A NaDES extract of Granville Rose displays pro-resolving and epidermal strengthening properties

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Abstract

Background: Specialized Pro-resolving Mediators (SPMs) are a class of lipid mediators capable of counter-acting inflammation. We previously observed that skin SPM pattern was affected by aging. A natural deep eutectic solvent (NaDES) extract of «Jardin de Granville» Rose flowers was evaluated for its ability to increase biosynthesis of SPMs both in old and young skin biopsies and to improve epidermal homeostasis.

Methods: «Jardin de Granville» rose flower extract was obtained by solid-liquid extraction of freeze-dried rose flowers in a fructose/propanediol/water NaDES mixture. The phytochemical profile was studied using UHPLC-UV methods. Skin explants were pre-treated by topical application of 1% NaDES Rose extract, before being challenged by PMA. A profile of 24 molecules including SPMs as resolvins and precursors was analyzed using LC-MS/MS methods. Besides, gene expressions of proteins involved in keratinocyte metabolism were analyzed.

Results: Flavonoids and free amino acids including astragalin and asparagine were identified as characteristic tracers of the extract. Lipidomic analysis showed increased production of

D-series resolvins, lipoxins A4 and B4 in both young and old skin biopsies further to PMA challenge, thus demonstrating an ability of «Jardin de Granville» Rose NaDES extract to facilitate resolution, the active signal for stopping inflammation at all age. The extract also significantly increased expressions of genes relevant to skin barrier functions such as filaggrin, cornifin, cytokeratin 10, desmoglein, occcludin and aquaporins.

Conclusion: Taken together, these results highlight this «Jardin de Granville» Rose NaDES extract as a potent ingredient to help resolve inflammation in both young and old skins, while improving epidermal vital functions.

Keywords: Natural Deep Eutectic Solvent; «Jardin de Granville» Rose; Inflammation; Specialized Pro-resolving Mediators; Epidermal homeostasis

Introduction.

Inflammation is a local reaction towards a disturbance of tissue homeostasis caused by damage to tissue structure and infection. It is normally self-limited and ends with its complete resolution, which involves the production of a class of lipid mediators capable of counter-acting inflammation, namely Specialized Pro-resolving Mediators (SPMs). Derived from poly-unsaturated fatty acids, such as arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid and, their biosynthesis involves different members of the cyclooxygenase and lipoxygenase families. Increasing attention has been paid lately to skin-relevant SPMs such as Resolvin D-series or Lipoxins A4, that have been reported to improve wound healing and alleviate skin conditions such as psoriasis or contact dermatitis [1-3] Comparing the SPM patterns in old vs. young biopsies, we previously observed an altered responsiveness to pro-inflammatory challenge by Phorbol 12-myristate 13-acetate (PMA), with a switch towards LOX-5 mediated SPM syntheses [4]

Deep eutectic solvents (DES) were originally described as mixtures of organic compounds that have a much lower melting point than either of the individual components and are liquids at ambient temperatures. NaDES are a particular type of DES obtained using bio-based compounds. They are composed of two or more compounds that are made of hydrogen donors and acceptors. NaDES have been shown to offer unique extraction properties as shown by the unique phytochemical profile of extracts obtained from commonly used herbal remedies [5, 6].

A natural deep eutectic solvent (NaDES) extract of "Jardin de Granville" Rose flowers was developed and evaluated for its ability to increase biosynthesis of SPMs both in old and young skin biopsies and to improve epidermal structure, cohesion and differentiation.

Materials and Methods

Plant extraction The "Jardin de Granville" Rose ("Evanrat") is a variety of Garden Rose created in France by hybridization between the 'Auscent' and 'Dorblan' varieties. The extract of "Jardin de Granville" Rose flowers was obtained by solid-liquid extraction of 5% of freeze-dried rose flowers in a natural deep eutectic solvent (NaDES) mixture comprised of

fructose, propanediol and water, in a 1/1/3 molar ratio, under moderate stirring, followed by solid-liquid separation and successive filtration steps.

Phytochemical profiling Flavonoids were quantified in the extract using reverse-phase ultra-high pressure liquid chromatography method with diode array detection (RP-UHPLC-DAD) and astragalin standard for calibration (λ: 350 nm) [7,8]. Free amino-acids were quantified using UHPLC, using a derivatization kit for external calibration (AccQ-Tag Ultra, Waters, Saint-Quentin-en Yvelines).

Skin samples and treatment Abdominal normal human skin biopsies were obtained from Caucasian female donors (62, 56 and 24 years old) undergoing surgical procedure after informed consent, in accordance with the ethical guidelines of the Bank for Tissues and Cells (Edouard Herriot Civil Hospital of Lyon, France). Ten-mm punch biopsies were sampled, transferred to Snapwell™ culture inserts (Corning, Boulogne-Billancourt, France) and cultured at the air-liquid interface in Prime-3D medium (CELLnTEC, Bern, Switzerland) supplemented with normocin™ (Invivogen) at 37°C and 5% CO_2. Skin explants were topically pre-treated for 16h with the NaDES extract of «Jardin De Granville» Rose lowers at 1% in a gel formulation before being challenged with gel formulation containing PMA at 1.5% (Sigma) and then cultured for 2h, 4h, 8h, 24h and 48h. Untreated skin explants were used as internal control. At each time point, skin explants were harvested, frozen in liquid nitrogen and stored at -80°C until quantification of bioactive lipids.

Metabololipidomic analysis Extraction and LC-MS/MS analysis were performed by Ambiotis SAS (Toulouse, France) using confidential in-house Standard Operating Procedures adapted from Le Faouder et al. (J Chromatogr B Analyt Technol Biomed Life Sci. 2013, 1, 932:123-33). Briefly, tissues were lyzed and solid phase extraction was performed to extract bioactive lipids from skin explants. LC-MS/MS analysis was performed using UHPLC system (Agilent LC1290 Infinity, Agilent Technologies, Les Ulis, France) coupled to Agilent 6490 triple quadrupole MS (Agilent Technologies) equipped with electro-spray ionization operating in negative mode.

Cumulative results after 48h of culture were expressed as quantity of lipids in µg/mg of skin tissue. The area under the curve (AUC) was calculated using trapezoidal rule. Data were expressed as mean ± standard error to the mean (SEM) for 3 independent experiments.

Genomic analysis Sub-confluent monolayers of normal human epidermal keratinocytes (NHEK) were cultured for 24h in the presence of the NaDES extract of «Jardin de Granville» Rose flowers at 0.03%. The NaDES solvant alone was tested at the same concentration. Retinoic acid (RA) at 1µM was used as positive control. Total RNA was extracted using Nucleospin RNA kit (Macherey-Nagel™, Fischer Scientific, Illkirch, France). cDNA were obtained using High Capacity Reverse Transcription Kit (Thermo-Fisher) according to the manufacturer's instructions. RT-PCR was then conducted using TaqMan Low Density Array according to the manufacturer's instructions associated with ABI Prism 7900HT Sequence detection system. Results are expressed as mean from 3 independent experiments. Statistical analysis was carried out using Student's t test.

Results

Mostly flavonol-type flavonoids were detected and more particularly kaempferol and quercetin heterosides: astragalin, nicotiflorin, isoquercitrin, rutin, afzelin. Astragalin appeared as the main flavonoids, making up nearly 50% of total flavonoids with a concentration of 435 mg/kg. As far as amino acids were concerned, asparagine represented around 50% of the total amount with 519 mg/kg.

SPM analysis showed increased production of D-series resolvins in both young and old skin biopsies further to PMA challenge. More specifically, resolvin D3 was increased in all three biopsies, both RvD3 and RvD4 were boosted in the 56-year-old biopsy and both RvD1 and RvD3 in the 62-year-old skin biopsy. Interestingly, LXA4 and LXB4 were also significantly increased in the 24-year-old skin biopsy

PCR Array results showed that several genes involved in vital epidermal pathways were upregulated further to the treatment with the NaDES rose flower extract. Filaggrin, transglutaminase 1, kallikrein related peptidase 7, cornifin, ceramide synthase and cytokeratin 1, all of which are involved either in epidermal differentiation or in barrier maintenance, were upregulated. Keratinocyte cohesion proteins desmoglein, occludin, and

claudin-1were also upregulated. Aquaporin-3, -9 and -10 were also upregulated, indicating a possible role of the NaDES rose extract in skin hydration. Finally, the extract appeared to favor skin antioxidant defense by boosting expression of methionine sulfoxide reductase B1.

Discussion.

Inflammation plays a key role in tissue homeostasis and repair. Inflammation dysfunction is associated with several chronic diseases, including skin conditions such as psoriasis, atopic dermatitis or acne. The resolution of inflammation is an active process that normally allows the return to tissue homeostasis after injury. On the opposite, low-level chronic inflammation creates tissue imbalance and accelerate the aging process, a phenomenon known as inflamm'aging. Increasing attention is paid to Specialized Pro-resolving Mediators, which are potent anti-inflammatory lipid mediators derived from poly-unsaturated fatty acids. Amongst them, D-series resolvins have been reported to be involved in wound-healing, re-epithelialization and tissue repair. In particular, RvD1 has been shown to improve skin resistance to UV-induced oxidative stress and inflammation [7]. Lipoxins and particularly lipoxin A4 (LXA4) have recently emerged as potent resolution mediators in skin inflammation [8, 9].

In our study, the ability of a newly designed NaDES extract of "Jardin de Granville" Rose flowers to enhance resolution capacities of human skin biopsies of different ages were evaluated. The NaDES mixture used was made of fructose, propanediol and water, in a 1/1/3 molar ratio. The addition of water to the hydrogen bond donor and receptor that are propanediol and fructose, respectively, has been shown to facilitate handling while preserving their extraction properties [10]. Because NaDES are capable of extracting unique phytochemical profiles [11], we first paid attention the phytochemical profile of the NaDES extract of "Jardin de Granville" Rose flowers. Significant amounts of flavonoids and free amino acids were detected, amongst which astragalin and arginine accounted for the majority of each family (50%).

The pro-resolving properties of the NaDES extract of "Jardin de Granville" Rose flowers were evaluated on 3 skin biopsies from 3 different donors of different ages (24, 56 and 62

years old, respectively). Further to NaDES extract treatment, D-series resolvins were upregulated in all three biopsies, RvD3 being the one that was upregulated in all samples. Interestingly, RvD3 has been shown to downregulated acute inflammation, notably by downregulating cytokine production and reducing granulocyte infiltration [12]. Of note is also that, LXA4 and LXB4 were also significantly increased in the young skin biopsy. Given that LXA4 also has potent properties in resolving skin inflammation, the NaDES extract exhibits high potential to resume inflammation at an early stage and avoid chronic inflammatory status that may disturb skin homeostasis.

Skin represents a vital barrier against physical aggressions and water evaporation. For this, the epidermis plays the most crucial role. From that perspective, proper structure, cohesion, differentiation and cornification process are key. Likewise, from a cosmetic point of view, epidermal hydration and homeostasis are of great importance to preserve a healthy and youthful skin appearance. Our genomic data have shown that the NaDES extract of "Jardin de Granville" Rose flowers could increase expression of key players in epidermal differentiation, barrier formation, tissue cohesion and antioxidant defense. Not only are those markers important for epidermal homeostasis but also for tissue repair in case of an aggression or disruption. Since inflammation is the first physiological response to tissue injury, those data also appear to be complementary to the pro-resolving effect previously observed with the extract.

Conclusion. Combining phytochemical data on one side, with metabololipidomic and genomic data on the other side, we identified a new NaDES extract of "Jardin de Granville" Rose flowers as a potent protector of skin integrity backed by a unique phytochemical signature.

Conflict of Interest Statement.

NONE.

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