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CENTRE FOR SCIENCE AND RESEARCH

Magnolol-honokiol as a novel TRPV1 channel-modulating complex? Impact on skin sensitivity symptoms.

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INTRODUCTION

The thermoreceptor TRPV1 (capsaicin receptor) in known to mediate skin sensitivity including sensation of pain, itch, warmth and afferent functions to chemical stimuli. The aim of this study was to evaluate the effect of magnolol and honokiol complex (**MaHo**) on TRPV1 channel inhibition in order to check its ability to fight with sensitive skin symptoms.

MATERIALS AND METHODS

TRPV1 activity was performed on: HBE (Human Bronchial Epithelium), HaCaT (Keratinocytes) and PBMC (Canine Peripheral Blood Mononuclear Cells) by calcium assay in presence of MaHo complex. Two face care products (**day and night cream no. 2581 and 2582**) containing MaHo (at concentration 1%) was examined in two groups (25 and 20 volunteers, respectively) displaying sensitive skin according to Sensi Scale-10[1]. Severity of several skin parameters like i.e. elasticity and smoothness were evaluated in instrumental analysis. Tests were completed with self-evaluation questioner.

RESULTS

MaHo activated calcium permeable channels, including TRPV1. Traces appeared to be dose-dependent and stronger than after capsaicin usage. MaHo also caused increase in calcium levels in cells kept in no-calcium buffer. In efficacy study of day and night creams, the overall skin sensitivity according to Sensi Scale-10 was reduced by 67% and 88%, respectively. Also, reduction in melanin content as well as improvement in skin elasticity and smoothness were observed.

Evaluation of skin condition of night cream no. 2582

Table 1. **Skin sensitivity evaluated by patients according to SensiScale-10[1]**. Results demonstrate reduction in skin sensitivity symptoms in average by 88%.

Degree of overall skin irritation during the past 3 days before and at the end of the test (n=20); score >13 - sensitive skin			
D0	**D21**	**difference**	**% of reduction**
15,9	1,56	-14	88%

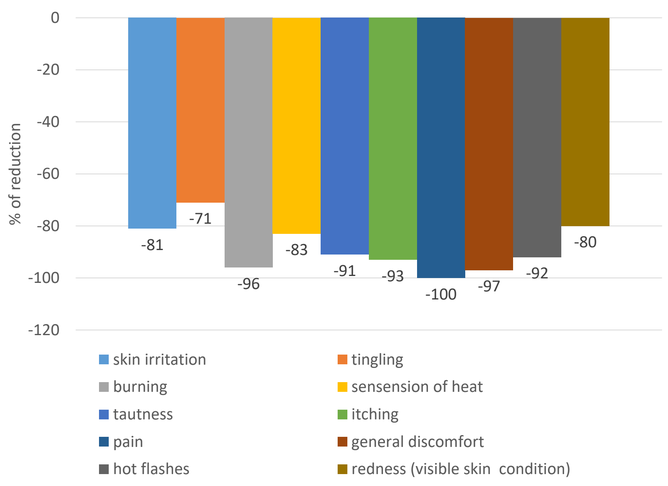
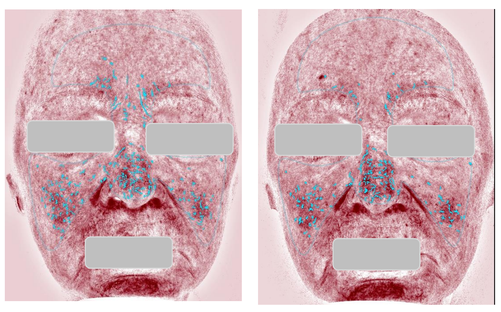


Table 2. **Instrumental evaluation of skin condition before and after 3 weeks of product usage**. Corner density, Sew and Volume were measured by Visioscan and UV spots and Red areas by Visia.

Instrumental evaluation of skin condition (n=11)	
Skin parameter	**Results**
Red areas	Reduction by 22% in 58% of volounteers and for whole group reduction by 9%
Corner Density – skin cross linking	Improvement by 12% in 58% of volounteers
Sew – wrinkles	Reduction in by 31% in 50% of volounteers
Volume – depth, volume and numer of irregularities	Improvement by 28% in 50% of volounteers and for whole group reduction by 5%
UV spots	Improvement by 11% in 50% of volounteers and for whole group reduction by 4%



Subject MK, age 67, reduction of red areas by 26%.

Figure 1. Visualisation of red areas by Visia before and after 3 weeks of product usage.

TRPV1 activation *in vitro*

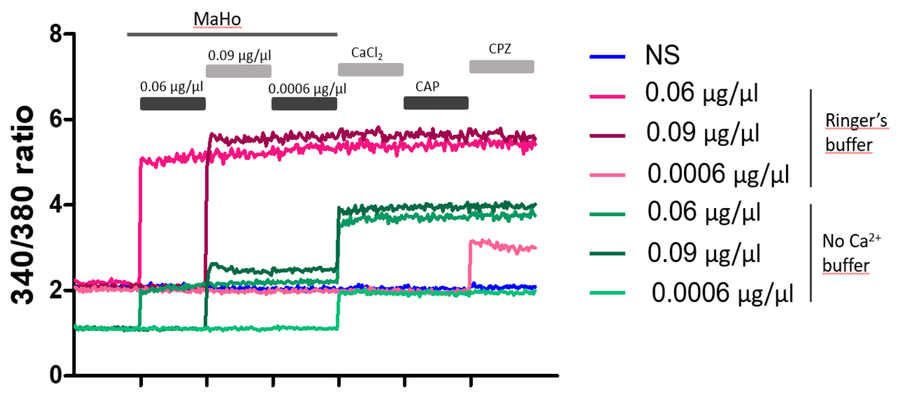


Figure 2. **Calcium assay in Ringer's solution and Ringer's without calcium as a control on HBE cell line.** Substances MaHo in different concentrations, receptor activator capsaicin (CAP, up to 20 µM), and receptor inhibitor capsazepine (CPZ, up to 30 µM). Fluorescent calcium indicator: Fura-2 AM (3 µM). **Significant increase in intracellular calcium levels was observed after administration of MaHo in concentrations: 0.06 µg/µL and 0.09 µg/µL in Ringer's buffer group and the increase (smaller) was also observed in no calcium buffer group – followed by further increase in the presence of 5 mM CaCl2. No effect was observed after administration of 0.0006 µg/µL, CAP (20µM into NS group) or CPZ (30 µM into NS and 0.06 µg/µL group).**

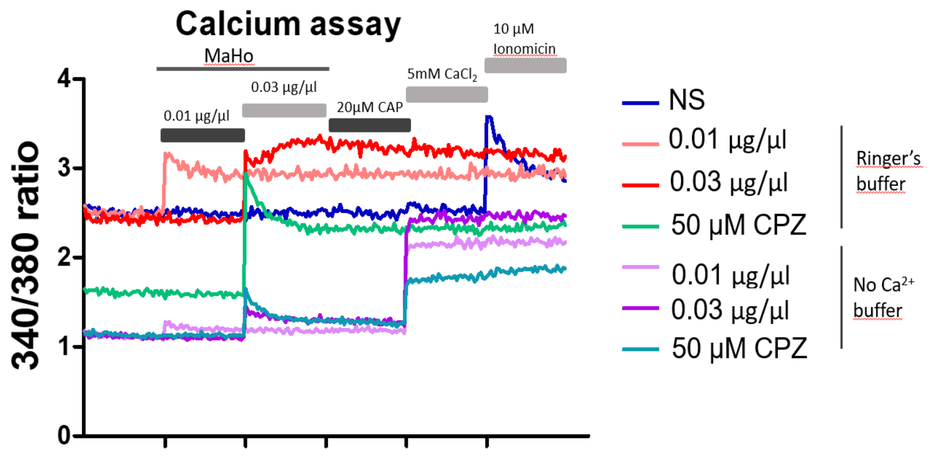


Figure 3. **Calcium assay in Ringer's solution and Ringer's without calcium as a control on HBE cell line.** Cells were pretreated with 50 µM CPZ for 30 minutes prior data acquisition (only groups denotes as 50 µM CPZ) additionally 0.03 µg/ µL of MaHo was added into CPZ pre-treated group during data acquisition. Concentration of 0.01 and 0.03 mg/ml elicit cell response in both ringer's and no-calcium buffer groups, no strong CAP response was detected. CPZ pretreatment did not inhibit the response completely - abberant calcium levels in ringer's group was observed and in no-calcium buffer, response was lower in CPZ pretreated group when compared to 0.03 mg/ml treated group alone. **HBE cells might express low levels of TRPV1 ion channel, which is in line with the available literature [2]. MaHo elicit cells response even in no-calcium buffer which indicate that observable effect might be an artifact not associated with calcium influx through calcium permeable ion channel. Such an effect was previously described in other studies [3]. Further increase of 340/380 ratio was observed after administration of calcium (CaCl2) in MaHo treated group, which suggests calcium dependent pathway associated with ion channel pathways (potentially TRPV1).**

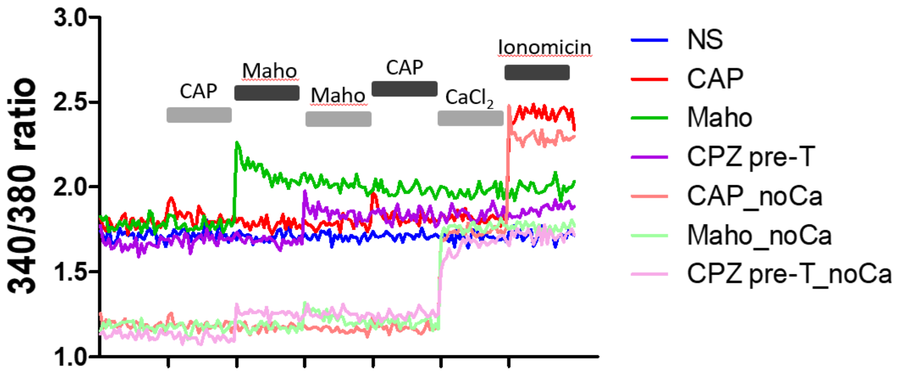


Figure 4. **Calcium assay in Ringer's solution and Ringer's without calcium as a control on HaCaT cell line.** CAP – 20 µM, Maho – 0,01 µg/µl, CPZ – 30 µM (pre-treatment, added 30 minut prior the acquisition), CaCl2 – 5 mM, Ionomicin – 1 µM. TRPV1 inhibitor Capsazepine (CPZ) partially abolished response to Maho in both Ringer's buffer and no-calcium buffer, cells appeared to respond to CAP (the response, however, was weak). **Cells respond strongly to 0.01 µg/µl of Maho – stronger response to Maho than to CAP might be due to difference in molarity or difference in BINDING affinity – Maho might have higher affinity to TRPV1 than CAP.**

Evaluation of skin condition of day cream no. 2581

Table 3. **Skin sensitivity evaluated by volountees according to SensiScale-10[1]**. Results demonstrate reduction in skin sensitivity symptoms in average by 67%.

Degree of overall skin irritation during the past 3 days before and at the end of the test (n=25) >13 - sensitive skin			
D0	**D21**	**difference**	**% of reduction**
17	5,56	-11,44	67%

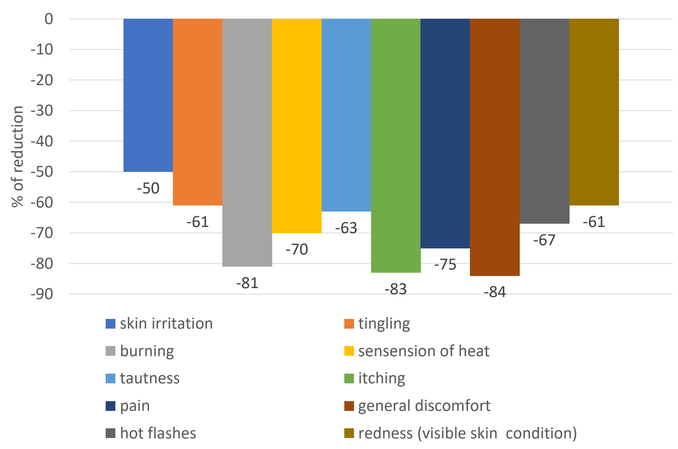


Table 4. **Instrumental evaluation of skin condition before and after 3 weeks test of product usage**. Melanin, moisturisation and elasticity were measured by Courage&Khazaka probes; Sesm, Ser and Volume measured by Visioscan.

Instrumental evaluation of skin condition (n=12)	
Skin parameter	**Results**
Melanin	Reduction by 9% in 50% of volounteers
Sesm – smothness with moisturisation	Improvement by 28% in 58% of volounteers
Ser - roughness	Reduction in by 20% in 50% of volounteers
Volume – depth, volume and numer of irregularities	Improvement by 20% in 75% of volounteers and for whole group reduction by 5%
Moisturisation	Improvement by 48% in 58% of volounteers and for whole group by 14%
Elasticity	Improvement by 17% in 58% of volounteers and for whole group by 5%
Firmness	Improvement by 52% in 83% of volounteers and for whole group by 38%

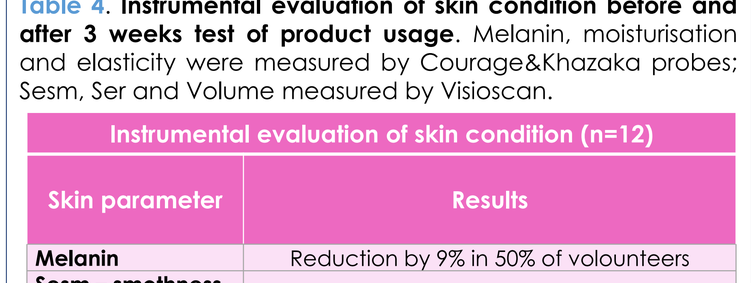


Figure 5. **Calcium assay on PBMC cell line.** Red trace – group treated with CAP and with Maho; Blue trace – group treated with Ionomicin. Result clearly shows that MaHo coused increse in calcium ions than CAP. Probably it activates strongly several channel at that same time or it interacts with Fura 2.

CONCLUSION

In conclusion, MaHo potentially increases intracellular calcium levels, which might be explained by activation of calcium-permeable ion channels, including TRPV1. Thus, It may behave similarly to capsaicin, which is known as "desensitizator" of TRPV channels. Moreover, *in vivo* study of creams with MaHo complex confirmed high efficacy in reducing sensitive skin's signs/ symptoms.

LITERATURE

1. Misery L, Loser K, Ständer S. Sensitive Skin. J Eur Acad Dermatol Venereol. 2016 Feb:30 Suppl 1:2-8
2. Harford TJ, Grove L, Rezaee F, Scheraga R, Olman MA, Piedimonte G. RSV infection potentiates TRPV1-mediated calcium transport in bronchial epithelium of asthmatic children. Am J Physiol - Lung Cell Mol Physiol [Internet] American Physiological Society; 2021 Jun 1 [cited 2022 Jun 13];320(6):L1074–L1084. PMID:33787326
3. Kopp RF, Leech CA, Roe MW. Resveratrol interferes with Fura-2 intracellular calcium measurements. J Fluoresc [Internet] NIH Public Access; 2014 Mar 1 [cited 2022 Jun 13];24(2):279. PMID:24151033