Effects of Bioactive Molecules and Natural Extracts with Antioxidant Action in the Modulation of Aging Mechanisms*

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Introduction

Increasing human life expectancy does not always translate into growing health span; in fact, for the majority of industrialized nations, the burden of age-related illnesses has been steadily rising over the past few decades[1;2]. Because of this, the ageing population has become a significant social and economic burden for modern civilization. Medical research and the healthcare sector must create therapeutic strategies that particularly target ageing pathways in order to maximize and preserve physical and psychological functionality[3-5]. Unraveling the molecular mechanisms of ageing will undoubtedly be crucial for the development of this rapidly expanding science[7].

One of the main causes of ageing is thought to be the production of reactive oxygen species (ROS) and other free radicals in mitochondria during metabolic processes[9,10]. Unquestionably, one of the factors most strongly influencing cellular senescence and ageing is the harm that ROS and other free radicals generated in mitochondria do to significant biomolecules like lipids, proteins (enzymes), or nucleic acids[8]. ROS have repeatedly been linked to key aging-related processes, such as telomere attrition[11], autophagy[12], and stem cell population fatigue[13].
The development of most age-related chronic diseases, including atherosclerosis[14], insulin resistance[15], and several cardio-metabolic and neurological diseases[16-18], is increasingly being linked to oxidative stress and the accompanying inflammation.

Based on these theoretical considerations, many authors think that dietary supplementation with bioactive molecules and natural extracts with antioxidant activities known to neutralize and scavenge free radicals represents a promising interventional strategy for delaying or preventing age-related pathological conditions.

Nutraceuticals for muscle protection and recovery (project 1: muscle aging)

Skeletal muscle tissue is essential for strength, mobility, and metabolic control[19]. Starting at the age of 30 to 40, muscle mass declines by up to 3 to 8% every decade, and after the age of 70, the rate of loss rises to 15% per decade[20]. Acute muscular fatigue may emerge as a result of the generation of ROS during difficult activities[21]. In addition, an inflammatory response is triggered when a muscle is damaged in order to direct repair. Despite the fact that excessive inflammation can be harmful,

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The National Review of Criminal Sciences, Volume 66, No.1, March 2023.

considerably reducing inflammation might not be the optimal strategy for optimum recovery[22;23]. For that reason, dietary antioxidants are widely included to diets because they function as free radical scavengers[24], minimizing or eliminating oxidative stress, easing physical stress and muscular aches, and enhancing athletic performance.

Curcumin regulates the NF-kB and PGC-1a pathways, lowers MDA levels, and increases SIRT1 protein in muscle, all of which help to promote mitochondrial biogenesis in skeletal muscle, especially during in vivo exercise testing[26, 27]. Curcumin is also necessary for muscle regeneration following injury. Vitamin D has an impact on how muscles regenerate, starting with satellite cell activation and continuing through myoblast proliferation, migration, and differentiation to support its role in skeletal muscle dynamic healing[28]. Magnesium and potassium are two minerals that can be used as micronutrients or supplements to help in recuperation. Magnesium aids in proper muscle relaxation and contraction and supports energy metabolism[29]. As muscles are activated, the extracellular potassium concentration increases[30].

Nutraceuticals for Retinae protection (project 2: retinal aging)

The primary factor in permanent blindness, glaucoma is characterised by selective loss of retinal ganglion cells (RGC)[32] as a result of oxidative stress, apoptosis, and neuroinflammation[31]. Increased intraocular pressure (IOP), which can be brought on by either an increase in aqueous fluid production or a decrease in its outflow, results in optic nerve injury in this situation[33]. Ischemic damage to the optic disc and vision loss could ensue from a significant increase in IOP[34].

It is well known that vitamin C is good for the eyes[35] since it helps to control IOP[36] and prevent cataracts[37].

In addition, serum vitamin D levels have been connected to glaucoma and/or IOP[38] because this vitamin works to reduce oxidative stress, boost vascularization, and modulate immunological response in addition to inhibiting angiogenesis in the eye[40].

A natural substance from the carotenoid family called lycopene can stop cell death by directly addressing oxidative stress, hence reducing inflammation and angiogenesis[41].

Blackcurrant anthocyanins have been proven to enhance blood flow in the optic nerve head (ONH) and alter plasma endothelin-1 concentrations linked to glaucoma pathogenesis, and they have the potential to cure hypertension as well as cardiovascular, neurological, and ophthalmic diseases[42, 43].

Furthermore, the active ingredient in Gastrodia Elata, gastrodin, has long been used to treat cerebrovascular injury because of its high-performance effects[44;45] based on its capacity to boost antioxidant enzyme activity, reduce oxidative stress, and combine these effects with caspase inhibition to delay apoptosis[46]. Moreover, it decreases iNOS expression considerably, TNF-a, and IL-6 production, demonstrating its remarkable anti-inflammatory properties[47].

Nutraceuticals for neuroprotection (project 3: brain aging)

As it has become extremely difficult to examine the mechanisms behind brain ageing in depth and to consider neuroprotective and therapeutic approaches because these mechanisms are numerous and interconnected. Recent research has shown that neurotrophins, a family of cell growth and survival proteins, play a significant role in both the central and peripheral nervous systems[48]. Nerve growth factor, neurotrophin-3, neurotrophin-4/5, and brain-derived neurotrophic factor (BDNF) are members of the neurotrophin family[49]. The most prevalent neurotrophin in the brain, BDNF, which is physically mediated by the tyrosine receptor kinase B (TrkB), appears to be necessary for neuronal survival during adult brain development and network creation[50].

Preclinical research suggests that BDNF signalling dysregulation plays a role in a variety of neurodegenerative diseases, including Alzheimer's disease. It interacts with oxidative stress, which has been linked to ageing, neurodegenerative diseases, and a number of neuropsychiatric disorders[51], as the brain's natural antioxidant capacity declines with ageing, making the brain more vulnerable to the negative effects of oxidative damage[52].

The use of exogenous BDNF as a treatment for neurodegenerative disorders has recently been introduced; however, exogenous BDNF supplementation has shown some limitations, such as the amount of BDNF that enters the brain and the possibility that it can cross the BBB or not[53;54]. Eventually, various solutions have been developed in an effort to overcome such delivery challenges, but they have not completely solved the problem[55]. Turmeric (Curcuma longa) contains curcumin, which has been used in eastern medicine for thousands of years[56].

Curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin are the three main curcuminoids that make up turmeric (BDMC). The most common curcumin, curcumin, has the lowest molecular weight of the three curcuminoids and excels them in terms of antioxidant and anti-inflammatory activities[57, 58].

The first time vitamin D achieved notoriety, it was as a steroid hormone that acts on target organs through the vitamin D receptor (VDR)[59]. Vitamin D can be obtained orally or through photolysis of its skin precursor[60;61]. The ability to produce and inactivate 1,25(OH)2D3 demonstrates that the brain can alter its levels and availability locally. The enzyme CYP27B1, which converts 25(OH)D3 to 1,25(OH)2D3[62], as well as the enzyme CYP24A1 for its catabolism into 24,25(OH)2D3[63], are both expressed in the human brain. Neurotrophic factors like NGF and GDNF are produced and released differently under the influence of vitamin D[64]. Furthermore, acetylcholine and other neuromediators, such as vitamin D, are produced with the help of vitamin D[65;66]. In order to maintain cellular calcium homeostasis, parvoalbumin and other proteins that bind Ca2+ ions are produced as part of vitamin D's neuroprotective effects[67–69]. Moreover, vitamin D blocks the production of iNOS to avoid a chain reaction that results in neurotoxicity and neuronal death[70;71].

The rhizome of ginger, Zingiber officinale Roscoe (Zingiberaceae family), is a common food ingredient and is widely used in traditional medicine to treat a variety of illnesses, such as the common cold, nausea, asthma, cough, bleeding, and muscular discomfort[72;73]. Ginger has also been used in combination with other prescription medications to treat brain disorders like paralysis from ischemic stroke and as a nerve relaxant[74]. The spicy components of ginger include gingerols, shogaols, zingerones, gingerdiols, gingerdione, and capsaicin[75].
The pungent compound in dried ginger is 6- shogaol, a dehydrated form of 6-gingerol. It is more stable than 6-gingerol and has stronger pharmacological effects because of the heat liability supplied by the existence of the -hydroxyl keto group[76;77;78]. Numerous studies have shown that 6-shogaol has strong anti-disease Alzheimer's effects, improves cognition, and boosts the antioxidant system. It also increases NGF levels and postsynaptic proteins in the hippocampus, suppresses inflammatory mediators, and enhances cognitive performance in both A (1-42) and scopolamine-induced dementia mouse models[79;80].

6-Shogaol inhibits H_2O_2-induced neuronal death in astrocytes by upregulating BDNF, GDNF, NGF, Bcl-2, and Bcl-xL and downregulating ROS, Bax, and caspase 3 through the ERK1/2 signalling pathway[81]. By blocking NO, iNOS, PGE2, IL-1, TNF-, Cox-2, P38 MAPK, and NF-B in BV2 and primary microglial cells that have been exposed to LPS, 6-shogaol has also been shown to be beneficial[82].

Objectives

The carried out studies were designed to investigate the various potential therapeutic and protective effects of various combinations of bioactive molecules and natural extracts to alleviate oxidative stress and inflammation during aging and degenerative diseases.

Project 1: Our goal was to use an in vitro exercise model to test the biological effects of a new magnesium and potassium formulation combined with vitamin D and curcumin developed to support muscle activity and prevent hypercontraction damage.

Project 2: Our goal was to create a new oral formulation based on gastrodin, vitamin D3, vitamin C, black currant, and lycopene that could counteract the early changes associated with glaucoma.

Project 3: Phase 1: investigated whether the use of low-dose BDNF sequentially kinetically activated (SKA) can counteract some mechanisms that underpin nerve tissue degeneration and aging by increasing endogenous protective mechanisms will; Phase 2: Exploring several papers and reviews on vitamin D3's varied actions and the underlying mechanisms to support future theories for using vitamin D3 alone or in combination with natural extracts to provide neuroprotection;

Phase 3: investigated the various biological effects of a new combination of curcumin, vitamin D3, and 6-shogaol to alleviate oxidative stress and neuroinflammation along with providing an endogenous source for inducing BDNF.

Methodology

Project 1: Vitamin D, buffered magnesium bisglycinate, curcumin, and potassium citrate were used to treat C2C12 mice myoblasts (MKVC). The effects of this combination were evaluated under both normal and caffeine-induced hypercontraction settings using cell survival, determining morpho-functional alterations, evaluating calcium and magnesium movements, defining the key kinases implicated, and determining glucose absorption, glycogen, and lactate levels.

Project 2: Retinal cells and tissues were treated with a mixture containing Gastrodin, vitamin D3 in addition to vitamin C, black currant, and lycopene (VGLCR). Experiments were carried out to study the biological mechanisms involved in glaucoma progression, as well as in conditions mimicking glaucoma to study the etiology of retinal degeneration. The various biological effects were assessed using cell viability and oxidative stress markers and endogenous antioxidants, as well as the most important activated intracellular signaling pathways and morphological changes in the retinal area.

Project 3: Phase 1: *In vitro* and *in vivo* experiments were performed to evaluate the ability of the BDNF-SKA formula to protect and regenerate survival molecular pathways, with intestinal absorption in vitro and brain function in vivo being examined. The various effects were investigated by measuring cell viability and proliferation, quantifying BDNF in vitro and using a blood-brain barrier model, and analyzing the intracellular signaling pathways that activated after BDNF-SKA treatment alone or under oxidative stress conditions. In vivo experiments were also used to determine BDNF bioavailability in rat serum and brain, as well as the various intracellular signaling pathways activated in the brain. Phase 3: Human neuroblastoma SH-SY5Y cell line was treated with a combination of curcumin, vitamin D3 and 6-shogaol named CVS, experiments were performed in oxidative stress and iron induced damage conditions. The effects were determined through cell viability, determining survival kinases and other intracellular markers of neurodegeneration. Following these results, astrocytes were treated with the same combination to check the possibility to induce BDNF expression and then studying the different intracellular pathways activated in astrocytes and human neuroblastoma SH-SY5Y treated with astrocyte conditioned medium.

Results

Findings from exploring the various effects of combinations of selected bioactive molecules and natural extracts with antioxidant activity in the modulation of different aging-related processes in several tissues in all three projects.

Project 1: Under physiological conditions, the combination of MKVC improved mitochondrial activity, oxygen consumption, ATP production, as well as the viability and physiological differentiation of C2C12 murine myoblasts. Controlling hypercontraction, restoring ion fluxes, reducing inflammation signaling, and supporting the main mechanisms involved in aerobic activity have all been shown

to have beneficial effects on skeletal muscles under conditions mimicking intense activity.

Project 2: The VGLCR combination has demonstrated a significant ability to reverse glaucoma- induced damage by inhibiting apoptosis and counteracting ROS production. The intracellular mechanism that was activated following the administration of the combination either before or after the glaucoma induction confirmed these effects. The main results were obtained as a glaucoma preventive action, showing a beneficial action on all selected markers both on cells and on eyecup preparations.

Project 3: in Phase 1, The findings were groundbreaking because they demonstrated that BDNF SKA can induce endogenous BDNF production via its receptor TrkB while also influencing apolipoprotein E expression. Furthermore, BDNF SKS had effects on β-amyloid and sirtuin-1 proteins, confirming the hypothesis of BDNF SKA having a fine endogenous regulatory effect in maintaining the health of both neurons and astrocytes.

While in Phase 3, using human neuroblastoma SHSY5Y cell line as a model of neurodegenerative diseases, CVS combination has indeed helped in cell viability and ROS production proving the safety of such combination under normal and neuroinflammation conditions. Furthermore, such treatment had beneficial effects on APP, β-amyloid, and sirtuin-1 proteins. CVS-treated astrocytes have shown an induced expression of BDNF as a neuroprotective mechanism, that when applied to neuroblastoma SHSY5Y could be a source of endogenous BDNF production and its receptor TrkB, as well as its effects on APP, β-amyloid, and sirtuin-1 proteins.

Conclusions and Future Prospective

Finally, the data from the three investigations show for the first time that such combinations have multiple favorable impacts in muscle protection and recovery, retinal protection, and neuroprotection.

In **project 1**, MKVC combination has favorable effects on skeletal muscle cells under physiological settings and during severe activity, showing that it might be a promising candidate for muscular exercise support. Further research in an *in vivo* model may be valuable to determine if the protective mechanism induced by MKVC can also have beneficial effects in the natural loss of muscle mass, known as sarcopenia, that happens with age.

In **project 2**, The capacity of VGLCR to adjust the major essential parameters associated in glaucoma has been discovered for the first time in this work. These findings support the concept that, in the future, VGLCR might be a new dietary supplement for slowing the degenerative process in human glaucoma by activating survival pathways even in the early stages of the illness. All of these findings show VGLCR's active function in the healing of glaucoma-related ocular damage for the first time; VGLCR appears to have larger favorable effects if administered preventively, becoming a key component in the battle against glaucoma.

In **project 3**, This study shows convincing evidence of BDNF's usefulness in cell models. Of course, as a further step, research on behavioral consequences will be required. If the favorable benefits are verified in this second phase, it is possible that BDNF may be a valuable supplement in the future for modifying a damaged brain environment to aid recovery during degenerative diseases. Further research utilizing CVS combination has demonstrated its capacity to promote BDNF expression as a method of neuroprotection, making astrocytes mediated medium with CVS combination a promising therapeutic intervention for future *in vivo* investigations.

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