Extended Longevity

Scientific White Paper

Pinetonal Formula. Pineal Aging Clock reversal, increasing Melatonin and Pinoline. Includes phytotherapeutic extracts of: *Pistacia vera, Scutellaria baicalensis, Passiflora incarnata, Panax quinquefolius, Elitaria cardamomum, and Cinnamomum verum*. Biological Actions, Molecular Mechanisms, and Their Effects.

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Pinetonal Formula is a Pineal Gland supporting herbal analog formulation of six (6) plant extracts known to increase melatonin, pineol, zinc and selenium, including: ***Pistacia vera, Scutellaria baicalensis, Passiflora incarnata, Panax quinquefolius, Elitaria cardamomum,*** **and** ***Cinnamomum verum*****.** This formula is a synergistic herbal analog to synthetic compounds that produce the Pineal gland age-reversing effect of Melatonin, Pineol, Zinc and Selenium.

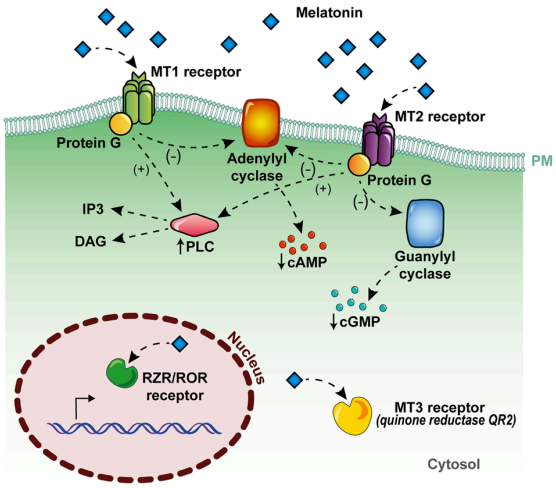
The Pineal Gland

The pineal gland is a unique organ that synthesizes melatonin as the signaling molecule of natural photoperiodic environment and as a potent neuronal protective antioxidant. A functional pineal gland is necessary for preserving optimal human health. Melatonin, along with its metabolites, have been known to significantly reduce the oxidative stress burden of aging cells or cells exposed to toxins. Known as the sleep hormone, melatonin also has antioxidant, anti-inflammatory, antiapoptotic, and other properties. In mammals, this multitasking indolamine is synthesized in the pineal gland in a circadian manner in response to the photoperiodic information received via the retino-hypothalamic pathway. It is directly released into the bloodstream, where it is distributed to all tissues. The pineal gland regulates the cyclic production of the hormones of our body by producing melatonin, and as we age it produces less and less.

Melatonin and Aging

As we age, our melatonin levels decrease, with the steepest decline occurring from about age fifty on. By age sixty, our pineal glands are producing half the amount of melatonin they did when we were twenty. As melatonin levels drop, we begin to exhibit serious signs of aging, because the pineal gland aging clock breaks down. When this happens, it signals other parts of the body that it is time to grow old. The disappearance of the nocturnal peak of melatonin is a specific sign that the organism is aging and with it the deterioration of the hormonal control of our essential functions. It is necessary to reverse this trend to recover and maintain the pineal gland at the state in which it was during youth. The nocturnal peak of melatonin is produced by the Pineal Gland between one and three a.m. Since the pineal gland produces melatonin only at night, giving your body **Pinetonal** at night, protects and rests the Pineal Gland. The Pineal Gland regenerates and maintains itself in a youthful state producing molecules that regulate the neuroendocrine system, resulting in the

normalization of immunological, metabolic and endocrine functions, slowing down aging.



Pathophysiological processes where melatonin plays important roles

Melatonin Overview

Melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitous molecule present in nature that carries out many functions. More commonly known as the sleep hormone, melatonin also has antioxidant, anti-inflammatory, and antiapoptotic properties. Due to its amphiphilic characteristics, melatonin can diffuse and easily cross all morpho-physiological barriers, such as the placenta or the blood–brain barrier and it can enter all cells of the body, influencing the function of a variety of tissues. Pineal synthesis is timed by the suprachiasmatic nucleus (SCN) of the hypothalamus, depending on the light–dark cycle over a 24-h period. Melatonin is mainly produced during the dark phase, and the maximal plasma concentration of this serotonin-derived hormone usually occurs 4 to 5 hours after dark. Light stimulus activates melanopsin breakdown in retinal photoreceptive ganglion cells that, via the retino-hypothalamic pathway, induce the inhibition of melatonin synthesis, as a consequence, during the daily light period, its level is low or even undetectable.

In the blood, once secreted from the pineal gland, melatonin is usually bound to albumin, and conjugated, in the liver, to produce the principal urinary metabolite, which is finally eliminated through the kidney. However, melatonin is not exclusively produced in the pineal gland, but it is also locally synthesized in several cells and tissues, such as the retina, the gastrointestinal tract, and the innate immune system. The synthesis in extra pineal sites presumably does not follow circadian rhythms, except for the retina, and mainly works as a local antioxidant.

The mitochondria are the primary sites of melatonin synthesis. Mitochondria are major sources of free radicals and in addition to being commonly used to treat disoriented circadian clocks due to jet lag and other disturbances (i.e., sleep inefficiency). Melatonin has been widely used as an antioxidative therapy. The direct antioxidant and free radical scavenging properties of melatonin are mainly due to its electron-rich aromatic indole ring, which makes it a potent electron donor that can significantly reduce oxidative stress. Over this direct action, melatonin can further activate melatonin MT 1 and MT2 receptors, upregulating antioxidative defensive systems by increasing the expression or activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase.

Melatonin and Inflammation

Melatonin is a potent and widespread anti-inflammatory agent. Several studies have shown that melatonin can regulate the activation of the immune system, reducing chronic and acute inflammation. Melatonin exerts its anti-inflammatory effects by modulating both pro- and anti-inflammatory

cytokines in various pathophysiological situations. Different cytokines are associated with inflammatory diseases, wherein the balance between pro-inflammatory and anti-inflammatory molecules determines the clinical outcome to some degree, melatonin could modulate serum inflammatory parameters. Melatonin is considered a potent molecule in the management of a large variety of inflammatory diseases.

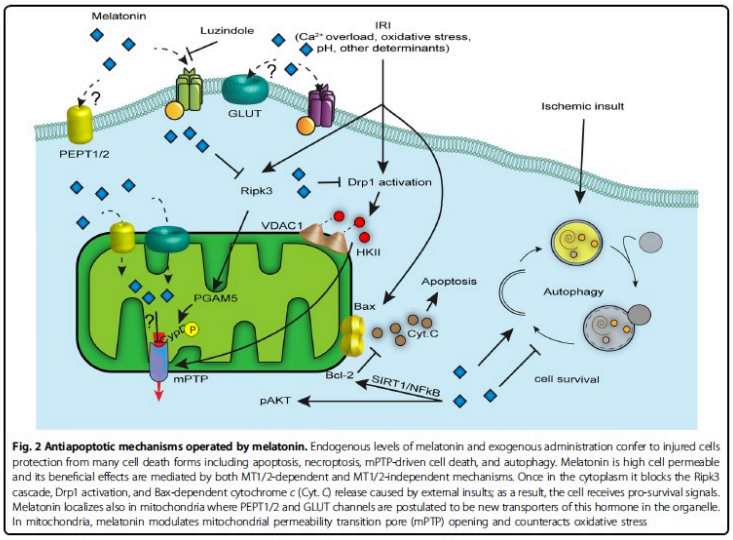


Fig. 2 Antiapoptotic mechanisms operated by melatonin. Endogenous levels of melatonin and exogenous administration confer to injured cells protection from many cell death forms including apoptosis, necroptosis, mPTP-driven cell death, and autophagy. Melatonin is high cell permeable and its beneficial effects are mediated by both MT1/2-dependent and MT1/2-independent mechanisms. Once in the cytoplasm it blocks the Ripk3 cascade, Drp1 activation, and Bax-dependent cytochrome *c* (Cyt. C) release caused by external insults; as a result, the cell receives pro-survival signals. Melatonin localizes also in mitochondria where PEPT1/2 and GLUT channels are postulated to be new transporters of this hormone in the organelle. In mitochondria, melatonin modulates mitochondrial permeability transition pore (mPTP) opening and counteracts oxidative stress

Active Herbal Ingredients

Pistacia vera, is a source of natural plant-based melatonin. 3 mg of melatonin can be found in 18 Pistachio nuts. Pistachios are rich in melatonin (2609 ± 3096 pg/g). One pistachio cultivar showed a melatonin content higher than 12,000 pg/g. *Pistacia vera* contains a trove of phenolic compounds, terpenoids, monoterpenes, flavonoids, alkaloids, saponins, fatty acids, and sterols. The Pistachio kernel and seed extracts showed significant antiviral activity.

Scutellaria baicalensis, flavones isolated from *Scutellaria baicalensis* root exhibit strong neuroprotective effects on the brain. Their neuroprotective potential has been shown in both oxidative stress-induced and amyloid-beta-induced neuronal death models. Baicalein, the main flavone present in *Scutellaria baicalensis* root, strongly inhibited aggregation of neuronal amyloidogenic proteins in vitro and induces dissolution of amyloid deposits. It stimulates brain tissue regeneration, inducing differentiation of neuronal precursor cells. The efficacy of *Scutellaria baicalensis* on TGF-β signaling pathway components has been demonstrated by the SB treatment of cells and resulted in a significant decrease in the expression of TGF-β isoforms, TGF-β receptors, and SMADs. *Scutellaria baicalensis* is an analog of the drug Alk5 kinase inhibitor, which is able to block the growth factor's receptors, stopping it from aging the body's stem cells. "This is the first demonstration that we can and a drug that makes the key TGF-beta1 pathway, which is elevated by aging, behave younger, thereby rejuvenating multiple organs".

Passiflora incarnata, is an important plant used in Ayurveda for the treatment of various disorders of the CNS and is a rich source of flavonoids. It has antioxidant, antiparkinsonian, and memory-enhancing activity. The extracts can be considered as an appropriate sleep inducer. It contains multiple bioactive metabolites such as, flavonoids (like, chrysin that show CNS depressant activity by agonizing GABA-benzodiazepine receptor), amino acids (like, GABA), and harmala alkaloids (reversible monoamine oxidase-A inhibitor).

Panax quinquefolius, a source of selenium, Panax ginseng root significantly extended life span via modulation of multiple longevity assurance genes, including genes involved in insulin signaling and stress response pathways. Ginseng extract had an anti-fibrosis effect via the regulation of the TGF-ß1/Smad signaling pathway in the CCl4-induced liver fibrosis model. It affected the inhibition of the expression of TGF-ß1, Smad2, and Smad3.

Elitaria cardamomum, is one of the highest sources of plant-based zinc. Extracts of cardamom may be effective against a variety of bacterial strains that contribute to fungal infections.

Cinnamomum verum, Cinnamon health benefits are attributed to its content of a few specific types of antioxidants, including polyphenols, phenolic acid and various flavonoids. These compounds work to fight oxidative stress in the body and aid in the prevention of chronic disease. the effects of cinnamon on life span implicated major longevity pathways. These include the DAF-16 transcription factor in the insulin signaling pathway, which promotes the expression of stress resistance and the longevity genes. Cinnamon activates the insulin signaling pathway, anti-oxidative pathway and serotonin signaling for its lifespan prolonging effect.

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Melatonin and the theories of aging: a critical appraisal of melatonin's role in antiaging mechanisms.

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Abstract

The classic theories of aging such as the free radical theory, including its mitochondria-related versions, have largely focused on a few specific processes of senescence. Meanwhile, numerous interconnections have become apparent between age-dependent changes previously thought to proceed more or less independently. Increased damage by free radicals is not only linked to impairments of mitochondrial function, but also to inflammaging as it occurs during immune remodeling and by release of proinflammatory cytokines from mitotically arrested, DNA-damaged cells that exhibit the senescence-associated secretory phenotype (SASP). Among other effects, SASP can cause mutations in stem cells that reduce the capacity for tissue regeneration or, in worst case, lead to cancer stem cells. Oxidative stress has also been shown to promote telomere attrition. Moreover, damage by free radicals is connected to impaired circadian rhythmicity. Another nexus exists between cellular oscillators and metabolic sensing, in particular to the aging-suppressor SIRT1, which acts as an accessory clock protein. Melatonin, being a highly pleiotropic regulator molecule, interacts directly or indirectly with all the processes mentioned. These influences are critically reviewed, with emphasis on data from aged organisms and senescence-accelerated animals. The sometimes-controversial findings obtained either in a nongerontological context or in comparisons of tumor with nontumor cells are discussed in light of evidence obtained in senescent organisms. Although, in mammals, lifetime extension by melatonin has been rarely documented in a fully conclusive way, a support of healthy aging has been observed in rodents and is highly likely in humans.

KEYWORDS: SAMP8; free radicals; inflammaging; metabolic sensing; mitochondria; senescence-associated secretory phenotype; stem cells

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ELSEVIER FULL-TEXT ARTICLE

Front Neuroendocrinol. 2004 Sep-Dec;25(3-4):177-95.

Human pineal physiology and functional significance of melatonin.

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Abstract

Descriptions of the pineal gland date back to antiquity, but its functions in humans are still poorly understood. In both diurnal and nocturnal vertebrates, its main product, the hormone **melatonin**, is synthesized and released in rhythmic fashion, during the dark portion of the day-night cycle. **Melatonin** production is controlled by an endogenous circadian timing system and is also suppressed by light. In lower vertebrates, the pineal gland is photosensitive, and is the site of a self-sustaining circadian clock. In mammals, including humans, the gland has lost direct photosensitivity, but responds to light via a multisynaptic pathway that includes a subset of retinal ganglion cells containing the newly discovered photopigment, melanopsin. The mammalian pineal also shows circadian oscillations, but these damp out within a few days in the absence of input from the primary circadian pacemaker in the suprachiasmatic nuclei (SCN). The duration of the nocturnal **melatonin** secretory episode increases with nighttime duration, thereby providing an internal calendar that regulates seasonal cycles in reproduction and other functions in photoperiodic species. Although humans are not considered photoperiodic, the occurrence of seasonal affective disorder (SAD) and its successful treatment with light suggest that they have retained some photoperiodic responsiveness. In humans, exogenous **melatonin** has a soporific effect, but only when administered during the day or early evening, when endogenous levels are low. Some types of primary insomnia have been attributed to diminished **melatonin** production, particularly in the elderly, but evidence of a causal link is still inconclusive. **Melatonin** administration also has mild hypothermic and hypotensive effects. A role for the pineal in human reproduction was initially hypothesized on the basis of clinical observations on the effects of pineal tumors on sexual development. More recent data showing an association between endogenous **melatonin** levels and the onset of puberty, as well as observations of elevated **melatonin** levels in both men and women with hypogonadism and/or infertility are consistent with such a hypothesis, but a regulatory role of **melatonin** has yet to be established conclusively. A rapidly expanding literature attests to the involvement of **melatonin**

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Cell Death & Disease

REVIEW ARTICLE Open Access

Melatonin as a master regulator of cell death and inflammation: molecular mechanisms and clinical implications for newborn care

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Abstract

Melatonin, more commonly known as the sleep hormone, is mainly secreted by the pineal gland in dark conditions and regulates the circadian rhythm of the organism. Its intrinsic properties, including high cell permeability, the ability to easily cross both the blood–brain and placenta barriers, and its role as an endogenous reservoir of free radical scavengers (with indirect extra activities), confer it beneficial uses as an adjuvant in the biomedical field. Melatonin can exert its effects by acting through specific cellular receptors on the plasma membrane, similar to other hormones, or through receptor-independent mechanisms that involve complex molecular cross talk with other players. There is increasing evidence regarding the extraordinary beneficial effects of melatonin, also via exogenous administration. Here, we summarize molecular pathways in which melatonin is considered a master regulator, with attention to cell death and inflammation mechanisms from basic, translational and clinical points of view in the context of newborn care.

Facts

- Melatonin is a ubiquitous molecule with natural and powerful antioxidant proprieties and administration of exogenous melatonin is safe
- Melatonin exerts anti-inflammatory effects mainly by inhibiting inflammasome activation
- Melatonin exerts its antiapoptotic activities mainly by blocking caspase 3 cleavage and mPTP opening
- "Oxygen radical diseases of neonatology" refers to the oxidative stress that has a leading role in the pathogenesis of neonatal morbidities and pathologic conditions

Open questions

- How endogenous melatonin contrast the oxidative stress that has a leading role in the pathogenesis of neonatal morbidities and pathologic conditions?
- Which are the intracellular targets of melatonin?
- How could melatonin improve the treatment of neonatal disease?
- What factors ultimately determine the melatonin efficacy as an adjunctive treatment in sepsis, chronic lung disease and hypoxic–ischemic encephalopathy of the term and preterm infants

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) is a ubiquitous molecule present in nature that carries out many

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Morphofunctional and signaling molecules overlap of the pineal gland and thymus: role and significance in aging

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Keywords: pineal, thymus, melatonin, neuroendocrine-immune, aging, Gerotarget

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ABSTRACT

Deficits in neuroendocrine-immune system functioning, including alterations in pineal and thymic glands, contribute to aging-associated diseases. This study looks at ageing-associated alterations in pineal and thymic gland functioning evaluating common signaling molecules present in both human and animal pinealocytes and thymocytes: endocrine cell markers (melatonin, serotonin, pCREB, AANAT, CGRP, VIP, chromogranin A); cell renovation markers (p53, AIF, Ki67), matrix metalloproteinases (MMP2, MMP9) and lymphocytes markers (CD4, CD5, CD8, CD20). Pineal melatonin is decreased, as is one of the melatonin pathway synthesis enzymes in the thymic gland. A further similarity is the increased MMPs levels evident over age in both glands. Significant differences are evident in cell renovation processes, which deteriorate more quickly in the aged thymus *versus* the pineal gland. Decreases in the number of pineal B-cells and thymic T-cells were also observed over aging. Collected data indicate that cellular involution of the pineal gland and thymus show many commonalities, but also significant changes in aging-associated proteins. It is proposed that such ageing-associated alterations in these two glands provide novel pharmaceutical targets for the wide array of medical conditions that are more likely to emerge over the course of ageing.

INTRODUCTION

A reduction in the functioning of the nervous, endocrine and immune systems form an appreciable component of aging-associated diseases. Age-associated changes in neuroendocrine and immune networks, particularly age-associated impairments in the functioning of the pineal and thymic glands, are now believed to pay an important role in the mechanisms of aging and the development of age-associated diseases [1-2]. Pinealectomized mice display a decrease in thymic weight, accompanied by cellular depletion and impaired secretory function [3-4]. Pharmacological blockage of the murine pineal gland decreases blood levels of the thymic hormone thymulin. The rhythmic pattern of several immune characteristics associated with glucocorticoid synthesis in the adrenal cortex, a structure tightly associated with pineal gland, is changed in thymectomized mice [5-6]. Such data highlight the reciprocated influences of these glands, with consequences for immune system regulation.

The FASEB Journal • FJ Express Full-Length Article

Pineal clock gene oscillation is disturbed in Alzheimer's disease, due to functional disconnection from the "master clock"

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ABSTRACT **The suprachiasmatic nucleus (SCN) is the "master clock" of the mammalian brain. It coordinates the peripheral clocks in the body, including the pineal clock that receives SCN input via a multisynaptic noradrenergic pathway. Rhythmic pineal melatonin production is disrupted in Alzheimer's disease (AD). Here we show that the clock genes *hBmal1*, *hCry1*, and *hPer1* were rhythmically expressed in the pineal of controls (Braak 0). Moreover, *hPer1* and *hβ1-adrenergic receptor* (*hβ1-ADR*) mRNA were positively correlated and showed a similar daily pattern. In contrast, in both preclinical (Braak I-II) and clinical AD patients (Braak V-VI), the rhythmic expression of clock genes was lost as well as the correlation between *hPer1* and *hβ1-ADR* mRNA. Intriguingly, *hCry1* mRNA was increased in clinical AD. These changes are probably due to a disruption of the SCN control, as they were mirrored in the rat pineal deprived of SCN control. Indeed, a functional disruption of the SCN was observed from the earliest AD stages onward, as shown by decreased vasopressin mRNA, a clock-controlled major output of the SCN. Thus, a functional disconnection between the SCN and the pineal from the earliest AD stage onward could account for the pineal clock gene changes and underlie the circadian rhythm disturbances in AD.—Wu, Y-H., Fischer, D. F., Kalsbeek, A., Garidou-Boof, M-L., van der Vliet, J., van Heijningen, C., Liu, R-Y., Zhou, J-N., Swaab, D. F. Pineal clock gene oscillation is disturbed in Alzheimer's disease, due to functional disconnection from the "master clock." *FASEB J.* 20, E1171–E1180 (2006)**

Key Words: circadian system · clock gene · vasopressin mRNA · superchiasmatic nucleus

In mammals, the master circadian clock is located within the suprachiasmatic nucleus (SCN) of the hypothalamus, which receives environmental light-dark information and orchestrates circadian rhythms at the organismal level (1, 2). Molecular components of the circadian oscillator in mammals are a set of clock genes that involve intracellular transcriptional/translational feedback loops with negative (Per1–3, Cry1–2) and positive limbs (Bmal1 and clock) (1, 3). Recent mammalian clock gene studies have revealed molecular clocks in many other brain regions and peripheral tissues, such as the pineal gland and liver, that are probably synchronized by the master clock in the SCN (4–6). Human clock genes are also expressed widely in the brain (7), although an analysis of rhythmic expression has so far been reported only in peripheral tissues such as oral mucosa, skin, and peripheral blood mononuclear cells (8–10).

A major output of the SCN in mammals, including humans, is the circadian rhythm of melatonin synthesis in the pineal gland, which is involved in the regulation of the circadian system (11–13). Sympathetic innervation of the mammalian pineal is activated at night via a multisynaptic pathway from the SCN to release noradrenalin, which acts on the β_1-adrenergic receptor (β_1-ADR) of the pinealocyte to trigger the cAMP signaling pathway (14) and thus leads to the activation of melatonin biosynthesis (15, 16). Clock gene Per1 is rhythmically expressed in the rodent pineal under the same noradrenergic control from the SCN as the one that regulates melatonin synthesis (17–20). Thus, the molecular clock of the rodent pineal seems to be synchronized by the central clock in the SCN. Although the role of the pineal molecular clock has not been fully elucidated, its involvement in the gated expression of N-acetyltransferase, the rhythm-generating enzyme of melatonin biosynthesis, has been proposed in rodents (21).

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molecules

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Review

Mitochondria: Central Organelles for Melatonin's Antioxidant and Anti-Aging Actions

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Abstract: Melatonin, along with its metabolites, have long been known to significantly reduce the oxidative stress burden of aging cells or cells exposed to toxins. Oxidative damage is a result of free radicals produced in cells, especially in mitochondria. When measured, melatonin, a potent antioxidant, was found to be in higher concentrations in mitochondria than in other organelles or subcellular locations. Recent evidence indicates that mitochondrial membranes possess transporters that aid in the rapid uptake of melatonin by these organelles against a gradient. Moreover, we predicted several years ago that, because of their origin from melatonin-producing bacteria, mitochondria likely also synthesize melatonin. Data accumulated within the last year supports this prediction. A high content of melatonin in mitochondria would be fortuitous, since these organelles produce an abundance of free radicals. Thus, melatonin is optimally positioned to scavenge the radicals and reduce the degree of oxidative damage. In light of the "free radical theory of aging", including all of its iterations, high melatonin levels in mitochondria would be expected to protect against age-related organismal decline. Also, there are many age-associated diseases that have, as a contributing factor, free radical damage. These multiple diseases may likely be deferred in their onset or progression if mitochondrial levels of melatonin can be maintained into advanced age.

Keywords: oxidative stress; free radicals; electron transport chain; oxidative phosphorylation; free radical theory of aging; melatonin uptake; melatonin synthesis

1. Introduction

A surplus of chemically-reduced oxygen derivatives, often referred to as reactive oxygen species (ROS), some of which are free radicals (with an unpaired valence electron), commonly leads to an augmented level of molecular damage identified as oxidative stress [1]. The excess of highly reactive oxygen metabolites overwhelms a complex antioxidant defense network such that it does not adequately defend against the consequent deleterious effects. All major molecular groups typically sustain damage when attacked by free radicals, but the level of oxidative stress is most frequently based on the quantities of damaged lipid products, protein carbonyls, and mutilated nucleic acids [2]. While many of the toxic derivatives of ground state oxygen are oxygen-based and therefore are referred to as reactive oxygen species (ROS), others are nitrogen (RNS) or chlorine (RCS)-based. For the purposes of the current report, these are all considered under the collective term of ROS. Likewise, the damage inflicted by ROS, depending on the species involved, is referred to as either oxidative stress or nitrosative stress. Herein, both are categorized as oxidative stress.

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Review

Pineal Calcification, Melatonin Production, Aging, Associated Health Consequences and Rejuvenation of the Pineal Gland

Dun Xian Tan *, Bing Xu, Xinjia Zhou and Russel J. Reiter *

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Abstract: The pineal gland is a unique organ that synthesizes melatonin as the signaling molecule of natural photoperiodic environment and as a potent neuronal protective antioxidant. An intact and functional pineal gland is necessary for preserving optimal human health. Unfortunately, this gland has the highest calcification rate among all organs and tissues of the human body. Pineal calcification jeopardizes melatonin's synthetic capacity and is associated with a variety of neuronal diseases. In the current review, we summarized the potential mechanisms of how this process may occur under pathological conditions or during aging. We hypothesized that pineal calcification is an active process and resembles in some respects of bone formation. The mesenchymal stem cells and melatonin participate in this process. Finally, we suggest that preservation of pineal health can be achieved by retarding its premature calcification or even rejuvenating the calcified gland.

Keywords: pineal gland; calcification; melatonin; aging; neurodegenerative diseases; rejuvenation

1. Introduction

Pineal gland is a unique organ which is localized in the geometric center of the human brain. Its size is individually variable and the average weight of pineal gland in human is around 150 mg [1], the size of a soybean. Pineal glands are present in all vertebrates [2]. Pineal-like organs are also found in non-vertebrate organisms such as insects [3–5]. It appears that the sizes of pineal glands in vertebrates are somehow associated with survival in their particular environments and their geographical locations. The more harsh (colder) their habitant, the larger their pineal glands are. A general rule is that the pineal gland increases in size in vertebrates from south to north or from the equator to the poles [6]. It is unknown whether if the same species moved to a different environment this would cause a change in the size of their pineal gland.

It was reported that several physiological or pathological conditions indeed alter the morphology of the pineal glands. For example, the pineal gland of obese individuals is usually significantly smaller than that in a lean subject [7]. The pineal volume is also significantly reduced in patients with primary insomnia compared to healthy controls and further studies are needed to clarify whether low pineal volume is the basis or a consequence of a functional sleep disorder [8]. These observations indicate that the phenotype of the pineal gland may be changeable by health status or by environmental factors, even in humans. The largest pineal gland was recorded in new born South Pole seals; it occupies one third of their entire brain [9,10]. The pineal size decreases as they grow. Even in the adult seal, however, the pineal gland is considerably large and its weight can reach up to approximately 4000 mg, 27 times larger than that of a human. This huge pineal gland is attributed to the harsh survival environments these animals experience [11].

Extended Longevity

RESEARCH ARTICLE

PLANT BIOCHEMISTRY

10.1126/sciadv.1501780

A specialized flavone biosynthetic pathway has evolved in the medicinal plant, *Scutellaria baicalensis*

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Wogonin and baicalein are bioactive flavones in the popular Chinese herbal remedy Huang-Qin (*Scutellaria baicalensis* Georgi). These specialized flavones lack a 4′-hydroxyl group on the B ring (4′-deoxyflavones) and induce apoptosis in a wide spectrum of human tumor cells in vitro and inhibit tumor growth in vivo in different mouse tumor models. Root-specific flavones (RSFs) from *Scutellaria* have a variety of reported additional beneficial effects including antioxidant and antiviral properties. We describe the characterization of a new pathway for the synthesis of these compounds, in which pinocembrin (a 4′-deoxyflavanone) serves as a key intermediate. Although two genes encoding flavone synthase II (FNSII) are expressed in the roots of *S. baicalensis*, FNSII-1 has broad specificity for flavanones as substrates, whereas FNSII-2 is specific for pinocembrin. FNSII-2 is responsible for the synthesis of 4′-deoxyRSFs, such as chrysin and wogonin, wogonoside, baicalein, and baicalin, which are synthesized from chrysin. A gene encoding a cinnamic acid–specific coenzyme A ligase (SbCLL-7), which is highly expressed in roots, is required for the synthesis of RSFs by FNSII-2, as demonstrated by gene silencing. A specific isoform of chalcone synthase (SbCHS-2) that is highly expressed in roots producing RSFs is also required for the synthesis of chrysin. Our studies reveal a recently evolved pathway for biosynthesis of specific, bioactive 4′-deoxyflavones in the roots of *S. baicalensis*.

INTRODUCTION

Scutellaria baicalensis Georgi is a species in the family Lamiaceae commonly used in traditional Chinese medicine, where it is known as Huang-Qin (Fig. 1, A and B). Huang-Qin has been used for more than 2000 years for the treatment of fever and lung and liver complaints and was first recorded in Shennong Bencaojing (written between 200 and 300 AD). The authoritative Materia Medica (Bencao Gangmu), written in 1593, describes the use of *S. baicalensis* for treatment of a wide range of disorders. Its author, Li Shizhen, reported successful self-administration to treat a severe lung infection (*1*). Modern day use of Huang-Qin has reported successful outcomes in combination therapies of non–small cell lung carcinomas (*2*–*4*). Huang-Qin has also been applied in the treatment of inflammation, respiratory tract infections, diarrhea, dysentery, liver disorders, hypertension, hemorrhaging, and insomnia (*5*).

Scutellaria is rich in flavones (Fig. 1, C and D), which are flavonoids widely distributed in the plant kingdom and most usually produced in flowers, where they serve as copigments with anthocyanins, giving bluer colors to flowers such as gentian. Dietary flavones have diverse beneficial properties for animal cells, including activities as free radical scavengers and anticancer properties (*6*, *7*). Baicalin and wogonoside, and their respective aglycones baicalein and wogonin, are the major bioactive flavones produced in large amounts by the roots of *S. baicalensis* [the root-specific flavones (RSFs)]. RSFs lack a 4′-hydroxyl group on their B ring compared to the widely distributed "classic flavones" associated with aerial tissues such as flowers (Fig. 1C). The 4′-deoxyRSFs provide a variety of specific health benefits in Huang-Qin, such as antifibrotic activity in the liver, and antiviral and anticancer properties (*8*–*13*). *Scutellaria* RSFs specifically promote apoptosis in tumor cells but have low or no toxicity in healthy cells (*13*, *14*). We are interested in elucidating the biosynthetic pathways for the RSFs for applications involving increased production of these bioactive compounds.

Flavones are synthesized by the flavonoid pathway, which is part of phenylpropanoid metabolism (*15*). Naringenin is a central intermediate in biosynthesis of normal 4′-hydroxyflavones (*16*). In the aerial parts of *Scutellaria*, the 4′-hydroxyflavones, scutellarin and scutellarein accumulates, derived from naringenin. However, *Scutellaria* roots accumulate large amounts of specialized RSFs lacking a 4′-OH group on their B rings (Fig. 1C) (*17*). These 4′-deoxyRSFs, which include baicalein and wogonin and their glycosides, are unlikely to be synthesized from naringenin because no dehydroxylase that removes hydroxyl groups from the B ring of flavonoids has been found in plants (Fig. 1C). This finding suggests that an alternative pathway recruits cinnamic acid to form cinnamoyl–coenzyme A (CoA) through a CoA ligase, which is then condensed with malonyl-CoA by chalcone synthase (CHS) to form a chalcone, and then isomerized by chalcone isomerase (CHI) to form pinocembrin, a 4′-deoxyflavanone. Pinocembrin could be converted by a flavone synthase (FNS) to form chrysin and subsequently decorated by hydroxylases, methyltransferases, and glycosyltransferases (GTs) to produce the different RSFs in *S. baicalensis* (Fig. 1C). To date, cDNAs encoding phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H), 4-coumaroyl–CoA ligase (4CL), CHS, and CHI have been reported from *S. baicalensis* (*18*, *19*). However, biochemical and genetic evidence indicating which, if any, of these genes are involved in the biosynthesis of RSFs is still lacking. It is also possible that specific isoforms of CoA ligase are required for the formation of cinnamoyl-CoA (*20*–*22*), and isoforms of CHS and CHI for the for-

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RESEARCH Open Access

Ethanol extract of *Scutellaria baicalensis* Georgi prevents oxidative damage and neuroinflammation and memorial impairments in artificial senescense mice

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Abstract

Aging is a progressive process related to the accumulation of oxidative damage and neuroinflammation. We tried to find the anti-amnesic effect of the *Scutellaria baicalens* Georgia (SBG) ethanol extract and its major ingredients. The antioxidative effect of SBG on the mice model with memory impairment induced by chronic injection of D-galactose and sodium nitrate was studied. The Y-maze test was used to evaluate the learning and memory function of mice. The activities of superoxide dismutase, catalase and the content of malondialdehyde in brain tissue were used for the antioxidation activities. Neuropathological alteration and expression of bcl-2 protein were investigated in the hippocampus by immunohistochemical staining. ROS, neuroinflammation and apoptosis related molecules expression such as Cox-2, iNOS, procaspase-3, cleaved caspase-3, 8 and 9, bcl-2 and bax protein and the products of iNOS and Cox-2, NO, PGE2, were studied using LPS-activated Raw 264.7 cells and microglia BV2 cells. The cognition of mice was significantly improved by the treatment of baicalein and 50 and 100 mg/kg of SBG in Y-maze test. Both SBG groups showed strong antioxidation, antiinflammation effects with significantly decreased iNOS and Cox-2 expression, NO and PGE2 production, increased bcl-2 and decreased bax and cleaved caspase-3 protein expression in LPS induced Raw 264.7 and BV2 cells. We also found that apoptotic pathway was caused by the intrinsic mitochondrial pathway with the decreased cleaved caspase-9 and unchanged cleaved caspase-8 expression. These findings suggest that SBG, especially high dose, 100 mg/kg, improved the memory impairments significantly and showed antioxidation, antiinflammation and intrinsic caspase-mediated apoptosis effects.

Background

Traditionally, *Scutellaria baicalensis* Georgi (SBG) has been widely used to treat high fever, jaundice and infection in the form of decoction or extracts. Several studies have reported that major compounds, such as baicalin and baicalein isolated from this medicinal herb showed antioxidative, antiinflammatory effects [1-5]. Those effects of baicalin and baicalein were could have originated from the traditional effects of the original herb of SBG. The brain is susceptible to free-radical damage due to its comparatively high levels of oxygen metabolism and also relatively deficient in both free-radical scavenging enzymes and antioxidant molecules as compared with other organs [6,7]. Oxidative stress by the imbalance between free radicals and the antioxidant system is a prominent and early feature in the pathogenesis of neuronal damage [8,9].

Until now, several models such as amyloid beta, aluminum-maltolate, senescence-accelerated, natural senescent model and D-galactose and sodium nitrate model have been used to mimic the pathophysiological alterations of senile dementia [10-13]. D-galactose can induce caspase-mediated apoptosis, inflammation and oxidative

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Passiflora incarnata L. (Passionflower) extracts elicit GABA currents in hippocampal neurons *in vitro*, and show anxiogenic and anticonvulsant effects *in vivo*, varying with extraction method

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Abstract

Potential mechanisms of *Passiflora incarnata* extracts and the effect of extraction methods on ingredients and biological effects were explored. Using the same batch of plant material, total flavonoid yields as measured by high performance liquid chromatography coupled to diode array detection (HPLC-DAD) increased substantially with hot vs. cold extraction methods.

Whole *Passiflora* extract induced prominent, dose-dependent direct $GABA_A$ currents in hippocampal slices, but the expected modulation of synaptic $GABA_A$ currents was not seen. GABA was found to be a prominent ingredient of *Passiflora* extract, and GABA currents were absent when amino acids were removed from the extract.

Five different extracts, prepared from a single batch of *Passiflora incarnata*, were administered to CF-1 mice for one week in their drinking water prior to evaluation of their behavioral effects. Anticonvulsant effects against PTZ induced seizures were seen in mice that received two of the five *Passiflora* extracts. Instead of the anxiolytic effects described by others, anxiogenic effects in the elevated plus maze were seen in mice receiving any of the five *Passiflora* extracts.

Keywords

Flavonoid; $GABA_A$ receptor; Epileptic seizure; Elevated plus maze; Rotarod; Pentylenetetrazol

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Disclosure of Conflict of Interest

A. S. is a part-time employee of Oregon's Wild Harvest, a company which manufactures botanical extracts and has provided *Passiflora incarnata* fresh herb and some of the extracts for these studies.

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ANTIRADICAL ACTIVITIES OF THE EXTRACT OF *PASSIFLORA INCARNATA*

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Abstract: The objective of this work was to investigate the aqueous and ethanolic extracts of passionflower and the influence of the flavonoids they contain on the antiradical activity by DPPH· and ABTS·+ methods. The data show that the *Passiflora* extract has not only sedative but also antiradical activity. The ethanol extract catches free radicals more effectively than the water extract. The strongest antiradical effect among the investigated flavonoids (chlorogenic acid, hyperosid, isovitexin, caffeic acid, quercetin, luteolin, orentin, rutin, scutelarein, vicenin and vitexin) was predetermined by vicenin, isovitexin and orentin. The antiradical activity increases with the increase of the concentration of the mentioned materials.

Keywords: *Passiflora incarnata,* flavonoids, free radical scavenging activities

Multiple studies show that herbal preparations of the passionflower are widely used in medicine as sedatives and tranquilizers (1). It has been determined that *Passiflora incarnata* lowers the level of HDL and can be used as a prophylactic means against atherosclerosis. It has an anti-atherogenic and cardioprotective effect (2, 3). The wide range of pharmacological action of passionflower is determined by its bioactive ingredients: alkaloids and flavonoids (4). Flavonoids have been shown to act as scavengers of various oxidizing species i.e. superoxide anion ($O_2^{\cdot}$), hydroxyl or peroxy radicals (5). They may also act as quenchers of singlet oxygen (6). Another possible contributory mechanism to the antioxidant activity of flavonoids is their ability to stabilize membranes by decreasing membraine fluidity (5). It is important to neutralize the free radicals, which are the products of the metabolic processes, since they may play a role in cardiac insufficiency. The neutralization can be performed not only by the antioxidant protective enzymes but also by different bioactive materials found in herbal preparations. There are multiple data in literature, which show that bioactive compounds neutralize free radicals in different ways. The data how free radicals interact with *Passiflora incarnata,* and the data how its antiradical activity is predetermined by flavonoid ingredients, were not found. Therefore, it is an important field of investigation, since *Passiflora incarnata* is a popular ingredient in numerous phytopharmaceutical preparations.

The objective of this work was to investigate the aqueous and ethanolic extracts of passionflower and the influence of the flavonoids they contain on the antiradical activity by DPPH· and ABTS·+ methods.

EXPERIMENTAL

Plant material

The herb of *Passiflora incarnata* was harvested from the collection of the medicinal plants in Kaunas Botanical Garden (Vytautas Magnus University, Lithuania). The raw material was sorted out and dried at ambient temperature in a dry room with active ventilation.

Flavonoids

These flavonoid standarts (chlorogenic acid, hyperosid, isovetixin, caffeic acid, quercitin, luteolin, orentin, rutin, scutelarein, vitexin) were used for this experiment (producer Carl Roth GmbH, Karlsruhe, Germany).

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Protective Effect of Standardized Extract of *Passiflora incarnata* Flower in Parkinson's and Alzheimer's Disease

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Abstract

Background:

Oxidative stress plays an important role in the pathogenesis of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Flavonoids exert their antioxidant effects by neutralizing all types of oxidizing radicals including the superoxide and hydroxyl radicals. *Passiflora incarnata* Linn. (Passifloraceae) is an important plant used in Ayurveda for the treatment of various disorders of the CNS and is a rich source of flavonoids.

Aim:

In the present study, we investigated the antioxidant, antiparkinsonian, and memory enhancing activity of flavonoid rich n-butanol extract of *P. incarnata* flowers (BEPIF).

Materials and Methods:

Antioxidant activity was assessed using DPPH and hydrogen peroxide scavenging assay. The antiparkinsonian activity was evaluated using haloperidol induced catalepsy and tacrine induced vacuous chewing movement and memory enhancing activity was assessed using elevated plus maze and object recognition test.

Statistical Analysis:

The results were analyzed by Analysis of Variance test followed by Dunnett's test.

Results:

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