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A novel PPARgamma agonist monascin potentially applied in diabetes prevention

Running title: Anti-diabetic effect of monascin

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Abstract

Edible fungi of the *Monascus* species have been used as traditional Chinese medicine in eastern Asia for several centuries. *Monascus*-fermented products possess a number of functional secondary metabolites, including the anti-inflammatory pigments monascin and ankaflavin. Monascin has been shown to prevent or ameliorate several conditions, including hypercholesterolemia, hyperlipidemia, diabetes, and obesity. Recently, monascin has been shown to improve hyperglycemia, attenuate oxidative stress, inhibit insulin resistance, and suppress inflammatory cytokines production. In our recent study, we have found that monascin is a peroxisome proliferator-activated receptor-gamma (PPARgamma) agonist. This PPARgamma agonist activity had been investigated and exerted benefits for inhibition of inflammation in methylglyoxal (MG)-treated rats, prevention of pancreas impairment caused advanced glycation endproducts (AGEs), promotion of insulin expression in vivo and in vitro, and attenuated carboxymethyllysine (CML)-induced hepatic stella cells (HSCs) activation in past several years. Moreover, our studies also demonstrated that monascin also activated nuclear factor-erythroid 2-related factor 2 (Nrf2) in pancreatic RIN-m5F cell line thereby invading methylglyoxal-resulted in pancreas dysfunction. In this review, we focus on the chemo-preventive properties of monascin against metabolic syndrome through PPARgamma and Nrf2 pathways.
Keywords: monascin, peroxisome proliferator-activated receptor-gamma (PPARgamma) agonist, methylglyoxal (MG), advanced glycation endproducts (AGEs), nuclear factor-erythroid 2-related factor 2 (Nrf2)
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1. Introduction

Monascus was classified and named in 1884 by the French scientist van Tieghem.¹ The genus Monascus belongs to the family Monascaceae, order Eurotiales, class Ascomycetes, phylum Ascomycota, and kingdom Fungi. Thus far, 58 Monascus strains have been deposited in the American Type Culture Collection; however, most strains belong to only 3 species: Monascus pilosus, Monascus purpureus, and Monascus ruber.² Monascus-fermented products, especially those produced by solid-state rice fermentation, have been used as food colorants and dietary material for more than 1,000 years. Monascus-fermented rice, also known as red mold rice, is a common foodstuff and traditional health remedy in Asian countries. Red mold rice, largely produced by M. purpureus contains various chemical components, some of which have been purified and identified, including monascolins,³,⁴ γ-aminobutyric acid,⁵ pigments such as monascin and ankaflavin,⁶ and antioxidant such as dimerumic acid.⁷ It was reported that monascin is the major constituent of the azaphilonoid compound. The structure of monascin is shown in Fig. 1a, and which has been recently reported to be a PPARgamma agonist in our study (Fig. 1b).⁸ It is suggests that monascin plays a role for PPARgamma activation.

Hyperglycemia is associated with protein glycation; advanced glycation end products (AGEs) are generated by the nonenzymatic interaction between
carbohydrates and proteins. AGEs have properties to generate free radicals and undergo autoxidation to generate other reactive intermediates, thereby resulting in the development of diabetes.\(^9\) Methylglyoxal (MG) is a highly reactive dicarbonyl metabolite produced during glucose metabolism\(^10\) and is a major precursor of AGEs involved in the pathogenesis of diabetes and inflammation. Studies suggest that AGEs and MG can generate large amounts of proinflammatory cytokines through receptor for AGES (RAGE) activation, and these results are related to the modulation of inflammatory molecules through oxidative stress.\(^10\)

PPAR\(\gamma\) ligands are reported to activate the phosphatidylinositol 3-kinase/Akt pathway, which can elevate insulin sensitivity to downregulate blood glucose.\(^11\) Moreover, PPAR\(\gamma\) ligands have been reported to exert anti-inflammatory activity by inhibiting inflammatory gene expression while PPAR\(\gamma\) agonists bind to PPARs.\(^12\) Many phytochemicals, including auraptene, resveratrol, 6-shogaol, and isoprenoid, are considered to function as PPAR\(\gamma\) agonists and demonstrate anti-inflammatory activity by interfering with nuclear factor-kappa B (NF\(\kappa\)B) signaling.\(^13\) Several flavonoids, such as rutin and quercetin, elevate PPAR\(\gamma\) mRNA expression, which attenuates inflammation and insulin resistance.\(^14,15\) The transcriptional activity of PPAR\(\gamma\) is modulated through phosphorylation by kinases such as c-Jun N-terminal kinases (JNK). PPAR\(\gamma\)
loses its transcriptional activity by JNK phosphorylation at serine 82, and is subsequently degraded by the ubiquitin pathway. Treating diabetes with PPARgamma ligands (agonists), such as pioglitazone, can prevent PPARgamma phosphorylation by altering its structure.\(^\text{16}\)

PPARgamma is expressed in islet beta cells\(^\text{17}\) and is important for a variety of pancreatic functions, including beta cell survival,\(^\text{18}\) pancreatic and duodenal homeobox-1 (PDX-1) and glucokinase (GCK) regulation,\(^\text{19}\) and glucose-stimulated insulin secretion.\(^\text{20}\) In addition, PPARgamma is known to affect pancreatic beta cell function and insulin production.\(^\text{21}\) Studies have reported that PPARgamma binds to the PDX-1 promoter to upregulate PDX-1 expression and insulin production.\(^\text{19}\) A recent acute study suggested that AGE injection can initiate beta cell dysfunction and demonstrated that dietary restriction of AGEs significantly improves insulin sensitivity.\(^\text{22}\) AGEs also decrease insulin synthesis in pancreatic beta cells by repressing PDX-1 protein expression and inhibiting glucose-stimulated insulin secretion.\(^\text{23}\) PDX-1 plays a significant role in both pancreatic development and maintenance of beta cell function, but the inhibition of beta cell function caused by AGEs was improved by pioglitazone (PPARgamma agonist) activating PPARgamma.\(^\text{24}\) Several lines of evidence indicate that PDX-1 binds to insulin and GCK and that GCK catalyzes the first step of glycolysis to regulate glucose
responsiveness for insulin release.\textsuperscript{25} These findings indicated that PPARgamma plays an important role for diabetes improvement. However, we had found that monascin is a PPARgamma agonist to up-regulate insulin sensitivity and inhibited hyperglycemia in AGEs- or MG-treated animals in our recent studies.

2. Anti-inflammation and antioxidation of monascin

High carbohydrate diets result in hyperglycemia and insulin resistance. In diabetic patients, there is a positive correlation between high methylglyoxal (MG) concentration in the blood and hyperglycemia. Recent studies have shown that MG administration results in inflammation.\textsuperscript{26}

Several literatures have reported the modulation of inflammatory cytokines through oxidative stress.\textsuperscript{27,28} Oxidative stress is increased during diabetes and hyperinsulinemia; reactive oxygen species have been reported to be generated as a result of hyperglycemia, which causes many of the secondary complications of diabetes.\textsuperscript{28}

We have indicated that monascin can suppress the production of inflammatory factors (tumor necrosis factor-alpha and interleukin-6) from monocytes induced by MG depending on PPARgamma regulation and these effects are abolished by
The anti-inflammatory capacity of monascin is mediated by the inhibition of JNK, extracellular signal-regulated kinase (ERK), and p38 kinases (Fig. 2). Inflammation is an independent risk factor of cardiovascular diseases and is associated with endothelial dysfunction. Monascus-fermented metabolites, including monascin, ankaflavin, and monacolin K, have been found to reduce TNF-α-stimulated endothelial adhesiveness as well as downregulating intracellular ROS formation, NF-κB activation, and VCAM-1/E-selectin expression in human aortic endothelial cells, supporting the notion that the various metabolites from Monascus-fermented products might have potential implications in clinical atherosclerosis disease.

Recently, our study also reports that monascin can extend the life span under high-glucose conditions and attenuate oxidative stress in Caenorhabditis elegans. Our results indicate that monascin enhanced expression of small heat shock protein (sHSP-16), superoxide dismutase (SOD), and glutathione S-transferase (GST). Monascin not only regulates stress response/antioxidant genes to improve oxidative stress resistance but also promotes antioxidation and avoid oxidative damage via regulation of the FOXO/DAF-16-dependent insulin signaling pathway. Furthermore, Nrf2 has been found to attenuate oxidative damage by expressions of heme oxygenase-1 (HO-1), and glutathione-cysteine ligase (GCL). Our study has
carried out the Nrf2 regulation by monascin in vivo and in vitro. Results indicated that
monascin inhibited inflammatory cytokine production in S100b (the receptor for
AGEs activator)-treated THP-1 monocytes via up-regulation of Nrf2 and alleviated
p47phox translocation to the membrane; and these effect were abolised by Nrf2
inhibitor treatment depending on retinoic acid receptor-alpha. We also found that
monascin markedly activated Nrf2 and attenuated insulin resistance in vitro and in
vivo pointing out as Fig. 3. These findings had pointed out that monascin
suppressed oxidative stress and inflammation by showing antioxidation.

3. Anti-diabetic effect of monascin

Diabetes mellitus, which is characterised by hyperglycemia, is an endocrine
disorder resulting from insulin deficiency that leads to high blood glucose
concentration. Type 2 diabetes and obesity are chronic diseases that promote the
development of insulin resistance, inflammation, and atherosclerosis. Type 2
diabetes is a chronic disease caused by deficient insulin secretion or ineffective
insulin activity, thereby negatively affecting carbohydrate metabolism. High
triacylglycerol levels in the blood tend to coexist with low levels of high-density
lipoprotein cholesterol (HDL-C), contributing to a condition called diabetic
dyslipidemia or hypertriglyceridemia. The total cholesterol (TC) and total
triacylglycerol (TG) cause an increased risk of heart disease, which should be controlled as tightly as possible in diabetes mellitus.\textsuperscript{38} Insulin resistance in type 2 diabetic patients is thought to be associated with the induction of inflammatory cytokines such as TNF-alpha and IL-6.\textsuperscript{39} The TNF-alpha impairs insulin-dependent signal transduction through a mechanism involving downregulation of the insulin receptor (IR) and IR substrate-1 protein (IRS-1), inhibition of IR and IRS-1 tyrosine phosphorylation, increased protein tyrosine phosphatase 1B (PTP1B) activity, and inhibition of the insulin-stimulated glucose transporter (GLUT), thereby resulting in hyperglycemia.\textsuperscript{38} Results of our recent study have shown that monascin can attenuate JNK phosphorylation and suppress PPARgamma phosphorylation in C2C12 myotubes treated with TNF-alpha and thereby improve insulin sensitivity.\textsuperscript{40} In addition, monascin also inhibits protein tyrosine (Tyr) phosphatase 1B (PTP1B) expression to attenuate insulin resistance, resulting in GLUT translocation to plasma membrane and subsequently promoting glucose uptake as shown in Fig. 4.\textsuperscript{40}

In vitro studies suggest that MG impairs insulin mediated glucose uptake in adipocytes\textsuperscript{41} and reduces insulin sensitivity for 30 min in L6 muscle cells treated with 2.5 mM MG.\textsuperscript{42} Moreover, 1 mM MG suppresses insulin secretion and production in INS-1E pancreatic islet \( \beta \)-cells.\textsuperscript{43} In vivo studies demonstrate that MG impairs insulin transcription factor pancreatic and duodenal homeobox-1 (PDX-1) to result in
Recently, monascin has been reported to act as PPARgamma agonist,\textsuperscript{8} and the in vitro (MG-treated RIN-m5F cells) and in vivo (MG-treated Balb/c mice) results indicated that MG leads to marked PPARgamma phosphorylation (serine 82); this effect led to reduction in PDX-1, GCK, and insulin expression. Monascin and rosiglitazone protected impairment of insulin expression in MG-treated animals confirmed by immunohistochemical stain for pancreatic insulin (Fig. 5).\textsuperscript{26} Moreover, monascin also prevented hyperglycemia and significantly downregulated blood glucose during oral glucose tolerance test (OGTT) in fructose-rich diet-induced C57BL/6 mice, and the potential mechanism was shown as Fig. 6.\textsuperscript{46}

Hepatic stellate cells (HSCs) express the receptor for AGEs (RAGE)\textsuperscript{47} and also express many components of the NADPH oxidase complex, such as p47phox. Importantly, one study has implicated p47phox-derived reactive oxygen species (ROS) in HSCs activation, suggesting that hepatic fibrosis is always involved in diabetes.\textsuperscript{48}

To gain better insights into the role of AGEs in HSCs, we investigated the effect of AGEs on ROS production by HSCs. Carboxymethyllysine (CML) is a key AGE with highly reactive dicarbonyl metabolites (e.g., methylglyoxal) and promotes lipid peroxidation to generate malondialdehyde (MDA).\textsuperscript{49} We had investigated the inhibitory effect of Monascus-fermented metabolite monascin on CML-induced
RAGE signaling in HSCs and its resulting antihepatic fibrosis activity. We found that monascin upregulated PPARgamma to attenuate alpha-smooth muscle actin (alpha-SMA) and ROS generation in CML-treated HSCs in a RAGE activation-independent pathway. Therefore, monascin may regulate PPARgamma to delay or inhibit the progression of liver fibrosis and may prove to be a major antifibrotic mechanism to prevent liver disease (Fig. 7).50

4. Conclusions

These health-promoting functions of monascin may be used to augment the anti-metabolic syndrome, antihypertensive and anti-atherogenic effects of current pharmacotherapeutics. The bioactivity of monascin is responsible for the previously described health benefits and for the prevention of numerous inflammation-related diseases. Together, these findings suggest that monascin can act as an antidiabetic and antioxidative stress agent, and thus, monascin may have therapeutic potential in the treatment or prevention of diabetes and diabetes-associated oxidative stress complications.
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Conflict of interest

The authors declare that there are no conflicts of interest.
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Figure legends

Figure 1. (a) Chemical structure of monascin. (b) Monascin is a PPARgamma agonist.8 The PPARgamma agonist activity of monascin was carried out by LanthaScreen™ TR-FRET PPARγ coactivator assay kit (Invitrogen, Carlsbad, CA, USA). PPARgamma: peroxisome proliferator-activated receptor-gamma.

Figure 2. The proposed mechanism of monascin on inflammation in THP-1 cell. Ovalbumin-induced inflammation was alleviated by monascin via inhibition of JNK phosphorylation and regulation of PPARgamma.30 MS: monascin. JNK: c-Jun N-terminal kinases. ERK: extracellular signal-regulated kinase.

Figure 3. The potential mechanism of monascin attenuated inflammation caused by RAGE activation. Monascin promotes Nrf2 activation to elevate antioxidant status, thereby attenuating oxidative stress and inflammation caused by RAGE signal.29 MS: monascin. AGEs: advanced glycation endproducts. RAGE: receptor for AGEs. TNF-α: tumor necrosis factor-alpha. IL-1β: interleukin-1beta. PKC: protein kinase C. Nrf2: nuclear factor-erythroid 2-related factor 2. HO-1: heme oxygenase-1. GCL: glutathione-cysteine ligase.
**Figure 4.** The inhibition of insulin resistance in C2C12 myotubes treated by monascin.\(^4^0\) IR: insulin receptor. IRS: insulin receptor substrate. GLUT: glucose transporter. TNF-α: tumor necrosis factor-alpha. PPARgamma: peroxisome proliferator-activated receptor-gamma.

**Figure 5.** Effects of monascin, rosiglitazone, AITC, or NAC treatment on pancreatic insulin level of methylglyoxal-injected Balb/C mice stained by immunohistochemical stain.\(^2^6\) Monascin promoted insulin expression and may protect impairment of pancreatic function in methylglyoxal-treated animals. MG: methylglyoxal. MS: monascin. Rosi: rosiglitazone. AITC: allyl isothiocyanate. NAC: N-acetylcysteine.

**Figure 6.** The potential anti-diabetic mechanism of monascin in mice fed high fructose diet.\(^4^6\) Monascin improved fructose-rich diet-induced glucose intolerance, hyperlipidemia, hyperinsulinemia, and hepatic fatty acid accumulation, presumably by inhibiting lipogenesis and ameliorating insulin resistance and inflammation in the liver through PPARgamma activation. PPARgamma: peroxisome proliferator-activated receptor-gamma. ChREBP: carbohydrate responsive element binding protein. SREBP-1c: sterol regulatory element-binding protein-1c. ACC:
acetyl-coA carboxylase. FAS: fatty acid synthase. PGC: peroxisome
proliferator-activated receptor-gamma coactivator.

Figure 7. Potential mechanism of monascin on antifibrosis in HSCs. Monascin and
rosiglitazone upregulated PPARgamma to attenuate fibrotic biomarker expression and
ROS generation in CML-treated HSCs. CML: carboxymethyllysine. ROS: reactive
oxygen species. RAGE: receptor for advanced glycation endproducts. α-SMA:
α-smooth muscle actin. TIMP: tissue inhibitor of metalloproteinase. MMP-13: matrix
metalloproteinase-13.
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6

Monascin (natural PPARγ agonist)

PPARα

FRD

ChREBP
SREBP-1c
ACC
FAS
PGC-1α
PGC-1β

Lipogenesis

Hyperglycemia and hyperlipidemia

Insulin sensitivity
Monascin/rosiglitazone

CML \(\rightarrow\) RAGE/p47phox

\(\downarrow\)

ROS

\(\downarrow\)

\(\alpha\)-SMA

TIMP

MMP-13

\(\downarrow\)

Hepatic fibrosis