

A NaDES extract of Rose 'Jardin de Granville®' displays pro-resolving and epidermal strengthening properties

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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 (figure 1), 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 human healthy skin 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]. Because low-level chronic inflammation creates tissue imbalance and accelerates the aging process, a phenomenon known as inflammaging, the altered responsiveness observed in old skin biopsies may participate in the inflammaging process.

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 homeostasis.

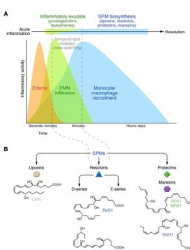


Figure 1: Acute inflammatory response and Specialized Pro-resolving Mediators (SPMs). C.N. Serhan and al., JCI2018

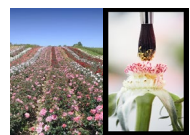


Figure 2: Rose 'Jardin de Granville®'

Results & Discussions:

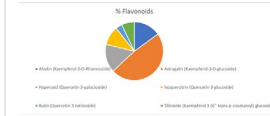


Figure 3: Flavonoids in NaDES FPW 113 Extract

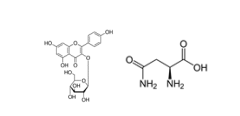


Figure 4: Astragalgin and Asparagin structures

Mostly flavonol-type flavonoids were detected, and Astragalgin appeared as the main flavonoids, making up nearly 50% of total flavonoids. As far as amino acids were concerned, asparagin represented around 50% of the total.

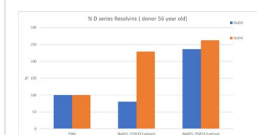


Figure 5: Serie D Resolvins RvD3 and RvD4

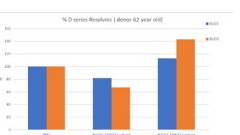


Figure 6: Serie D Resolvins RvD1 and RvD3

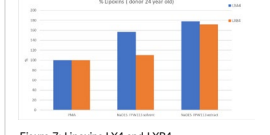


Figure 7: Lipoxins LX4 and LXB4

More specifically, resolvin D3 was increased in all three biopsies, both RvD3 and RvD4 were boosted in the 56-year-old biopsy (figure 5) and both RvD1 and RvD3 in the 62-year-old skin biopsy (figure 6). Interestingly, LX4 and LXB4 were also significantly increased in the 24-year-old skin biopsy (figure 7).

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

Materials & Methods:

Plant extraction & phytochemical profiling
 NaDES FPW113 extract is an extract of 'Jardin de Granville®' Rose flowers by 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. Flavonoids were quantified using reverse-phase ultra-high pressure liquid chromatography method with diode array detection (RP-UHPLC-DAD) and astragalgin standard for calibration (λ: 350 nm). Free amino-acids were quantified using UHPLC, using a derivatization kit for internal 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). Ten-mm punch biopsies were sampled, transferred to Snapwell® culture inserts (Corning, Bologne-Biellancourt, France) and cultured at the air-liquid interface in Prime-3D medium (CELLnTEC, Bern, Switzerland) supplemented with normocin™ (InvivoGen) at 37°C and 5% CO₂. Skin explants were typically pre-treated for 16h with the NaDES extract 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.

Metabolomic analysis
 Tissue samples were lysed 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 ± SEM for 3 independent experiments.

Genomic analysis
 Sub-confluent monolayers of normal human epidermal keratinocytes were cultured for 24h in the presence of the NaDES extract of 'Jardin de Granville®' Rose flowers at 0.03%. The NaDES solvent 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 (Machery-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.

Conclusions:

Combining phytochemical data on one side, with metabolomic 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. Indeed, significant amounts of flavonoids and free amino acids were detected in the fructose/propanediol/water (FPW113) extract of 'Jardin de Granville®' Rose flowers, amongst which astragalgin and arginine accounted for the majority of each family (50%).

D-series resolvins, particularly RvD3, were upregulated in skin biopsies treated with NaDES extract in inflammatory conditions (PMA). Interestingly, RvD3 has been shown to downregulate acute inflammation, notably by downregulating cytokine production and reducing granulocyte infiltration [7]. LX4, which has potent properties in resolving skin inflammation, was also significantly increased in the young skin biopsy. Our genomic data have shown that the NaDES extract of 'Jardin de Granville®' Rose flowers could also increase expression of key players in epidermal differentiation, barrier formation, tissue cohesion and antioxidant defense, all of which are involved in epidermal homeostasis and tissue repair.

Ultimately, the fructose/propanediol/water (NaDES FPW113) extract of 'Jardin de Granville®' Rose flowers has demonstrated great potential in vitro and ex vivo to support epidermal homeostasis and counteract the inflammaging process.

Acknowledgements:

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