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A Hypothetical Anti-Aging Mechanism of "Yang-Invigorating" Chinese Tonic Herbs*

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ABSTRACT

Chinese tonic herbs are generally classified into Yin and Yang categories based on their health-promoting action. Emerging evidence has suggested that in addition to up-regulating mitochondrial functional status, Yang tonic herbs also enhance cellular/mitochondrial antioxidant capacity, and may thus prevent age-related diseases and prolong the healthy part of lifespan (*i.e.* healthspan). The proposed biochemical mechanism underlying the antioxidant action of Yang tonic herbs involves a sustained and low level of mitochondrial reactive oxygen species production, which is secondary to the increased activity of the electron transport chain, with the possible involvement of mitochondrial uncoupling. "Yang invigoration" improves antioxidant defense in the body in the long term and thereby offers a promising prospect for preventing or possibly delaying age-related diseases and the detrimental effects of aging.

Keywords: Chinese Medicine; Yang; Mitochondrial Decay; Age-Related Diseases

1. Mitochondrial Decay and Age-Related Diseases

Aging is a process characterized by a general decline in physiological functions, and it is also considered as a major risk factor for many age-related diseases, including, but not limited to, neurodegenerative diseases, cardiovascular disorders, and metabolic diseases [1-3]. The "Mitochondrial Free Radical Theory of Aging" (MFRTA) attempts to explain the role of reactive oxygen species (ROS) arising from mitochondrial electron transport in the aging process [4]. At odds with the MFRTA is the observation that the significant loss-of-function mitochondrial DNA (mtDNA) mutations accumulated only to low levels in most tissues, even by very old age. To address this anomaly, Aubrey de Grey proposed the "Reductive Hotspot Hypothesis of Mammalian Aging" as a supplement of the MFRTA. This theory attempts to explain how the relatively few cells that have lost oxidative phosphorylation capacity due to mtDNA mutations may be toxic to the rest of the body and result in the development of age-related diseases [5,6]. Since then, mitochondrial decay in aging has been implicated in a broad spectrum of degenerative and metabolic diseases [1,7-9]. The "Double-Agent Theory", which endeavors to provide a unifying view of aging and diseases, postulated that mitochondrial ROS generation produces a genetic response mimicking that triggered by infection associated increase in intracellular oxidative stress [10]. However, the continuous mitochondrial ROS production would lead to a persistent shift in gene expression, with resultant chronic inflammation which is commonly involved in age-related diseases. In this connection, in order to decrease the vulnerability to age-related diseases, we must find ways to attenuating the mitochondrial ROS production [10]. Despite conflicting views concerning the primary role of mitochondrial ROS as a cause of aging [11], the generation of ROS within mitochondria remains the most viable theory to explain the process of aging. Increased levels of ROS within mitochondria are the principal trigger not only for mitochondrial dysfunction, but also for diseases associated with aging in general [12]. Several studies have revealed a complex network of signaling pathways modulated by nutrients, such as IGF-1, TOR, sirtuins, AMP kinase and PGC-1α that are connected and then converge to inhibit oxidative stress within the mitochondrion [13-17].

Under normal physiological conditions, mitochondria not only serve as the powerhouse of cells by generating ATP through oxidative phosphorylation, but also regulate a wide range of cellular processes, including cellular signaling, differentiation and proliferation, as well as cell survival and death [18]. Experimental studies showed that mitochondria from liver tissue of elderly rats generated a larger amount of ROS than liver mitochondria of young rats, indicative of increasing oxidative stress as a function of aging [19]. The age-related increase in mitochondrial oxidative stress can disrupt mitochondrial structural and

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functional integrity, thereby triggering a vicious cycle of ROS generation. Experimental findings indicate that the age-related decrease in mitochondrial respiratory efficiency was associated with the significant decline in respiratory complex (I-V) activities, presumably mediated by self-inflicted oxidative damage [20-22]. In addition, the extent of oxidative damage on key metabolic enzymes increases with age, with consequent decreases in substrate binding affinity and mitochondrial ATP generation capacity [23,24]. The oxidation of DNA, RNA, protein and lipid molecules in mitochondria and other cellular components can culminate in functional impairment in cells, tissues, and ultimately in vital organs such as brain, heart and liver [19,25-27]. Taken together, the capacity to produce ATP and respond to cellular stress decrease as a function of age during the age-associated deterioration of mitochondrial structure and function. The mitochondrial dysfunction results in increased ROS generation, which tilts the cellular environment towards an oxidative state (*i.e.*, impairment of cellular redox balance) and increases the susceptibility to diseases associated with aging [18,28,29].

2. Pharmacological Basis of "Yang-Invigoration" in Chinese Medicine

According to traditional Chinese medicine (TCM) theory, tonic herbs are classified into four categories, based on their health-promoting actions: Yang-invigorating; Yin-nourishing; Qi-invigorating and Blood-enriching. The Qi-invigorating and Blood-enriching herbs are further grouped under the Yang and Yin categories, respectively. Holistically, it is believed that the Yang-invigorating herbs enhance physiological cellular activities, which is in turn critically dependent on mitochondrial ATP generation through the oxidative phosphorylation process at the cellular level. A previous study in our laboratory has shown that short-term oral treatment with the methanol extract of Yang-invigorating herbs, including Cortex Eucommiae, Herba Cistanches, Herba Cynomorii, Rhizoma Curculiginis, Herba Epimedii, Radix Dipsaci, Rhizoma Drynariae, Fructus Psoraleae, Semen Cuscutae, Radix Morindae, and Semen Alliion, enhanced myocardial ATP generation and produced significant stimulatory action on pyruvate-supported mitochondrial electron transport in mice [30]. This finding is corroborated by a recent study involving Yang and Yin tonic herbs using a cell-based assay of ATP- generating capacity, which showed that Yang but not Yin tonic herbs enhanced mitochondrial ATP generation capacity in H9c2 cardiomyocytes [31]. Activity-directed fractionation of three Yang tonic herbs (namely, Herba Cistanches, Herba Cynomorii, and Semen Cuscutae) indicated that the active ingredient(s) appeared to reside in the butanol and/or ethylacetate fraction [31]. Moreover, long-term treatment with a Yang-invigorating Chinese herbal formula (VI-28; composed of Radix Ginseng, Cornu Cervi, Cordyceps, Radix Salviae, Semen Allii, Fructus Cnidii, Fructus Evodiae and Rhizoma Kaempferiae) was found to enhance mitochondrial ATP generation in brain, heart, liver and skeletal muscle tissues of male and female rats [32]. Although studies on isolated mitochondria may not directly reflect the *in vivo* mitochondrial function, it provided important information to support the rationale that Yang-invigorating herbs may enhance the functional capacity of mitochondria. Interestingly, in addition to stimulating mitochondrial ATP generation, VI-28, a proprietary Chinese herbal formula, also increased the levels/activities of mitochondrial antioxidant components such as reduced glutathione (GSH), α-tocopherol (α-TOC) and manganese-superoxide dismutase (Mn-SOD), indicative of up-regulation of mitochondrial redox status by "Yang-invigoration" [32]. In connection with this, several Yang herbs have been shown to possess antioxidant activity both *in vitro* and *in vivo* [33]. Studies from various laboratories showed that Yang tonic herbs produced antioxidant actions by free radical-scavenging [34], inhibition of oxidant production [35], inhibition of NADPH-dependent lipid peroxidation [36] and increase of antioxidant enzyme activities [37], with a resultant protection against oxidative tissue damage. These findings were consistent with our earlier study which showed that Yang tonic herbs possessed stronger free radical scavenging activity than that of tonic herbs of other functional categories [38]. As mentioned earlier, ROS are unavoidably generated during the oxidative phosphorylation process, particularly under conditions of increased electron transport activity. Given that increased formation of ROS within mitochondria can cause an adaptive response that leads to the enhancement of mitochondrial antioxidant capacity [39], the induction of mitochondrial antioxidant components by the Yang-invigorating VI-28 may, at least in part, be mediated by an increase in ROS production from complex I and/or complex III resulting from the stimulation of mitochondrial electron transport (see **Figure 1**).

3. Protection against Oxidant-Induced Tissue Injury by Yang-Invigorating Tonic Herbs/Formulae

"Yang-invigoration", which can up-regulate cellular/mitochondrial antioxidant components, may protect against oxidant-induced injury. In a study using rodent models of cerebral/myocardial ischemia/reperfusion injury, carbon tetrachloride hepatotoxicity, and gentamicin nephrotoxicity, long-term treatment with VI-28 significantly ameliorated oxidative stress-induced tissue damage in various organs, including brain, heart, liver and kidney. The tissue protection afforded by VI-28 pretreatment was associated with increases in the levels/activities of mitochondrial antioxidant components (GSH, α-TOC and

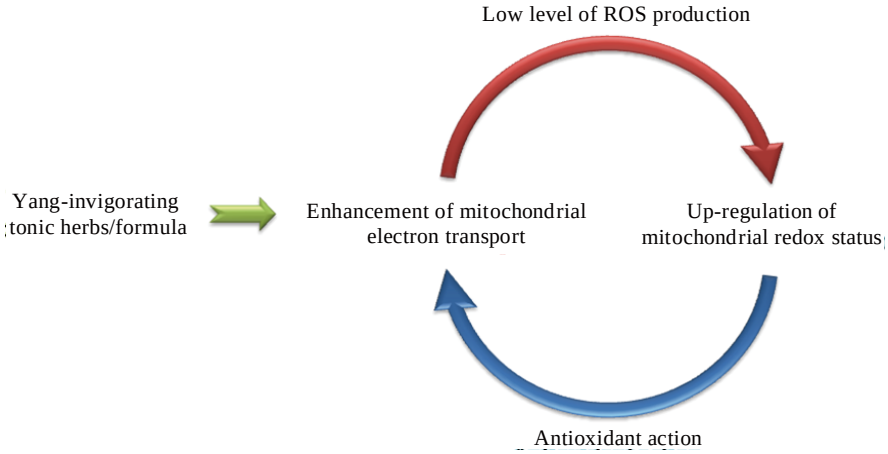

Figure 1. Pharmacological basis of "Yang-invigoration" in Chinese medicine. Yang-invigorating herbs/formulae enhance mitochondrial functional capacity with a concomitant production of ROS which triggers an adaptive antioxidant response that enhances mitochondrial antioxidant defense and safeguards mitochondrial function.

Mn-SOD), as well as the preservation of mitochondrial structural integrity [40]. In addition, Wu-Zi-Yan-Zhong-Wan (WZ), a Chinese herbal formula containing five herbs, namely, Fructus Lycii, Semen Cuscutae, Fructus Rubi, Semen Plantaginis and Fructus Schisandrae, is prescribed for treating "Yang deficiency" in TCM. Studies showed that WZ decreased the extent of ethanol-induced ROS production and lipid peroxidation as well as prevented ethanol-induced decreases in cellular/mitochondrial GSH levels and mitochondrial membrane potential in CYP2E1 cDNA-transfected human HepG2 (E47) cells [41] and chronic ethanol-intoxicated rats [42]. Taken together, both cell-based and animal studies provide convincing evidence to support the role of Yang tonic herbs/formulae in protecting the body against oxidant-induced injury, presumably by up-regulating cellular/mitochondrial antioxidant defense components in a tissue non-specific manner.

4. "Yang-Invigoration" and Prolongation of Healthspan

According to the United Nations organization, the world's elderly population (60 years of age or older) is currently 650 million, and it is forecasted to reach 2 billion by the year 2050 [43]. The rapid increase in the elderly population will entail major challenges in societies, particularly in the area of healthcare expenditures for age-related diseases. Preventive measures for achieving a healthy lifespan are therefore instrumental in relieving the financial burden in aging societies. A growing body of evidence has revealed the crucial involvement of mitochondrial dysfunction and impaired antioxidant status in the pathogenesis of various age-related diseases and the aging process in general [2,44,45]. However, outcomes of experiments which attempt to increase tissue antioxidants levels through dietary supplementation, pharmacological induction or transgenic techniques were unsatisfactory in terms of extending longevity [46-48]. Numerous randomized, double-blinded clinical trials have also shown that antioxidant supplements cause no beneficial effect and occasionally harmful to human [49]. Yang tonic herbs/formulae, which can induce endogenous mitochondrial antioxidant status and functional capacity enhancement [30,40], may therefore offer a promising prospect for preventing or possibly delaying age-related diseases and the detrimental effects of aging. With respect to Chinese medicine, more than 50% of the elderly people in China were found to show a deficiency of Yang (or Qi) in body function [50], and Yang tonic herbs/formulae are therefore commonly used for retarding the adverse consequences of aging in the practice of Chinese medicine. According to TCM theory, a deficiency of Yang is believed to be one of the causative factors for the development Parkinson's disease (PD), a common neurodegenerative disease that severely compromises the quality of life in many elderly individuals [51]. This notion is paralleled by a clinical study which revealed the gradual development of "Yang-deficiency" symptoms prior to the clinical manifestations of PD [52]. In this connection, a pilot study investigating whether a Yang-invigorating Chinese herbal suppository preparation (ViNeuro; a VI-28-related product) could produce any symptom-relieving effect in patients suffering from PD was conducted [53]. The results indicated that all PD patients (40 - 69 years of age; 6 males and 3 females) showed improvement in various Parkinsonian symptoms, particularly relieving reduction in rigidity and tremor, after taking ViNeuro for 6 months. The relief of "Yang-deficiency" symptoms such as aversion to cold, poor appetite, frequent urination, and constipation were also observed [53]. Based on the finding that ViNeuro can enhance the mitochondrial ATP generation

capacity (a "Yang-invigoration" property), it is plausible that the relief of Parkinsonian symptoms involves an improvement of cellular energy status that eventually leads to an enhancement of neuronal function. A recent study showed that long-term dietary supplementation with VI-28, the aforementioned Yang-invigorating herbal formula, can extend the median lifespan of both male and female C57BL/6J mice [54]. The enhanced survival was associated with the mitigation of age-dependent progressive impairments in mitochondrial antioxidant status and functional capacity [54]. Conceivably, the retardation of mitochondrial decay in structure and function by "Yang invigoration" delays the onset of age-related diseases, thereby enhancing longevity and thus increasing the healthy part of lifespan (*i.e.* healthspan) of animals. In this regard, the finding of myocardial protection with various interventions on mitochondrial oxidative stress suggested the prominent role of mitochondrial decay in cardiac aging [12].

5. "Yang-Invigoration" Enhances Mitochondrial Functional Capacity and Antioxidant Status

Preliminary studies in our laboratory using animal and cell models showed that a semi-purified fraction of Herba Cistanches not only stimulated state-3 respiration, but also caused an increase in state-4 respiration in rat mitochondria and H9c2 cardiomyocytes, with the latter being reversed by GDP, an uncoupling protein inhibitor (unpublished data). The experimental findings suggested the involvement of mitochondrial uncoupling in the "Yang-invigoration" produced by Herba Cistanches. Taken together with our previous observations, we hypothesize that "Yang-invigoration" can produce both short-term and long-term beneficial effects in the body (see **Figure 2**). In the short term, "Yang-invigoration" stimulates mitochondrial electron transport, presumably by increasing both complex I and III activities, with a resultant increase in ATP generation activity (*i.e.*, functional capacity). The stimulation of electron transport is accompanied by an increase in ROS generation and a rise in membrane potential, with the latter also inhibiting further electron transport but favouring ROS production [51]. On the one hand, the increased mitochondrial ROS generation triggers a retrograde response to up-regulate cellular/mitochondrial antioxidant defense components [52]. On the other hand, mitochondrial ROS also stimulate the uncoupling protein activity and thus lower the membrane potential through dissipation of the proton gradient [53,54]. This restoration of membrane potential allows the re-activation of electron transport and the associated ROS production at low level. As such, with a recurring "Yang-invigoration", a sustained and low level of mitochondrial ROS production can result in both the up-regulation of antioxidant defense components characteristic of mitohormesis [52,55,56] and the prolonged activation of mitochondrial uncoupling. The reduced level of mitochondrial ROS generation associated with increased electron transport due to uncoupling may be an effect secondary to the up-regulation of antioxidant defense or the decrease in membrane potential [55,56]. A low level of mitochondrial ROS generation has also been shown to be a potential key factor that contributes to the low rate of aging in almost all long-lived species [57]. While the role of mitochondrial uncoupling in influencing lifespan remains to be established, experimental investigations in transgenic mice have ascribed a role for uncoupling protein-1 dependent uncoupling in increasing healthspan [57]. Therefore, in addition to the enhancement of mitochondrial function capacity, "Yang invigoration" improves antioxidant defense in the body in the long term and thereby offers a promising prospect for preventing or possibly delaying age-related diseases and the detrimental effects of aging.

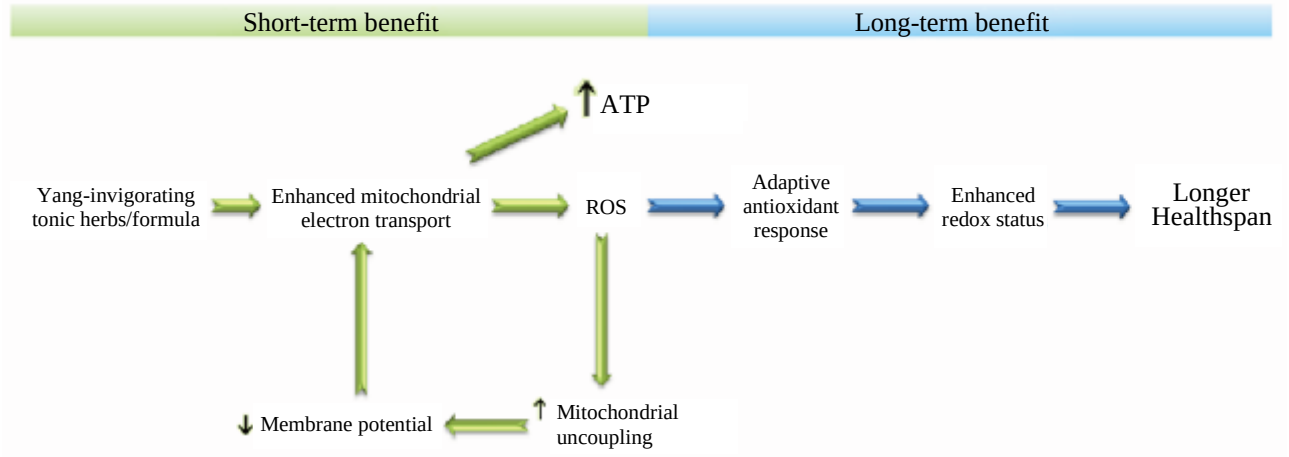

Figure 2. Up-regulation of cellular/mitochondrial antioxidant defense mechanism by "Yang-invigoration". "Yang-invigoration" may produce both short-term and long-term beneficial effects (please refer to the text for details).

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