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Photoaging and chronological aging profile: Understanding oxidation of the skin

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ABSTRACT

The impact of chronological aging and photoaging on the skin is particularly concerning, especially when oxidative stress is involved. This article provides evidence of quantitative and qualitative differences in the oxidative stress generated by chronological aging and photoaging of the skin in HRS/J hairless mice. Analysis of the results revealed an increase in lipid peroxides as the skin gets older and in photoaged skin (10.086 ± 0.70 η MDA/mg and 14.303 ± 1.81 η MDA/mg protein, respectively), although protein oxidation was only verified in chronological aged skin (15.449 ± 0.99 η protein/mg protein). The difference between both skin types is the decay in the capacity of lipid membrane turnover revealed by the dislocation of older skin to the left in the chemiluminescence curve. Imbalance between antioxidant and oxidation processes was verified by the decrease in total antioxidant capacity of chronological and photoaged skins. Although superoxide dismutase remained unchanged, catalase increased in the 18 and 48-week-old skin groups and decreased in irradiated mice, demonstrating that neither enzyme is a good parameter to determine oxidative stress. The differences observed between chronological and photoaging skin represent a potential new approach to understanding the phenomenon of skin aging and a new target for therapeutic intervention.

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1. Introduction

Aging of the skin occurs through two independent, biologically divergent mechanisms, sun exposure related aging and chronological aging. Among several oxidative stressors, reactive oxygen species (ROS) have been associated with the process of UV-induced skin damage, including photoaging, immunomodulation, melanogenesis and, ultimately, photocarcinogenesis [1]. Besides direct photochemical reactions, such as the generation of specific pyrimidine dimers, UVB generates a high level of ROS in the skin resulting in photooxidative damage of the cells and extracellular matrix [2]. The aging process has been the subject of numerous theories that fall into two groups: genetic and damage-accumulation theories. It has been proposed that oxidative damage is a common link between all the aging theories. Age-related oxidative phenomenology continues to provide evidence that there is a decline in the antioxidant enzymes and antioxidant defenses leading to an accumulation of oxidative damage end products [3]. These products are all markers of oxidative stress [4], and the influence of the environment, most notably solar UV irradiation, is of considerable importance for skin aging [5–7].

It is now well known that to counteract oxidative stress and maintain a redox balance within the cells, the skin is equipped with a network of antioxidant systems [8]. Ascorbic acid, α-tocopherol, uric acid and glutathione, are well-known nonenzymatic antioxidant molecules. Previous studies have also demonstrated the presence of several major enzymatic antioxidants, i.e. glutathione peroxidase (GPx), glutathione reductase, superoxide dismutase (SOD) and catalase (CAT), in the epidermis [8]. Among these enzymes, SOD and CAT are the major antioxidant enzymes protecting the epidermis [7]. SOD converts superoxide anions into hydrogen peroxide (H_2O_2), while CAT degrades H_2O_2 into water [9].

Despite the entire antioxidant defence, the skin is intensively subjected to exogenous and endogenous aggression; consequently, unsolved problems remain concerning chronological aging and photoaging. One of these is how oxidative stress is involved in each one of these aging processes. In the present work, evidence of quantitative and qualitative differences in the oxidative stress generated by chronological aging and photoaging was verified. Furthermore, the role of such differences in oxidative stress and antioxidant content between chronological aging and photoaging was investigated in the skin of HRS/J hairless mice.

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2. Materials and methods

2.1. Animals and UVB irradiation

Male and female hairless mice HRS/J were obtained from the University of São Paulo (USP). The mice were provided with water and balanced commercial feed (Nuvilab CR1; Nuvital Nutrients Ltda., Curitiba, Brazil) *ad libitum*, as recommended by National Research Council and National Institute of Health, USA. The mice used for the experiments were 8, 18 and 48-week-old, with 8 mice in each group. They were treated in accordance with the institutional ethical guidelines for animal experiments. A second group of 8-week-old mice was irradiated three times a week for 10 weeks, receiving a suberythemogenic dose of 54 mJ/cm^2 in week 1 and erythemogenic doses of 72 mJ/cm^2 in week 2, 90 mJ/cm^2 in week 3 and 108 mJ/cm^2 from weeks 4 to 10 [10], according to the method described by Moloney et al. [11]. The irradiation chamber was adjusted with a PHILIPS TL/12 40 W UVB fluorescent lamp, which emits irradiation from 270 to 400 nm with maximum peak around 313 nm. UVB output was measured using a Research Radiometer model IL-1700 (International Light, USA; calibrated by IL service staff) with a radiometer sensor for UV (SED005) and UVB (SED240), which detected that UVB was 73% of the total UV irradiation in the present experimental conditions. The UVB irradiation rate was 0.47×10^{-4} mW/cm^2.

The lamp was embedded in a 1.30 m × 0.43 m × 0.45 m box, in which the caged mice were placed, 35 cm beneath the lamp. Dorsal skin samples from control and experimental mice were removed 6 h after the last irradiation in the irradiated group and the homogenate was prepared in phosphate buffer was used for analysis. Control groups consisted of caged mice placed in the chamber with no exposure to irradiation.

2.2. Free radical formation analysis

The destabilization of cell membrane lipids formed peroxides that were measured by a very sensitive chemiluminescence (CL) method, which was initiated by the addition of tert-butyl hydroperoxide to the tissue homogenate in a Turner Designs luminometer, model TD-20/20, with a response range of 300–650 nm [12]. The CL generates curves that demonstrate the quantity of membrane peroxides formed as it shifts towards the *y* axis. The final lipoperoxidation products were analyzed by measuring the formation of thiobarbituric acid reactive species (TBARS), as described by Oliveira and Cecchini [13]. Briefly, low molecular weight aldehydes, such as malondialdehyde (MDA), react with thiobarbituric acid (TBA), generating a colored product that absorbs light at 532 nm. Carbonyl proteins were assayed using the method for detecting protein hydrazones, followed by reaction with dinitrophenylhydrazine, according to Reznick and Parker [14].

2.3. Chemiluminescence of skin oxidized with 2-azo-bis-2-amidinopropane (ABAP)

To obtain a reference for oxidized tissue, a sample of control skin was prepared using exactly the same process as hairless mice skin. Test skin was incubated with ABAP for 30 min at 37 °C, before initiating the experiments. The CL generates curves which demonstrate the quantity of lipid peroxides formed in the cell membrane as it shifts towards the *y* axis [12].

2.4. Antioxidant activity analysis

The total antioxidant capacity (TRAP) was also detected by CL. Initially, light emission in a reaction medium containing 2-azo-bis-(2-amidinopropane) and luminol, an alkoxyl generating system, was measured. Then, another curve was measured with the addition of a standard antioxidant, Trolox, a hydrosoluble vitamin E, which hinders the curve peak due to its antioxidant property. Finally, Trolox was replaced by the tissue homogenate and the peak hinder time was determined in comparison with the standard and results were expressed in μM Trolox [15].

Superoxide dismutase (SOD) and catalase (CAT) activities were assayed spectrophotometrically, as previous described [16]. Briefly, SOD quantification was based on the inhibition of pyrogallol autoxidation in aqueous solution by SOD and CAT present in the skin homogenate was determined using a standard H_2O_2 system.

2.5. Protein concentration

All protein concentrations were measured by the method of Lowry et al. [17], modified by Miller [18], except for protein carbonyl contents, which were quantified directly at 280 nm. Bovine serum albumin (BSA) was used as standard in both situations.

2.6. Statistical analysis

Reported values are presented as the mean ± SEM. All differences between groups were analyzed by the Student *t* test, except for the CL curves, which were qualitatively analyzed by two-way ANOVA and quantitatively analyzed by the Bonferroni *post hoc* test. Significance level was set as $p < 0.05$.

3. Results

Table 1 shows different results for each analysis in the different groups in terms of protein oxidation (CP), late lipid peroxide formation (TBARS) and the antioxidant enzyme activities (CAT and SOD). CP content was significantly lower in the 18-week-old group (3.847 ± 0.50 η protein/mg protein) compared to the 8-week-old group (7.919 ± 0.65 η protein/mg protein), but not compared to the irradiated (6.525 ± 0.96 η protein/mg protein) counterpart. In contrast, protein content oxidation was higher when comparing the 48-week-old group (15.449 ± 0.99 η protein/mg protein) to the remaining groups tested.

TBARS formation was greater in the irradiated group (14.303 ± 1.81 η MDA/mg protein) compared to the remaining groups. Although the 48-week-old group showed significantly lower lipid peroxide content (10.086 ± 0.70 η MDA/mg protein) compared to the irradiated group, it was still significantly higher than the 8 (3.650 ± 0.15 η MDA/mg protein) and 18-week-old groups (2.378 ± 0.67 η MDA/mg protein), which were not significantly different from each other.

The enzymatic profile of the skin samples showed an increase in catalase activity for 18-week-old (0.650 ± 0.08 abs/mg protein) and 48-week-old skin (0.577 ± 0.03 abs/mg protein) compared to 8-week-old skin (0.391 ± 0.01 abs/mg protein). Irradiated skin (0.489 ± 0.08 abs/mg protein) showed no significant difference compared to 8-week-old skin. SOD content was not significantly different in any of the groups analyzed.

Membrane lipid peroxide exposure (Fig. 1B) was significantly increased for 18-week-old skin (2.75 RLU/mg protein ± 0.32) compared to 8-week-old (2.01 RLU/mg protein ± 0.28), 48-week-old (1.83 RLU/mg protein ± 0.19) and irradiated skin samples (1.54 RLU/mg protein ± 0.13). Irradiated skin was also different from all the other groups, though in contrast, it showed a reduction in membrane lipid peroxide exposure formation. A different pattern of early lipid peroxide formations was observed for 48-week-old skin, which was significantly different from 8 and 18-week-old and irradiated skin, showing peculiar displacement of the curve

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