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Phytopharmaceuticals in the Therapy of Younger Alzheimer Patients

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Abstract

Commercially available medicinal plant extracts such as *Ginkgo biloba* leaf extract show no consistent pattern of clinical benefit for people with dementia or cognitive impairment, and have been suggested to be toxic to cells at higher doses. However, medicinal plants may contain other more efficient bioactive molecules apart from the well-known flavonoids and terpenoids. Recombinant therapeutic proteins, plant-made copper chaperone for superoxide dismutase (CCS) derived from *Ginkgo biloba* leaves, may establish and maintain physiologic Cu levels through restoration and modulation of biometal metabolism in organ systems of younger Alzheimer patients (> 50 years). Medications developed from plant-made copper chaperone proteins may delay progression during early disease stages or even be a basis for a possible causal treatment of preclinical stages of Alzheimer's disease by preventing formation of A β plaques in the brain, a major putative factor involved in Alzheimer's disease etiopathogenesis.

Introduction

The dysregulation of biometal (Cu, Zn, Fe) homeostasis and oxidative stress in brain cells have been found to impact on the interaction between metal ions and amyloid β (A β), a major putative factor involved in early Alzheimer's disease (AD) pathogenesis [1,2]. The regulation of metal ion homeostasis in the cytoplasm is strongly influenced by the copper chaperone for superoxide dismutase (CCS) and Cu,Zn-superoxide dismutase (SOD-1) [3]. The dynamic interplay of properly folded CCS and SOD-1 guarantees that free Cu and Zn ions are being complexed by these metal proteins and do not catalyze oxidation processes of proteins, lipids, DNA or other molecules in the cells [3]. When these physiological complexation mechanisms do not function properly, oxidative stress and dyshomeostasis of Cu and Zn metabolism may give rise to misfolding, accumulation and aggregation of amyloid β peptides [3]. The outcome of these pathological processes may lead to different incurable chronically progressive neurodegenerative diseases such as Alzheimer's disease [1-3]. Several therapeutic strategies and nearly all medications used or suggested as A β inhibitors, including metal-chelating agents or radical scavengers, at present time, aim at the treatment of AD symptoms only and may either be toxic, lack specificity or have unknown mechanisms of action *in vivo* [3,4].

The aim of this article is to give a short review on the interaction of metal ions with novel and early herbal compounds derived from *Ginkgo biloba*, and their possible role in the treatment of early Alzheimer's disease. Though *Ginkgo biloba* leaf extracts are generally administered to treat dementia syndroms in older AD patients (> 65 years old), no data is available on possible effects of recombinant plant-made copper chaperones from *Ginkgo biloba* in younger AD patients (> 50 years old) with preclinical stages of disease.

Interaction of Herbal Drugs with Metal Ions

The medical community, including the pharmaceutical industry, as well as AD patients, have become aware of the well-known antioxidant effects of ancient herbal medications, namely *Ginkgo biloba* leaf extracts [5]. A complex mixture of flavonoids and terpenoids are thought to represent the main bioactive compounds of these plant extracts [6]. For example, the flavonol quercetin, a major compound of *Ginkgo* extract, was found to interact with Cu^{2+} and Fe^{3+} [7]. However, an ideal therapeutic drug to dissolve amyloid β peptides would involve a compound selective for Cu^{+}, Zn^{2+} and Fe^{3+} [8]. A meta-analysis by *Birks and Evans* revealed that commercially available medicinal plant extracts (EGb 761® *Ginkgo biloba* leaf extract) have no consistent pattern of clinical benefit associated with *Ginkgo biloba* for people with dementia or cognitive impairment [5]. Furthermore, the results from the study of *He and colleagues* suggested that high doses of herbal remedies can even be toxic to cells [9]. Thus, *Ginkgo biloba* extracts may induce unwanted side-effects and may also lack specificity as to the

binding and delivering of Cu^{+} and Zn^{2+} in the cytoplasm. Likely, these extracts neither reduce metal-mediated oxidative stress efficiently nor contribute to the homeostatic control of biometals in humans, though *in vitro* studies have shown that gingkolides may protect against synapse damage and cognitive loss seen during early stages of AD [6]. However, medicinal plants may contain other, more efficient antioxidants apart from the well-known flavonoids, terpenoids and ginkgolides, namely metallochaperones [10].

Efficacy of Plant-Made Copper Chaperones

As a basis for a new drug development involving metal-chelating agents, the following facts may be important. Endogenous biomolecules such as Cu,Zn-superoxide dismutase, are one of the major means by which cells counteract the deleterious effects of reactive oxygen species (ROS). For proper functioning SOD-1 has to be activated by the metallochaperone protein, copper chaperone for SOD [10-13]. Copper ions are required for enzymatic activity whereas the zinc ion helps to stabilize the enzyme [11]. Studies by *Choi and colleagues* suggest that recombinant human CCS molecules produced in bacteria provide a potential strategy for therapeutic delivery of these compounds in various human diseases related to ROS and SOD [11]. Drugs derived from recombinant proteins potentially have greater efficacy and fewer side-effects than small organic molecules (e.g., Cu orotate, quercetin) because their action can be more precisely targeted towards the A β plaque formation as a major putative factor in the etiopathogenesis of Alzheimer's disease, rather than the treatment of AD symptoms [12].

The dysfunction of the CCS-SOD-1 interaction may be the initial event in the etiopathogenesis of Alzheimer's disease because loss of bioactive CCS and SOD-1 may increase A β production, elicit dyshomeostasis of biometal metabolism and generate reactive oxygen species [14-20]. It is a well-known fact that biometals (Fe, Zn, Cu) are accumulated in the brain with normal ageing [17]. Important factors affecting the balance between metal ion accumulation and deficiency are, for example, environmental exposure, ageing or drug interaction [15,18]. Conclusively, the deterioration of the intracellular metal regulatory systems involving inactive CCS and SOD-1 molecules in brain, is suggested as causal for preclinical stages, development and progression of AD and other neurodegenerative diseases.

For the restoration and modulation of metal ion homeostasis in the treatment of AD, some researchers have proposed a novel class of pharmacologically active plant ingredients (APIs) as antioxidants: copper chaperone for superoxide dismutase derived from medicinal plants (e.g., *Ginkgo biloba*) [10,12]. In plant molecular farming approaches transgenic plants (e.g., tobacco) may serve as an efficient production platform for medications in regard to protein yield, quality and stability [21]. Recombinant CCS proteins produced in transgenic plants may cross the blood-brain barrier and are relatively free from side-effects [10,12]. Being properly folded, plant-made CCS may have the ability to bind and deliver Cu^{+}/Cu^{2+}, Zn^{2+} and Fe^{3+} ions and to normalize the SOD-1 activity via specific protein-protein interactions in the central nervous system and peripherally [10,12].

In contrast to plant-made pharmaceuticals (PMPs), organisms like yeast, mammals or bacteria used for molecular farming approaches, express properly folded as well as improperly folded recombinant therapeutic proteins. These medications may lack stability and pharmacological efficiency in protein-misfolding diseases, e.g., Alzheimer's disease or prion diseases [21]. Furthermore, PMPs may have several advantages in terms of cost, scalability or safety issues compared to the other genetically modified organisms [21].

Because of their specific biochemical behavior, plant-made CCS from medicinal plants may be efficient in the treatment of patients with preclinical stages of AD. The endogenous levels of this essential Cu protein may be important, since a mild Cu deficiency has been described in AD patients [22]. Furthermore, the expression level of CCS has been found to reflect the Cu status of patients and thus, may serve as a marker for *in vivo* copper levels [23]. The metal levels in patients with Alzheimer's disease compared with healthy individuals are important parameters for developing metal-based medications that may restore intracellular metal ion metabolism [18]. It is anticipated that plant-made CCS may establish physiologic copper levels through restoration and modulation of biometal (Cu, Zn, Fe) metabolism in diseased organ systems of AD patients [10,12]. Yet, to the present knowledge, no systematic studies have addressed the possible role of plant-made CCS in copper homeostasis.

Drug Delivery and Plaque Degradation

The effects of recombinant CCS and a possible role in the treatment of AD can be characterized as follows: unfolded SOD-1 in diseased blood involving neurotoxic soluble A β oligomers [2,24], may be activated by copper ion incorporation via specific CCS-SOD-1 interaction [10,12]. Copper-demetallated CCS and the Cu cofactor-containing CCS complexes may pass through the blood-brain barrier and activate unfolded SOD-1 by Cu ion transfer or bind free Cu, Zn and Fe ions in brain cells, respectively [10,12].

Since copper homeostasis is regarded as an important possible factor in the etiopathogenesis of AD, restoring homeostasis of copper metabolism may positively affect early disease stages of AD patients by balancing oxidative and anti-oxidative processes and reducing intracellular protein-misfolding processes leading to formation of extracellular A β plaque deposits [25,26]. The dynamic interactions of plant-derived CCS medications with unfolded human SOD-1 and free redox-active metal ions (e.g., Cu^{+}) may decrease A β production [10,12] and upregulate a cascade of essential biochemical reactions, such as the degradation of misfolded proteins and A β plaque deposits by molecular chaperones and the ubiquitin proteasome system [27].

A mulitifaceted approach involving analytical and biophysical methods for the determination of dose, bioavailability and mechanisms of action of recombinant plant-made CCS in younger AD patients, as well as sophisticated purification protocols for resolving properly folded and misfolded metalloproteins, have been developed recently [10,12]. In particular, QPNC-PAGE (quantitative preparative native continuous polyacrylamide gel electrophoresis) and GPC (gel permeation chromatography) are efficient methods for isolating CCS and SOD-1 in complex protein mixtures [10]. As part of this holistic approach, targeted drug delivery [3,8], status of copper ion metabolism [25], and the relative biochemical impact of metalloproteins [12] can be evaluated by using nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) methods [10]. A promising technology platform involving the fields of biotechnology, bioinformatics and plant molecular farming, may contribute to a sustainable production of chaperone-based medications for AD patients and a large scale expression of recombinant therapeutic proteins by transgenic plants in future [10,21,28].

Conclusions

For the restoration and modulation of metal ion homeostasis and for balancing intracellular prooxidative and anti-oxidative processes in the treatment of Alzheimer's disease, plant-made copper chaperone for superoxide dismutase (CCS) proteins potentially have greater efficacy and fewer side-effects compared to small organic molecules (e.g., quercetin) from medicinal plant extracts or recombinant therapeutic proteins produced in bacteria, fungi or mammals. Possibly suitable for use in younger AD patients (> 50 years), CCS proteins derived from medicinal plants may be targeted more towards prevention of A β plaque formation as a major putative factor involved in AD etiopathogenesis, rather than the treatment of AD symptoms in older patients (> 65 years). I suggest that recombinant CCS derived from *Ginkgo biloba* leaves might be promising in the treatment of patients suffering from preclinical symptoms of Alzheimer's disease.

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