Niacinamide: a versatile anti-aging molecule against environmental stressors – new learnings from the mitochondrial physiology

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Introduction & Objectives

- Niacinamide, also called nicotinamide, is a form of vitamin B3 used for decades in cosmetic and pharmaceutical products for the treatment of rosacea, acne, sensitive skin and signs of skin photoaging.[1,2]
- It contributes to the improvement of skin barrier integrity through the biosynthesis pathway of ceramides.[3]
- Recently, niacinamide has been shown to alleviate the induction of inflammatory and senescence-related phenotypes induced by environmental stressors in cellular models.[4,5]
- Mitochondrion plays a critical role in skin development[7] and the response to environmental stressors, being at the crossroads of the cellular response between pollution and aging.[8,9]
- Especially, mitochondria form specific structures with subdomains of the endoplasmic reticulum membrane, called mitochondria-associated membranes (or MAMs), a molecular platform essential for NLRP3 inflammasome formation[10].
- Recent publications evidenced a role of niacinamide in the transcriptomic regulation of subunits of the mitochondrial respiratory chains[11]. In this context, we wanted to explore further the action of niacinamide on the mitochondrial physiology and dynamics in a context of "inflamm'aging".

Methods

- Human primary dermal fibroblasts from a mature female donor (55 years old, Caucasian) validated for pollution susceptibility and mitochondrial poisoning were submitted to standardized urban pollutant particles and with or without niacinamide 0.75ppm or 5ppm.

NADPH oxidase activity

- The antioxidant effects of test elements were measured after pre-incubation of the cells at 37°C with the test elements at the determined concentrations for 30 minutes. At the end of the incubation period cell viability was determined using a fluorometric method. The NADPH oxidase enzymatic activity was immediately dynamically measured using a kinetic loop with a luminescence method in a microplate reader (Luminoskan-Thermo).

Fusion/fission balance

- The fusion/fission methodology derives from the Mitostream technology in which the 48 variables, defining mitochondrial behavior, are measured dynamically in live cells. To assess the fusion/fission balance, image analysis focuses on the descriptors related to mitochondria dynamics.

MAMs detection

- Proximity ligation assay was used for the detection of protein interactions (<40 nm). These interactions were visualized on a fluorescence microscope equipped with a-computer aided image acquisition and image analysis as an individual fluorescent dot.

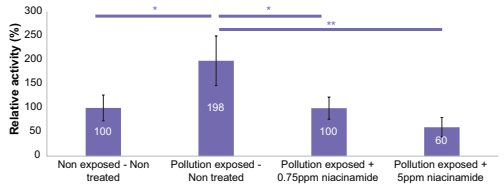
Autophagy / Mitophagy assessment

- The Autophagy/Mitophagy analysis was performed with the use of a lysosomal marker and a dye tracking damaged mitochondria. Several outcome measures were recorded: Cell number and cell size, Number of lysosomal objects per field, Number of damaged mitochondria per field, density of damaged mitochondria co-localizing with lysosomal objects.

Results

- Pollution exposure in non-treated cells significantly induced by 97% the activity of the NADPH oxidase, demonstrating the pro-oxidant effect of pollution (p=0.015). Co-incubation of pollution-exposed cells with niacinamide decreased NADPH oxidase activity cells by 50% with 0.75ppm niacinamide (p=0.015) and 70% with 5ppm niacinamide (p=0.002), to a level comparable to that seen in non-exposed, non-treated cells (p>0.05) (**Figure 1**).

Figure 1: Niacinamide alleviates pollution-induced NADPH oxidase activity

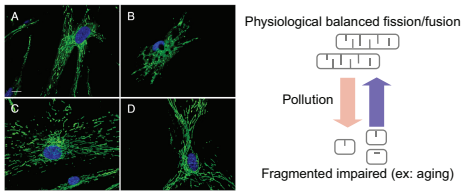


Significance level compared to pollution-exposed & non-treated cells: *p<0.05, **p<0.01. Bars indicates SD. Normality Test: Passed (P=0.411). Equal Variance Test: Passed (P=0.064). ANOVA: (P=0.002)

All Pairwise Multiple Comparison Procedures (Tukey-Kramer Test).

- Pollution induced a significant hyperfission in the mitochondrial network. At the lower concentration, addition of niacinamide protected the mitochondrial network from pollution-induced modification in the mitochondrial dynamics, restoring both fusion and diminishing the pollution-induced fission and hyper-fission in exposed cells. At the highest concentration, niacinamide restored the complexity and branching of mitochondrial population in urban pollutants-exposed cells (**Figure 2**).
- An increase in ER-mitochondria interactions in pollution exposed cells was clearly visible and quantified compared to non-exposed cells. Niacinamide at the highest concentration reduced pollution-induced increase in MAMs density below the basal level, providing an anti-inflammatory effect (**Figure 3**).

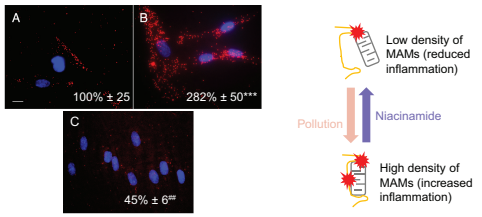
Figure 2: Niacinamide restores a balanced mitochondrial network fighting hyperfission



On the left: Representative microphotographs for the different experimental conditions: (A) Non-exposed, non-treated control; (B) Pollution-exposed, non-treated control; (C) Pollution-exposed, niacinamide 0.75ppm-treated cells; (D) Pollution-exposed, 5ppm niacinamide-treated cells. See the punctuated network in B compared to A, C and D. Numbers indicate the percentage of structures in hyperfission, *=p<0.05 compared to non-exposed, non-treated control. Bar in microphotograph A represents 10µm. Quantification of fusion-fission dynamics was performed using Micv1.1-Mitostream® technology).
On the right: Interpretation of the results. Pollution impairs the mitochondrial dynamics and increases the odds of hyperfission. In the contrary, niacinamide restores a balanced mitochondrial network.

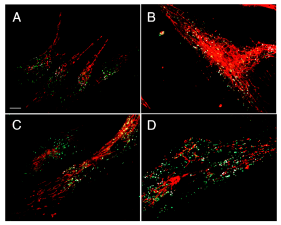
- Pollution increased the occurrence of damages on mitochondrial membrane by 153% (p=0.0059) and the lysosome population by 186% (p=0.0328). This effect was partially restored by niacinamide, especially at the lowest concentration. Reduced mitochondrial damage may represent a more efficient mitophagy, enhancing the recycling of damaged mitochondria or a direct protection of the mitochondrial network against damages (**Figure 4**).
- Co-localizing mitochondria and lysosomes are interpreted as mitophagosomes. Pollution increased the mitophagosome population by 339% (p=0.022). This effect was partially & non-significantly modified by niacinamide, which diminished the pollution-induced formation of mitophagosomes by 50% (**Figure 4**).
- Pollution exposure increased by 20% (p=0.003) the distance of interaction between objects in the mitophagosomes, suggesting a deficiency in the mitophagy. Pollution exposed cells treated with niacinamide displayed a significantly reduced distance of interaction between objects in the mitophagosomes (for 0.75ppm by 0.65-fold, p≤0.0001 and for 5ppm by 0.77-fold p≤0.0001), normalizing the interaction and restoring the efficiency of mitochondria recycling (**Figure 4**).

Figure 3: Niacinamide reduces pollution-induced MAMs density



Visualization of the mitochondria-ER interactions at MAMs by in situ proximity ligation assay. Cell nuclei appear in blue and interactions between the two-targeted proteins are depicted in red. (A) Non-exposed non-treated cells; (B) Pollution exposed non-treated cells; (C) Pollution exposed cells treated with 5ppm niacinamide. Numbers indicate the density of MAMs relative to the condition Non-treated non-exposed. ***=p<0.001 compared to Non-exposed non-treated cells. ##=p<0.01 compared to Pollution-exposed non-treated cells. Staining specificity was ascertained with a negative control in which ligase was omitted (not shown). Bar in A is 10µm.

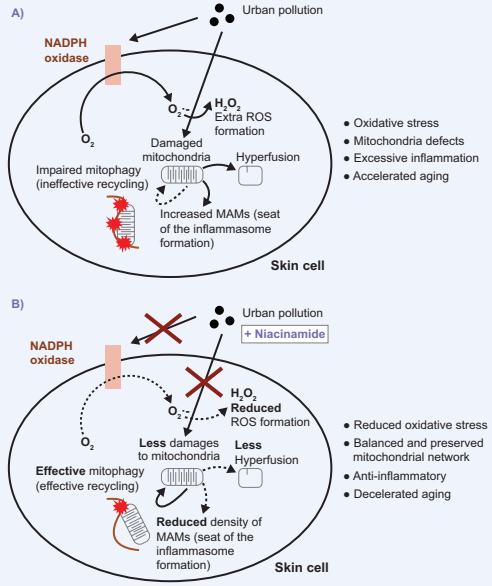
Figure 4: Niacinamide restores pollution-impaired mitophagy efficiency



Visualization of the occurrence of damages to mitochondria, lysosomal density & mitophagic activity. (A) Non-exposed, non-treated cells; (B) Pollution-exposed, non-treated cells; (C) 0.75ppm niacinamide-treated cells; (D) 5ppm niacinamide-treated cells. Red signal represents damaged mitochondria, green signal represents lysosomal compartments, yellow represents dually stained objects and white represents colocalized objects. Bar in A represents 10µm. Note the high level of damaged mitochondria with exposure to pollution, reduced with the treatment with niacinamide and the high degree of colocalization with the 5ppm niacinamide treatment, indicating effective mitophagy.

Conclusions

Figure 5: A) Summary of effects of pollution B) Summary of the protective actions of niacinamide against pollution



- Niacinamide, dose-dependently, reduced the pollution-induced NADPH-oxidase activity. At the lowest tested dose, niacinamide only reduced the NADPH-oxidase activity. At the highest tested dose, however, niacinamide abolished both NADPH-oxidase activity and MAM formation below the level seen in non-exposed & non-treated cells. Both doses reduced pollution-induced fission in the mitochondrial network and increased efficiency of autophagy/mitophagy, enhancing the recycling of damaged mitochondria. Hence Niacinamide protected the mitochondrial network against pollution-induced impairments (**Figure 5**).
- **Overall, we evidenced niacinamide is a directly operative and potent antioxidant for the mitochondrion. Niacinamide counteracts all the signs of pollution-induced mitochondrial defects, also observed during aging. These results depicter some of the mechanisms by which niacinamide alleviates the signs of intrinsic and pollution-aggravated aging, of highest interest for the care of sensitive skin premature aging**.

References

1. Matts, P, et al. Int Fed Soc Cosmet Chem Mag 5, 285–289 (2002). **2.** Forbat, E, et al. Clin. Exp. Dermatol. 42, 137–144 (2017). **3.** Tanno, O, et al. Br. J. Dermatol. 143, 524–531 (2000). **4.** Bierman JC, et al. Int. J. Cosmet. Sci. 42, 501–511 (2020). **5.** Boo YC. Antioxidants 10, 1315 (2021). **6.** Shen K, et al. Annu. Rev. Cell Dev. Biol. 38, 179–218 (2022). **7.** Mangez C, et al. Exp. Dermatol. 31, 622–627 (2022). **8.** López-Otín C, et al. Cell 153, 1194–1217 (2013). **9.** Tigges J, et al. Mech. Ageing Dev. 138, 26–44 (2014). **10.** Shao B, et al. Front. Pharmacol. 6, (2015). **11.** Oblong JE, et al. Aging Cell 19, e13248 (2020).

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