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PEER REVIEWED

Tetra-carboxy-methyl-naringenin-chalcone, a new active to treat rosacea

KEYWORDS: Tetra-carboxy-methyl-naringenin-chalcone, rosacea, cathelicidin, cytokines, blood flow reduction.

Abstract Tetra-carboxy-methyl-naringenin-chalcone (TCM-NC) is a new cosmetic active derived from naringenin, a flavanone naturally occurring in the peel of citrus fruit and tomato skin. The naringenin isomer naringenin-chalcone has been reported to have potent anti-allergic properties. For this reason the effect of TCM-NC was studied in an *in vitro* assay mimicking the inflammation pathway of rosacea skin and skin redness.
In rosacea skin, an overexpression and release of cathelicidin by surface keratinocytes leads to an inflammatory reaction in neighboring keratinocytes. If not treated, a chronic inflammation can develop inducing increased local blood flow and angiogenesis. In this paper, the inhibitory effect of the new TCM-NC on the cathelicidin-induced inflammatory reaction in human epidermal keratinocytes is reported. In a clinical study performed on volunteers with rosacea skin, TCM-NC clearly diminishes capillary blood flow and thereby the appearance of facial redness.

INTRODUCTION

Naringenin-chalcone is a polyphenol occurring naturally in plants e.g. the skin of tomato and citrus fruits. Recent studies have reported that naringenin-chalcone has potent anti-allergic and anti-inflammatory properties (1). The chalcone spontaneously isomerizes in aqueous solutions from the flavanone naringenin (Figure 1), which, however, exhibits only weak anti-allergic activity (1). The equilibrium between the chalcone and the flavanone depends on the pH of the solution, a condition not suitable for cosmetic formulations. Apart from this instability, naringenin-chalcone has a low solubility in water and other cosmetic solvents. Our first goal was to modify the naringenin-chalcone in order to block cyclisation and also to increase the solubility in water. To meet these challenges, tetra-carboxy-methyl-naringenin-chalcone (TCM-NC) (Figure 2) was designed and synthesized using the commercially available naringenin as a starting material. TCM-NC exhibits improved pH-stability and solubility in water. Having achieved this goal, we demonstrate the efficacy of TCM-NC on chronic skin inflammations, such as rosacea skin.

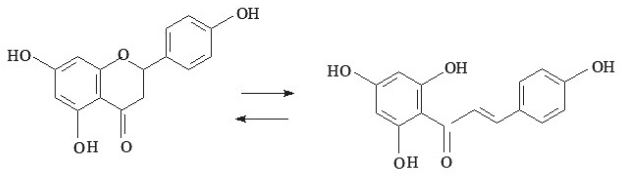

Figure 1. Equilibrium between Naringenin and Naringenin-chalcone

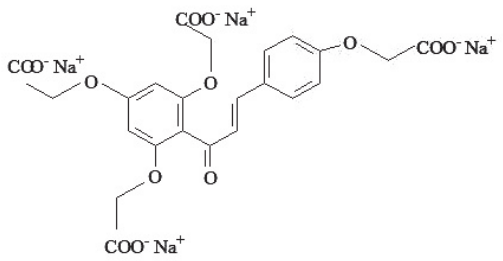

Figure 2. Sodium salt of Tetra-carboxy-methyl-naringenin-chalcone

Rosacea is a chronic inflammatory disease, characterized by transient or persistent central facial erythema, visible blood vessels, papules and pustules (2). Until recently the pathophysiology of this skin disorder was poorly understood and limited to trigger factors that induce or increase rosacea symptoms, such as UV radiation, heat, spicy food or stress (3). Recent studies by Yamasaki and coworkers show, that the antimicrobial peptide cathelicidin plays a major role in the development of rosacea (4). Cathelicidin is an inactive precursor peptide transformed by serine proteases of the kallikrein family to the biologically active form. The main peptide is the 37 amino acid long cathelicidin LL37 (5). In healthy skin, the cathelicidin LL37 level in the epidermis is barely detectable. However, upon skin injury or infection, the expression of cathelicidin is highly upregulated in in order to protect the damaged epidermis from microbe invasion (6). In addition to its antimicrobial activity,

Cathelicidin LL-37 plays a role in the activation and control of immune response: LL 37 triggers the release of pro-inflammatory metabolites such as cytokines and interleukins in mast cells and keratinocytes. Furthermore, it was shown to induce neovascularization (7). Interestingly, individuals with rosacea were shown to express abnormally high levels of cathelicidin and serine proteases in comparison to the skin of non-affected individuals (8). Injection of higher concentrations of cathelicidin LL37 into murine skin resulted in skin inflammation resembling pathological changes in rosacea and moreover, in hyper vascularity and vasodilatation. To summarize, recent research on rosacea reveals that the expression and processing of cathelicidin, the release of pro-inflammatory mediators and the sign of rosacea are linked.
In the present study we investigated the ability of the new molecule TCM-NC to decrease the amount of LL-37-induced-cytokines in keratinocytes. Furthermore, the anti-inflammatory activity of TCM-NC was demonstrated in a clinical study on volunteers with rosacea skin.

MATERIALS AND METHODS

Synthesis of tetra-carboxy-methyl-naringenin-chalcone (sodium salt)

In a first step, naringenin (5,7-dihydroxy-2-(4-hydroxyphenyl) chroman-4-one) is completely etherified using methylchloroacetate and potassium carbonate. This intermediate is transformed in a second hydrolytic reaction with sodium hydroxide to tetra-carboxy-methyl-naringenin-chalcone (2-(3,5-bis(carboxymethoxy)-4-((2E)-3-(4 carboxymethoxy)phenyl)prop-2-anoyl)phenoxy)acetic acid. This compound is dissolved in water, neutralized with sodium hydroxide and precipitated with ethanol to yield the TCM-NC sodium salt. TCM-NC is commercialized by Mibelle Group Biochemistry (Switzerland) under the trade name CM-Naringenin-Chalcone.

In vitro anti-inflammatory study

Normal human keratinocytes (NHEK) used at the 3rd passage, were seeded in 24-well plates in culture medium and incubated for 24 hours. The medium was then removed and replaced with the assay medium either containing the test compound (TCM-NC 0.033%) or nothing for control. After 24 hours of pre-incubation, the assay medium was replaced with medium containing the test compound and the stress-inducing factors LL-37 (20 µg/mL) + Calcitriol (10^{-7} M) + IL-17 (10ng/mL). The cells were then incubated for 48 hours. Non-stimulated controls were performed in parallel.
Afterwards, the cells were washed in phosphate buffered saline (PBS) solution and immediately frozen at -80°C. The expression of inflammation markers was analyzed using the RT-qPCR method.

Clinical vehicle-controlled anti-couperose study

An O/W emulsion containing 0.1% tetra-carboxy-methyl-naringenin-chalcone (or a vehicle) was formulated with water, glyceryl stearate (3%), stearic acid (2%), sodium stearoyl lactylate (2%) stearyl alcohol (1.2%), acetyl alcohol (1.2%) and phenoxyethanol (0.8%). The formulations (verum and vehicle) were tested in a double-blind half-face study on 11 female caucasian volunteers (Phototype I – III) aged 30 to 63 with visible rosacea on the cheekbones. The cream containing TCM-NC or the placebo were applied twice daily for 56 days, one to each side of the face. On days 28 and 56, capillary blood flow was determined by means of the DRT4® Laser Doppler device (Moor Instruments, UK) and photographic analysis of the face using the VisioFace® camera (Canon). All volunteers completed the study.

RESULTS AND DISCUSSION

In order to stabilize the highly active naringenin-chalcone and to increase the hydro solubility, the structure of the molecule was modified. The new cosmetic active,

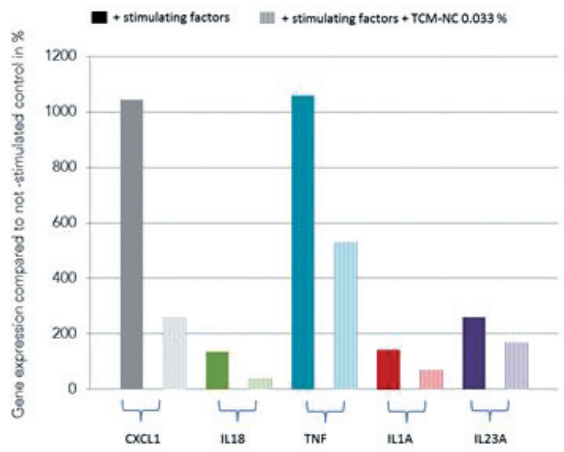

Figure 3. *in vitro* anti-inflammatory effect of TCM-NC (0.033%) under stressed conditions induced by LL-37, Calcitriol and IL17

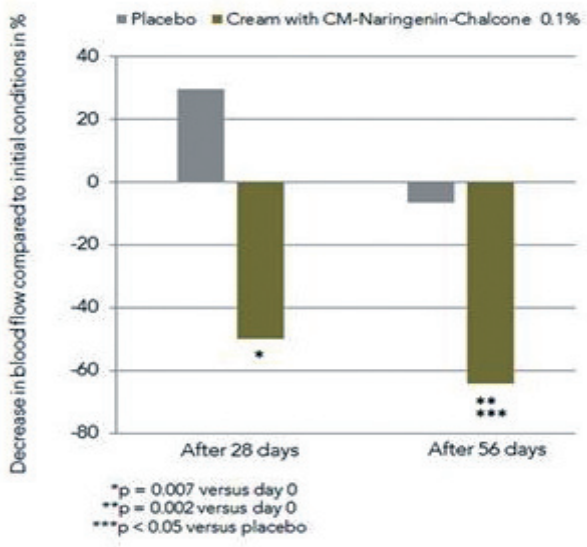

Figure 4. *in vivo* modulation of blood flow in the presence of TCM-NC (0.1%)

the tetra-carboxy-methyl-naringenin-chalcone was synthesized in high purity (90%) based on the natural flavanone naringenin. The TCM-NC shows water solubility greater than 10% without pH shift. The safety of the ingredient was attested in the standard tests used in the cosmetic industry (cell viability, human photo patch test, ocular irritation, reverse mutation assay). TCM-NC was tested up to 3.3% on keratinocyte viability and showed no effect on number or morphological modifications.

In a set of experiments, normal human keratinocytes were incubated with the stress stimulating factors cathelicidin LL-37, calcitriol and IL-17. Figure 3 shows that these stressors greatly enhance the expression and release of inflammatory mediators, such as CXCL1, IL23A, IL18, IL1A and TNF. The addition of 0.033% TCM-NC to the stressed keratinocyte culture however significantly decreases the amount of released cytokines (Figure 3). Thus TCM-NC exhibits anti-inflammatory properties by inhibiting the production of pro-inflammatory cytokines in keratinocytes.

In order to demonstrate the effect of the newly developed

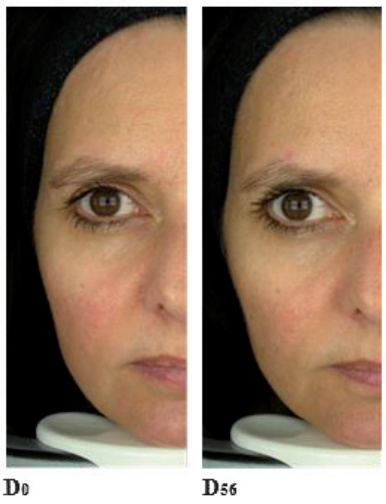

Figure 5. Decrease in skin redness (couperose) on the cheekbone following application of TCM-NC (0.1%) for 56 days

active *in vivo*, a placebo controlled clinical study was performed on 11 female Caucasian volunteers with rosacea skin. After 56 days of treatment with a cream containing TCM-NC, rosacea redness was visibly reduced compared to the untreated skin area (Figure 5). Statistical analysis reveals that the capillary blood flow is reduced by 57**%** compared to placebo (Figure 4).

CONCLUSIONS

The present work shows that tetra-carboxy-methyl-naringenin-chalcone is a promising new active ingredient for cosmetic and dermatological formulations with proven efficacy on rosacea skin. It exhibits anti-inflammatory properties by reducing the LL37 induced release of inflammatory cytokines. The newly developed compound was tested in a clinical study on volunteers with mild rosacea. It is shown to decrease capillary blood flow and visibly reduce facial redness. Thus, TCM-NC is an effective active ingredient suitable for sensitive and itchy skin, such as rosacea skin.

TCM-NC is a semisynthetic active derived from the natural product naringenin, which is isolated from orange peel. The modified molecule is stable and of improved solubility. This example demonstrates how natural products can be improved for application as cosmetic active ingredients.

REFERENCES AND NOTES

1. Yamamoto, T. et al. Anti-allergic activity of naringenin chalcone from a tomato skin extract. *Biosci Biotechnol Biochem* 68, 1706-11 (2004).
2. Crawford, G.H., Pelle, M.T. & James, W.D. Rosacea: I. Etiology, pathogenesis, and subtype classification. *J Am Acad Dermatol* 51, 327-41; quiz 342-4 (2004).
3. Buechner, S.A. Rosacea: an update. *Dermatology* 210, 100-8 (2005).
4. Yamasaki, K. & Gallo, R.L. The molecular pathology of rosacea. *J Dermatol Sci* 55, 77-81 (2009).
5. Zaiou, M., Nizet, V. & Gallo, R.L. Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. *J Invest Dermatol* 120, 810-6 (2003).
6. Dombrowski, Y. & Schauber, J. Cathelicidin LL-37: a defense molecule with a potential role in psoriasis pathogenesis. *Exp Dermatol* 21, 327-30 (2012).
7. Reinholz, M., Ruzicka, T. & Schauber, J. Cathelicidin LL-37: an antimicrobial peptide with a role in inflammatory skin disease. *Ann Dermatol* 24, 126-35 (2012).
8. Yamasaki, K. et al. Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nat Med* 13, 975-80 (2007).