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WATER AS ANTI-AGEING AGENT, THANKS TO OSMOLYTES FUNCTIONS.

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Eric Gasparotto, Julie Mercier, Loic Pentecouteau, Céline Laperdrix, Pierre-Yves Morvan & Romuald Vallée

CODIF Technologie Naturelle, 35400 Saint-Malo, France.

Introduction:

Osmolytes structure offer numerous free terminations –OH, and/or pairs of electrons in doublet (-O-; -S-; -N=) to stabilize proteins but also water molecules. Osmolytes family can be divided into three groups: (1) small-sized carbohydrates (trehalose, polyols, glycerol, inositol etc.); (2) amino-acids (glycine, proline, taurine etc.); (3) methylamines (glycine betaine). We focused on myoinositol and taurine, that are really conserved and the more represented in epidermal keratinocytes [1].

Myoinositol is an isomer from the simple sugar inositol (C6H12O6), presenting 6 hydroxyl terminations. It is named phytic acid in plant physiology. The sodium-dependent myoinositol transporter (SMIT) is known to be expressed by human keratinocytes under different stress conditions (osmolar stress and Ultra-Violet, UV). In another hand, taurine is a sulphur containing derivative from amino acid (NH_2-CH_2-CH_2-SO_3H). Literature indicates the presence of taurine and of its transporter (TAUT) in the epidermis and relates that the accumulation of taurine as one potential mechanism protecting epidermal keratinocytes from dehydration and oxydation [2]. Cultured human keratinocytes accumulated taurine in a concentration- and osmolarity-dependent manner. A high level of taurine protects cultures of keratinocytes from both osmotically induced and UV-induced apoptosis [3]. Ageing and especially photo-ageing is characterized by the deterioration of tissue structure and function. Reduction in keratinocyte cell size with age and downregulation of osmolytes transporters SMIT and TAUT with UV exposure were reported.

In this study we present results concerning a biotechnological active ingredient obtained from butterfly lavender (Lavandula stoechas) dedifferentiated cells.

Materials & Methods:

Obtaining of the extract:
The extract was obtained using propane-1-3-diol on butterfly lavender (Lavandula stoechas) dedifferentiated cells ground with high-pressure homogenizer. It was described as cell pulp for its viscous turbid liquid aspect.

Biological material:
2D cultures : All the experiments were performed on normal human keratinocytes cultivated in monolayer conditions (used in passage 2 to 3). The cells were cultivated over 3 to 5 days on a plastic support in the presence of complete growing environment, at 37°C, 5% CO2 and saturated humidity. The Lavandula stoechas extract was added (0.05% in culture medium) on cultures presenting 70% to 80% confluency.

3D cultures in CODIF reconstructed epidermis according to our knowledge.
Normal human epidermal keratinocytes (NHEK) from donor aged 7 year were used at third passage and seeded for two days in 12 mm diameter inserts with complete medium (EPI Life) at 37°C and 5% of CO2. Cells were air-lifted to induced differenciation in a multi-strata epidermis. Further to 18 days of incubation certain epidermises were pre-treated with the active substance for 24 hours. Then, at Day 19, the epidermises were stressed for 24 hours at 400 mosm before being set in formaldehyde for the preparation of histological sections. The histological sections were coloured with hematein-eosin (H.E.).

Molecular biology:
We analysed gene expression using the qPCR performed following the manufacturer's instructions and this various steps: RNA extraction using Qiagen's RNeasy Plus Mini ® kit, conversion of RNAm to DNAc using Invitrogen's Superscrip IV, real time DNAc amplification using Applied Biosystems's PowerUp SYBR Green Master Mix kit and primer couples to genes of interest, real time amplification is performed and monitored on Applied Biosystems's QuantStudio 3.
We analysed gene expression using the whole transcriptome method following the manufacturer's instructions : RNA extraction using Qiagen's RNeasy Plus Mini ® kit, amplification and labelling using Agilent's Low input Quick Amp Labeling® kit, Hybridisation of fragmented RNAc on the SurePrint G3 Human Gene Exp V3 chip (approx. 60,000 probes) performed on the Agilent hybridisation station, images of each SurePrint G3 Human Gene Exp V3 probe acquired using Agilent's SureScan DX Microarray Scanner. We used R statistical language and R Studio software for fold change analysis.

ELISA test:
For the ELISA analysis, the cell pellets were thawed and taken up in the assay buffer, sonicated and refrozen until freeze-drying. The lyophilisates were taken up in 1 ml of assay buffer and centrifuged. Quantification of taurine was performed using a taurine assay kit (Cell Biolabs, ref. MET- 5071) and the reading acquired using the software Fluostar OMEGA.

Results & Discussion:

In the presence of Lavandula stoechas extract, used at 0.05%, molecular analysis demonstrated increases of expression of 3 enzymes implicated in taurine metabolism: BAAT of +75% (Bile Acid-CoA: Amino Acid N-Acyltransferase), CDO1 of +94% (Cysteine Dioxygenase Type 1) and GADL1 of +125% (Glutamate Decarboxylase Like 1). In addition, the taurine receptor (TAUT) was also increased by +51% in presence of the active substance compared to the untreated cells (detection by qPCR).

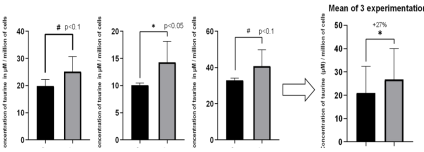

Figure 1:
ELISA quantitation of taurine in NHEK cultures after 400 mosm stress (+/- the Lavandula stoechas extract grey/black), 3 different experiments on different donors and mean of experiments.

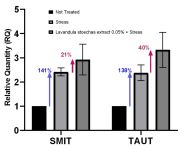

Figure 2:
SMIT and TAUT gene expression after 24 hours with the Lavandula stoechas extract treatment and 1 night of hyperosmolar stress on NHEK

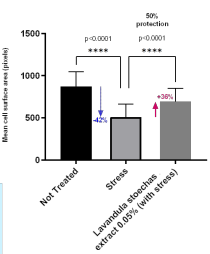

Figure 4:
Analysis of mean cell surface of human epidermal keratinocytes

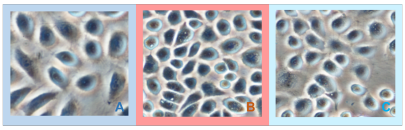

Figure 3:
Microscopic observation of the surface area of epidermal keratinocytes after 24 hours of the different treatments: A-Unstressed, untreated cells. B-stressed, untreated cells. C-stressed cells, pre-treated with the Lavandula stoechas extract used at 0.05%.

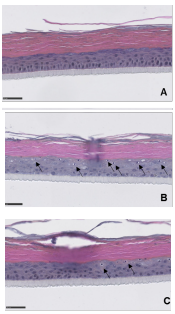

Figure 5:
Microscopic observations of pyknotic cells (arrows) in reconstructed human epidermis in different conditions: A-unstressed, untreated. B-stressed, untreated. C-stressed, pre-treated with the Lavandula stoechas extract (scale bar indicates 50µm).

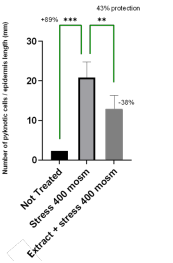

Figure 6:
Analysis of the number of pyknotic cells in the reconstructed human epidermis in different conditions (untreated, stressed and untreated or pre-treated with the Lavandula stoechas extract and then stressed (Experiment n=6).

First, using full transcriptomic and PCR analysis after 24h of contact on human keratinocytes, we demonstrated an increase of cluster genes linked to osmolytes metabolism SBAAT, CDO1, GADL1. It increased the transcription of taurine but also the expression of the corresponding protein by 27% (fig 1), even in osmotic stress conditions. Using RT-qPCR (fig 2), we demonstrated the improvement of TAUT and SMIT (osmolytes transporters) in parallel [2]. Thus, in a first point of view, the Lavandula stoechas extract could be presented as an active ingredient for hydration. Literature on osmolytes and taurine evoke also a decrease of synthesis and transport with ageing and environmental stress.

Secondly, after analysis of 3D models, we measured increases of cellular surface up to 36%*** (p<0.001) in condition of treatment (fig 3-4). If keratinocytes were put in dehydrated conditions, water lost corresponded to 42%*** and if they were also treated by the extract the loss of water was reduced by 50%, to reach only 21% of loss. The treatment with the extract induced a modification of cell surface and shape thanks to water retention and osmolytes function as described [1]. Reconstructed epidermis submitted to osmotic stress and treated with the extract exhibited a fully increased thickness of living epidermis but also of stratum corneum, a stimulated viability by 43%*** and an alteration of nuclei (pyknotic cells) reduced by 38%** (p<0.01), see fig 5-6. Those results indicates that there are not only water retention but also an improvement of keratinocytes metabolism and differentiation as well, to provide a better barrier function, even in stress conditions. This is definitely a sign of anti-ageing properties, as indicated by bibliography [6].

Taking the example of the development of our extract and its cosmetics properties, we demonstrated once again the importance of water management in skin cells biology, thanks to osmolytes function. Water is held surrounding cells to be available for all functions for viability, morphology, differentiation and protection, even in stress conditions exacerbating ageing dysfunctions.

Conclusions:

The importance of osmolytes in skin homeostasis was confirmed. Water management by osmolytes and by our active induced reduction of markers for stress and ageing. That's why water and its retention into cells or skin tissue could be considered as the first anti-ageing active. The extract that we developed from Lavandula stoechas stem cells acts on water management by osmolytes and particularly by taurine to induce anti-ageing properties.

References:

1. Warskulat U, Reinen A, Grether-Beck S, Krutmann J, Häussinger D (2004) The osmolytes strategy of normal human keratinocytes in maintaining cell homeostasis. J Invest Dermatol Sep;123(3):516-21. 2. Balou S, Adamaki M, Ioannou P, Pappa A, Panayiotidis MI, Spandidos DA, Christodoulou I, Kyriakopoulos AM, Zoumpourlis V (2021) Protective role of taurine against oxidative stress Mol Med Rep. Aug;24(2):605. 3. Jarekie G, Siefken W, Cantensen S, Sprigmann G, Bleck O, Steinhart H, Höger P, Wittern KP, Wenck H, Stäb F, Gauermann G, Schreiner V, Doering T (2000) Role of Taurine accumulation in keratinocyte hydration. J Invest Dermatol Aug;121(2):354-61. 4. Koppes SA, Kemperman P, Calkoen-Kwa E, Engebertsen KA, Puppels GJ, Caspers PJ, Kezic S (2017) Determination of natural moisturizing factors in the skin corneo microspectroscopy versus HPLC. Biomarkers Sep;22(6):502-507. 5. Tfayli A, Jamal D, Vyumvuhore R, Marfak M, Baillet-Guffroy A (2013) Hydration effects on the barrier function of stratum corneum lipids: Raman analysis of ceramides 2, III and 5. Analyst Nov 7;138(21):6302-8. 6. Tsatsou F, Trakatelli M, Patsatsi A, Kalokasidis K, Sotiriadis D (2012) Extrinsic ageing, Dermato-Endocrinology, 4:3, 285-297.