The Prevention of Alzheimer’s Disease and Parkinson’s Disease by Monascus purpureus NTU 568-Fermented Compounds

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Abstract

Alzheimer’s disease (AD) and Parkinson’s disease (PD), which are senile neurodegenerative diseases, have become more common in recent years. An important cause for AD is currently the deposition of amyloid β (Aβ) in the brain, leading to severe oxidative stress and inflammation, as well as neuronal death and memory damage in AD patients. PD is a chronic degeneration of the central nervous system that mainly affects the motor system. Its symptoms typically appear gradually, and are most obviously tremors, body stiffness, slowed movement, and gait abnormality during early stages; though the mechanism for the death of dopaminergic neurons is not yet completely understood, oxidative stress has been shown to be a very important factor in triggering the development of PD in many human and animal studies. As has been revealed in past research, fermented products of Monascus and their extracts can effectively improve the course of AD and PD, possessing significant preventative and alleviating effects. The main effectors are confirmed to be monascin and ankaflavin for AD treatment, and dimeric acid and deferricoprogen for PD treatment. This review, therefore, focuses on the analysis and comprehensive discussion of these four active components, for an overall understanding of their role in the AD and PD improvement function of Monascus fermented products.

Keywords: Alzheimer’s disease; Parkinson’s disease; Monascus

Introduction

As the aging population grows each year, Alzheimer’s disease (AD) and Parkinson’s disease (PD), which are senile neurodegenerative diseases, have become more common in recent years. An important cause for AD is currently the deposition of amyloid β (Aβ) in the brain, leading to severe oxidative stress and inflammation, as well as neuronal death and memory damage in patients with AD [1]. In a number of in vitro and in vivo studies, it has been found that the use of antioxidants can effectively prevent the memory disability due to nerve impairment, caused by Aβ [1-3]. Epidemiology data also indicate the reduction in occurrence rate of AD by non-steroidal anti-inflammatory drugs [4]. On the other hand, a high cholesterol and high caloric diet increases the lipid content of the brain and causes lipid oxidation [5,6]. As such, a high caloric diet and Aβ deposition heighten the oxidation stress of the brain. Studies have pointed out that red mold rice also inhibits the increase in brain cholesterol caused by a high caloric diet, and lowers the expression of the risk factor apolipoprotein E (ApoE) and activity of β-secretase, ultimately reducing the accumulation of Aβ40 [7].

PD is a chronic degeneration of the central nervous system that mainly affects the motor system [8]. Its symptoms typically appear gradually and are most commonly tremors, body stiffness, slowed movement and gait abnormality during early stage [9]; these symptoms may be accompanied by cognitive and behavioral problems. Though the mechanism for the death of dopaminergic neurons is not yet completely understood, oxidative stress has been shown to be a very important factor in triggering the development of PD in many human and animal studies [10]. Antioxidants such as vitamins E and D are thought to protect brain cells from PD. Relevant research however, has not reached consistent conclusions, with some studies indicating that fats and fatty acids also have protective effect on neurons, and can reduce the risk of disease. Furthermore, studies have tentatively shown that estrogen and non-steroidal anti-inflammatory drugs may also have protective functions [11-13]. Based on the above, inhibition of oxidative stress may be an important way of alleviating and preventing PD.

Monascus fermented product is a traditional natural food coloring used in many food products. The secondary metabolite of Monascus – monacolin K (MK) have been proven to have cholesterol lowering, blood pressure regulating, and anti-oxidative effects. As functional foods are receiving increasing attention, Monascus fermented products, with their many physiological benefits are becoming important. As has been revealed in past research, fermentation products of Monascus and their extracts can effectively improve the course of AD and PD, possessing significant preventative and alleviating effects [7,14-19]. The functional compounds are confirmed to be the novel yellow pigment monascin (MS) and ankaflavin (AK) for AD treatment, and novel anti-oxidative agent dimereric acid (DMA) and deferricoprogen (DFC) for PD treatment. Their structures are shown in Figure 1 [16,20]. This review, therefore, focuses on the analysis and comprehensive discussion of these four active components, for an overall understanding of their role in the AD and PD improvement function of Monascus fermented products.

The Pathogenesis of AD and PD

The pathogenesis of AD

As the aging population increases year by year, there is also an increase in patients with dementia. Dementia can be divided into...
multiple types, with Alzheimer's disease being the most common. AD is a degenerative disease of the cranial nerves, with common clinical symptoms of memory impairment, spatial operation disorder, language disorder, dysgraphia, loss of self-cognition, damaged judgment ability, attention dispersion, and others. The senile plaques accumulated around nerve cells are mainly composed of a peptide with 39-43 amino acids [21], called the Aβ, which is hard to dissolve. Amino acid sequence analysis of the amyloid separated from senile plaques shows that it is of the same type as the β-protein from the cerebrovascular plaques of patients with AD. Aβ is expressed in the neurons and glia of the central nervous system, and other body tissues [22,23], it is a peptide of 39-43 amino acids formed by the cleavage of amyloid precursor protein (APP) by the enzymes β-secretase and gamma-secretase. Aβ could be produced in large excess due to genetic mutation or other oxidative stresses. Its deposition in the brain causes nerve cells to lose their functions and die [24]. The main structural component of paired helical filament (PHF) in tangle cells is the tau protein, which cannot combine with microtubules as normal due to its high level of phosphorylation, thus forming PHF. Microtubules also lose their physiological functions due to the lack of tau protein binding [25]. The accumulation of these two abnormal proteins causes death of nerve cells and glial proliferation [26]. These pathological changes could be the results of genetic defects, aging, brain cell injury, local inflammation and Aβ toxicity. A production of Aβ in large quantities or a reduction in its scavenging rate leads to its aggregation. This is followed by the polymerization and deposition of Aβ to form diffuse plaques. Aβ oligomers act on synapses and activate neuronal cells and astrocytes, causing progressive synapse and nerve damage. This changes the ionic balance of the neurons and brings about oxidative damage and alteration in kinase phosphatase activity, leading to PHF, and shortage of neurotransmitters, followed by extensive loss of neuronal function and nerve cell death and eventually AD [27].

The pathogenesis of PD

Parkinson’s disease is a chronic degeneration of the central nervous system that mainly affects the motor system [8]. Its symptoms usually appear gradually with time, which are most obviously tremors, body stiffness, slowed movement and gait abnormality during early stage [9] and may be accompanied by cognitive and behavioral problems.

Although the mechanism of dopaminergic neuronal death remains unclear, it has been shown through human trials and animal studies that oxidative stress is an important factor for triggering the development of PD [10]. Vitamins E and D are thought to protect brain cells from the disease. Relevant research, however, has not reached consistent conclusions, with some indicating that fats and fatty acids also have protective effect on neurons, and can reduce the risk of disease. Furthermore, studies have tentatively shown that estrogen and non-steroidal anti-inflammatory drugs may also have protective functions [11-13]. Based on the above, inhibition of oxidative stress may be an important way of alleviating and preventing PD. Numerous studies have developed a number of PD animal models to be used as a testing platform for screening neuroprotective drugs. Many research-related neurotoxins, such as methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) are used in these animal models. 6-OHDA is commonly used to induce PD in both in vitro and in vivo models through oxidative stress [28], which is shown to enhance the generation of ROS, a key player in 6-OHDA induced PD models [29]. In addition, mitogen-activated protein kinases (MAPKs) are usually associated with neural cell death and apoptosis [30] and the Ak mouse strain thymoma (Akt) pathway is, thus, believed to be a necessity in protecting the neurons from cell death and safeguarding cell survival [30,31]. Various studies have revealed the regulation of 6-OHDA induced neuronal cell damage by MAPK pathway activation and Akt inhibition [32].

Past Researches on the Pathogenesis of AD and the Improvement by Monascus Fermented Product

Neuroprotective effects of Monascus fermented product and its relation to AD improvement

Inhibition of Aβ toxicity in neurons: Research has shown that
increase in cholesterol mevalonate concentrations in IMR-32 and PC-12 cells leads to rise in risk factor levels such as APPβ and Aβ, and the decrease in the production of neurotrophic factor sAPPα [18]. The ethanol extract of red mold rice fermented by M. purpureus NTU 568 could significantly inhibit the expression of the inflammation factor iNOS induced by Aβ40, and could reduce NO and ROS production, as well as oxidative stress. From this review, we conclude that the ethanol extract inhibits the cytotoxicity mechanism of Aβ40 (Figure 2) [7].

Inhibition of Aβ deposition via anti-oxidation and anti-inflammation: Introduction of Aβ40 raises the level of AChE activity in the brain, as well as levels of active oxygen atoms and lipid peroxidation, while reducing the total anti-oxidation power and superoxide dismutase activity. Oxidative stress and inflammatory response lead to the formation of more Aβ fibers and greater severity in the pathogenesis of AD, but these damages are reduced prominently by dietary supplementation of red mold rice (RMR). This is mainly due to the anti-oxidation and anti-inflammation abilities of Monascus fermented products and the protection offered by RMR against the formation of fibrous Aβ and its deposition in the hippocampus, thereby improving memory learning ability [17]. As such, metabolites from Monascus fermentation should be effective in preventing the advancement of Aβ-induced memory impairment into AD.

Suppression of Aβ formation and promotion of sAPPα formation: The AD risk factor Aβ is predominantly formed by the cholesterol catalyzed β-secretase lysis of the precursor protein APP in the brain, forming the toxic Aβ. As indicated by previous studies, a high caloric diet with high cholesterol increases the chance of AD and worsens the condition of the disease, via elevated formation of apoE and β-secretase activity, which increase the deposition of Aβ in the brain, worsening the damage in memory learning ability. Research shows that AD and formation of Aβ are related to lipid metabolism in cells [33-35]. The ethanol extracts of NTU 568 RMR reduce the amount of Aβ and sAPPβ synthesized [36], and increase the production of the neuroprotective sAPPα. The expression and activity of β-secretase are both inhibited, avoiding its cleavage of APP to form APPβ. The inhibition of Aβ synthesis by the alcohol extracts of RMR is mainly through the reduction in cholesterol formation in nerve cells (Figure 2) [7], which could prevent the secretion of β-secretase induced by cholesterol and block the paths for Aβ and APPβ generation, leading to enhanced α-secretase activity and the level of neuroprotective sAPPα. RMR improves the memory learning ability in the water maze task, significantly. The simultaneous introduction of Aβ40 and high caloric diet causes brain damage, including rise in acetylcholine and more intense oxidative and inflammatory responses. RMR could also inhibit the increase in cholesterol concentration in the brain caused by a high caloric diet, and lower the expression of the risk factor apoE and activity of β-secretase, ultimately reducing the accumulation of Aβ40 in hippocampal tissue, and raising the production of the neuroprotective factor sAPPα [18]. In addition, RMR has also been shown to suppress the increase in cholesterol concentration caused by a high caloric diet, minimizing the expression of risk factor apoE and β-secretase activity.

Past studies on PD improvement by Monascus fermented products

PD improvement by Monascus-fermented products: Past research has indicated that M. purpureus NTU 568-fermented rice extracts could improve neural toxicity in SH-SY5Y cells and PD in rat models, both induced by 6-OHDA [15]. This research confirms the attenuation of PD by M. purpureus NTU 568-fermented rice extracts using the in vitro and in vivo neural toxicity models of 6-OHDA induced PD. The basic mechanism of these processes includes the suppression of oxidative stress and damage by the down-regulation of NOX expression and reduction of inflammatory factors activated by 6-OHDA. This result is of importance for the development of novel PD therapy, and shows that the neural protective function of the ethanol extract of M. purpureus NTU 568-fermented rice could come from the anti-oxidative and anti-inflammatory response abilities of its bioactive components. Thus, M. purpureus NTU 568-fermented rice extracts can be used as a dietary constituent in the prevention and treatment of PD. This is the first confirmation that Monascus-fermented products exhibit neural protective effects in PD cells and animal models [15].
Anti-inflammatory and anti-oxidative metabolites produced from Monascus purpureus NTU 568

Anti-inflammatory yellow pigment–MS and AK: MS and AK are the yellow pigments in Monascus fermented product. As shown in Table 1, they have been identified as anti-inflammatory components and their health effects have been applied to the prevention and improvement of various conditions as follows:

In an experiment studying the TPA-induced inflammation of mouse, MS was found to have effective anti-inflammation results [37]. For the inflammation triggered by lipopolysaccharide (LPS) induction of murine RAW 264.7 macrophages, AK could achieve anti-inflammation through the reduction of nitrite content, and inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) expressions [38]. MS could suppress the inflammation caused by ovalbumin (OVA) induction of human THP-1 monocytes. MS also effectively attenuates the expression of TNF-α, interleukin-6 (IL-6) protein and mRNA. For the OVA-induced inflammation, MS could downregulate the phosphorylation of mitogen-activated protein kinase (MAPK) of c-Jun NH2-terminal kinase (JNK), but not that of ERK or p-38 MAPK. In addition, the assay of PPAR-γ antagonist GW9662 shows that MS suppresses JNK phosphorylation by increasing PPAR-γ expression. Therefore, it can be used as a functional component for anti-inflammation [39].

MS has been confirmed by previous research to possess inhibitory effects on the receptor for advanced glycation end products (RAGE) of hepatic stellate cells (HSCs). MS could increase PPAR-gamma activity to reduce the expression of α-smooth muscle actin (α-SMA) in HSCs and production of ROS; thus, delaying or inhibiting the development of fibrosis through activation of PPAR-gamma [40]. Past research has also shown the regulation on the expression of anti-oxidation enzyme Nrf-2 by AK, which upregulates the signal transduction pathway of Nrf-2 in a methylyglyoxal-induced diabetes rat model to enhance the activity of anti-oxidation enzymes, and promotes the activity of PPAR-α, improving insulin sensitivity [41].

Anti-oxidative agents-dimereric acid (DMA) and deferricoprogen (DFC): DMA and DFC are the anti-oxidative agents in M. purpureus NTU 568 fermented products. As shown in Table 1, they have been identified as anti-oxidative agents and their health effects have been applied to the prevention and improvement of various conditions as follows:

Aniya et al. discovered the anti-oxidation effect of Monascus fermented product. Upon further purification, it is known that this anti-oxidation effect comes from DMA, which is capable of removing α,α-diphenyl-β-picyrhydrazyl (DPPH) free radical, even at low concentration, as well as reducing the level of ROS [42]. Taira et al. proposed a mechanism for the anti-oxidative ability of DFC in 2002, which states that DMA could inhibit the peroxidation of NADPH and Fe2+-dependent lipid in rat liver microsomes at a concentration of 20-200 μM. Through clearing OH·, ferryl-Mb and peroxyl radicals, and inhibiting lipid peroxidation (LPO), it donates an electron to the oxidizing agent to be oxidized to nitroxide radical, which is removed to achieve the anti-oxidation effect [43].

Yamashiro et al. in 2008 explored the effects of DMA on rat liver microsomes and hepatocyte oxidative stress, i.e., cytotoxicity, induced by salicylic acid (SA) and tert-butylhydroperoxide (t-BHP) and found that DMA could prevent the damage of hepatocytes by inhibiting oxidative stress [44]. As research indicates, the large quantity of carboxymethyllysine (CML) present in patients with diabetes is the cause for hepatic fibrosis. As such, the effects of DMA on RAGE signal transduction and HSCs activation were discussed by the authors, which lead to the finding that DMA could suppress collagen production in HSCs, and mRNA expression for α-SMA, platelet derived growth factor-β receptor (PDGF-BR) and procollagen 1α1 (proCol-1α1). These results show that DMA achieves anti-fibrosis in HSCs by increasing glutamate-cysteine ligase activity and suppressing oxidative stress [45].

Past research has uncovered the high anti-oxidative activity of DFC. Hsu et al. found that DFC could reduce the oxidative damage caused to the human embryonic kidney cell line HEK-293 by citrinin, effectively preventing renal cell apoptosis [46]. Upon further investigation of the antagonistic mechanism of DFC against renal cell toxicity induced by citrinin, the authors found that while citrinin increases the number of renal cells detained in the sub-G1 phase, causing their death, the introduction of DFC could effectively reduce this phenomenon. In terms of the proteins involved in apoptosis, citrinin enhances the activity of caspase-3 and caspase-9 in renal cells, which is significantly diminished by the action of DFC, showing that DFC safeguards kidney cells from damage by suppressing the mitochondrial apoptosis pathways [46].

Past researches on the improvement of AD and PD by Monascus fermented functional compounds

Active components of Monascus fermented product for AD improvement and their mechanism: In the cortex cerebra and hippocampus, the performance of the donepezil group is not as efficient as MS and AK in inhibiting β-secretase, p-tau and apo E. Similar trends are observed in the water maze test as well as MDA and ROS levels in the cortex cerebra and hippocampus of rats introduced with Aβ40 (Figure 3) [47].

The influence of MS and AK on the deposition of the protein Aβ40 in the cortex cerebra and hippocampus: From these results and previous studies, it can be seen that the Aβ40 administered

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<td>Deferricoprogen</td>
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Table 1: The application of Monascus fermented metabolite in the preventive medicine.
could stimulate oxidation stress and inflammatory response in the brain, resulting in deposition of Aβ40 [23]. As deposition continues with time, oxidative stress and inflammation of greater severity are triggered. This vicious cycle repeats and leads to continued worsening of brain damage. MS and AK lower Aβ40 accumulation in the cortex cerebra by their suppression of oxidation stress and inflammation, so that the Aβ40 introduced into the brain will not be stimulated by oxidants or inflammatory factors to deposit or cause damage to the brain; thus, effectively improving memory learning competence. The inhibition in Aβ40 deposition by oral feeding of MS and AK leads to reduced expression of MDA, ROS and AD related proteins, as well as inflammation-related proteins in the cortex cerebra and hippocampus (Figure 3) [47].

**Table 3:** Active components monascin and ankaflavin of Monascus purpureus NTU 568 for AD improvement and their mechanism. The mechanisms includes inhibiting BACE levels by lowering the cholesterol level, inhibiting the synthesis of apo E, a risk factor that promotes Aβ deposition and inhibiting the Aβ-induced inflammatory response [47].

The influence of MS and AK on the deposition of the protein sAPPα in the cortex cerebra and hippocampus: sAPPα is a protective factor for brain nerve cells, capable of promoting synapse growth and protecting nerve cells. In the previous study, the animals in the vehicle group are provided with a series of nerve cell protections to lower the deposition tendency of the Aβ40 introduced, and to inhibit Aβ formation caused by oxidation stress and inflammatory response [23]. The deposition of the protein sAPPα in the donepezil group is not as significant as in the MS and AK groups, which confirms the expression enhancement of the nerve cell protective factor sAPPα by MS and AK feeding. The synthesis route of sAPPα is opposite to that of Aβ. Studies have shown that cholesterol could elevate β-secretase activity and increase Aβ formation, which, together with oxidative stress, will cause the production of more Aβ and inhibit the synthesis of sAPPα. MS and AK could reduce the occurrence of risk factors such as oxidation stress in the brain and the deposition of Aβ40; thus, effectively promoting the expression of sAPPα. As such, the improvement in memory learning ability could be attributed to the elevation of sAPPα expression by MS and AK (Figure 3) [47].

**Table 4:** Dimerumic acid and deferricoprogen as the active ingredients in Monascus-fermented products for PD improvement

As proven in another ongoing study on red mold rice, the active ingredients DMA and DFC can prevent 6-OHDA induced apoptosis and oxidative stress in SH-SY5Y cells [16]. In addition, DMA and DFC activated Akt phosphorylation could stimulate the expression of HO-1 and reduce the phosphorylation of JNK and p-38 to offset the neurotoxicity in SH-SY5Y cells induced by 6-OHDA. In addition, DMA and DFC can regulate the gene expression of grin2c, xtc2 and fcgr2a to improve neuronal damage (Figure 4). These results of mechanism-based therapies are critical for progress in PD prevention and treatment. In addition, we deduce that the DMA and DFC from Monascus purpureus fermentation can be used as functional components in preventing and treating PD [16].

The previous finding that 6-OHDA triggers cell oxidative stress has been widely used in the generation of PD cells and animal models [28]. Past research has suggested that the ROS involved initiate auto-oxidation [28], and that NADPH oxidase (NOX)-derived ROS plays a vital role in the 6-OHDA induced cell death [48] by mediating the mitochondrial caspase cascade to activate the caspase for 6-OHDA-induced apoptosis [48]. However, the details on the molecular mechanism of cytoxicity induced by 6-OHDA are not fully understood yet. Research results show that in differentiated pheochromocytoma PC-12 cells treated with 6-OHDA, DMA, and DFC could suppress extracellular auto-oxidation and the formation of intracellular ROS through the downregulation of NOX-2 expression, and suppress the activation of caspase-3 by increasing Bcl2 expression and reducing Bax expression. Through these two mechanisms, DMA and DFC lower the number of apoptotic cells in 6-OHDA-induced differentiated PC-12 cells (Figure 5) [14]. These results are significant for the development of anti-oxidation therapy for PD. As such, metabolites from Monascus purpureus NTU 568 fermentation can be used as functional foods in the prevention or treatment of PD.

**Prospects of Monascus Fermented Constituents for AD and PD**

According to the results of the presented studies, Monascus purpureus NTU 568-fermented products contain anti-oxidative and anti-inflammatory substances. The anti-oxidative components DMA and DFC could improve PD pathogenesis by combating oxidative stress and reducing apoptosis. The anti-inflammatory agents MS and AK can inhibit the brain inflammatory response induced by Aβ40 and dampen the occurrence of related risk factors. In terms of future development, the amount of active components present will affect the treatment of AD and PD. Relevant studies have indicated that to be...
effective for AD and PD improvement, the content of active ingredients needs to reach a certain level, thus requiring advanced extraction and enrichment techniques for their production in high concentration, while still maintaining effectiveness. Though Monascus is a health-benefiting genus of fungus commonly seen in recent years, not all its species are able to produce DFC, DMA, MS and AK. The \textit{M. purpureus} NTU 568 often used in past research are able to produce these active compounds, with speed accelerated by solid-state fermentation, and in large quantities. Therefore, the fermentation products obtained from this strain can possibly become functional products for the prevention and improvement of PD and AD in the future.

References


