Extended Longevity

Scientific White Paper

CMEnhance Formula. Supports Cellular Metabolic Efficiency by increasing Resveratrol, Sirtuins, NAD, and NMN. Includes phytotherapeutic extracts of *Polygonum cuspidatum, Scutellaria baicalensis, Tabebuia avellanedae, Curcuma longa, and Cinnamomum verum*. Biological Actions, Molecular Mechanisms, and Their Effects.

Steven M Schorr
***Extended Longevity, Inc**., Department of Scientific Research. P.O. Box 448 Puunene, HI 96784 USA*

CMEnhance Formula is designed to support Cellular Metabolic Efficiency, increase expression of Resveratrol, Sirtuins, NR, and NMN and contains extracts of: ***Polygonum cuspidatum, Scutellaria baicalensis, Tabebuia avellanedae, Curcuma longa, and Cinnamomum verum***. This formula is a synergistic herbal analog to synthetic resveratrol and NAD precursors NMN and NR.

Overview

(ATP) is a multifunctional nucleoside triphosphate used in cells as a coenzyme. It is often called the "molecular unit of currency" of intracellular energy transfer. ATP transports chemical energy within cells for metabolism. It is one of the end products of photophosphorylation and cellular respiration and is used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division. ATP can be produced by redox reactions using simple and complex sugars (carbohydrates) or lipids as an energy source. For complex fuels to be synthesized into ATP, they first need to be broken down into smaller, simple molecules. Carbohydrates are hydrolyzed into simple sugars, such as glucose and fructose. Fats (triglycerides) are metabolized to give fatty acids and glycerol. The overall process of oxidizing glucose to carbon dioxide is known as cellular respiration and can produce about 30 molecules of ATP from a single molecule of glucose. ATP can be produced by several distinct cellular processes; the three main pathways used to generate energy in eukaryotic organisms are glycolysis and the citric acid cycle/oxidative phosphorylation, both components of cellular respiration; and beta-oxidation. The majority of this ATP production by a non-photosynthetic aerobic eukaryote takes place in the mitochondria, which can make up nearly 25% of the total volume of a typical cell.

Oxidative phosphorylation

Oxidative phosphorylation is a metabolic pathway that uses energy released by the oxidation of nutrients to produce adenosine triphosphate (ATP). Although the many forms of life on earth use a range of different nutrients, almost all aerobic organisms carry out oxidative phosphorylation to produce ATP, (the molecule that supplies energy to the metabolism.) This pathway is probably so pervasive because it is a highly efficient way of releasing energy. During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP. In eukaryotes, these redox reactions are carried out by a series of protein complexes

within mitochondria. The energy released by electrons flowing through this electron transport chain is used to transport protons across the inner mitochondrial membrane, in a process called *chemiosmosis*. This generates potential energy in the form of a pH gradient and an electrical potential across this membrane. This store of energy is tapped by allowing protons to flow back across the membrane and down this gradient, through a large enzyme called ATP synthase. This enzyme uses this energy to generate ATP from adenosine diphosphate (ADP), in a phosphorylation reaction. This reaction is driven by the proton flow, which forces the rotation of a part of the enzyme; the ATP synthase is a rotary mechanical motor. ATP synthase releases this stored energy by completing the circuit and allowing protons to flow down the electrochemical gradient, back to the N-side of the membrane. This kinetic energy drives the rotation of part of the enzyme's structure and couples this motion to the synthesis of ATP.

In eukaryotes, the enzymes in this electron transport system use the energy released from the oxidation of NADH to pump protons across the inner membrane of the mitochondrion. This causes protons to build up in the intermembrane space and generates an electrochemical gradient across the membrane. The energy stored in this potential is then used by ATP synthase to produce ATP. This coenzyme contains electrons that have a high transfer potential. They will release a large amount of energy upon oxidation. Although oxidative phosphorylation is a vital part of metabolism, it produces reactive oxygen species such as superoxide and hydrogen peroxide, which lead to the creation of free radicals, damaging cells and contributing to disease and aging (senescence). The electron transport chain in the mitochondrion is the site of oxidative phosphorylation in eukaryotes. The NADH and succinate generated in the citric acid cycle are oxidized, releasing energy to power the ATP synthase. This process also produces radical oxygen species (free radicals) the cause of oxidative stress. Molecular oxygen is an ideal terminal electron acceptor because it is a strong oxidizing agent. The reduction of oxygen does involve potentially harmful intermediates. Although the transfer of four electrons and four protons reduces oxygen to water, which is harmless, the transfer of one or two electrons produces superoxide or peroxide anions, which are dangerously reactive.

Reactive Oxygen Species (Free Radicals)

These reactive oxygen species and their reaction products, such as the hydroxyl radical, are very harmful to cells, as they oxidize proteins and cause mutations in DNA. This cellular damage might contribute to disease and is proposed as one cause of aging. As the production of reactive oxygen species by these proton-pumping complexes is greatest at high membrane potentials, it has been proposed that mitochondria regulate their activity to maintain the membrane potential within a narrow range that balances ATP production against oxidant generation. For instance, oxidants can activate uncoupling proteins that reduce membrane potential. To counteract these reactive oxygen species, cells contain numerous antioxidant systems, including antioxidant vitamins such as vitamin C and vitamin E, and antioxidant enzymes such as superoxide dismutase, catalase, and peroxidases, which detoxify the reactive species, limiting damage to the cell.

Oxidative stress

Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.

Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Some reactive oxidative species can even act as messengers in redox signaling. In humans, oxidative stress is thought to be involved in the development of many diseases or may exacerbate their symptoms. Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses, such as glutathione. The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis.

Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. Such species include free radicals and peroxides. The major portion of long-term effects is inflicted by damage to DNA. Most of these oxygen-derived species are produced at a low level by normal aerobic metabolism. Normal cellular defense mechanisms destroy most of these and any damage to cells is constantly repaired. However, under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and causing the cell to simply fall apart.

Cellular Metabolic Efficiency (CME)

Within the cell mitochondria, ATP oxidative phosphorylation produces radical oxygen species (ROS) (including free radicals) as a by-product of the cell's energy creation reaction. **CMEnhance** passes through the cellular and then mitochondrial membrane to facilitate the neutralization of the radical oxygen species that is the cause of oxidative stress within the cell. When oxidative stress is minimized Cellular Metabolic Efficiency (CME) is achieved, thus increasing the efficiency of the cell mitochondria's ATP energy-producing reaction. This releases more energy in the cell and this reaction is multiplied by our bodies 100 trillion cells resulting in the experience of increased energy, health and well-being. High antioxidant levels combined with high-bioavailability are the key to establishing Cellular Metabolic Efficiency (CME). High-bioavailability is a function of penetration through the cellular and mitochondria membranes. Small molecules are more efficient in penetrating the cell and mitochondrial walls, thus are more available to effect oxygen radical absorbance. By this process, **CMEnhance** increases Cellular Metabolic Efficiency (CME), thus increasing energy and reducing metabolic oxidative stress at the cellular level. This will also have the effect of reducing inflammation, increasing health and well-being and slowing down cell apoptosis, thus slowing down the aging process.

Sirtuin overview

Post-translational modifications play an important role in cells, such as DNA recognition, protein-protein interactions, catalytic activity, and protein stability. Protein acetylation/deacetylation is a histone covalent modification that is mainly catalyzed by histone acetylase and histone deacetylase, respectively. The Sirtuin protein family plays an important role in different cellular processes such as apoptosis, mitochondrial biosynthesis, lipid metabolism, fatty acid oxidation, cellular stress response, insulin secretion, and aging.

Sirtuin family

The yeast silencing regulator 2 (Sir2) protein and sirtuin in other prokaryotic and eukaryotic organisms are a class of proteins that belong to deacetylases and/or ADP ribose that is highly conserved depending on NAD^+. *The Sir2* gene plays a very important role in maintaining the mating type of yeast, the length of telomeres, and the generation of rDNA-encoded DNA repeats. Sir2 plays an important role in life regulation. The *sir2* gene can extend the lifespan of the yeast by inhibiting genomic instability. An additional *sir2* gene copy can extend yeast life by about 40%. The Sirtuin protein family also plays an important role in the life-extension process caused by caloric restriction. The Sirtuin protein family is an NAD^+ dependent protein deacetylase and/or ADP ribosyltransferase, suggesting that the sirtuin protein family may act as a receptor for NAD^+ and is closely related to glycolipid metabolism. The Sirtuin protein family is involved in a series of physiological and pathological processes in living organisms and is closely related to glycolipid metabolism, lifespan regulation, stress response, inflammatory response, and tumor formation. The mammalian sirtuin protein family has seven members (SIRT1~SIRT7), all of which have highly conserved NAD^+ binding domains and catalytic domains.

Molecular compounds, such as resveratrol, increase the enzymatic activity of SIRT1. These compounds inhibit the expression of downstream genes SREBP. SIRT1 in the liver plays an important role in glycolipid metabolism, and activation of SIRT1 may be useful in the treatment of metabolic diseases. The brain can regulate the metabolic balance of the whole body through the nervous and endocrine systems. Calorie restriction and starvation both increase the expression of SIRT1 in the hypothalamus. Neuronal activity in the hypothalamus is enhanced after mouse brain-specific overexpression of SIRT1. This suggests that SIRT1 in the brain affects the secretion of hypothalamic/pituitary hormones and participates in the process of calorie restriction to delay aging in mammals. SIRT1 is involved in the regulation of rhythms. SIRT1 binds to CLOCK/BMAL1 and regulates the expression of rhythmic. NADT, the rate-limiting enzyme of NAD^+ biosynthesis, can be directly regulated by CLOCK/BMAL1, which may be the molecular mechanism of NAD^+ and SIRT1 enzyme activity rhythm changes.

Pathway regulation

SIRT1 is involved in several metabolic pathways including fat production, insulin secretion, glucose synthesis, and mitochondrial biosynthesis. Energy limitation can increase the expression of SIRT1 and prolong life. Several new SIRT1 agonists, including resveratrol, have been discovered, which appears to mimic certain effects of energy limitation in a variety of organisms. SIRT1 activators have good application prospects in many therapeutic fields including type 2 diabetes, inflammation, neurodegenerative diseases, and heart diseases. Sirtuins also play an important role in vascular biology, which may regulate age-related atherosclerosis.

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Active Herbal Ingredients

Polygonum cuspidatum, is abundant in Resveratrol a multi-biofunctional phytochemical, stilbenes and anthraquinones, such as polydatin, anthraglycoside B, and emodin. *P. cuspidatum* possesses wound healing activity and has been effective for inflammatory diseases, hepatitis, tumors, and diarrhea.

Scutellaria baicalensis, flavones exhibit strong neuroprotective effects on the brain. Their neuroprotective potential has been shown in both oxidative stress-induced and amyloid-beta neuronal death models. Baicalein, the main flavone present strongly inhibited aggregation of neuronal amyloidogenic proteins in vitro and induces dissolution of amyloid deposits. It has been shown to stimulate 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. The herb effectively inhibited basal and TGF-β1-induced cancer cell proliferation. It is an analog of the drug Alk5 kinase inhibitor and blocks the growth factor's receptors, stopping it from aging the body's stem cells. *Scutellaria baicalensis* blocks the key TGF-beta1 pathway, which is elevated by aging, thereby rejuvenating multiple organ systems.

Tabebuia avellanedae demonstrates anti-inflammatory, antibacterial, and anticancer activity. Active pharmacological compounds such as naphthoquinones, furanonaphthoquinones, anthraquinones, β -lapachone, benzoic acid derivatives, benzaldehyde derivatives, cyclopentene derivatives, iridoids, coumarins, anthraquinone-2-carboxlic acid, and flavonoids have been extracted. Pau d'Arco demonstrated inhibitory activity on inflammatory cytokines, tumor-necrosis factor-α, and interleukin-1β.

Curcuma longa, Turmeric root, and it's curcuminoid constituents have demonstrated properties consistent with decreases in inflammatory stress signaling and increases in protective signaling. Curcumin is known to have anti-aging, anti-oxidant, anti-inflammatory, anti-arthritic, and anti-cancer effects and increases BDNF while having a positive effect on Alzheimer's disease and depression. It is also anti-rheumatic, and anti-microbial.

Cinnamomum verum, Cinnamon health benefits are attributed to its content of a few specific types of antioxidants, including polyphenols, phenolic acid, and flavonoids. These compounds work to fight oxidative stress in the body and aid in the prevention of chronic disease.

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REVIEW ARTICLE OPEN

It takes two to tango: NAD^+ and sirtuins in aging/longevity control

Shin-ichiro Imai[1] and Leonard Guarente[2,3]

The coupling of nicotinamide adenine dinucleotide (NAD^+) breakdown and protein deacylation is a unique feature of the family of proteins called 'sirtuins.' This intimate connection between NAD^+ and sirtuins has an ancient origin and provides a mechanistic foundation that translates the regulation of energy metabolism into aging and longevity control in diverse organisms. Although the field of sirtuin research went through intensive controversies, an increasing number of recent studies have put those controversies to rest and fully established the significance of sirtuins as an evolutionarily conserved aging/longevity regulator. The tight connection between NAD^+ and sirtuins is regulated at several different levels, adding further complexity to their coordination in metabolic and aging/longevity control. Interestingly, it has been demonstrated that NAD^+ availability decreases over age, reducing sirtuin activities and affecting the communication between the nucleus and mitochondria at a cellular level and also between the hypothalamus and adipose tissue at a systemic level. These dynamic cellular and systemic processes likely contribute to the development of age-associated functional decline and the pathogenesis of diseases of aging. To mitigate these age-associated problems, supplementation of key NAD^+ intermediates is currently drawing significant attention. In this review article, we will summarize these important aspects of the intimate connection between NAD^+ and sirtuins in aging/longevity control.

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IT'S SO LONG AGO

Since the first discovery of nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase activity of the silent information regulator 2 (Sir2) family ('sirtuins'),[1] the field of sirtuin biology has been evolving rapidly over the past 16 years. Many researchers from different fields have encountered sirtuins in their own research, enriching our knowledge of this fascinating family of enzymes. It is now clear that sirtuins are involved in the regulation of many fundamental biological processes throughout the body.[2,3] Furthermore, it has been revealed that sirtuins possess much broader enzymatic activities, namely, deacylases, including deacetylase, desuccinylase, demaloynylase, deglutarylase, long-chain deacylase, lipoamidase, and ADP-ribosyltransferase.[4,5] All these enzymatic activities specifically require NAD^+, and the catalytic mechanism of this NAD^+ dependency has been studied extensively.[6] Clearly, sirtuins have evolved to respond to the availability of NAD^+, an essential currency of cellular metabolism and DNA damage repair, and convert this information to many different biological outputs. In this particular review article, we will focus on this intimate connection between sirtuin function, aging/longevity control in particular, and their indispensable co-substrate, NAD^+.

The origin of the connection between NAD^+ and sirtuins is ancient. For instance, vibriophage KVP40 possesses a minimal set of genes for NAD^+ biosynthesis and consumption, namely, the genes encoding two key NAD^+ biosynthetic enzymes, nicotinamide phosphoribosyltransferase (NAMPT) and nicotinamide/nicotinic acid mononucleotide adenylyltransferase (NMNAT),[2,3] and a sirtuin family protein (Figure 1).[7] Although why such a minimalistic organism keeps these three genes in its genome remains unclear, one potential explanation is that controlling the host cell's metabolism and proliferation in a NAD^+-dependent manner could provide benefits for this particular vibriophage to efficiently produce progeny in the host cell. Such a NAD^+/sirtuin-mediated virus–host relationship might be a prototype for the much more complex inter-tissue communication mediated by NAMPT and the mammalian sirtuin SIRT1.[8] As discussed later in this review, NAMPT and SIRT1 comprise multiple layers of feedback regulatory loops inside cells and between tissues and organs, and contribute to the systemic regulation of mammalian aging and longevity.[9–12] Another interesting example is the connection between the nicotinamidase Pnc1 and Sir2 proteins in yeast, worms, and flies. Whereas vertebrates and a limited number of bacterial species mainly use NAMPT to synthesize NAD^+ from nicotinamide, invertebrates and most bacterial species use nicotinamidase to convert nicotinamide to nicotinic acid and synthesize NAD^+ from nicotinic acid (Figure 1).[13] Pnc1 regulates NAD^+ biosynthesis and affects lifespan in those organisms.[14–16]

Although all genetic, pathophysiological, and pharmacological studies point out that sirtuin activity can be affected by changes in NAD^+ levels, whether sirtuin activity is indeed regulated by the physiological fluctuation of NAD^+ has been of great debate. However, a recent detailed kinetic study has demonstrated that at least for deacetylase activities of SIRT1-3, each K_m for NAD^+ is consistent with the notion that changes in NAD^+ levels in each subcellular compartment can directly regulate sirtuin activity.[17] These findings further affirm the functional connection between

[1]Department of Developmental Biology, Washington University School of Medicine, St Louis, MO, USA; [2]Department of Biology and Glenn Laboratories for the Science of Aging, Massachusetts Institute of Technology, Cambridge, MA, USA and [3]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA. Correspondence: S Imai (imaishin@wustl.edu) or L Guarente (leng@mit.edu)

Subtitles are cited from Todd Rundgren's lyrics of 'It Takes Two To Tango (This Is For The Girls)' in his album 'Something/Anything?' released in 1972.

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REVIEW ARTICLE

Sirtuins, a promising target in slowing down the ageing process

Wioleta Grabowska · Ewa Sikora · Anna Bielak-Zmijewska

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Abstract Ageing is a plastic process and can be successfully modulated by some biomedical approaches or pharmaceutics. In this manner it is possible to delay or even prevent some age-related pathologies. There are some defined interventions, which give promising results in animal models or even in human studies, resulting in lifespan elongation or healthspan improvement. One of the most promising targets for anti-ageing approaches are proteins belonging to the sirtuin family. Sirtuins were originally discovered as transcription repressors in yeast, however, nowadays they are known to occur in bacteria and eukaryotes (including mammals). In humans the family consists of seven members (SIRT1-7) that possess either mono-ADP ribosyltransferase or deacetylase activity. It is believed that sirtuins play key role during cell response to a variety of stresses, such as oxidative or genotoxic stress and are crucial for cell metabolism. Although some data put in question direct involvement of sirtuins in extending human lifespan, it was documented that proper lifestyle including physical activity and diet can influence healthspan via increasing the level of sirtuins. The search for an activator of sirtuins is one of the most extensive and robust topic of research. Some hopes are put on natural compounds, including curcumin. In this review we summarize the involvement and usefulness of sirtuins in anti-ageing interventions and discuss the potential role of curcumin in sirtuins regulation.

Keywords Sirtuins · Ageing · Senescence · Curcumin

W. Grabowska · E. Sikora · A. Bielak-Zmijewska (✉)
Laboratory of Molecular Bases of Aging, Department of Biochemistry, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Pasteur Str. 3, 02-093 Warsaw, Poland
e-mail: w.grabowska@nencki.gov.pl

E. Sikora
e-mail: e.sikora@nencki.gov.pl

A. Bielak-Zmijewska
e-mail: a.bielak@nencki.gov.pl

Introduction

In the year 1979 a paper announcing discovery of mating-type regulator 1 (MAR1) in *Saccharomyces cerevisiae* was published (Klar et al. 1979). Lack of this protein resulted in the inhibition of silencing of HM loci, which control the mating type and sterility in yeast. Three more proteins with similar function were discovered later in 1979 and the nomenclature was unified thus creating a family of Sir (silent information regulator) proteins (Michan and Sinclair 2007). Shortly, it was shown that sirtuins are evolutionarily conserved from bacteria to humans (Vaquero 2009). We now know a number of processes sirtuins are involved in and we still discover their new functions. In bacteria phosphoribosyltransferases cobT and cobB

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Sirtuins, cell senescence, and vascular aging

Yujiro Kida, MD, PhD and **Michael S. Goligorsky, MD, PhD**
Renal Research Institute, Departments of Medicine, Pharmacology, and Physiology, New York Medical College, Valhalla, New York, U.S.A

Abstract

The sirtuin (SIRTs) constitute a class of proteins with NAD+-dependent deacetylase or ADP-ribosyltransferase activity. Seven SIRT family members have been identified in mammals, from SIRT1, the best studied for its role in vascular aging, to SIRT7. SIRT1 and SIRT2 are localized both in the nucleus and cytoplasm. SIRT3, SIRT4, and SIRT5 are mitochondrial, while SIRT6 and SIRT7 are nuclear. Extensive studies have clearly revealed that SIRT proteins regulate diverse cell functions and responses to stressors. Vascular aging involves the aging process (senescence) of endothelial and vascular smooth muscle cells. Two types of cell senescence have been identified: (1) replicative senescence with telomere attrition and (2) stress-induced premature senescence without telomere involvement. Both types of senescence induce vascular cell growth arrest and loss of vascular homeostasis, contributing to the initiation and progression of cardiovascular diseases. Previous mechanistic studies have revealed in detail that SIRT1, SIRT3, and SIRT6 demonstrate protective functions against vascular aging, while definite vascular function of other SIRTs is under investigation. Thus, direct SIRT modulation and NAD+ stimulation of SIRT are promising candidates for cardiovascular disease therapy. A small number of pilot studies have been conducted to assess SIRT modulation in humans. These clinical studies have not yet provided convincing evidence that SIRT proteins alleviate morbidity and mortality in patients with cardiovascular diseases. The outcomes of multiple ongoing clinical trials are awaited to define the efficacy of SIRT modulators and SIRT activators in cardiovascular diseases, along with the potential adverse effects of chronic SIRT modulation.

1. Introduction

1.1 Sirtuins

Sirtuin (SIRT), or silent information regulator 2 (Sir2) proteins, are a class of proteins that possess nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase activity or ADP-ribosyltransferase activity. In mammals, seven SIRT proteins have been identified, from SIRT1, the best studied for its role in vascular aging, to SIRT7. They have different tissue

Corresponding author: Yujiro Kida, MD, PhD, Department of Medicine, Renal Research Institute, New York Medical College, 15 Dana Road, Valhalla, NY 10595, U.S.A. Phone: 914-594-4731, Fax: 914-594-4732, yujiro_kida@nymc.edu.

Disclosures
None.

biomolecules

MDPI

Review

Nicotinamide Mononucleotide: Exploration of Diverse Therapeutic Applications of a Potential Molecule

Saikat Kumar Poddar [1,*], Ali Ehsan Sifat [1], Sanjana Haque [1], Noor Ahmed Nahid [1], Sabiha Chowdhury [1] and Imtias Mehedi [2]

1 Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh; ali.ehsan.sifat@gmail.com (A.E.S.); haquesanjana@gmail.com (S.H.); noor.nahid@gmail.com (N.A.N.); sabihanabila@gmail.com (S.C.)

2 Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh; imtiasmehedi@gmail.com

* Correspondence: saikatpoddar@du.ac.bd; Tel.: +880-1723009070

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Abstract: Nicotinamide mononucleotide (NMN) is a nucleotide that is most recognized for its role as an intermediate of nicotinamide adenine dinucleotide (NAD+) biosynthesis. Although the biosynthetic pathway of NMN varies between eukaryote and prokaryote, two pathways are mainly followed in case of eukaryotic human—one is through the salvage pathway using nicotinamide while the other follows phosphorylation of nicotinamide riboside. Due to the unavailability of a suitable transporter, NMN enters inside the mammalian cell in the form of nicotinamide riboside followed by its subsequent conversion to NMN and NAD+. This particular molecule has demonstrated several beneficial pharmacological activities in preclinical studies, which suggest its potential therapeutic use. Mostly mediated by its involvement in NAD+ biosynthesis, the pharmacological activities of NMN include its role in cellular biochemical functions, cardioprotection, diabetes, Alzheimer's disease, and complications associated with obesity. The recent groundbreaking discovery of anti-ageing activities of this chemical moiety has added a valuable essence in the research involving this molecule. This review focuses on the biosynthesis of NMN in mammalian and prokaryotic cells and mechanism of absorption along with the reported pharmacological activities in murine model.

Keywords: ageing; Alzheimer's disease; diabetes; ischemic preconditioning; nicotinamide mononucleotide; obesity

1. Introduction

Nicotinamide mononucleotide (NMN) or Nicotinamide-1-ium-1-β-D-ribofuranoside 5′-phosphate is a type of bioactive nucleotide which is naturally formed by the reaction between a phosphate group and a nucleoside containing ribose and nicotinamide [1]. Generally, it exists in two anomeric forms namely alpha and beta. The beta anomer is the active form between these two with a molecular weight of 334.221 g/mol [2]. NMN is naturally abundant in various types of food [3]. Vegetables like broccoli, cabbage contain 0.25–1.12 and 0.0–0.90 mg NMN/100 gm, fruits like avocado, tomato contain 0.36–1.60 and 0.26–0.30 mg NMN/100 gm, whereas raw beef has 0.06–0.42 mg NMN/100 gm [3]. NMN is also used as a substrate for prokaryotic enzymes like NadM in *Methanobacterium thermoautotrophicum*, NadR in *Haemophilus influenza*, NadM/Nudix in *Francisella tularensis* [4].

In human cells, NMN is available as a source of cellular energy. Not long ago, this molecule was only known for its activity as an intermediate in nicotinamide adenine dinucleotide (NAD+) biosynthesis. During this biosynthetic process of NAD+, NMN acts as an important substrate for

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Sirtuin activators and inhibitors

José M. Villalba[1] and **Francisco J. Alcaín**[2,*]

[1]Departamento de Biología Celular, Fisiología e Inmunología, Facultad de Ciencias, Campus Universitario de Rabanales, Edificio Severo Ochoa, Universidad de Córdoba, Campus de Excelencia Internacional Agroalimentario, ceiA3, 14014-Córdoba, Spain

[2]Departamento de Ciencias Médicas, Facultad de Medicina, Campus de Ciudad Real, Universidad de Castilla la Mancha, 13071-Ciudad Real, Spain

Abstract

Sirtuins 1-7 (SIRT1-7) belong to the third class of deacetylase enzymes, which are dependent on NAD^+ for activity. Sirtuins activity is linked to gene repression, metabolic control, apoptosis and cell survival, DNA repair, development, inflammation, neuroprotection and healthy aging. Because sirtuins modulation could have beneficial effects on human diseases there is a growing interest in the discovery of small molecules modifying their activity. We review here those compounds known to activate or inhibit sirtuins, discussing the data that support the use of sirtuin-based therapies. Almost all sirtuin activators have been described only for SIRT1. Resveratrol is a natural compound which activates SIRT1, and may help in the treatment or prevention of obesity, and in preventing tumorigenesis and the aging-related decline in heart function and neuronal loss. Due to its poor bioavailability, reformulated versions of resveratrol with improved bioavailability have been developed (resVida, Longevinex®, SRT501). Molecules that are structurally unrelated to resveratrol (SRT1720, SRT2104, SRT2379, among others) have been also developed to stimulate sirtuin activities more potently than resveratrol. Sirtuin inhibitors with a wide range of core structures have been identified for SIRT1, SIRT2, SIRT3 and SIRT5 (splitomicin, sirtinol, AGK2, cambinol, suramin, tenovin, salermide, among others). SIRT1 inhibition has been proposed in the treatment of cancer, immunodeficiency virus infections, Fragile X mental retardation syndrome and for preventing or treating parasitic diseases, whereas SIRT2 inhibitors might be useful for the treatment of cancer and neurodegenerative diseases.

2. Introduction

The benefits of the Fountain of Youth, able to extend human lifespan, have been a general goal, appearing in writings by the ancient Greeks and also in tales among the indigenous peoples of the Caribbean. The discovery that overexpressing the Silent information regulator (Sir2) prolonged the lifespan of *Caenorhabditis elegans* (1) and *Drosophila melanogaster* (2) attracted a lot of interest in sirtuins. This interest was even reinforced by reports that calorie restriction (CR) could extend lifespan in mammals by inducing sirtuin 1 (SIRT1) expression and promoting the long-term survival of irreplaceable cells (3). A role for sirtuins in promoting longevity is now questioned due to the recent demonstration that high-level expression of Sir2 alone was not sufficient to increase lifespan relative to the transgenic controls, both in worms and flies, and all genotypes responded similarly and normally to CR (4). However, a great interest has indeed emerged in the discovery of and in developing molecules able to regulate sirtuin activity.

*Correspondence and reprints: Francisco J. Alcaín, Departamento de Ciencias Médicas, Facultad de Medicina, Campus de Ciudad Real, Universidad de Castilla la Mancha, 13071-Ciudad Real, Spain, Phone: + 34 926 295300 ext 6638, franciscoj.alcain@uclm.es.

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Sirtuins and NAD+ in the Development and Treatment of Metabolic and Cardiovascular Diseases

Alice E. Kane[1] and **David A Sinclair**[1,2]

[1]Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA.

[2]Department of Pharmacology, The University of New South Wales, Sydney 2052, Australia.

Abstract

The sirtuin family of nicotinamide adenine dinucleotide (NAD^+)-dependent deacylases (SIRT1–7) are thought to be responsible, in large part, for the cardiometabolic benefits of lean diets and exercise and when upregulated can delay key aspects of aging. SIRT1, for example, protects against a decline in vascular endothelial function, metabolic syndrome, ischemia-reperfusion (IR) injury, obesity and cardiomyopathy, and SIRT3 is protective against dyslipidemia and IR injury. With increasing age, however, NAD^+ levels and sirtuin activity steadily decrease and the decline is further exacerbated by obesity and sedentary lifestyles. Activation of sirtuins or NAD^+ repletion induces angiogenesis, insulin sensitivity and other health benefits in a wide range of age-related cardiovascular and metabolic disease models. Human clinical trials testing agents that activate SIRT1 or boost NAD^+ levels are in progress and show promise in their ability to improve the health of cardiovascular and metabolic disease patients.

Keywords

STACs; NAD^+ booster; atherosclerosis; ischemia-reperfusion injury; obesity; metabolic syndrome; cardiomyopathy; dyslipidemia; insulin resistance; aging

1. Introduction

The lifespan extending abilities of the sirtuins were first discovered in yeast in the 1990s[1,2] when the silent information regulator (SIR2) gene was shown to increase the replicative lifespan of yeast when upregulated[2]. Sir2 is a yeast gene silencing protein, which silences transcription at the HM mating-type loci in young yeast but re-localizes to the ribosomal DNA as cells age to prevent DNA damage that contributes to yeast aging[3]. Upregulating the expression of Sir2 reduces DNA damage and increases the lifespan of yeast[2]. Subsequently, Sir2 was shown to have histone deacetylase activity which requires nicotinamide adenine dinucleotide (NAD^+)[4,5] and sirtuins in mammals (SIRT1–7) were identified. Mammalian sirtuins were also shown to have important beneficial roles in aging, longevity and stress

Corresponding author: Alice Kane, Ph.D., Paul F. Glenn Center for the Biological Mechanisms of Aging, Department of Genetics, Harvard Medical School, 77 Avenue Louis Pasteur, Boston MA 02115 USA, Telephone: (617) 432-3931, Fax: (617) 432-6225, alice_kane@hms.harvard.edu.

Extended Longevity

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Curcumin elevates sirtuin level but does not postpone *in vitro* senescence of human cells building the vasculature

Wioleta Grabowska[1], Małgorzata Suszek[1], Maciej Wnuk[2], Anna Lewinska[3], Emilia Wasiak[1], Ewa Sikora[1] and Anna Bielak-Zmijewska[1]

[1] Department of Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

[2] Department of Genetics, University of Rzeszow, Rzeszów, Poland

[3] Department of Biochemistry and Cell Biology, University of Rzeszow, Rzeszów, Poland

Correspondence to: *Anna Bielak-Zmijewska,* ***email***: *a.bielak@nencki.gov.pl*

Keywords: curcumin, senescence, sirtuins, VSMC, EC, Gerotarget

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ABSTRACT

It is believed that curcumin, a component of the turmeric that belongs to hormetins, possesses anti-aging propensity. This property of curcumin can be partially explained by its influence on the level of sirtuins. Previously, we have shown that relatively high (2.5-10 μM) doses of curcumin induce senescence of cancer cells and cells building the vasculature. In the present study we examined whether curcumin at low doses (0.1 and 1 μM) is able to delay cell senescence and upregulate the level of sirtuins in human cells building the vasculature, namely vascular smooth muscle (VSMC) and endothelial (EC) cells. To this end we used cells senescing in a replicative and premature manner. We showed that low doses of curcumin in case of VSMC neither postponed the replicative senescence nor protected from premature senescence induced by doxorubicin. Moreover, curcumin slightly accelerated replicative senescence of EC. Despite some fluctuations, a clear increasing tendency in the level of sirtuins was observed in curcumin-treated young, senescing or already senescent cells. Sirtuin activation could be caused by the activation of AMPK resulting from superoxide elevation and ATP reduction. Our results show that curcumin at low doses can increase the level of sirtuins without delaying senescence of VSMC.

INTRODUCTION

Curcumin, a natural compound derived from *Curcuma longa*, is considered as a potent anti-aging factor [1, 2]. There is a lot of data concerning its beneficial activity for the whole organism, including elongation of the life of model organisms. Curcumin is able to reduce the negative influence of some factors and proved beneficial in alleviating the symptoms of some diseases [3, 4, 5]. The most important activity of curcumin stems from its anti-inflammatory properties but there are also data suggesting the role of curcumin in sirtuin stimulation [6, 7, 8]. Sirtuins, NAD-dependent deacetylases, are involved in DNA repair, genome stability, telomere structure maintenance but also in epigenetic modifications of histones [9]. Their activity is considered as potentially anti-aging, therefore activators of sirtuins could be regarded as potential anti-aging compounds. It is believed that sirtuins are responsible for lifespan elongation of model organisms and are the key elements elevated during caloric restriction [10, 11]. They are also necessary for the proper functioning of the cardiovascular system [12, 13, 14]. With age, the level of sirtuin 1 and 6 decreases [15]. The lack of sirtuin 6 caused premature aging [16] and of sirtuin 1 promoted expression of genes, which are normally expressed during aging [17]. Sirtuin 1 prevented replicative senescence of normal human umbilical cord fibroblasts and antagonized both replicative and premature senescence in stem cells and differentiated cells under conditions of oxidative stress [18, 19]. Also sirtuin 3 is strongly correlated with the aging process and there are data which link this protein with longevity [20, 21, 22]. Although there are suggestions that curcumin acts by sirtuin activation [6,

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Review Article

A Review of the Pharmacological Effects of the Dried Root of *Polygonum cuspidatum* (Hu Zhang) and Its Constituents

Huan Zhang,[1] Chang Li,[2] Sin-Tung Kwok,[3] Qing-Wen Zhang,[4] and Shun-Wan Chan[1,2,5]

[1] *Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China*
[2] *Food Safety and Technology Research Centre, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China*
[3] *Industrial Liaison Office, Technological and Higher Education Institute of Hong Kong, Hong Kong, China*
[4] *Institute of Chinese Medical Sciences, University of Macau, Macau, China*
[5] *State Key Laboratory of Chinese Medicine and Molecular Pharmacology, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China*

Correspondence should be addressed to Shun-Wan Chan; bcswchan@polyu.edu.hk

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Traditional Chinese medicine (TCM) has been widely used in China for thousands of years to treat and prevent diseases. TCM has been proven safe and effective, and it is being considered as one of the important types of complementary and alternative medicine and receives increasing attention worldwide. The dried root of *Polygonum cuspidatum* Sieb. et Zucc. (also known as "Hu Zhang" in Chinese) is one of the medicinal herbs listed in the Pharmacopoeia of the People's Republic of China. Hu Zhang is widely distributed in the world. It can be found in Asia and North America and is used as folk medicine in countries such as Japan and Korea. In China, Hu Zhang is usually used in combination with other TCM herbs. The therapeutic uses of those Hu Zhang-containing TCM prescriptions or formulations are for treating cough, hepatitis, jaundice, amenorrhea, leucorrhea, arthralgia, burns and snake bites. Recent pharmacological and clinical studies have indicated that Hu Zhang has antiviral, antimicrobial, anti-inflammatory, neuroprotective, and cardioprotective functions. This review gives a summary of the reported therapeutic effects of the active compounds and the different extracts of Hu Zhang.

1. Introduction

The definition of complementary and alternative medicine (CAM) is broad. In general, CAM refers to a group of health care systems, practices, and medications that are not considered conventional or orthodox. CAM includes traditional Chinese medicine (TCM), acupuncture, Ayurveda, massage therapies, and mind-body therapies (such as yoga). It is often used together with conventional medicine. It is common that patients with chronic diseases turn to CAM therapies for better treatment effects, fewer side effects, or for relieving side effects of drugs. TCM, a well-known CAM, has been used to treat a variety of diseases for thousands of years [1–3]. *Panax ginseng*, *Pinella ternate*, *Salviae miltiorrhizae* and *Arisaema japonicum* are some commonly known TCMs [1, 4, 5].

As one of the important types of CAM, TCM is receiving increasing attention among scientists worldwide. For treating some complex diseases such as diabetes mellitus and cancer, TCM is one of the common alternatives of conventional medications. In recent decades, researchers from mainland China, Hong Kong, and Taiwan have focused on the investigation of various TCM herbs and their active compounds and have discovered therapeutics that are based on single compounds, such as salvicine for anticancer activity and artemisinin for malaria treatment [6].

Polygonum cuspidatum Sieb. et Zucc. is a herbaceous perennial plant. It is a member of the genus *Polygonum* in the family Polygonaceae, which grows in Asia and North America. In China, there are about 80 species of *Polygonum* used in TCM [7]. Its dried root (Figure 1) is officially listed in the Pharmacopoeia of the People's Republic of China under the name "Hu Zhang" [7]; it is also used as folk medicine in Japan and Korea. From the perspective of TCM theory, Hu

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Research Article

Tabetri™ (*Tabebuia avellanedae* Ethanol Extract) Ameliorates Osteoarthritis Symptoms Induced by Monoiodoacetate through Its Anti-Inflammatory and Chondroprotective Activities

Jae Gwang Park,[1] Young-Su Yi,[2] Yo Han Hong,[1] Sulgi Yoo,[1] Sang Yun Han,[1] Eunji Kim,[1] Seong-Gu Jeong,[1] Adithan Aravinthan,[3] Kwang Soo Baik,[4] Su Young Choi,[4] Young-Jin Son,[5] Jong-Hoon Kim,[3] and Jae Youl Cho[1]

[1]*Department of Genetic Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea*
[2]*Department of Pharmaceutical Engineering, Cheongju University, Cheongju 28503, Republic of Korea*
[3]*College of Veterinary Medicine, Chonbuk National University, Iksan 54596, Republic of Korea*
[4]*R&I Planning Department, Nutribiotech Co. Ltd., Seoul 06132, Republic of Korea*
[5]*Department of Pharmacy, Sunchon National University, Suncheon 57922, Republic of Korea*

Correspondence should be addressed to Young-Jin Son; sony@sunchon.ac.kr, Jong-Hoon Kim; jhkim1@jbnu.ac.kr, and Jae Youl Cho; jaecho@skku.edu

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Although osteoarthritis (OA), a degenerative joint disease characterized by the degradation of joint articular cartilage and subchondral bones, is generally regarded as a degenerative rather than inflammatory disease, recent studies have indicated the involvement of inflammation in OA pathogenesis. *Tabebuia avellanedae* has long been used to treat various diseases; however, its role in inflammatory response and the underlying molecular mechanisms remain poorly understood. In this study, the pharmacological effects of Tabetri (*Tabebuia avellanedae* ethanol extract (Ta-EE)) on OA pathogenesis induced by monoiodoacetate (MIA) and the underlying mechanisms were investigated using experiments with a rat model and *in vitro* cellular models. In the animal model, Ta-EE significantly ameliorated OA symptoms and reduced the serum levels of inflammatory mediators and proinflammatory cytokines without any toxicity. The anti-inflammatory activity of Ta-EE was further confirmed in a macrophage-like cell line (RAW264.7). Ta-EE dramatically suppressed the production and mRNA expressions of inflammatory mediators and proinflammatory cytokines in lipopolysaccharide-stimulated RAW264.7 cells without any cytotoxicity. Finally, the chondroprotective effect of Ta-EE was examined in a chondrosarcoma cell line (SW1353). Ta-EE markedly suppressed the mRNA expression of matrix metalloproteinase genes. The anti-inflammatory and chondroprotective activities of Ta-EE were attributed to the targeting of the nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) signaling pathways in macrophages and chondrocytes.

1. Introduction

Osteoarthritis (OA) is a time- and age-dependent progressive degenerative joint disease characterized by the degradation of joint cartilage and the underlying bones. OA is characterized by joint stiffness and pain caused by articular cartilage damage, the alteration of subchondral bones, formation of osteophytes, and thickening of synovial linings [1–4]. One of the major risk factors for OA is age. Most OA patients are over 45 years of age, and the highest morbidity attributed to OA is observed in patients over 60 years of age [5]. Among adults over 60 years of age, the prevalence of OA is approximately 10% in males and 13% in females [6]. Given the current worldwide demographic trend in which the older population is growing quickly, OA patients are expected to increase in the future. Despite this large number of OA patients, no disease-modifying drugs have been developed to effectively treat OA, and the available drugs only alleviate

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Analgesic and anti-inflammatory effects in animal models of an ethanolic extract of Taheebo, the inner bark of *Tabebuia avellanedae*

MU HONG LEE[1,2], HYUN MI CHOI[1], DAE-HYUN HAHM[3], ERK HER[4], HYUNG-IN YANG[5], MYUNG CHUL YOO[1] and KYOUNG SOO KIM[1]

[1]East-West Bone and Joint Research Institute, Kyung Hee University, Seoul 134-727; [2]Korea Apiculture Agricultural Cooperative, Ansung 456-843; [3]Acupuncture and Meridian Science Research Center, Kyung Hee University, Seoul 130-701; [4]Department of Immunology, School of Medicine, Konkuk University, Chungju 380-701; [5]Department of Internal Medicine, Kyung Hee University Hospital at Gangdong, Seoul 134-727, Republic of Korea

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Abstract. Taheebo, the purple inner bark of the Bignoniaceae tree *Tabebuia avellanedae* Lorentz ex Griseb, which is found in tropical rain forests in northeastern Brazil, has been used as a traditional medicine for various diseases for more than 1,500 years. In the current study, various animal models were used to demonstrate the analgesic and anti-inflammatory properties of its ethanolic extract, thereby investigating its potential as a therapeutic treatment for diseases with pain and inflammation. In the hot plate and writhing tests for the *in vivo* analgesic effect test of Taheebo, a 200 mg/kg dose of the extract induced a significant anti-nociceptive effect and increased the pain threshold by approximately 30% compared with the control. In vascular permeability and tetradecanoylphorbol acetate (TPA)-, arachidonic acid- and carrageenan-induced paw edema tests for anti-inflammatory effects, treatment with 200 mg/kg Taheebo led to significant anti-inflammatory effects and inhibited inflammation by 30-50% compared with the control. At 100 mg/kg, the extract decreased the levels of pain and inflammation in all tested models, but the degree of inhibition was not statistically significant. The results suggest that the ethanolic extract of the inner bark of *Tabebuia avellanedae* has the potential to be developed as a therapeutic or supportive drug against diseases with accompanying pain and inflammation, including osteoarthritis.

Introduction

Among the elderly, osteoarthritis is the most common joint disease and a significant cause of physical illness (1). Symptoms include joint pain, stiffness, limited movement, joint deformity and varying degrees of joint inflammation (2,3). Current therapeutic strategies for osteoarthritis focus on alleviating symptoms, particularly pain and inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are the mainstream treatments for osteoarthritis (4). Although NSAIDs are recommended as an initial drug therapy to reduce joint inflammation and pain, their chronic use is limited by gastrointestinal-related toxicities, including nausea, dyspepsia, upper gastrointestinal bleeding and ulcer perforation (5). To minimize these toxicities, a new generation of NSAIDs, the cyclooxygenase (COX)-2 selective inhibitors (celecoxib, rofecoxib and valdecoxib), have been developed in an attempt to improve gastrointestinal tolerance. However, reported cardiovascular risks, including myocardial infarction and stroke, have led to the removal of rofecoxib from the market (6-8). Although other COX-2 inhibitors provide effective symptomatic relief, their substantial toxicities limit long-term use. Additionally, this therapeutic approach is not curative, but relieves clinical signs and symptoms of the disease; thus, a more effective and safe drug is necessary for the curative treatment of osteoarthritis.

Joins™, a herbal drug combining the extracts of *Clematis mandshurica*, *Trichosanthes kirilowii* and *Prunella vulgaris*, is commonly used for the curative treatment of osteoarthritis in Korea. Clinical studies have demonstrated that Joins relieves joint pain and improves functionality in osteoarthritis patients. Its efficacy may be attributed to cartilage protection and anti-inflammation (9); however, its lack of an immediate analgesic effect is a major drawback. The screening of herbs and natural products for a more efficient compound may lead to the development of a superior therapeutic drug, particularly one with more immediate analgesic effects.

Tabebuia avellanedae Lorentz ex Griseb, a Bignoniaceae, is a tree found in tropical rain forests in northeastern Brazil. Taheebo, a product obtained from the purple bark of the tree, has been traditionally used for over 1,500 years in South America to treat a variety of diseases (10). Its various fractions have been previously reported to exhibit anti-inflammatory, anti-bacterial, anti-fungal, diuretic, anti-coagulant and laxative properties in addition to an anticancer effect (11-14). In

Correspondence to: Dr Kyoung Soo Kim, East-West Bone and Joint Research Institute, Kyung Hee University, 149 Sangil-dong, Gangdong-gu, Seoul 134-727, Republic of Korea
E-mail: labrea46@yahoo.co.kr

Key words: *Tabebuia avellanedae* Lorentz ex Griseb, Taheebo, anti-inflammatory effect, anti-nociceptive effect

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Quercetin content in some food and herbal samples

Anna Wach, Krystyna Pyrzyńska, Magdalena Biesaga *

Department of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland

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Abstract

Quercetin is a typical flavonoid ubiquitously present in vegetables and fruits, and its antioxidant effect is implied to be helpful for human health. The efficiency of extraction process and acidic hydrolysis parameters for HPLC analysis of quercetin present in glycosides and aglycone forms was investigated. Hydrolysis for 5 min in the presence of 2.8 M HCl as well as for 10 min with 1.1 M HCl efficiently released quercetin from rutin. The method developed in this study was applied for quantitative determination of quercetin in some food (onion, apple) and herbal (*Hypericum perforatum* and *Sambucus nigra*) products.

Keywords: Flavonoids; Quercetin; Hydrolysis; HPLC

1. Introduction

Flavonoids, and particularly quercetin derivatives, have received special attention as dietary constituents during the last few years. The epidemiological studies point out to their possible role in preventing cardiovascular diseases and cancer (Chu, Chang, & Hsu, 2000; Hertog, Hollman, & Katan, 1992a; Hertog, Feskens, Hollmann, Katan, & Kronthout, 1993). This health-promoting activity seems to be related to the antioxidant (free-radical scavenging) activity to flavonoids (Murota & Terao, 2003). Flavonoids are widely distributed in plants and are categorized as flavonol, flavanol, flavanone, flavone, anthocyanidin and isoflavone.

Quercetin, 3,3′,4′,5,7-pentahydroxylflavone, is one of the most abundant flavonoids present in fruits and vegetables. In plants, it occurs mainly in leaves and in the outher parts of the plants as aglycones and glycosides, in which one or more sugar groups is bound to phenolic groups by glycosidic bond. Glucose is the most common sugar, with galactose and rhamnose also frequently found in composition with flavonoids. In general, quercetin glycosides contain a sugar group at the 3-position. A considerable amount of isoquercetin (quercetin-3-*O*-β-glucoside) has been found in apple and pear peels (Schieber, Hilt, Conrad, Beifuss, & Carle, 2002; Spanos & Wrolatad, 1992) as well as in *Hypericum perforatum* leaves or flowers (Urbánek, Blechtová, Pospišilová, & Polášek, 2002). Almost 180 different glycosides of quercetin have been described in nature, with rutin (quercetin-3-*O*-rutinoside) being one of the most common (Hollman & Arts, 2000). In onion, however, phenolic group at the 4′-position is necessarily bound by a sugar group and thus its major glycosides are quercetin 4′-*O*-β-glucoside and quercetin 3,4′-*O*-β-diglucoside (Murota & Terao, 2003). They together account for about 80% of the total content of flavonoids (Bonaccorsi, Caristi, Gargiulli, & Leuzzi, 2005). Vegetables, fruits and beverages are the main dietary sources of quercetin. Onion (*Allium cepa* L.) ranked highest in quercetin content in a survey of 28 vegetables and nine fruits (Hertog et al., 1992a). Amounts of this flavonoid in onions vary with bulb color and type, being distributed mostly in the outer skins and rings (Bonaccorsi et al., 2005; Crozier, Lean, McDonald, & Black, 1997).

Determination of individual flavonoid glycosides in plant materials is difficult, due to their large number. In many cases, for example for the development of useful database of monomeric flavonoid values, the knowledge of the total aglycone content for each flavonoid is required.

* Corresponding author. Tel.: +48 22 82 20 211; fax: +48 22 822 3532.
E-mail address: mbiesaga@chem.uw.edu.pl (M. Biesaga).

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