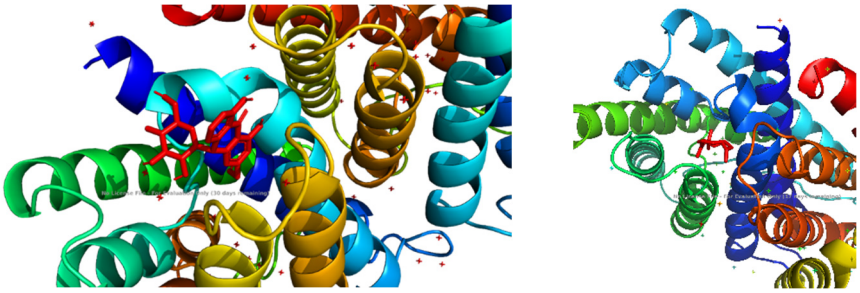
2.5. In Vitro Research of the Amount of AQP3 in Epidermal Cells

To determine the amount of AQP3 in epidermal cells, a commercially available kit with the sandwich-type enzyme-linked immunoassay was used (MatTek Europe, Bratislava, Slovakia). Cells of the HaCaT line were seeded in 96-well plates (96 Well EDGE Cell Culture Plates, Nest Scientific Biotechnology, Wuxi, China) at a concentration of 1×10^5 cells per well. On the next day, the cell media was removed and replaced with fresh DMEM media + 5% FBS 50 µL to maintain cell growth. Then, 50 µL of samples of the composition were added, and cultivation was performed for 24 h. After incubation, the cell supernatant was collected, and the amount of AQP3 in skin keratinocytes was determined by ELISA assay. The standards and samples were added to the corresponding microplate wells with specific biotin-conjugated AQP3 antibodies. Horseradish peroxidase-conjugated avidin was then added to each microplate well and incubated. After the addition of TMB substrate solution to each microplate well, the enzyme-substrate reaction was stopped by adding a sulfuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450 ± 10 nm. There was a negative control (purified water) and a positive control (glyceryl glucoside).

3. Results and Discussion

3.1. Molecular Docking with AQP3

Using AutoDock computational modelling to predict the affinity for skin AQP3, aloin from an *Aloe barbadensis* leaf extract (Figure 2b) and trimethylglycine (Figure 2a) were chosen as new potential candidates. These natural molecules demonstrated a good affinity towards the active site of AQP3 with an estimated docking score of −6.2 kcal/mol to −7.7 kcal/mol. To improve the affinity and stabilize the structure of AQP3, trimethylglycine could be useful as natural osmolyte. The glyceryl glucoside as a positive control had a moderate affinity with an estimated docking score −4.0 kcal/mol.



(**a**) (**b**)

Figure 2. (**a**) Detailed docking of AQP3 with aloin from an *Aloe Barbadensis* leaf extract; (**b**) detailed docking of AQP3 with trimethylglycine (the targeted molecules are visualized in a red color to show the binding with AQP3).

3.2. Drug-Likeness Activity

Thorough Phyto4Health modelling to predict the pharmacological properties, it was established that aloin from *Aloe barbadensis* leaf extract and trimethylglycine could have anti-psoriatic, antioxidant, anti-inflammatory, and immunosuppressant activities that are useful in the treatment of atopic skin diseases [17]. The main biological activities useful for skin disorders and skin hydration are presented in Table 1.

Table 1. Biological activities of Aloin and Trimethylglycine using the Phyto4Health database.

Compound	PASS Activities Type	Pa Value
Aloin	Antioxidant	0.676
	Anti-inflammatory	0.674
	Immunosuppressant	0.524
Trimethylglycine	Antieczematic atopic	0.806
	Anti-psoriatic	0.570
	Antioxidant	0.259

3.3. In Vitro Research of the Amount of AQP3 in Epidermal Cells

It was revealed that the addition of the *Aloe barbadensis* leaf extract standardized for aloin, trimethylglycine, and the combination of both of these phytochemicals in a 1:1 mass ratio to the epidermal cells increased the AQP3 amount (Table 2). The combination of *Aloe barbadensis* leaf extract and trimethylglycine increased the amount of AQP3 to 12.21 ± 0.91 ng/mL compared to the negative control—5.58 ± 0.24 ng/mL. The glyceryl glucoside in a mass concentration of 1% increased the amount of AQP3 in epidermal cells, but this influence was less than that of the novel plant-based combination.

Table 2. The effects of the compounds on the amount of AQP3.

Compound	AQP3, ng/mL	Changes Compared with the Negative Control, %
Negative control (purified water)	5.58 ± 0.24	-
Aloe barbadensis leaf extract with aloin, 1.0 weight %	6.73 ± 0.69 *	+20.61% *
Trimethylglycine, 1.0 weight %	6.58 ± 1.08	+17.92%
Aloe barbadensis leaf extract and trimethylglycine in a 1:1 mass ratio, 1.0 weight %	12.21 ± 0.91 **	+118.82% ***
Glyceryl glucoside, 1.0 weight %	6.89 ± 082 *	+23.48% *

Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4. Conclusions

A novel plant-based combination, with the investigated AQP3 targeted activity, was developed for the treatment of skin xerosis. The combination of *Aloe barbadensis* leaf extract and trimethylglycine in a 1:1 mass ratio resulted in a significant increase in the amount of AQP3 in epidermal cells compared to the negative and positive controls. Thus, the investigated plant-based substance has a promising potential for the treatment of skin disorders. However, additional research regarding toxicity, dermal tolerance, allergic potential, and clinical efficiency is needed to confirm this activity and the beneficial effect on the skin.

Author Contributions: Conceptualization, V.F. and A.V.; methodology, V.F.; software, V.F.; validation, V.F.; formal analysis, V.F.; investigation, V.F., A.V. and E.I.; resources, V.F., A.V. and E.I.; data curation, V.F.; writing—original draft preparation, V.F., A.V. and E.I.; writing—review and editing, V.F., A.V. and E.I.; visualization, V.F. and E.I.; supervision, V.F.; project administration, V.F.; funding acquisition, V.F. and A.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by SkyLab AG (Switzerland; 03-ZP21-122, 03-ZP22-122; 03-ZP22-182).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors are grateful to SkyLab AG for their administrative support and partial funding of the experiments.

Conflicts of Interest: The authors declare no conflict of interest. The SkyLab AG had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. The funder, SkyLab AG had a role in the funding. V.F. and A.V. are employees of SkyLab AG.

References

1. Augustin, M.; Kirsten, N.; Körber, A.; Wilsmann-Theis, D.; Itschert, G.; Staubach-Renz, P.; Maul, J.T.; Zander, N. Prevalence, predictors and comorbidity of dry skin in the general population. *J. Eur. Acad. Dermatol. Venereol.* **2019**, *33*, 147–150. [CrossRef] [PubMed]
2. Pohar, R.; McCormack, S. *Emollient Treatments for Atopic Dermatitis: A Review of Clinical Effectiveness, Cost-Effectiveness, and Guidelines [Internet]*; Canadian Agency for Drugs and Technologies in Health: Ottawa, ON, Canada, 2019.
3. Tricarico, P.M.; Mentino, D.; De Marco, A.; Del Vecchio, C.; Garra, S.; Cazzato, G.; Foti, C.; Crovella, S.; Calamita, G. Aquaporins Are One of the Critical Factors in the Disruption of the Skin Barrier in Inflammatory Skin Diseases. *Int. J. Mol. Sci.* **2022**, *23*, 4020. [CrossRef] [PubMed]
4. Bollag, W.B.; Aitkens, L.; White, J.; Hyndman, K.A. Aquaporin-3 in the epidermis: More than skin deep. *Am. J. Physiol. Cell Physiol.* **2020**, *318*, 1144–1153. [CrossRef]
5. Ikarashi, N.; Kon, R.; Kaneko, M.; Mizukami, N.; Kusunoki, Y.; Sugiyama, K. Relationship between Aging-Related Skin Dryness and Aquaporins. *Int. J. Mol. Sci.* **2017**, *18*, 1559. [CrossRef] [PubMed]
6. Boury-Jamot, M.; Sougrat, R.; Tailhardat, M.; Le Varlet, B.; Bonté, F.; Dumas, M.; Verbavatz, J.M. Expression and function of aquaporins in human skin: Is aquaporin-3 just a glycerol transporter? *Biochim. Biophys. Acta* **2006**, *1758*, 1034–1042. [CrossRef] [PubMed]
7. Qin, H.; Zheng, X.; Zhong, X.; Shetty, A.K.; Elias, P.M.; Bollag, W.B. Aquaporin-3 in keratinocytes and skin: Its role and interaction with phospholipase D2. *Arch. Biochem. Biophys.* **2011**, *508*, 138–143. [CrossRef] [PubMed]
8. Ikarashi, N.; Ogiue, N.; Toyoda, E.; Kon, R.; Ishii, M.; Toda, T.; Aburada, T.; Ochiai, W.; Sugiyama, K. Gypsum fibrosum and its major component $CaSO_4$ increase cutaneous aquaporin-3 expression levels. *J. Ethnopharmacol.* **2012**, *139*, 409–413. [CrossRef] [PubMed]
9. Kim, N.-H.; Lee, A.-Y. Reduced aquaporin3 expression and survival of keratinocytes in the depigmented epidermis of vitiligo. *J. Investig. Dermatol.* **2010**, *130*, 2231–2239. [CrossRef] [PubMed]
10. Choudhary, V.; Olala, L.O.; Kagha, K.; Pan, Z.Q.; Chen, X.; Yang, R.; Cline, A.; Helwa, I.; Marshall, L.; Kaddour-Djebbar, I.; et al. Regulation of the Glycerol Transporter, Aquaporin-3, by Histone Deacetylase-3 and p53 in Keratinocytes. *J. Investig. Dermatol.* **2017**, *137*, 1935–1944. [CrossRef] [PubMed]

11. Hara, M.; Ma, T.; Verkman, A.S. Selectively reduced glycerol in skin of aquaporin-3 deficient mice may account for impaired skin hydration, elasticity and barrier recovery. *J. Biol. Chem.* **2002**, *277*, 46616–46621. [CrossRef] [PubMed]
12. Yap, P.S.X.; Yang, S.K.; Lai, K.S.; Lim, S.H.E. Essential Oils: The Ultimate Solution to Antimicrobial Resistance in *Escherichia coli*? In *Recent Advances on Physiology, Pathogenesis and Biotechnological Applications*, 1st ed.; Samie, A., Ed.; IntechOpen: London, UK, 2017; Volume 15, pp. 299–313.
13. Ribeiro, A.S.; Estanqueiro, M.; Oliveira, M.B.; Sousa Lobo, J.M. Main Benefits and Applicability of Plant Extracts in Skin Care Products. *Cosmetics* **2015**, *2*, 48–65. [CrossRef]
14. Sánchez, M.; González-Burgos, E.; Iglesias, I.; Gómez-Serranillos, M.P. Pharmacological Update Properties of Aloe Vera and its Major Active Constituents. *Molecules* **2020**, *25*, 1324. [CrossRef] [PubMed]
15. Arumugam, M.K.; Paal, M.C.; Donohue, T.M., Jr.; Ganesan, M.; Osna, N.A.; Kharbanda, K.K. Beneficial Effects of Betaine: A Comprehensive Review. *Biology* **2021**, *10*, 456. [CrossRef] [PubMed]
16. Huang, Y.H.; Rose, P.W.; Hsu, C.N. Citing a Data Repository: A Case Study of the Protein Data Bank. *PLoS ONE* **2015**, *28*, 10. [CrossRef] [PubMed]
17. Ionov, N.; Druzhilovskiy, D.; Filimonov, D.; Poroikov, V. Phyto4Health: Database of Phytocomponents from Russian Pharmacopoeia Plants. *J. Chem. Inf. Model.* **2023**, *63*, 1847–1851. [CrossRef] [PubMed]