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Melatonin decreases the expression of inflammation and apoptosis markers in the lung of a senescence-accelerated mice model

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ABSTRACT

Aging is associated with an increase in oxidative stress and inflammation. The aging lung is particularly affected since it is continuously exposed to environmental oxidants while antioxidant machinery weakens with age. Melatonin, a free radical scavenger, counteracts inflammation and apoptosis in healthy cells from several tissues. Its effects on the aging lung are, however, not yet fully understood. This study aimed to investigate the effect of chronic administration of melatonin on the expression of inflammation markers (TNF-α, IL-1β, NFκB2, HO-1) and apoptosis parameters (BAD, BAX, AIF) in the lung tissue of male senescence-accelerated prone mice (SAMP8). In addition, RNA oxidative damage, as the formation of 8-hydroxyguanosine (8-OHG), was also evaluated. Young and old animals, aged 2 and 10 months respectively, were divided into 4 groups: untreated young, untreated old, old mice treated with 1 mg/kg/day melatonin, and old animals treated with 10 mg/kg/day melatonin. Untreated young and old male senescence accelerated resistant mice (SAMR1) were used as controls. After 30 days of treatment, animals were sacrificed. Lungs were collected and immediately frozen in liquid nitrogen. mRNA and protein expressions were measured by RT-PCR and Western blotting, respectively. Levels of 8-OHG were quantified by ELISA. Mean values were analyzed using ANOVA. Old nontreated SAMP8 animals showed increased ($p < 0.05$) mRNA and protein levels of TNF-α, IL-1β, NFκB2, and HO-1 compared to young mice and SAMR1 mice. Melatonin treatment with either dose reversed the aging-derived inflammation ($p < 0.05$). BAD, BAX and AIF expressions also rose with aging, the effect being counteracted with melatonin ($p < 0.05$). Aging also caused a significant elevation ($p < 0.05$) in SAMP8 8-OHG values. This increase was not observed in animals treated with melatonin ($p < 0.05$). In conclusion, melatonin treatment was able to modulate the inflammatory and apoptosis status of the aging lungs, exerting a protective effect on age-induced damage.

1. Introduction

Aging is a universal process through which organisms become more vulnerable to disease until they eventually die (Harman, 1981). Aging mechanisms and how to elude them have always fascinated the human being. The first theories that relate this process to oxidative stress were born in the 1950s (Harman, 1956), when the dysfunction of several biomolecules was attributed to the action of free radicals.

Free radicals are highly reactive molecules due to an unpaired electron in their outer orbital, as they tend to combine with other molecules and therefore modify their structure. Free radicals are bound to be formed in every living being, as a result of ionizing radiation, as well as enzymatic and non-enzymatic reactions necessary for life. The main source of free radicals in mammals is oxidative phosphorylation (Harman, 1972), where reactive oxygen species (ROS) appear because of the incomplete reduction of O_2: some examples are superoxide anion (O_2^-), hydroxyl radical (OH•) or hydrogen peroxide (H_2O_2). These appear in acute inflammation as a protective response against an internal or external insult. However, chronic inflammation leads to continuous oxidative stress and to cell damage and dysfunction, both seen through the aging process (Reuter et al., 2010).

The lung is especially exposed to free radicals because of its direct contact with environmental pollutants, such as tobacco smoke, ozone or even the O_2 content of the air. On top of that, respiratory diseases, widely common, induce an inflammatory response, which increases oxidative stress (Kinnula and Crapo, 2003). Sustained inflammation and oxidative stress are implicated in the pathophysiology of several pulmonary diseases, such as asthma or chronic obstructive pulmonary disease (COPD) (Angelis et al., 2014). Most organisms have developed

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0531-5565/

Table 1
Primers used in real-time PCR experiments. 18S was used as a housekeeping gene to compare the samples.

	Primers	Sequence (5′–3′)
18S	Forward	GGTGCATGGCCGTTCTTA
	Reverse	TCGTTCGTTATCGGAATTAACC
TNF-α	Forward	ATGAGAAGTTCCCAAATGGC
	Reverse	CTCCACTTGGTGGTTTGCTA
IL-1β	Forward	TGTGATGAAAGACGGCACAC
	Reverse	CTTCTTCTTTGGGTATTGTTTGG
NFκB2	Forward	TGGAACAGCCCAAACAGC
	Reverse	CACCTGGCAAACCTCCAT
HO-1	Forward	GTCAAGCACAGGGTGACAGA
	Reverse	ATCACCTGCAGCTCCTCAAA
BAD	Forward	GCCCTAGGCTTGAGGAAGTC
	Reverse	CAAACTCTGGGATCTGGAACA
BAX	Forward	GTGAGCGGCTGCTTGTCT
	Reverse	GGTCCCGAAGTAGGAGAGGA
AIF	Forward	AGTCCTTATTGTGGGCTTATCAAC
	Reverse	TTGGTCTTCTTTAATAGTCTTGTAGGC

enzymatic and non-enzymatic antioxidant systems, able to counteract the effects of free radicals. In the lung, several superoxide dismutases are especially important. They convert superoxide anion into H_2O_2, which is in turn eliminated through other systems, such as catalase, glutation peroxidase, peroxiredoxins or glutaredoxins (Holmgren, 2000; Kinnula and Crapo, 2003).

Melatonin is a molecule found in bacteria, unicellular eukaryotes, macroalgae, fungi, plants and animals (Cuesta et al., 2013; Reiter et al., 2010; Tan et al., 2010). In mammals, circulating melatonin produced in the pineal gland works as a circadian chronobiotic. However, it is also synthetized in many other cell types, where it exerts other functions: melatonin, and even its metabolites work directly as highly potent free radical scavengers capable of counteracting inflammation and apoptosis in healthy cells. Melatonin also works through at least two known membrane receptors and nuclear receptors, regulating the immune response and activating antioxidant enzymes (Reiter et al., 2010; Tan et al., 2010).

The aim of the present study was to investigate the effects of chronic administration of exogenous melatonin (at two different doses) on the mRNA and protein expression of inflammation markers and apoptosis parameters, as well as on RNA oxidative damage, in pulmonary tissue of senescence-accelerated prone male mice (SAMP8) aged 2 and 10 months, with lungs of SAMR1 male mice as controls.

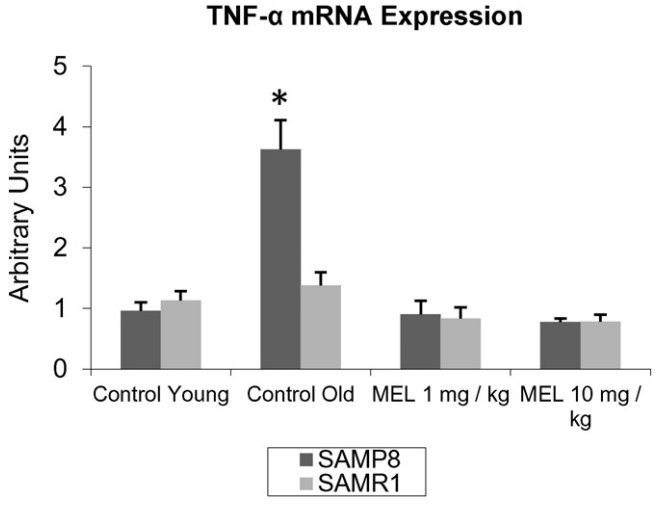

Fig. 1. Effect of aging reversed by the administration of melatonin in the lungs of male SAMP8 and SAMR1 on the mRNA expression of TNFα. *p < 0.05 as compared to control young SAMP8 mice, control old SAMR1 mice, and SAMP8 mice treated with 1 mg or 10 mg melatonin per kg per day.

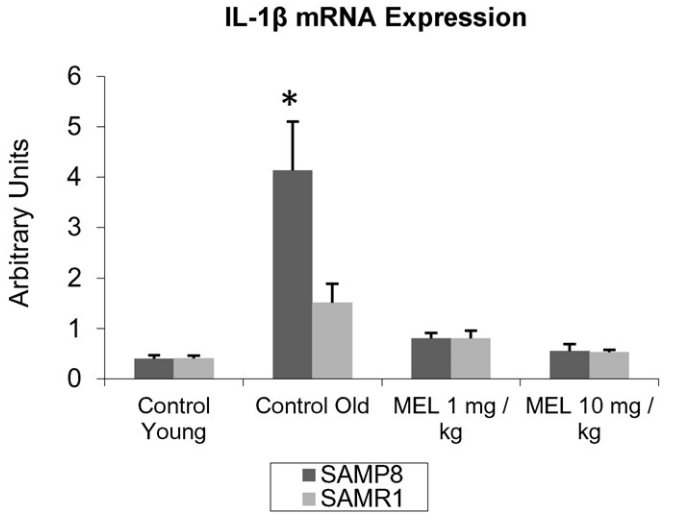

Fig. 2. Effect of aging reversed by the administration of melatonin in the lungs of male SAMP8 and SAMR1 on the mRNA expression of IL-1β. *p < 0.05 as compared to control young SAMP8 mice, control old SAMR1 mice, and SAMP8 mice treated with 1 mg or 10 mg melatonin per kg per day.

2. Material and methods

2.1. Animals

Male senescence-accelerated prone (SAMP8) and resistant (SAMR1) mice of 2 months of age (young) and 10 months of age (old) were used in the study (Butterfield and Poon, 2005; Cuesta et al., 2011; Takeda, 1999; Takeda et al., 1981). They were housed in cages under controlled environmental conditions (22 °C; 70% humidity), kept under a 12/12 h light/dark photoperiod, and fed ad libitum (food and water). The study was approved by the Ethical Committee of the Complutense University of Madrid (Madrid, Spain) in accordance with the Guidelines of Ethical Care of Experimental Animals of the European Union.

2.2. Treatment

Animals were divided into 4 experimental groups: untreated young and untreated old mice, as well as old mice treated with 1 mg/kg/day

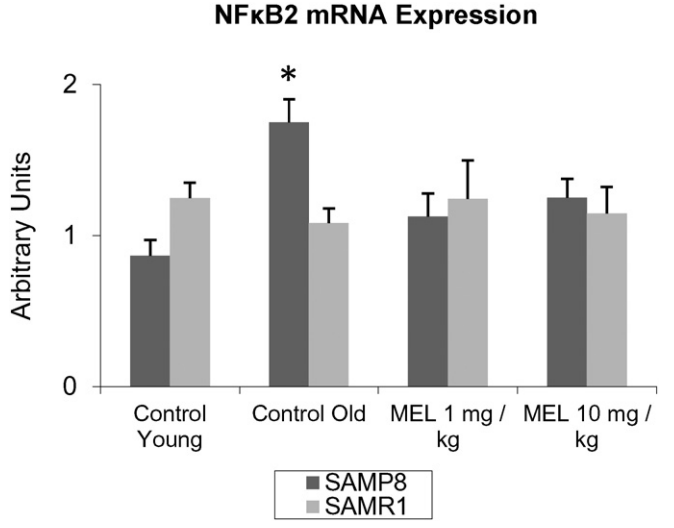

Fig. 3. Effect of aging reversed by the administration of melatonin in the lungs of male SAMP8 and SAMR1 on the mRNA expression of NF-κB. *p < 0.05 as compared to control young SAMP8 mice, control old SAMR1 mice, and SAMP8 mice treated with 1 mg or 10 mg melatonin per kg per day.