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Short Communication

Role of SIRT3/SOD2 signaling in mediating the antioxidant actions of melatonin in mitochondria

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ABSTRACT

Melatonin is a multifaceted antioxidant that is unevenly distributed in body fluids and within subcellular organelles. Moreover, melatonin is synthesized in most, if not all, taxa of the animal and plant kingdoms. Fortuitously, melatonin levels seem to be in especially high concentrations in the mitochondria, the major site of reactive oxygen species (ROS) generation. ROS normally inflict extensive molecular damage to mitochondria and other cellular organelles. Melatonin efficiently neutralizes ROS in mitochondria by direct scavenging and indirectly *via* stimulation of antioxidative enzymes involving a SIRT3/SOD2 signaling pathway as summarized herein; these actions protect this critical energy-producing organelle from malfunction, which would compromise survival of the cell and eventually of the organism.

KEYWORDS: mitochondria, oxidative stress, melatonin, superoxide dismutase 2, histone deacetylase, NAD^+-dependent enzymes, SIRT3

INTRODUCTION

Sirtuins are a family of enzymes which were initially defined as NAD^+-dependent deacetylases that silence gene expression by histone deacetylation in the nucleus of cells [1]. Subsequent to their discovery, however, it was found that sirtuins were distributed throughout the cell and that they not only targeted histones but proteins resident in the cytosol and mitochondria as well. In mammalian cells, seven sirtuins have been identified; they are conventionally referred to as SIRT1-7. NAD^+ is a required co-factor for each of the sirtuins. Since NAD^+ rises as a result of energy stress, this in part determines the adaptive response of cells to low energy by promoting the activities of sirtuins which results in the activation of their downstream targets.

For the purpose of the current report, SIRT3 is of primary interest. While SIRT3 is commonly considered to be located exclusively in mitochondria, some have reported its presence in the nucleus and cytoplasm as well. In mitochondria, SIRT3 is found in the inner mitochondria membrane and in the matrix [2, 3]. This sirtuin is most obviously expressed in cells that are highly metabolically active, e.g., cardiomyocytes, neural cells, hepatocytes, etc. Animals where SIRT3 has been knocked out are predisposed to cardiovascular problems, cancer and metabolic disorders. Upregulation of SIRT3 has been experimentally linked to improved mitochondrial physiology and biogenesis and a delay in cellular and organismal aging [4]. SIRT3 is also closely related to the control of mitochondrial oxidative stress levels [5]. The association of SIRT3 activity with oxidative stress reduction stems from its multiple functions in mitochondria. SIRT3 promotes oxidative metabolism due to the deacetylation of several mitochondrial enzymes [6]. SIRT3 also influences the generation

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of ROS by the electron transport chain [7] and controls the detoxification of ROS *via* the stimulation of antioxidant enzymes [8]. Thus, its combined actions as an enhancer of the efficiency of oxidative phosphorylation along with its ability to clear excessive ROS makes SIRT3 a major player in preserving mitochondrial morphology and function.

Like SIRT3, melatonin is a powerful suppressor of mitochondrial oxidative damage [9-11]. While historically thought to be exclusively originating from the pineal gland, recent studies show that melatonin is produced in the mitochondria, perhaps of all cells [12, 13]. Additionally, melatonin is believed to be avidly taken up by mitochondria *via* specific transporters in the mitochondrial membrane when it is exogenously administered [14].

As antioxidants, melatonin and its metabolites are remarkably efficient in reducing oxidative stress due to their ability to quench a wide variety of ROS [15, 16], to prevent their formation at the level of the electron transport chain (ETC) [17], to chelate transition metals which limits the formation of the highly destructive hydroxyl radical (•OH) [18] and to modulate large number of enzymes which either produce or detoxify ROS [16].

Melatonin: SIRT3 interactions in mitochondria

SIRT3 has a variety of functions in mitochondria that involve the metabolism of fatty acids and acetyl-CoA, mitochondrial-mediated programmed cell death and, importantly, antioxidant defense [8, 19]. This last function is one that links SIRT3 to both imported and locally-produced melatonin. While melatonin is undoubtedly capable of enhancing the reductive power of the mitochondria and limiting the molecular damage meted out by locally-produced ROS and free radicals, its ability to stimulate the activities of superoxide dismutases, especially mitochondria-based SOD2, may involve SIRT3. SOD2 is stimulated by SIRT3 when the latter deacetylates lysines 122, 68, 53 and 89; this leads to the elevation of SOD2 activity (Figure 1). Moreover, SIRT3 interacts with Foxo3a to augment the transcription of SOD2 and another important antioxidant enzyme, catalase (CAT). After SOD2 catalyzes the dismutation of the superoxide anion (O_2•-) to hydrogen peroxide (H_2O_2), CAT removes H_2O_2 by metabolizing it to ground state oxygen and water. This reduces the likelihood that H_2O_2 will be converted to the highly-damaging •OH, thereby curtailing oxidative stress and maintaining a healthy redox state in mitochondria (Figure 1). In addition to its direct free radical scavenging actions, the upregulation of the SIRT3/SOD2 pathway may be an essential means by which melatonin forestalls mitochondrial oxidative damage. At this point, it is not possible to ascertain what percentage of the total antioxidant protection afforded by melatonin results from its direct free radical scavenging or from its indirect effects on antioxidant enzyme processes.

That the inhibitory actions of melatonin against oxidative stress involve SIRT3 is provided by the work of several groups. In a study in which cadmium was used to induce oxidative damage to hepatocytes, melatonin at least in part *via* the SIRT3/SOD2/mROS pathway was clearly shown to be involved [20]. Cadmium, a highly toxic heavy metal that is a common environmental contaminant, normally promotes free radical-mediated oxidative damage. Melatonin has been shown to reduce the toxic consequences of cadmium at the molecular level [21]. However, when SIRT3 activity was pharmacologically inhibited in the hepatocytes, melatonin's ability to prevent the associated oxidative damage was largely lost suggesting that SIRT3 upregulation was a prerequisite for melatonin's protective actions. The SIRT3/SOD2/mROS was clearly shown to relate to melatonin's action in reducing mitochondrial oxidative damage. Cadmium, a highly toxic heavy metal, normally promotes free radical-mediated molecular destruction and apoptotic cell death not only in hepatocytes but many other cells as well. While melatonin reduced the toxic consequences of cadmium in liver cells when SIRT3 activity was pharmacologically inhibited, the ability of melatonin to limit cadmium-mediated mitochondrial destruction was conspicuously ameliorated. Thus, the molecular pathway by which melatonin suppresses the oxidizing environment of the mitochondrial milieu obviously involves SIRT3.

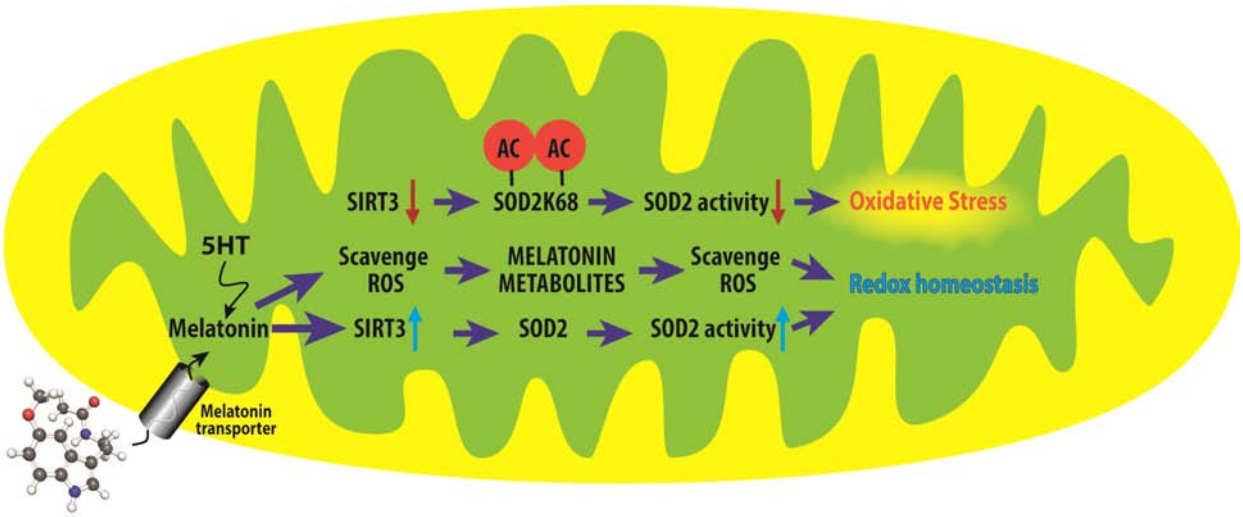

Figure 1. This illustration summarizes the actions of melatonin in mitochondria as they relate to regulation of the deacetylating enzyme, SIRT3, and the modulation of mitochondrial oxidative stress. Melatonin is in higher concentrations in the mitochondria than in other cellular organelles. It is likely taken up by mitochondria through the oligopeptide transporters (PEPT 1/2; melatonin transporter) and it is also synthesized locally in the mitochondria (5 HT → melatonin) of all cells. These two processes account for the high concentration of melatonin in this organelle. A variety of conditions cause the downregulation (↓) of SIRT3; this allows superoxide dismutase 2 (SOD2) to remain acetylated which results in a suppressed enzyme activity and an elevated level of oxidative stress. Melatonin promotes (↑) SIRT3 activity which leads to the deacetylation of SOD2 (among other molecules) as well as several enzymes of the tricarboxylic acid cycle. Via the stimulation of SIRT3 melatonin reduces oxidative damage and prevents the release of cytochrome c from the mitochondria thereby attenuating cellular apoptosis. Additionally, melatonin (and its metabolites) are highly effective direct ROS scavengers which contribute to the ability of melatonin to maintain cellular redox homeostasis.

We recently documented another situation in which melatonin interacts with SOD2 *via* SIRT3 to attenuate the response to free radicals [22]. In that study, obesity was the causative factor of the elevated oxidative stress in the oocytes of obese mice that had been fed a high fat diet (HFD). This renders the oocyte incapable of developing a viable zygote or fetus and obesity is a frequent cause of infertility in humans. HFD-induced obese mice exhibited high ROS generation in their oocytes and the spindle apparatus required for normal meiosis was damaged; this resulted in chromosomal abnormalities and faulty development of the embryo. Melatonin supplementation in the drinking fluid of obese mice reduced oocyte oxidative stress and improved oocyte quality.

When oocytes of obese mice were collected and matured *in vitro*, melatonin added to the culture medium improved the quality of the oocytes that were otherwise inferior. Moreover, with the aid of morpholino knockdown and acetylation-mimetic mutant overexpression pathways, we documented that the ability of melatonin to reduce defective phenotypes in oocytes involved the SIRT3/SOD2 pathway [22]. Thus, at least part of the mechanism which accounts for the ability of obesity to compromise oocyte quality was shown to involve the acetylation/deacetylation of SOD2. Importantly, this information may aid in resolving the problem of the high infertility level in human females with the use of melatonin [23].

Using a model of myocardial ischemia/reperfusion injury in streptozotocin-induced diabetic rats, Yu and co-workers [24] showed that melatonin preserved mitochondrial function *via* the involvement of the AMPK/PGC-1α/SIRT3/SOD2 signaling pathway. In that comprehensive study, both *in vivo* and *in vitro* data documented that melatonin-mediated phosphorylation of AMPK is an early step in this signaling sequence. Thus, blocking AMPK activation reduced melatonin's efficacy in counteracting ROS formation and lipid

breakdown in mitochondria. Similarly, SIRT3 siRNA prevented the mitoprotective actions of melatonin without impacting the phosphorylated AMPK:AMPK ratio or the expression of PGC-1α.

CONCLUDING REMARKS

The high concentrations and efficacy of melatonin in the mitochondria [25] along with its rapid uptake [14, 26] and its synthesis [13, 27] in this organelle justify melatonin being classified as a mitochondria-targeted antioxidant [11, 16, 28, 29]. In this location, it is optimally situated to neutralize ROS and free radicals generated due to electron leakage from the complexes of the electron transport chain. Melatonin, along with its metabolites [16, 30, 31], directly incapacitate ROS by electron donation [32] and *via* stimulation of antioxidant enzymes by mechanisms that likely involve SIRT3 [33] (Figure 1). As a result, melatonin is a powerful modulator of mitochondrial molecular damage in this highly critical energy-generating organelle. It is left to future researchers to define the percentage of the total protection provided by the direct and indirect antioxidant actions of melatonin at the mitochondria level.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interests.

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