M89, A DERMOCOSMETIC COMBINING 89% VICHY MINERAL WATER AND HYALURONIC ACID, DOES NOT MODIFY CUTANEOUS PENETRATION OF TOPICAL IVERMECTIN *EX VIVO*

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INTRODUCTION

Rosacea is a chronic inflammatory dermatosis with an unpredictable course and the characteristic features of persistent erythema associated with periodic intensification or 'flares'.[1-3]

Clinical trials with ivermectin 1% cream have demonstrated greater rapidity and overall superiority in decreasing inflammatory lesions when compared to vehicle in papulopustular rosacea.[1,4]

Mineral 89 (M89, Vichy Laboratoires) contains 89% of Vichy mineral water (VMW) recognized to be a volcanic mineralizing water, and 0.4% hyaluronic acid (HA), an extracellular matrix component with viscoelastic and hygroscopic properties, and is able reinforce skin barrier and improve signs and symptoms in rosacea among other indications.[5]

VMW is highly enriched in minerals which strengthen the skin's natural defenses, e.g. restoring the skin barrier, and enhancing innate immunity.[5-9] In vitro and in vivo studies showed that VVW induced a statistically significant increase ($p<0.05$) in catalase enzyme activity.[10] These primary antioxidant enzymes convert 2 potentially harmful reactive oxygen species: superoxide and hydrogen peroxide, to water.[6, 11] It has also been shown to have anti-inflammatory activity.[8, 12]

In vitro and *in vivo* studies on M89 and its ingredients have confirmed its potential to reinforce skin barrier function and even accelerate skin barrier recovery.[13] Salsberg *et al.* showed that M89 was very well tolerated, with no reported safety issues and was very much appreciated by subjects with sensitive skin and investigators.[14]

Moreover, results from a large, international observational study showed that M89 significantly improves skin signs and symptoms after 4 weeks of continued use with no tolerance issues in subjects with dermatological conditions such as rosacea, sensitive and reactive skin, as well as in subjects who had recently undergone esthetic procedures. M89 was well-tolerated and had high patient satisfaction.[15]

AIM

This *ex vivo* assay assessed the skin absorption and distribution in the skin of ivermectin 1% applied before or after M89 application.

METHODS

The assay was conducted using full thickness human skin samples from 3 donors (2 women, 1 man), who underwent abdominal plastic surgery. Subcutaneous fat was removed before storage of the skin samples at −20°C. The skin was thawed at room temperature and cleaned with distilled water. Transepidermal water loss (TEWL) was measured after about one hour of setting on Franz cells and was between 0.5 and 5 g/m²/h confirming skin barrier integrity. Skin surface temperature was maintained at 32±1°C using a water bath connected to the diffusion cells. The application area was 2 cm² and the receptor compartment contained 7 mL of PBS 0.01M pH 7.4 + 0.25% Tween® 80 as receptor fluid. Receptor fluid was stirred continuously during the assay. Each formulation was spread on the skin sample using a glass rod and with a 30 second-massage. The experimental design is given in Table 1.

Each condition was tested on 2 replicates for the 3 skin donors under non-occlusive conditions. Twenty-four hours after application, formulations that remained on the skin surface were removed with 2 kimwipes (2 x 2 cm) wetted with PEG 400 / ethanol (70/30 v/v) and skin surface was dried with 3 kimwipes (2 x 2 cm). Cells were dismantled, receptor fluid was sampled and *stratum corneum* was collected by tape-stripping. Skin corresponding to the application area was collected. Samples were stored at -80°C until analysis. Samples were analyzed using an HPLC method with MS/MS detection.

RESULTS

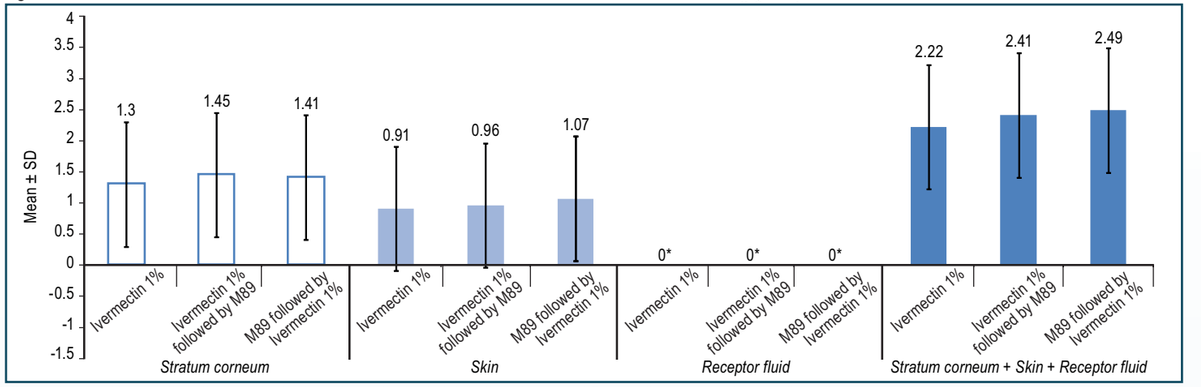
Ivermectin was not detected in the receptor fluid (LOD 1.5 ng/mL) and mainly found in the *stratum corneum*.

The mean total ivermectin amount in the *stratum corneum*, skin and receptor fluid was 2.22 µg/cm² (representing 4.15% of the applied dose) after application of ivermectin alone, 2.41 µg /cm² (4.54%) after application of ivermectin followed by M89 and 2.49 µg /cm² (4.83%) after application of M89 followed by ivermectin (Figure 1).

Table 1 **EXPERIMENTAL ASSAY DESIGN**

Condition	• Application of ivermectin 1% cream	• Application of ivermectin 1% cream • 20-minutes delay between applications • Application of M89	• Application of M89 • 20-minutes delay between applications • Application of ivermectin 1% cream
Total amount of ivermectin 1%/cm²	5 mg		
Total amount of M89/cm²	none	5 mg	
Skin thickness	Full skin thickness		
Number of cells per donor	2	2	2
Number of donors	3	3	3
Total number of cells	18	18	18
Receptor fluid	PBS 0.01M pH7.4 (data given by the supplier) + 0.25% tween 80		
Receptor fluid sampling	total volume of receptor fluid : 7 mL; collected after 24 hours		
Washing and dismantling of cells	24h		
Washing of formulations	2 kimwipes (2 x 2 cm) wetted with PEG 400 / EtOH (70/30 v/v) and 3 dried kimwipes (2 x 2 cm) or equivalent		
Strips	20* strips Polypropylene adhesive tape roll 3M (Sensitive®) size 19*5mm were performed on each skin. Strips were pooled by 4 (1-4, 5-8, 9-12, 13-16, 17-20).		
RCD extraction solvent	MeOH		

Figure 1 **CUTANEOUS DISTRIBUTION OF IVERMECTIN (µg/cm²)**



**Concentration of ivermectin in the receptor fluid was below the limit of detection - Number of Franz cells per application condition: 6*

CONCLUSIONS

Cutaneous absorption of topical ivermectin is not affected by the application of M89. M89 may be a beneficial adjuvant to current treatment of patients with papulo-pustular rosacea.

REFERENCES

1. Feldman SR, Huang WW, Huynh TT. Current drug therapies for rosacea: a chronic vascular and inflammatory skin disease. J Manag Care Spec Pharm. 2014;20(6):623-9.
2. Del Rosso JQ, Tanghetti EA, Baldwin HE, Rodriguez DA, Ferrusi IL. The Burden of Illness of Erythematotelangiectatic Rosacea and Papulopustular Rosacea: Findings From a Web-based Survey. J Clin Aesthet Dermatol. 2017;10(6):17-31.
3. Tan J, Almeida LM, Bewley A, Cribier B, Dlova NC, Gallo R, et al. Updating the diagnosis, classification and assessment of rosacea: recommendations from the global ROSacea COnsensus (ROSCO) panel. Br J Dermatol. 2017;176(2):431-8.
4. Cardwell LA, Nyckowski T, Uwakwe LN, Feldman SR. Coping Mechanisms and Resources for Patients Suffering from Rosacea. Dermatol Clin. 2018;36(2):171-4.
5. Nusgens BV. [Hyaluronic acid and extracellular matrix: a primitive molecule?]. Ann Dermatol Venereol. 2010;137 Suppl 1:S3-8.
6. Burke KE. Mechanisms of aging and development-A new understanding of environmental damage to the skin and prevention with topical antioxidants. Mech Ageing Dev. 2018;172:123-30.
7. Hughes MC, Williams GM, Baker P, Green AC. Sunscreen and prevention of skin aging: a randomized trial. Ann Intern Med. 2013;158(11):781-90.
8. Nonotte I, Montastier C, Boisnic S, Branchet-Gumila MC, Breton L. Inhibitory effect of Lucas spring water on substance P induced inflammation in organ culture of human skin1998. 535-42 p.
9. Moyal D, Tricaud C, Pham DM, Ngyen QL. P2619 Efficacy of a spa water in preventing UVA-induced catalase degradation. Journal of the American Academy of Dermatology. 2006;AB190.
10. Bruneau F, Bernard D, Ragueneau N, Montastier C. Effect of Vichy water on catalase activity in the stratum corneum. Int J Cosmet Sci. 1996;18(6):269-77.
11. Witorsch RJ, Thomas JA. Personal care products and endocrine disruption: A critical review of the literature. Crit Rev Toxicol. 2010;40 Suppl 3:1-30.
12. Poli F, Flechet ML, Egasse I, Auffret N, Reveret A, Montastier C, et al. Soothing effect of Vichy thermal spa water after glycolic acid peelings: A clinical randomized study. Nouvelles Dermatologiques. 2003;22:501-6.
13. Tacheau C, Weisgerber F, Fagot D, Bastien P, Verdier MP, Liboutet M, et al. Vichy Thermal Spring Water (VTSW), a cosmetic ingredient of potential interest in the frame of skin ageing exposome: an in vitro study. Int J Cosmet Sci. 2018;40(4):377-87.
14. Salsberg J, Andriessen A, Abdulla S, Ahluwalia R, Beecker J, Sander M, et al. A review of protection against exposome factors impacting facial skin barrier function with 89% mineralizing thermal water. J Cosmet Dermatol. 2019 Jun;18(3):815-820.
15. Tan J, Spada J, Orlandi C, Kerscher M, Anfilova M, Abdulla S et al. Vichy mineralizing water with hyaluronic acid is effective and well tolerated as an adjunct to the management of various dermatoses and after esthetic procedures. J Cosm Dermatol.2019. In press.

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Conflict of interest: *J. Tan is a consultant for Vichy Laboratoires, D. Kerob is an employee of Vichy Laboratoires. I. Durand, S. Gregoire and L. Roussel-Berlier are employees of l'Oréal Research and Development and M. Breisse, E. Raynaud and N. Merle are employees of Eurofins ADME bioanalyses and performed the assay for Vichy Laboratoires.*

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