

1 Can Plant-Made Copper Chaperones Heal Early Alzheimer's Disease?

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13 **Running title:**

14 Phytopharmaceuticals for restoring biometal homeostasis in human cells

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16 **Key words:**

17 Alzheimer's disease (AD), recombinant plant-made pharmaceuticals (PMPs), CCS, *Ginkgo*

18 *biloba*, SOD, A β , molecular chaperones

19

20 **Abbreviations:**

21 CCS: copper chaperone for superoxide dismutase

22 SOD-1: Cu,Zn-superoxide dismutase

23 A β : amyloid β

24 AD: Alzheimer's disease

25 ROS: reactive oxygen species

26 PMPs: plant-made pharmaceuticals

1 **Abstract:**

2 Therapeutic recombinant plant-made copper chaperone for superoxide dismutase (CCS)
3 derived from *Ginkgo biloba* leaves may establish and maintain physiologic Cu levels through
4 restoration and modulation of biometal metabolism in organ systems of younger Alzheimer
5 patients (> 50 years). Medications developed from plant-made copper chaperone proteins may
6 delay progression during early disease stages or even be a basis for a possible causal treatment
7 of preclinical stages of Alzheimer's disease by restoring cellular function of the CCS-SOD-1
8 mechanism and by preventing formation of A β plaques, a major putative factor involved in
9 Alzheimer's disease pathogenesis.

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1 INTRODUCTION

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

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

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25 INTERACTION OF HERBAL DRUGS WITH METAL IONS

26 The medical community, including the pharmaceutical industry, as well as AD patients, have
27 become aware of the well-known antioxidant effects of ancient herbal medications, namely
28 *Ginkgo biloba* leaf extracts [4]. A complex mixture of flavonoids and terpenoids are thought
29 to represent the main bioactive compounds of these plant extracts [5]. For example, the
30 flavonol quercetin, a major compound of *Ginkgo* extract, was found to interact with Cu^{2+} and
31 Fe^{3+} [6]. However, an ideal therapeutic drug to dissolve $A\beta$ peptides would involve a
32 compound selective for Cu^+ , Zn^{2+} and Fe^{3+} [7]. A meta-analysis by Birks and Evans revealed
33 that commercially available medicinal plant extracts (EGb 761[®] *Ginkgo biloba* leaf extract)
34 have no consistent pattern of any clinical benefit associated with *Ginkgo biloba* for people

1 with dementia or cognitive impairment [4]. Furthermore, the results from the study of He and
2 colleagues suggested that high doses of herbal remedies can even be toxic to cells [8]. Thus,
3 *Ginkgo biloba* extracts may induce unwanted side-effects and may also lack specificity as to
4 the binding of Cu^+ and Zn^{2+} in the cytoplasm. Likely, these extracts neither reduce metal-
5 based oxidative stress efficiently nor contribute to the homeostatic control of biometals in
6 human cells, though *in vitro* studies have shown that ginkgolides may protect against the
7 synapse damage and cognitive loss seen during the early stages of AD [5]. However,
8 medicinal plants may contain other, more efficient bioactive molecules apart from the well-
9 known flavonoids and terpenoids, namely metallochaperones [9].

11 EFFICACY OF PLANT-MADE COPPER CHAPERONES

12 As a basis for a new drug development involving metal-chelating agents, the following facts
13 may be important. Endogenous biomolecules such as Cu,Zn-superoxide dismutase, are one of
14 the major means by which cells counteract the deleterious effects of reactive oxygen species
15 (ROS). For proper functioning SOD-1 has to be activated by the metallochaperone protein,
16 copper chaperone for SOD [9-12]. Copper ions are required for enzymatic activity whereas
17 the zinc ion helps to stabilize the enzyme [10]. Studies by Choi and colleagues suggest that
18 recombinant human CCS molecules produced in bacteria provide a potential strategy for
19 therapeutic delivery of these compounds in various human diseases related to ROS and SOD
20 [10]. Drugs derived from recombinant proteins potentially have greater efficacy and fewer
21 side-effects than small organic molecules (e.g., Cu orotate, quercetin), because their action
22 can be more precisely targeted towards the $\text{A}\beta$ plaque formation as a major putative factor in
23 the pathogenesis of Alzheimer's disease rather than the treatment of AD symptoms [11]. The
24 dysfunction of the CCS-SOD-1 interplay may be one specific but major mechanism in the
25 pathogenesis of Alzheimer's disease. It is a well-known fact that biometals (Fe, Zn, Cu) are
26 accumulated in the brain with normal ageing [13]. Important factors affecting the balance
27 between metal ion accumulation and deficiency are, for example, environmental exposure,
28 ageing or drug interaction [14]. The inability of the human organism to maintain the metal ion
29 homeostasis due to improperly folded CCS and SOD molecules in brain cells is suggested as
30 causal for preclinical stages, development and progression of AD and other neurodegenerative
31 diseases [9,11,15].

32
33 For the restoration and modulation of metal ion homeostasis in the treatment of AD, we have
34 proposed a novel class of pharmacologically active plant ingredients as antioxidants: copper

1 chaperone for superoxide dismutase derived from medicinal plants (e.g., *Ginkgo biloba*)
2 [9,11]. In molecular farming approaches transgenic plants (e.g., tobacco) may serve as an
3 efficient production platform for medications in regard to protein yield, quality and stability
4 [16]. Recombinant CCS proteins produced in transgenic plants may cross the blood-brain
5 barrier and are relatively free from side-effects [9,11]. Being properly-folded, plant-made
6 CCS may have the ability to bind and deliver $\text{Cu}^+/\text{Cu}^{2+}$, Zn^{2+} and Fe^{3+} ions and to normalize
7 the SOD-1 activity via specific protein-protein interactions in the central nervous system and
8 peripherally [9,11].

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10 In contrast to plant-made pharmaceuticals (PMPs), organisms like yeast, mammals or bacteria
11 used for molecular farming approaches, express properly folded as well as improperly folded
12 recombinant therapeutic proteins. These medications may lack stability and pharmacological
13 efficiency in protein-misfolding diseases, e.g., Alzheimer's disease [16]. Furthermore, PMPs
14 may have several advantages in terms of cost scalability or safety issues compared to the other
15 genetically modified organisms [16].

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17 Because of their specific biochemical behavior, plant-made CCS from medicinal plants may
18 be efficient in the treatment of patients with preclinical stages of AD. The endogenous levels
19 of this essential copper protein may be important, since a mild copper deficiency has been
20 described in AD patients [17]. Furthermore, the expression level of CCS has been found to
21 reflect the Cu status of patients and thus, may serve as a marker for *in vivo* copper levels [18].
22 The metal levels in patients with Alzheimer's disease compared with healthy individuals are
23 important parameters for developing drugs that may restore the intracellular metal ion
24 metabolism [14]. It is anticipated that plant-made CCS may establish physiologic copper
25 levels through restoration and modulation of biometal metabolism in diseased organ systems
26 of AD patients [9,11]. Yet, to our knowledge, no systematic studies have addressed the
27 possible role of plant-made CCS in copper homeostasis.

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29 The effects of recombinant CCS and a possible role in the treatment of AD can be
30 characterized as follows: unfolded SOD-1 in diseased blood involving denatured proteins and
31 peptides [19], may be activated by copper ion incorporation via specific CCS-SOD interaction
32 [9,11]. Copper-demetalated CCS and Cu cofactor-containing CCS complexes may pass
33 through the blood-brain barrier and activate unfolded SOD-1 by Cu ion transfer or bind free
34 Cu, Zn and Fe ions in brain cells, respectively [9,11]. Since copper homeostasis is regarded as

1 an important possible factor in the complex pathogenesis of AD [20] restoring homeostasis of
2 Cu metabolism may positively affect early disease stages of AD patients by balancing
3 oxidative and anti-oxidative processes and by reducing protein-misfolding processes leading
4 to formation of A β plaque deposits [9, 11, 20]. The interactions of plant-derived CCS
5 medications with unfolded human SOD-1 and free metal ions (Cu, Zn, Fe) may trigger a
6 cascade of other biochemical reactions, such as the degradation of A β plaque deposits by
7 molecular chaperones and the ubiquitin proteasome system [21].

8
9 Further studies to determine the dose, bioavailability and mechanisms of action of plant-made
10 pharmaceuticals seem warranted in younger AD patients (> 50 years) as previously proposed
11 [9, 11].

12 13 **CONCLUSION**

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

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