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Mini Review

scyllo-Inositol, a Therapeutic Agent for Alzheimer's Disease

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Abstract

An inositol stereoisomer, *scyllo*-inositol (SI), has been regarded as a promising therapeutic agent for Alzheimer's disease (AD), because it is an orally available natural compound that penetrates into the brain and coats the surface of amyloid β-proteins (Aβ) to inhibit their lateral stacking into toxic amyloid fibrils. SI is relatively rare in nature, and we developed a *Bacillus subtilis* cell factory for the efficient production of SI from abundant *myo*-inositol (MI).

Keywords: Alzheimer's disease; Amyloid β-protein; *Bacillus subtilis*; *scyllo*-inositol

Abbreviations

AD: Alzheimer's disease; Aβ: Amyloid β-protein(s); MI: *myo*-inositol; SI: *scyllo*-inositol

Introduction

Alzheimer's disease (AD) is the most common and problematic form of dementia. In 2010, 35 million people worldwide suffered from AD, and the number is expected to rise to 115 million in 2050 [1]. Currently, there is no known cure for AD, but only a few medications that address the symptoms of the disease.

On the basis of the amyloid hypothesis, the initial cause of the disease is believed to be abnormal aggregation of amyloid β-protein (Aβ) into fibrillar polymers, which are hallmark lesions in the AD brain [2]. Although it is not yet precisely elucidated how the aggregation of Aβ is initiated [3,4], the excessive Aβ aggregation disrupts the calcium ion homeostasis in neurons, which finally induces apoptosis [5]. Aβ aggregates build up in the mitochondria and inhibit enzymes metabolizing glucose in neurons [6]. Therefore, AD has been regarded as a simple neurodegenerative disease. However, accumulating evidence implies that the development of the disease may involve more intricate events. For instance, Aβ fibrils in AD brain tissues present structural variations that may correlate with phenotypic variations of the disease [7]. In addition, the sustained formation of Aβ aggregates causes chronic activation of the innate immune system and disturbs microglial clearance functions [8].

On the other hand, another protein, named tau, is also involved in the disease development [9]. The tau proteins form neurofibrillary tangles inside nerve cells [10] that disturb the cytoskeleton, and thus, the transport system required for biochemical communication among neurons [11,12]. Furthermore, there are a number of hypotheses that attempt to explain the cause of the disease by involving other factors, including herpes simplex virus type 1 [13], cellular homeostasis of ionic copper, iron, zinc, and aluminum [14,15], extremely low frequency electromagnetic fields [16], smoking [17], age-related myelin breakdown [18-20], oxidative stress [21-23], and air pollution [24]. In any case, it is true that all the pathogenic events of the disease are tightly connected to the aggregation of Aβ [25-27]. Therefore, for any therapy for AD to show promise, aggregation of Aβ must be blocked earlier, before neurodegeneration and brain atrophy develop.

Accordingly, various chemical inhibitors targeting Aβ aggregation have been developed, including amyloid-binding dyes [28], catechols [29], curcumin [30], flavonoids [31], and polyphenols [32,33]. Some of these compounds originate in foods, and can be generally regarded as safe. Such inhibitors, including *scyllo*-inositol (SI) [34], (−)-epigallocatechin-3-gallate (EGCG) [35], and resveratrol [32] were shown to stabilize nontoxic oligomers of Aβ, and some of them are already in clinical or preclinical trials [36]. However, some of the preemptive clinical trials were not concluded successfully, which indicates that we need to understand the molecular mechanisms of the disease in depth and to devise improved ways of designing trials to accurately evaluate the compounds [37].

In this mini review, we focus on SI and summarize the current status of the studies on its mechanism of action on Aβ, effectiveness in animal models, ongoing clinical trials, and the efficient production of the compound itself.

What is SI?

Inositol stands for a group of compounds of a six-fold alcohol of cyclohexane. The epimerization of the six hydroxyl groups generates nine stereoisomers. *myo*-Inositol (MI) is the most prominent stereoisomer in nature and plays an important role as the structural basis for a number of secondary messengers that are various inositol phosphates. In addition, it serves as an important component of the membrane structural phospholipids, phosphatidylinositol. On the other hand, SI is another stereoisomer that is relatively rare in nature but has been regarded as a possible therapeutic agent for AD, and has received a fast-track designation from the US Food and Drug Administration for the treatment of AD. SI is a naturally occurring molecule that readily crosses the blood–brain barrier. It was shown that the human brain had the highest concentration of inositol in the body, with approximately 5mM MI and 0.5mM SI [38]. The concentration of SI in the brain was elevated in patients with AD [39]. In addition, high cerebral SI was proposed as a new marker of brain metabolism disturbances induced by chronic alcoholism [40]. Furthermore, a higher concentration of SI was found in the normal

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aging human brain [41]. These facts imply that cerebral SI levels may be controlled in response to functional defects in the brain caused by diseases and aging. Internal SI may be derived from MI through possible inter-conversion between the two inositol stereoisomer's, which was suggested in a previous study where SI was administered in mice [42].

How does SI work?

In 2000, SI was first reported to stabilize the non-toxic oligomers of Aβ and to inhibit their toxic aggregation [34]. To elucidate the mechanism by which SI blocks the self-aggregation of Aβ, molecular dynamics simulations of the interaction between SI and simple peptide models were conducted. It was observed that SI was able to bind to the surface of Aβ protofibrils to prevent their aggregation but could not break up the preformed aggregates [43]. In addition, SI preferentially bound to the β-sheet-containing Aβ protofibrils with affinities of 0.2–0.5mM commensurate with its *in vitro* inhibitory concentrations, and exhibited a higher binding specificity for phenylalanine-lined grooves on the Aβ protofibril surface, indicating that SI obviously coats the surface of Aβ protofibrils and disrupts their stacking into fibrillar aggregates [44]. A series of SI derivatives were synthesized and the effects of these compounds were investigated to reveal that all six hydroxyl groups of SI were involved in the complete inhibition of the fibrillar aggregation of Aβ [45].

Studies with animal models and clinical trials

SI is an orally available natural product that penetrates into the brain *in vivo*, and dose-dependently rescues the memory impairment produced by cerebroventricular injection of soluble Aβ in rats [46]. SI blocked the development of aggregation of Aβ in the brain of transgenic AD mice, and was able to reverse defect of memory and alleviated other symptoms [47,48]. There is a patent that claims the use of SI for treating AD [49], and some clinical investigations of orally-administered SI have been conducted. A phase 2 clinical study was recently conducted on 353 patients with mild to moderate AD for 18 months [50]. The clinical trial helped establish its safety profile, but the higher dose groups (1000 and 2000 mg dosed twice daily) showed greater rates of adverse events, including 9 deaths. Therefore, only the lower dose (250 mg twice daily) will be continued further, although the decision may reduce the ability of the study to establish the potential role of SI in the treatment of serious cases [51].

Efficient production of SI

As described above, SI is a promising therapeutic agent for AD. However, it is relatively rare in nature, and thus, is not sufficient to satisfy possible demand. At present, SI is produced by an expensive two-step enzymatic conversion [52] from MI, which (including its derivatives including phytic acid, as described below) is provided by fruits, beans, grains, and nuts [53]. To enable a more efficient production of SI, we devised a bacterial cell factory for the bioconversion of MI into SI.

Bacillus subtilis has the ability to metabolize both MI and SI, and the complete gene set necessary for their utilization has been characterized [54]. The *iolABCDEFGHIJ* operon encodes enzymes involved in multiple steps of the inositol metabolism, and the transcription of the operon is regulated by the IolR transcriptional repressor [55]. In the first step, MI is converted to *scyllo*-inosose by the IolG enzyme. *B. subtilis* possesses two additional inositol dehydrogenases, IolX and IolW, both of which act specifically on SI to convert it to *scyllo*-inosose [56]. IolX plays a major role in SI catabolism, whereas IolW efficiently reduces *scyllo*-inosose into SI. *scyllo*-Inosose is metabolized sequentially in multiple steps involving the IolE, IolD, IolB, IolC, IolJ, and IolA enzymes to give common intermediates, dihydroxyacetone phosphate and acetyl-CoA [57]. In the *B. subtilis* chromosome, we deleted all the "useless" genes including *iolABCDEFHIJ*, *iolX*, and *iolR* and overexpressed *iolG* and *iolW* under the control of a strong and constitutively active promoter to establish the cell factory with a complete bioconversion of 10 g/L MI into the same amount of SI secreted into the culture medium within 48 h [58].

Phytic acid (MI-1,2,3,4,5,6-hexaphosphate) is the principal storage form of phosphorus in plants, in particular bran and seeds. Phytases are a class of phosphatases that catalyze the hydrolysis of phytic acid to liberate MI and phosphate [59-61]. Because *B. subtilis* has a high ability to secrete enzymes [62,63], the cell factory could be modified to secrete phytases, and SI may be produced directly from agricultural waste materials such as rice bran, rich in phytic acid.

MI is synthesized from glucose-6-phosphate in two steps in many organisms [64]. In *B. subtilis*, glucose-6-phosphate is the starting compound of glycolysis, appearing when glucose is incorporated into the cell via the phosphotransferase system [65]. Glucose-6-phosphate is converted by inositol-3-phosphate synthase to MI 1-phosphate, which is then dephosphorylated by inositol monophosphatase to yield MI. Once we could manipulate the two enzymes function efficiently in *B. subtilis*, a novel cell factory could be devised to produce SI from glucose.

Conclusion

SI is a promising therapeutic agent for AD because of its ability to inhibit aggregation of Aβ in the brain. SI is relatively rare in nature, and we established the *B. subtilis* cell factory for production of SI from MI, which allowed 100% conversion of 10 g/L MI into the same amount of SI. By applying our *B. subtilis* cell factory concept, SI may be produced from raw and cheap materials in the future.

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