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# Monascin and AITC Attenuate Methylglyoxal-Induced PPAR $\gamma$ Phosphorylation and Degradation through Inhibition of the Oxidative Stress/PKC Pathway Depending on Nrf2 Activation

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## S Supporting Information

**ABSTRACT:** Abnormal cellular accumulation of the dicarbonyl metabolite methylglyoxal (MG) results in cell damage, inflammation, and oxidative stress. It is also associated with increased protein linkage to form advanced glycation end products (AGEs) or induce DNA strand breaks. The association between peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) and nuclear factor-erythroid 2-related factor 2 (Nrf2) is unclear. This study investigated Nrf2 activator protection against PPAR $\gamma$  phosphorylation and degradation to maintain pancreatic function. MG was used at a noncytotoxic concentration (200  $\mu$ M) to induce protein kinase C (PKC) and PPAR $\gamma$  phosphorylation in pancreatic RINm5F cells. For *in vivo* studies, MG (60 mg/kg bw) was intraperitoneally (IP) injected into Balb/C mice for 28 d to induce pancreas damage, at which point we investigated the effect of monascin protection (PPAR $\gamma$  and Nrf2 activator), rosiglitazone (PPAR $\gamma$  activator), allyl isothiocyanate (AITC; Nrf2 activator), or *N*-acetylcysteine (NAC) on pancreatic function. The *in vitro* and *in vivo* results indicated that MG leads to marked PPAR $\gamma$  phosphorylation (serine 82); this effect led to reduction in pancreatic and duodenal homeobox-1 (PDX-1), glucokinase (GCK), and insulin expression. However, monascin and rosiglitazone may protect PPAR $\gamma$  degradation by elevating PDX-1, GCK, and as a result, insulin expression. Monascin and AITC can attenuate PKC activation to suppress PPAR $\gamma$  phosphorylation caused by oxidative stress through the Nrf2 pathway. Similarly, the *N*-acetylcysteine (NAC) antioxidant also improved oxidative stress and pancreatic function. This study examined whether MG caused impairment of PDX-1, GCK, and insulin through PPAR $\gamma$  phosphorylation and degradation. MG and AGE accumulation improved on Nrf2 activation, thereby protecting against pancreas damage. Taken together, PPAR $\gamma$  activation maintained pancreatic PDX-1, GCK, and insulin expression levels to regulate blood glucose levels.

## KEYWORDS:

## INTRODUCTION

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. Abnormal cellular accumulation of MG occurs invariably in diabetes. MG decreases glucose tolerance in rodents,<sup>1</sup> suggesting that postprandial MG production in normoglycemic individuals could result in glucose intolerance and consequently greater MG accumulation. Recently, several types of food consumption including coffee, cream, and cake, have been known to result in high MG level in the plasma, thus causing both nutritional and health concerns. Coffee has been reported to contain high level of MG by 230  $\mu$ M (17.7 mg/L).<sup>2</sup> In addition, approximate 5100  $\mu$ g/g of MG was determined in honey.<sup>3</sup> In Spain, dietary exposure of the Spanish population to glyoxal and MG from cookies was estimated to be 213 and 216  $\mu$ g/person/day, respectively.<sup>4</sup> Wine and beer were found to have 1556  $\mu$ g/L and 1000  $\mu$ g/L of MG, respectively.<sup>5,6</sup> Plasma of diabetic patients contain high level of MG (200  $\mu$ g/L) when comparing to normal human (5  $\mu$ g/L).<sup>7</sup> These suggest that MG from dietary foods is accumulated in the plasma in normal humans, and the level may be equal to that of diabetic patients.

Recent studies have shown that MG administration results in inflammation.<sup>8</sup> *In vitro* studies suggest that MG impairs insulin-mediated glucose uptake in adipocytes<sup>9</sup> and reduces insulin sensitivity for 30 min in L6 muscle cells treated with 2.5 mM MG.<sup>10</sup> Moreover, 1 mM MG suppresses insulin secretion and production in INS-1E pancreatic islet  $\beta$ -cells.<sup>11</sup> *In vivo* studies demonstrate that MG impairs insulin transcription factor pancreatic and duodenal homeobox-1 (PDX-1) to result in diabetes.<sup>12,13</sup>

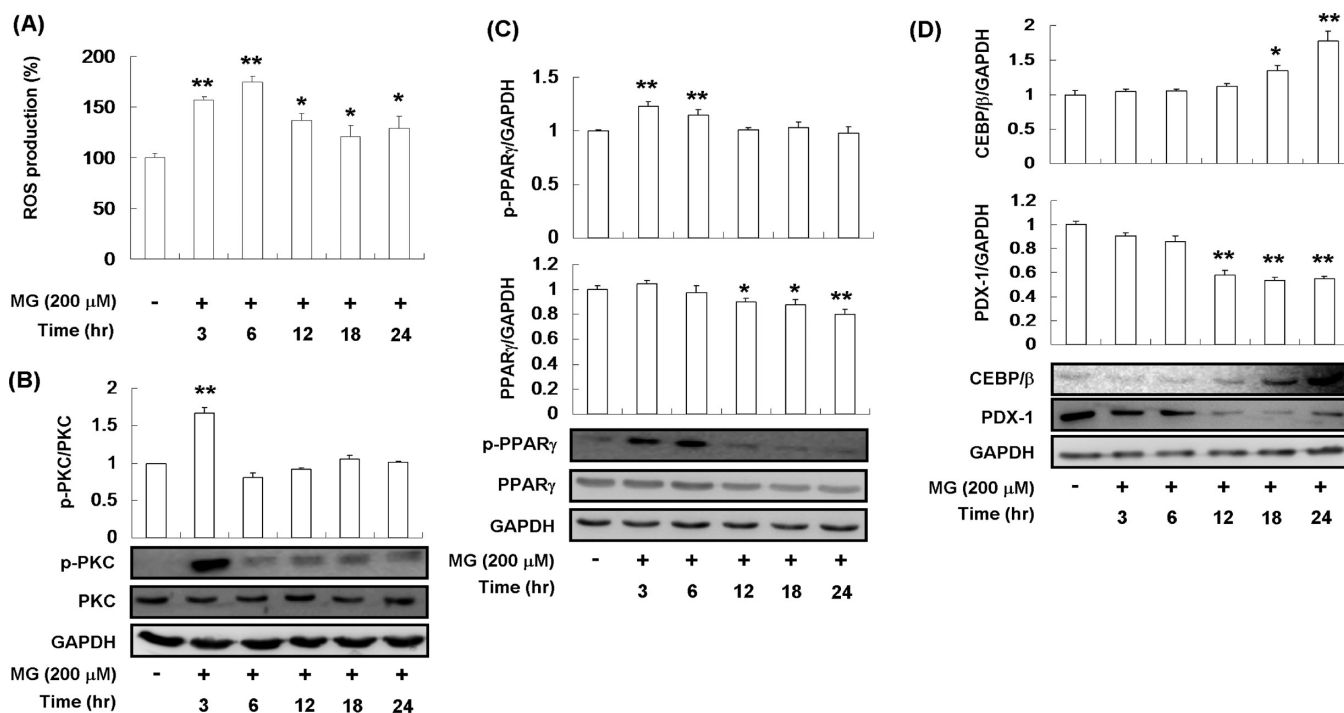
Peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) is known to affect pancreatic beta cell function and insulin production.<sup>14</sup> Studies have reported that PPAR $\gamma$  binds to the PDX-1 promoter to upregulate PDX-1 expression and insulin production.<sup>15,16</sup> However, MG impairs pancreatic function, but whether this involves suppression of PPAR $\gamma$  activation is unclear. Nrf2 is essential for antioxidant responsive element (ARE)-mediated induction.<sup>17</sup> Many antioxidants derived from dietary and medicinal plants induce nuclear factor-erythroid 2-

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**Figure 1.** Effect of MG (200 μM) on (A) ROS production, (B) PKC phosphorylation, (C) PPARγ phosphorylation, (D) CEBPβ and PDX-1 levels in pancreatic RINm5F cells treated with MG for various times (3–24 h). Data are shown as mean ± SD ( $n = 3$ ). \*Significantly different from the control group  $p < 0.05$ . \*\*Significantly different from the control group  $p < 0.01$ . MG: methylglyoxal.

related factor 2 (Nrf2) phosphorylation (serine 40), thereby activating the Nrf2 pathway to attenuate inflammatory factor expression and oxidative stress.<sup>18,19</sup> Recently, we found that MG induced inflammation, hyperglycemia, and pancreas damage and that these effects are improved by Nrf2 activation *in vivo*.<sup>20</sup>

Monascin is a PPARγ and Nrf2 activator and has been reported to improve insulin resistance.<sup>21</sup> Rosiglitazone, which belongs to the thiazolidinedione family, has been used as an antidiabetic drug and activates PPARγ.<sup>21</sup> Allyl isothiocyanate (AITC) is an Nrf2 activator that reduces oxidative stress and therefore improves hyperglycemia symptoms.<sup>22,23</sup> The aim of this study is to investigate the mechanism by which Nrf2 activation prevents MG-suppressed pancreatic function through PPARγ inhibition *in vitro* and *in vivo*. To test this, monascin, AITC, and rosiglitazone were used as positive controls.

## METHODS

**Materials.** Glucose, methylglyoxal (MG) (40%), 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), allyl isothiocyanate (AITC), insulin, *N*-acetylcysteine (NAC), and rosiglitazone were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2-[*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG) was from Invitrogen (Carlsbad, CA, USA). Monascin was isolated from *Monascus*-fermented rice (red mold rice) by Dr. Y. W. Hsu and was provided from SunWay Biotechnology Company (Taipei, Taiwan). Anti-GAPDH, anti-p-PKC (T497), and anti-PKC antibodies were purchased from Epitomics Inc. (Burlingame, CA, USA). Anti-p-Nrf2 (S40) and anti-Nrf2 antibodies were purchased from Bioss Inc. (Woburn, MA, USA). Anti-PPARγ antibody was purchased from Cayman (Ann Arbor, MI, USA). Anti-p-PPARγ (S82) and anti-AGEs antibodies were purchased from Abcam (Cambridge, MA, USA). Anti-insulin antibody was purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-CEBPβ, anti-PDX-1 and anti-PPARγ for immunoprecipitation (IP) antibodies were purchased from Santa Cruz Biotechnology Inc. (Burlingame, CA, USA).

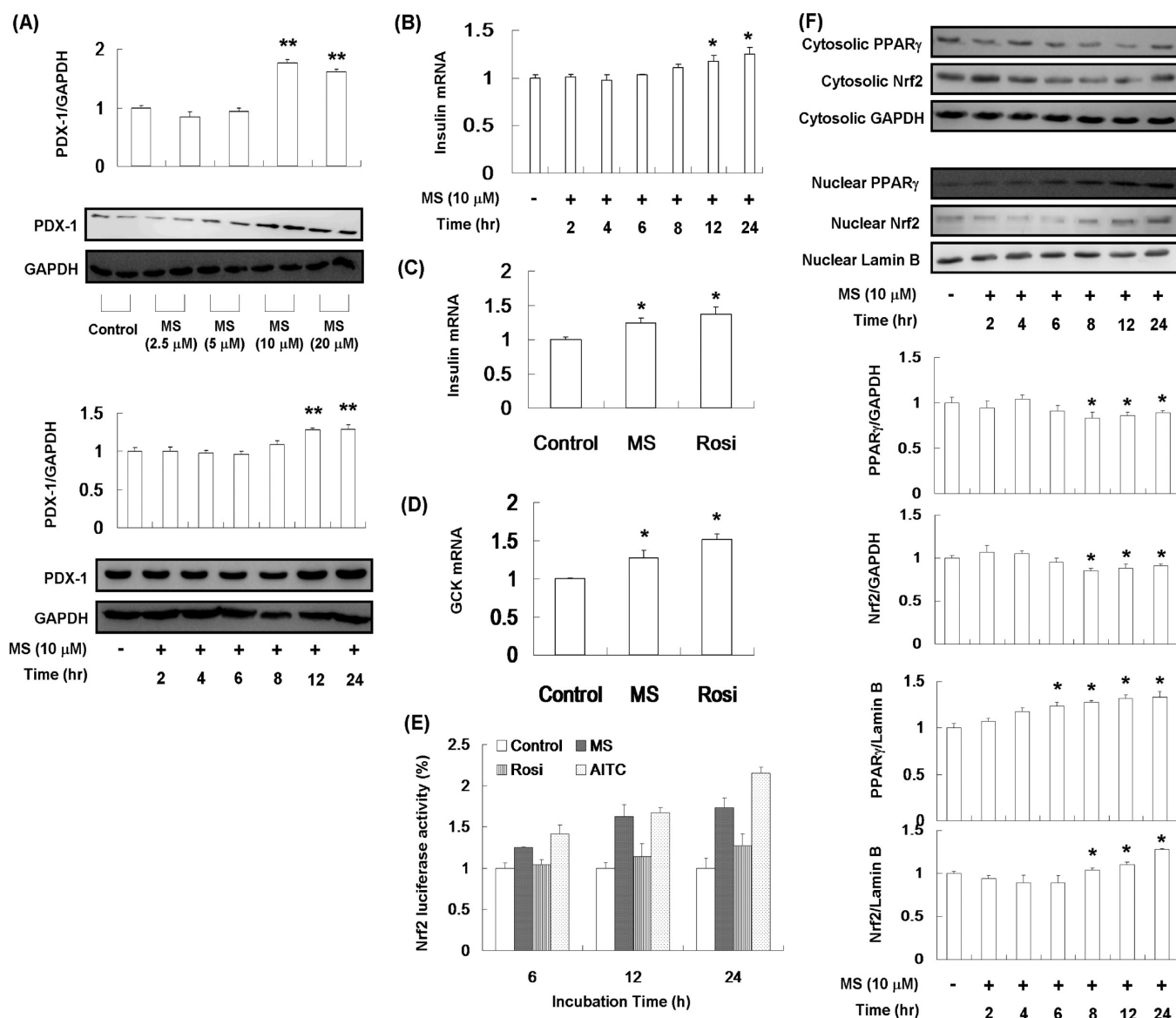
**Cell Culture.** RINm5F cells were maintained in RPMI-1640 medium supplemented with 10% FBS, streptomycin (100 mg/mL), and penicillin (100 U/mL), in a 5% CO<sub>2</sub> incubator at 37 °C.

**Reactive Oxygen Species (ROS) Measurement.** The level of oxidative stress was monitored by the measurement of ROS. Collected cells were suspended in 500 μL of PBS and mixed with 10 μM (final concentration) DCFH-DA to incubate for 20 min at 37 °C. The cells were washed three times with PBS to remove redundant DCFH-DA. The cell pellet was mixed with 500 μL of PBS, and the ROS level was assayed by flow cytometry (Becton-Dickinson, San Jose, CA, USA).<sup>24</sup>

**Nrf2 Luciferase Assay.** A DNA fragment containing three copies of the ARE4 from glutamate-cysteine ligase (GCL) gene was subcloned into a pGL3-promoter vector to construct pGL3-ARE4-Luc. Cells were transiently transfected with a DNA mixture containing 2 μg of pGL3-ARE4-Luc and 0.5 μg of control plasmid pRL-TK (Promega, Madison, WI, USA) using the lipofectamine-2000 transfection reagent in serum-free medium (Invitrogen, Carlsbad, CA, USA). Luciferase activity was conducted utilizing the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). Luciferase activity of pRL-TK was used to normalize the transfection efficiency.

**Animals.** Balb/C mice (4-week old) were obtained from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Animals were acclimatized for 1 week prior to use, divided at random into one control group and six treatment groups, and provided with food and water *ad libitum*. Animals were subjected to 12 h light/dark cycle with a maintained relative humidity of 60% and a temperature at 25 °C (Protocol complied with guidelines described in the "Animal Protection Law", amended on Jan. 17, 2001 Hua-Zong-(1)-Yi-Tzi-9000007530, Council of Agriculture, Executive Yuan, Taiwan, ROC). Animals were divided into 6 groups, including (a) control, (b) MG (60 mg/kg bw), (c) MG + monascin (10 mg/kg bw), (d) MG + rosiglitazone (10 mg/kg bw), (e) MG + AITC (10 mg/kg bw), (f) MG + NAC (200 mg/kg bw). MG, monascin, rosiglitazone, AITC, and NAC were administered by intraperitoneal injection for continuous 28 days.

**Oral Glucose Tolerance Test (OGTT).** OGTT was performed at day 28.<sup>12</sup> After overnight fasting, an OGTT was performed. Briefly, rats were anesthetized, a basal blood sample was collected, and an oral



**Figure 2.** (A) Effect of monascin on PDX-1 of RINm5F cells in dosage- and time-dependent manners. (B) Improvement of insulin mRNA level in RINm5F cells by monascin (10 μM) treatment for various times (3–24 h). PPARγ agonists (monascin and rosiglitazone) treatment for 24 h elevated (C) insulin and (D) GSK mRNA levels in RINm5F cells. (E) Effect of monascin, rosiglitazone, and allyl isothiocyanate (AITC) on Nrf2 luciferase activity in RINm5F cells. (F) Promotion of monascin on nuclear translocation of Nrf2 and PPARγ. Data are shown as mean ± SD (*n* = 3). \*Significantly different from the control group *p* < 0.05. \*\*Significantly different from the control group *p* < 0.01. MS: monascin. Rosi: rosiglitazone.

glucose load (2 g/kg bw) was given with a oral administration. Subsequently, blood was collected (0–120 min), blood glucose levels were determined using a glucose assay kit (BioAssay Systems, Hayward, CA, USA), and insulin levels were measured with a rat insulin ELISA assay kit (Merckodia, Winston Salem, NC, USA).

**Western Immunoblotting.** Protein was resolved on 10% SDS-PAGE gel and transferred to polyvinylidene fluoride membrane. The membranes were blocked with 5% nonfat dry milk solution for 1 h and incubated overnight with primary antibodies for 4 h. Subsequently, the membrane was washed three times each for 5 min in PBST and shaken in a solution of HRP-linked secondary antibody for 1 h, and the expressions of proteins were detected by enhanced chemiluminescent reagent (Millipore, Billerica, MA, USA).

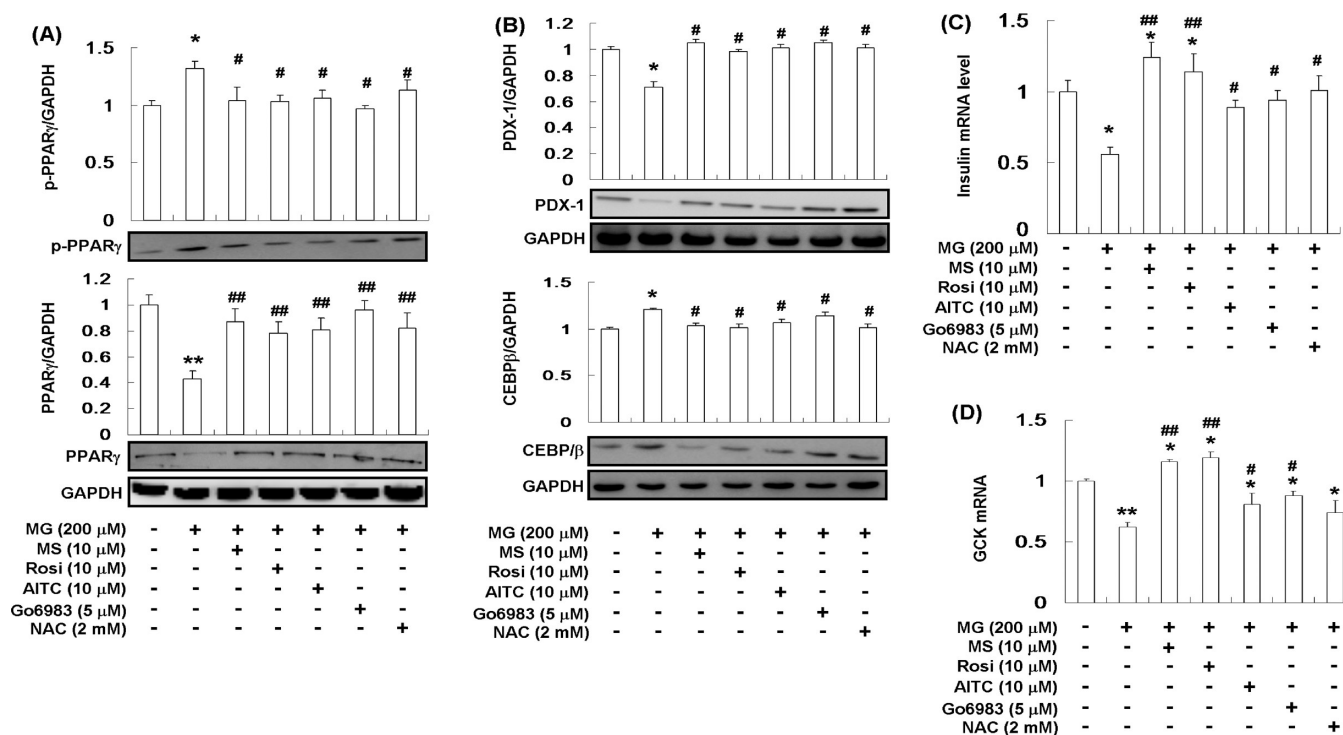
**Immunoprecipitation (IP).** Pancreatic tissue of mice was washed twice with ice-cold Hank's balanced salt solution (HBSS) and lysed in the lysis buffer on ice. After centrifugation at 12,000g, the pellet was discarded, and 1 mg of protein was reacted with 1 μg of various antibodies at 4 °C for 16 h. The immunoprecipitates were mixed with 30 μL of protein A-Sepharose beads at 4 °C for 1 h. The beads were

then washed three times with the lysis buffer and added with 5X sample buffer. The mixture was then boiled at 100 °C for 5 min. The supernatant was subjected to SDS-PAGE.

**Real-Time PCR.** cDNA from 3 μg of RNA was generated using SuperScript III First-Strand Synthesis System for RT-PCR (Life Technologies) according to the manufacturer's instructions. The reverse-transcription product was diluted in water, and a volume corresponding to 30 ng of original RNA was used for real-time PCR according to the manufacturer's protocol. Primers: rat GAPDH sense, 5'-ACT CCC ATT CCT CCA CCT TT-3', and antisense, 5'-TTA CTC CTT GGA GGC CAT GT-3'; rat GSK sense, 5'-GCT TCA CCT TCT CCT TCC C-3', and antisense, 5'-CCC ATA TAC TTC CCA CCG A-3'; rat insulin sense, 5'-TTC TTC TAC ACA CCC AAG AC-3', and antisense, 5'-CTA GTT GCA GTA GTT CTC CA-3'; rat HO-1 sense, 5'-TCT ATC GTG CTC GCA TGA AC-3', and antisense, 5'-CAG CTC CTC AAA CAG CTC AA-3'.

**Immunohistochemistry (IHC) Stain.** Frozen sections of 6 μm in thickness were cut in a cryostat and thaw mounted onto silane-coated slides. The sections were incubated with 3% H<sub>2</sub>O<sub>2</sub> for 20 min to





**Figure 3.** (A) Inhibition of PPAR $\gamma$  phosphorylation (serine 82) (6 h), (B) elevated PDX-1 and reduced CEBP $\beta$  (24 h), (C) insulin mRNA expression (24 h), and (D) GSK mRNA (24 h) expression in MG-treated pancreatic RINm5F cells by monascin, rosiglitazone, AITC, NAC, or Go6983 treatment. Data are shown as mean  $\pm$  SD ( $n = 3$ ). \*Significantly different from the control group  $p < 0.05$ . \*\*Significantly different from the control group  $p < 0.01$ . #Significantly different from the MG group  $p < 0.05$ . ##Significantly different from the MG group  $p < 0.01$ . MG: methylglyoxal. MS: monascin. Rosi: rosiglitazone. AITC: allyl isothiocyanate. NAC: N-acetylcysteine. Go6983: PKC inhibitor.

quench the endogenous peroxidase activity. After being rinsed twice with PBS, the sections were incubated with skim milk (5%) for 1 h and primary monoclonal antibody for 12 h at 4 °C. After being washed in PBS, the sections were incubated with the secondary antibody (1:200) in PBS for 1 h. After the sections were rinsed twice with PBS, immunoreactions were visualized by incubation with 3,3'-diaminobenzidine tetrahydrochloride for 10 min. The sections were counterstained with hematoxylin.

**Statistical Analysis.** Experimental results were averaged triplicate analyses. The data were recorded as mean  $\pm$  SD and analysis by statistical analysis system (SAS Inc., Cary, NC, USA). One-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests. Results were considered statistically significant at  $p < 0.05$ .

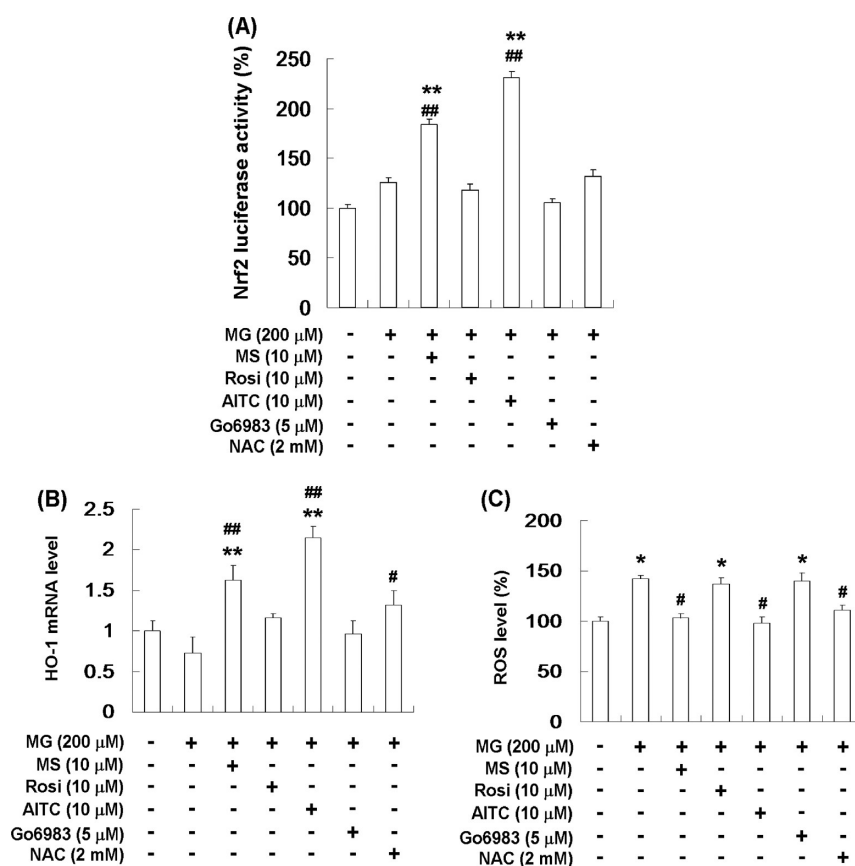
## RESULTS

### MG-Induced PPAR $\gamma$ Phosphorylation in RINm5F Cells.

To determine whether the generation of reactive oxygen species (ROS) results in pancreatic dysfunction, we used RINm5F pancreatic cells as a model. We found that treatment with 100  $\mu$ M MG did not induce oxidative stress (data not shown); however, treatment with 200  $\mu$ M MG for 3 h significantly increased ROS generation (Figure 1A). Similarly, after 3 h of MG treatment, PKC phosphorylation markedly increased (Figure 1B). PPAR $\gamma$  is reported to be phosphorylated at serine 82 by PKC, thereby resulting in its degradation or dysfunction.<sup>25,26</sup> We investigated the effects of MG on PPAR $\gamma$  phosphorylation with MG treatment. As shown in Figure 1C, p-PPAR $\gamma$  levels were enhanced in RINm5F cells treated with MG for 3–6 h, and PPAR $\gamma$  levels decreased in MG-treated RINm5F pancreatic cells treated for 6–24 h. CEBP $\beta$  is a negative regulator for PDX-1, and studies have shown that MG induction results in CEBP $\beta$  elevation in the pancreas.<sup>13,15</sup>

The results of the current showed that MG treatment for 18 h significantly increased CEBP $\beta$ , while PDX-1 decreased with 12-h treatment (Figure 1D).

**Effects of Monascin on Pancreatic Function.** As shown in Figure 2A, monascin (10  $\mu$ M and 20  $\mu$ M) treatment for 24 h markedly increased the PDX-1 levels in RINm5F pancreatic cells in a time-dependent manner. Moreover, monascin also increased insulin mRNA expression, similar to the results for rosiglitazone (PPAR $\gamma$  agonist) treatment (Figure 2B and C). To test pancreatic function, we employed glucokinase (GCK) as a glucose sensor. GCK is a regulator of glucose uptake in pancreatic cells and a diabetes mellitus candidate gene that is a critical determinant of pancreatic beta cell intermediary metabolism.<sup>27</sup> Interestingly, PPAR $\gamma$  activation has been found to upregulate GCK gene expression,<sup>28</sup> and rosiglitazone has been used to stimulate insulin synthesis and release by enhancing GCK.<sup>29</sup> In the current study, the monascin and rosiglitazone treatment enhanced GCK mRNA levels in RINm5F pancreatic cells (Figure 2D). Recently, apart from acting as a PPAR $\gamma$  agonist, monascin has been shown to promote Nrf2 activity, thereby attenuating inflammation and oxidative stress.<sup>30</sup> Therefore, we investigated whether monascin caused Nrf2 and PPAR $\gamma$  activation. The results indicated that monascin and allyl isothiocyanate (AITC, Nrf2 activator) both increased Nrf2 luciferase activity in a time-dependent manner, but rosiglitazone did not have this effect (Figure 2E). Additionally, monascin also promoted Nrf2 and PPAR $\gamma$  translocation from the cytoplasm into the nucleolus in a time-dependent manner in RINm5F pancreatic cells (Figure 2F). These findings indicate that Nrf2 and PPAR $\gamma$  were both activated by monascin treatment.



**Figure 4.** Effects of (A) Nrf2 luciferase activity, (B) HO-1 mRNA expression, and (C) ROS level in MG-treated pancreatic RINm5F cells treated by monascins, rosiglitazone, AITC, NAC, or Go6983 treatment for 24 h. Data are shown as mean  $\pm$  SD ( $n = 3$ ). \*Significantly different from the control group  $p < 0.05$ . \*\*Significantly different from the control group  $p < 0.01$ . #Significantly different from the MG group  $p < 0.05$ . ##Significantly different from the MG group  $p < 0.01$ . MG: methylglyoxal. MS: monascin. Rosi: rosiglitazone. AITC: allyl isothiocyanate. NAC: N-acetylcysteine. Go6983: PKC inhibitor.

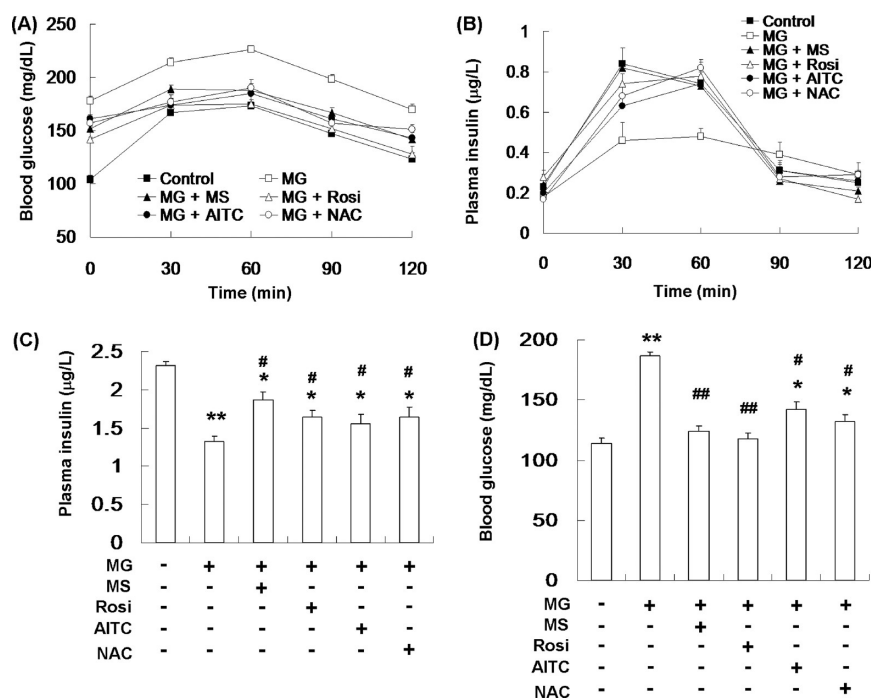
**Anti-PPAR $\gamma$  Phosphorylation and Antipancreatic Dysfunctions.** As shown in Figure 3A, RINm5F pancreatic cells that were treated with MG for 6 h exhibited significantly elevated PPAR $\gamma$  phosphorylation (serine 82) levels while nonphosphorylated PPAR $\gamma$  was reduced. These results may be due to the degradation of p-PPAR $\gamma$ , thus leading to a reduction in PPAR $\gamma$  as a result of MG treatment. However, monascin, rosiglitazone, AITC, Go6983 (PKC inhibitor), and NAC (antioxidant) markedly inhibited PPAR $\gamma$  phosphorylation. These data suggest that MG treatment increases ROS levels and activates PKC, which phosphorylates PPAR $\gamma$ . Rosiglitazone and PKC inhibitors have been reported to prevent PPAR $\gamma$  phosphorylation,<sup>31,32</sup> and studies involving Nrf2 activation or NAC treatment have shown that reducing oxidative stress also suppresses PKC activation, thereby preventing PPAR $\gamma$  phosphorylation.<sup>20,21,25</sup> Moreover, the inhibition of PPAR $\gamma$  phosphorylation by AITC was attenuated by knockdown of Nrf2 in MG-treated RINm5F cells. Similarly, Nrf2 silencing also resulted in PPAR $\gamma$  phosphorylation, suggesting that Nrf2 protects PPAR $\gamma$  from phosphorylation. Importantly, monascin markedly attenuates PPAR $\gamma$  phosphorylation in Nrf2 knockdown-MG-treated RINm5F cells (Supplemental Figure 1). These findings showed that monascin suppresses p-PPAR $\gamma$  via agonist activity.

The level of the downstream molecule of PPAR $\gamma$ , that is, PDX-1, was reduced by MG treatment for 24 h, but monascin, rosiglitazone, AITC, Go6983, and NAC clearly rescued PDX-1

levels and reduced CEBP $\beta$  levels in MG-treated RINm5F pancreatic cells (Figure 3B). Therefore, the monascin- and rosiglitazone-mediated PPAR $\gamma$  activation regulated PDX-1 and insulin expression (Figure 3C). Another downstream molecule of PPAR $\gamma$ , GSK, was also markedly rescued by monascin and rosiglitazone treatments, and these effects were greater than those seen with AITC, Go6983, and NAC treatment (Figure 3D).

**Elevation of Nrf2 by Monascin in MG-Treated RINm5F Pancreatic Cells.** To confirm if the inhibition of oxidative stress by monascin was through an Nrf2-related mechanism, we investigated Nrf2 luciferase activity in MG-treated RINm5F pancreatic cells. As shown in Figure 4A, monascin and AITC markedly increased Nrf2 luciferase activity with 24 h treatment, but rosiglitazone, Go6983, and NAC treatment did not show this effect. Moreover, a downstream target of Nrf2, heme oxygenase-1 (HO-1) mRNA, also increased on 24-h monascin and AITC treatment (Figure 4B), thereby suppressing MG-induced ROS generation (Figure 4C).

**Antidiabetic Effects of Monascin in MG-Treated Balb/C Mice.** MG injection at a dose of 60 mg/kg bw has been shown to induce pancreatic damage and dysfunction;<sup>13</sup> therefore, we used this dosage of MG to induce pancreas impairment by intraperitoneal injection for 28 d. The results indicated that monascin, rosiglitazone, AITC, and NAC significantly improved OGTT (0–120 min) in MG-injected mice (Figure 5A). This hypoglycemic effect was dependent on



**Figure 5.** Effect of monascin, rosiglitazone, AITC, or NAC treatment on (A) blood glucose and (B) plasma insulin in OGTT in MG-injected Balb/C mice at day 28. Recovery effects of monascin, rosiglitazone, AITC, or NAC treatment on (C) plasma insulin and (D) blood glucose levels in MG-injected Balb/C mice after sacrifice at day 29. Data are shown as mean  $\pm$  SEM ( $n = 6$ ). \*Significantly different from the control group  $p < 0.05$ . \*\*Significantly different from the control group  $p < 0.01$ . #Significantly different from the MG group  $p < 0.05$ . ##Significantly different from the MG group  $p < 0.01$ . MG: methylglyoxal. MS: monascin. Rosi: rosiglitazone. AITC: allyl isothiocyanate. NAC: N-acetylcysteine.

monascin, rosiglitazone, AITC, and NAC promoting insulin secretion from the pancreas into the plasma (Figure 5B). Following overnight fasting, the animals were sacrificed, and the plasma insulin was measured using an insulin ELISA kit. The results demonstrated that the plasma insulin level in MG-injected mice was lower than in the control group, but this reduction was recovered by monascin, rosiglitazone, AITC, or NAC treatment (Figure 5C). These findings indicate that MG injection induced pancreatic damage that led to hyperglycemia. Interestingly, monascin and rosiglitazone rescued MG-induced damage to levels greater than those seen with AITC and NAC treatment (Figure 5D). These results are likely due to the fact that monascin and rosiglitazone effectively activate PPAR $\gamma$ . Moreover, blood glucose was downregulated by the ITT assay in MG-injected mice, suggesting that MG did not impair the insulin sensitivity in each group (Supplemental Figure 2).

**Monascin-Induced Upregulation of Nrf2 Reduces the Generation of AGEs in the Pancreas of MG-Injected Mice.** Nrf2 has been found to suppress oxidative stress, thereby protecting pancreatic damage caused by MG treatment.<sup>17–20</sup> Interestingly, glyoxalase-1 is a downstream target of Nrf2. Glyoxalase-1 catalyzes the conversion of MG to S-D-lactoylglutathione, while glyoxalase-2 hydrolyses this glutathione thiolester to D-lactic acid and glutathione, thereby reducing the generation of AGEs.<sup>33,34</sup> We found that monascin and AITC significantly increase Nrf2 activation (serine 40 phosphorylation) by using IHC staining for p-Nrf2 in the pancreas of MG-injected mice, and this effect was found to be stronger than that obtained using rosiglitazone and NAC treatment (Figure 6). Since MG interacts with this protein, it results in the production and accumulation of AGEs in the organs. Therefore, we evaluated AGE generation by IHC staining (Figure 7) and found that monascin, AITC, and NAC

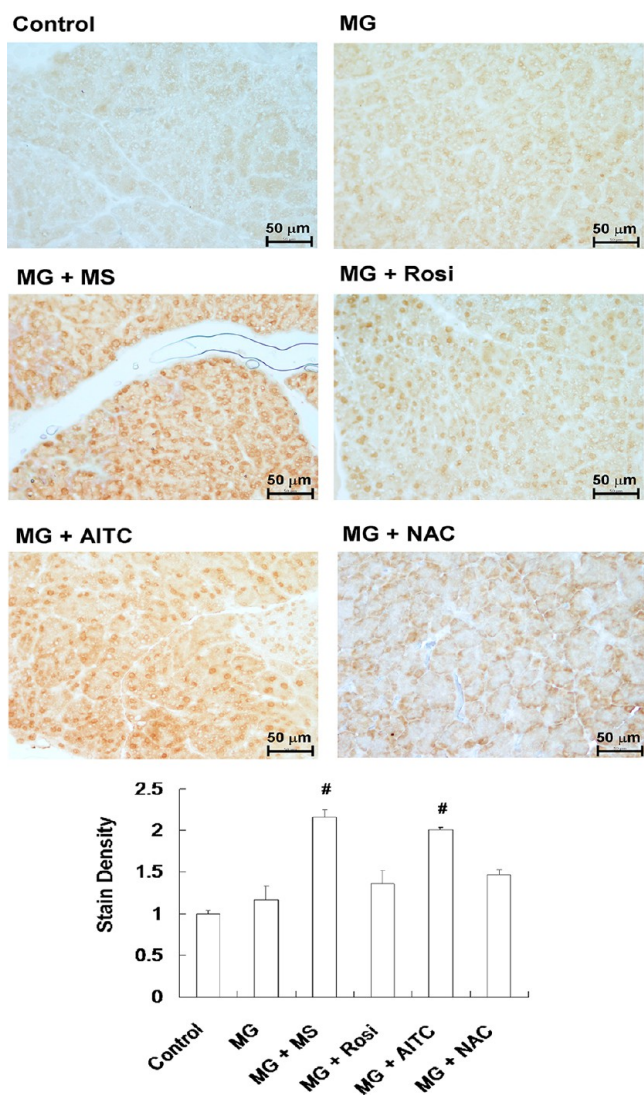
markedly reduced AGE levels in the pancreas of MG-injected mice.

**Effect of Monascin Regulation on Insulin Expression and a Potential Mechanism.** Nrf2 activation and AGE reduction were seen in the pancreas of MG-injected mice treated with monascin; hence, the product of lipid peroxidation was also suppressed by monascin, rosiglitazone, AITC, or NAC treatment (Figure 8A). Similarly, MG-induced PKC phosphorylation (oxidative stress signal) was also inhibited by monascin, rosiglitazone, AITC, or NAC treatment (Figure 8B). The enhanced CEBP $\beta$  levels in the pancreas of MG-injected mice were also markedly reduced by monascin and rosiglitazone treatment as compared to that for the AITC and NAC groups, suggesting that PPAR $\gamma$  is an antagonist of CEBP $\beta$  (Figure 8C). In contrast, GCK mRNA and PDX-1 levels in the pancreas of MG-injected mice were rescued with monascin and rosiglitazone treatments, and this effect was greater than those observed with AITC and NAC treatments (Figure 8D and E). MG-induced phosphorylation of pancreatic PPAR $\gamma$  (serine 82) was inhibited and rescued by monascin, rosiglitazone, AITC, or NAC treatment (Figure 8F) and promoted insulin expression (Figure 9).

## DISCUSSION

PPAR $\gamma$  is expressed in islet beta cells<sup>35</sup> and is important for a variety of pancreatic functions, including beta cell survival,<sup>36</sup> PDX-1 and GCK regulation,<sup>15,16,28,37</sup> and glucose-stimulated insulin secretion.<sup>38,39</sup> PPAR $\gamma$  is regulated at the protein level by several mechanisms, including phosphorylation (serine 82, serine 84, serine 110, serine 112, or serine 273) and reduced PPAR $\gamma$  activity, leading to this transcription factor being degraded via the ubiquitination pathway; however, a PPAR $\gamma$  agonist may prevent PPAR $\gamma$  phosphorylation.<sup>31,32</sup> Serine 82

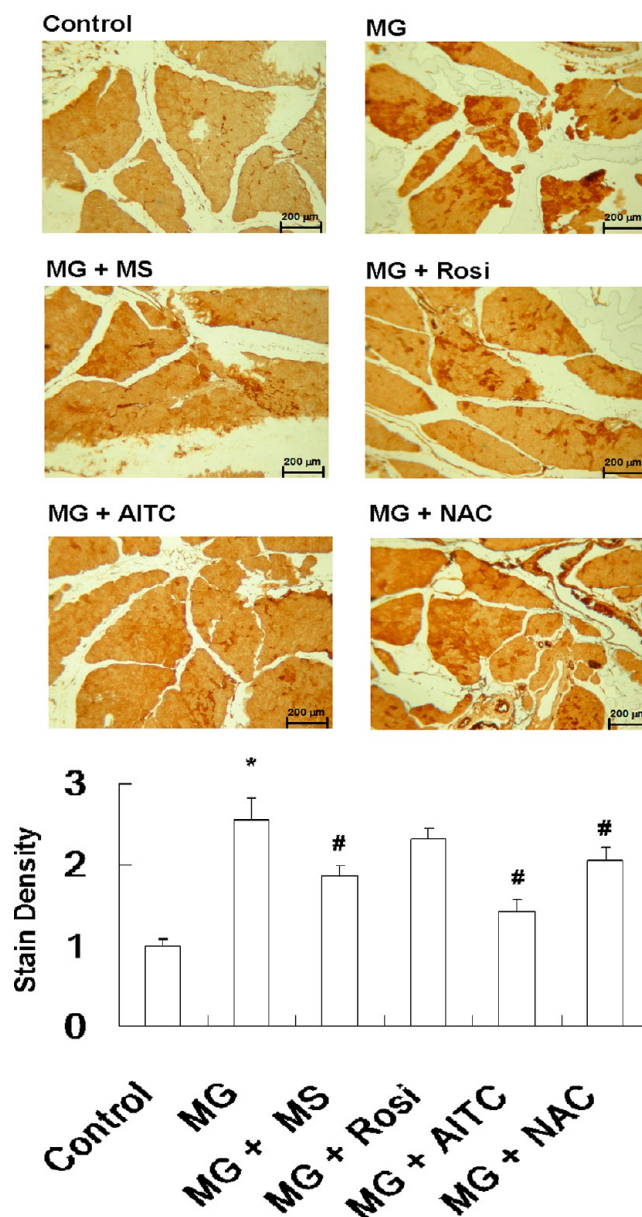




**Figure 6.** Elevation by monascin, rosiglitazone, AITC, or NAC treatment of pancreatic p-Nrf2 (serine 40) level in MG-injected Balb/C mice stained by IHC. Data are shown as mean  $\pm$  SEM ( $n = 6$ ). <sup>#</sup>Significantly different from the MG group  $p < 0.05$ . MG: methylglyoxal. MS: monascin. Rosi: rosiglitazone. AITC: allyl isothiocyanate. NAC: N-acetylcysteine.

phosphorylation of PPAR $\gamma$  by PKC has been shown to negatively regulate PPAR $\gamma$  activity.<sup>40</sup> In addition to rosiglitazone, monascin may act as a PPAR $\gamma$  agonist to inhibit PPAR $\gamma$  phosphorylation. Importantly, our results showed that Nrf2 activation attenuated MG-induced PPAR $\gamma$  phosphorylation (Figures 1, 3, 8), thereby protecting PPAR $\gamma$  degradation and increasing Nrf2 (Figures 2, 6). These findings suggested that monascin, rosiglitazone, and AITC might affect pancreatic functions.

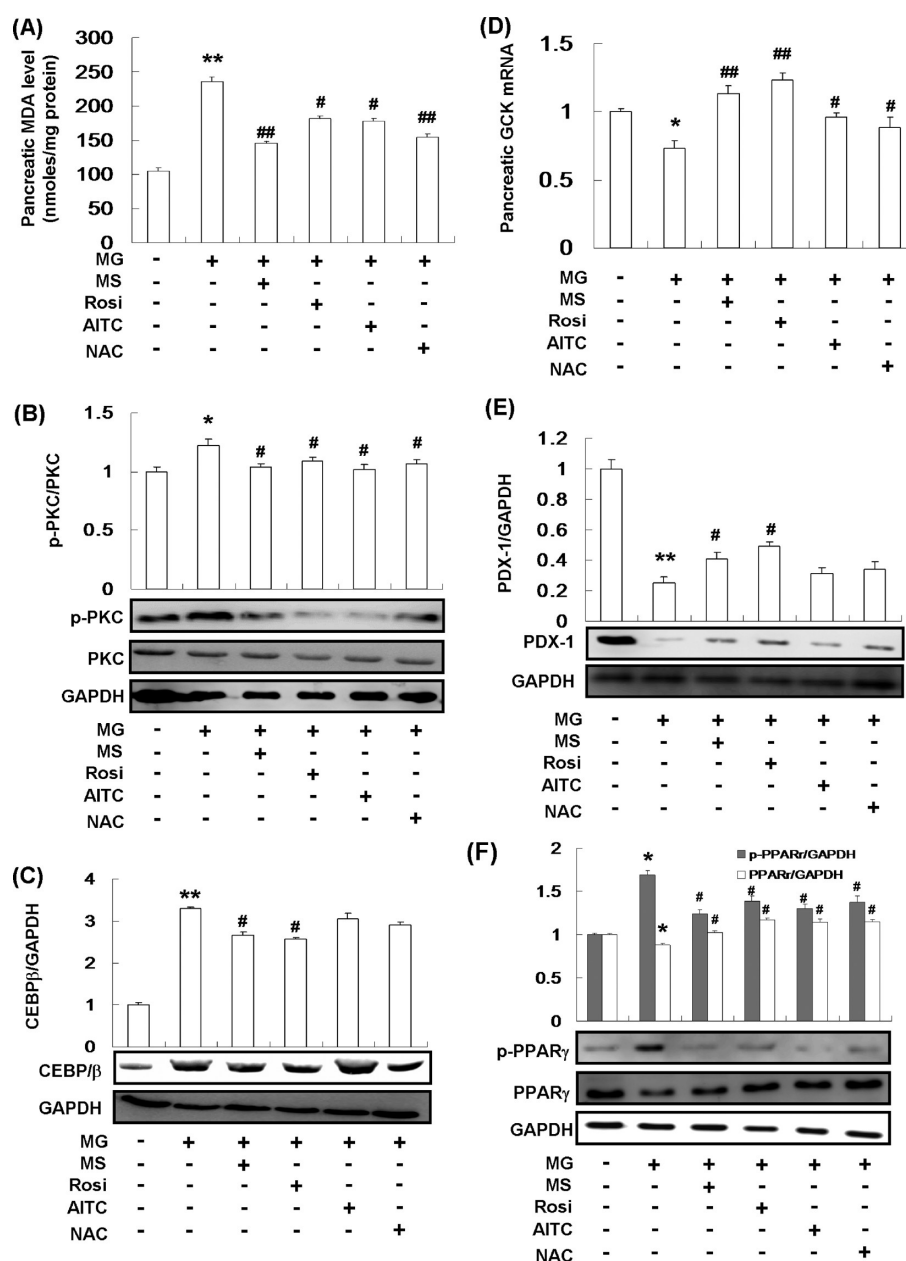
Nrf2 is a transcription factor for antioxidant enzyme<sup>17</sup> and glyoxalase<sup>41</sup> expression. A positive correlation between oxidative stress and hyperglycemia has been confirmed.<sup>42</sup> In addition, MG induction results in oxidative stress,<sup>12,13,43</sup> but this effect is attenuated by Nrf2-mediated upregulation of glyoxalase, which counters dicarbonyl glycation.<sup>44</sup> Glyoxalase converts MG to D-lactic acid, thereby reducing the generation of AGEs.<sup>41,44</sup> For pancreatic functions, Nrf2 has been found to protect against pancreatic beta cell apoptosis through the upregulation of several antioxidants.<sup>45–47</sup> In addition, Nrf2



**Figure 7.** Suppression by monascin, rosiglitazone, AITC, or NAC treatment of pancreatic AGEs accumulation in MG-injected Balb/C mice stained by IHC. Data are shown as mean  $\pm$  SEM ( $n = 6$ ). <sup>\*</sup>Significantly different from the control group  $p < 0.05$ . <sup>#</sup>Significantly different from the MG group  $p < 0.05$ . MG: methylglyoxal. MS: monascin. Rosi: rosiglitazone. AITC: allyl isothiocyanate. NAC: N-acetylcysteine.

deletion results in impaired glucose tolerance and exacerbates hyperglycemia *in vivo*,<sup>48</sup> suggesting that Nrf2 plays an important role in pancreatic function. The relationship between Nrf2 and PPAR $\gamma$  expression remains unclear. In previous studies, Nrf2 elevated PPAR $\gamma$  expression,<sup>49</sup> and Nrf2 knockout also decreased PPAR $\gamma$  expression.<sup>50</sup> Conversely, PPAR $\gamma$  knockout decreases Nrf2 expression.<sup>51</sup> Recently, we found that ankaflavin, isolated from *Monascus*-fermented rice, markedly increased Nrf2 and PPAR $\gamma$  to metabolize MG to D-lactic acid, thereby attenuating MG toxicity and the development of diabetes *in vivo*.<sup>20</sup> Currently, the relationship between MG and PPAR $\gamma$  is still poorly defined. We found that monascin administration significantly increased Nrf2 activation, inhibited





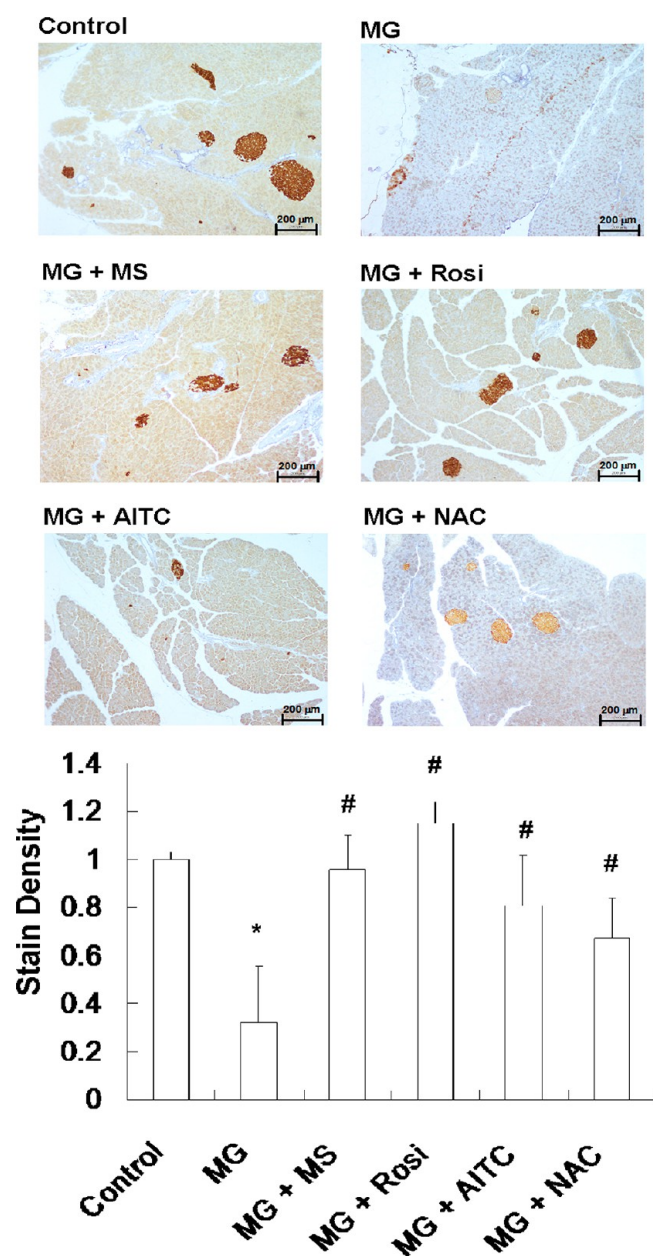
**Figure 8.** Effects of monascin, rosiglitazone, AITC, or NAC on (A) MDA level (lipid peroxidation product), (B) PKC phosphorylation, (C) CEBP $\beta$  level, (D) GCK mRNA, (E) PDX-1 level, and (F) PPAR $\gamma$  phosphorylation in pancreas of MG-injected Balb/C mice. Data are shown as mean  $\pm$  SEM ( $n = 6$ ). \*Significantly different from the control group  $p < 0.05$ . \*\*Significantly different from the control group  $p < 0.01$ . #Significantly different from the MG group  $p < 0.05$ . ##Significantly different from the MG group  $p < 0.01$ . MG: methylglyoxal. MS: monascin. Rosi: rosiglitazone. AITC: allyl isothiocyanate. NAC: N-acetylcysteine.

AGE generation, and attenuated PPAR $\gamma$  phosphorylation (Figures 6, 7).

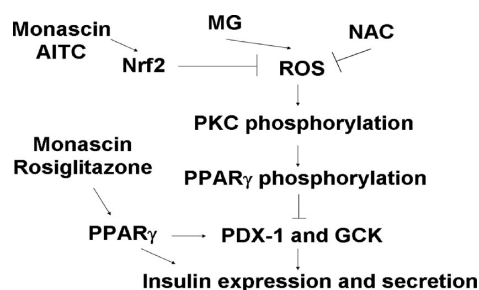
MG may react with proteins to generate AGEs in diabetic patients, and these products might result in inflammation caused by the AGE receptors.<sup>1</sup> MG can change insulin structure and modulate beta cell function.<sup>43</sup> MG also induces oxidative stress and inhibits glucose-stimulated insulin secretion.<sup>11</sup> A recent acute study suggested that AGE injection can initiate beta cell dysfunction and demonstrated that dietary restriction of AGEs significantly improves insulin sensitivity.<sup>52</sup> AGEs also decrease insulin synthesis in pancreatic beta cells by repressing PDX-1 protein expression and inhibiting glucose-stimulated insulin secretion.<sup>53,54</sup> PDX-1 plays a significant role in both pancreatic development and maintenance of beta cell

function,<sup>35</sup> but the inhibition of beta cell function caused by AGEs was improved by pioglitazone (PPAR $\gamma$  agonist) activating PPAR $\gamma$ .<sup>55</sup> 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.<sup>56</sup>

Taken together, these data strongly indicate that toxic reactive aldehydes, MG, negatively affect Nrf2 and PPAR $\gamma$  activation. Therefore, insulin, GCK, and PDX-1 expression in pancreatic-RINm5F cells is negatively regulated. However, monascin (novel PPAR $\gamma$  and Nrf2 activator), rosiglitazone (PPAR $\gamma$  agonist), and AITC (Nrf2 activator) clearly prevent PPAR $\gamma$  phosphorylation and improve pancreatic function (Figure 10).



**Figure 9.** Effects of monascin, rosiglitazone, AITC, or NAC treatment on pancreatic insulin level of MG-injected Balb/C mice stained by IHC. Data are shown as mean  $\pm$  SEM ( $n = 6$ ). \*Significantly different from the control group  $p < 0.05$ . #Significantly different from the MG group  $p < 0.05$ . MG: methylglyoxal. MS: monascin. Rosi: rosiglitazone. AITC: allyl isothiocyanate. NAC: N-acetylcysteine.



**Figure 10.** Potential protective mechanism of pancreatic function by PPAR $\gamma$  and Nrf2 activators.

Monascin is reported to show anti-inflammatory activities.<sup>57</sup> We previously demonstrated monascin can suppress E-selectin expression caused by TNF- $\alpha$  treatment in human umbilical vein endothelial cells (HUVECs).<sup>58</sup> Monascin also exerts antioxidant activity that attenuates oxidative stress and protects against pancreatic damage in streptozotocin-induced rats.<sup>59</sup> In conclusion, the development of new strategies to improve insulin secretion in beta cell function in type 2 diabetic patients suffering from decreased circulating levels of MG or AGEs due to chronic hyperglycaemia.

## ■ ASSOCIATED CONTENT

### § Supporting Information

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### Notes

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## ■ REFERENCES

- (1) Ankrah, N. A.; Appiah-Opong, R. Toxicity of low levels of methylglyoxal: depletion of blood glutathione and adverse effect on glucose tolerance in mice. *Toxicol. Lett.* **1999**, *109*, 61–67.
- (2) Wang, J.; Chang, T. Methylglyoxal content in drinking coffee as a cytotoxic factor. *J. Food Sci.* **2010**, *75*, H167–H171.
- (3) Adams, C. J.; Manley-Harris, M.; Molan, P. C. The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr. Res.* **2009**, *344*, 1050–1053.
- (4) Arrbias-Lorenzo, G.; Morales, F. J. Analysis, distribution, and dietary exposure of glyoxal and methylglyoxal in cookies and their relationship with other heat-induced contaminants. *J. Agric. Food Chem.* **2010**, *58*, 2966–2972.
- (5) De Revel, G.; Bertrand, A. A method for the detection of carbonyl compounds in wine: glyoxal and methylglyoxal. *J. Sci. Food Agric.* **1993**, *61*, 267–272.
- (6) Barros, A.; Rodrigues, J. A.; Almeida, P. J.; Oliva-Teles, M. T. Determination of glyoxal, methylglyoxal and diacetyl in selected beer and wine by HPLC with UV spectrophotometric detection after derivatization with o-phenylenediamine. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22*, 2061–2069.
- (7) Khuhawar, M. Y.; Kandhro, A. J.; Khand, F. D. Liquid chromatographic determination of glyoxal and methylglyoxal from serum of diabetic patients using meso-stilbenediamine as derivatizing agent. *Anal. Lett.* **2006**, *39*, 2205–2215.
- (8) Wu, C. H.; Huang, S. M.; Yen, G. C. Silymarin: A novel antioxidant with antiglycation and antiinflammatory properties *in vitro* and *in vivo*. *Antioxid. Redox Signaling* **2011**, *1*, 353–366.
- (9) Jia, X.; Olson, D. J.; Ross, A. R.; Wu, L. Structural and functional changes in human insulin induced by methylglyoxal. *FASEB J.* **2006**, *20*, 1555–1557.
- (10) Riboulet-Chavey, A.; Pierron, A.; Durand, L.; Murdaca, J.; Giudicelli, J.; Van Obberghen, E. Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. *Diabetes* **2006**, *55*, 1289–1299.
- (11) Fiory, F.; Lombardi, A.; Miele, C.; Giudicelli, J.; Beguinot, F.; Van Obberghen, E. Methylglyoxal impairs insulin signalling and insulin

action on glucose-induced insulin secretion in the pancreatic beta cell line INS-1E. *Diabetologia* **2011**, *54*, 2941–2952.

(12) Dhar, A.; Desai, K. M.; Wu, L. Alagebrium attenuates acute methylglyoxal-induced glucose intolerance in Sprague-Dawley rats. *Br. J. Pharmacol.* **2010**, *159*, 166–175.

(13) Dhar, A.; Dhar, I.; Jiang, B.; Desai, K. M.; Wu, L. Chronic methylglyoxal infusion by minipump causes pancreatic  $\beta$ -cell dysfunction and induces type 2 diabetes in Sprague-Dawley rats. *Diabetes* **2011**, *60*, 899–908.

(14) Irwin, N.; McKinney, J. M.; Bailey, C. J.; McClenaghan, N. H.; Flatt, P. R. Acute and long-term effects of peroxisome proliferator-activated receptor- $\gamma$  activation on the function and insulin secretory responsiveness of clonal beta-cells. *Horm. Metab. Res.* **2011**, *43*, 244–249.

(15) Moibi, J. A.; Gupta, D.; Jetton, T. L.; Peshavaria, M.; Desai, R.; Leahy, J. L. Peroxisome proliferator-activated receptor- $\gamma$  regulates expression of PDX-1 and NKX6.1 in INS-1 cells. *Diabetes* **2007**, *56*, 88–95.

(16) Gupta, D.; Jetton, T. L.; Mortensen, R. M.; Duan, S. Z.; Peshavaria, M.; Leahy, J. L. In vivo and in vitro studies of a functional peroxisome proliferator-activated receptor- $\gamma$  response element in the mouse pdx-1 promoter. *J. Biol. Chem.* **2008**, *283*, 32462–32470.

(17) Keum, Y. S.; Owuor, E. D.; Kim, B. R.; Hu, R.; Kong, A. N. Involvement of Nrf2 and JNK1 in the activation of antioxidant responsive element (ARE) by chemopreventive agent phenethyl isothiocyanate (PEITC). *Pharm. Res.* **2003**, *20*, 1351–1356.

(18) Surh, Y. J. NF $\kappa$ B and Nrf2 as potential chemopreventive targets of some anti-inflammatory and antioxidative phytonutrients with anti-inflammatory and antioxidative activities. *Asia Pac. J. Clin. Nutr.* **2008**, *17*, 269–272.

(19) Weng, C. J.; Chen, M. J.; Yeh, C. T.; Yen, G. C. Hepatoprotection of quercetin against oxidative stress by induction of metallothionein expression through activating MAPK and PI3K pathways and enhancing Nrf2 DNA-binding activity. *New Biotechnol.* **2011**, *28*, 767–777.

(20) Lee, B. H.; Hsu, W. H.; Chang, Y. Y.; Kuo, H. F.; Hsu, Y. W.; Pan, T. M. Ankaflavin: a natural novel PPAR $\gamma$  agonist upregulates Nrf2 to attenuate methylglyoxal-induced diabetes in vivo. *Free Radical Biol. Med.* **2012**, *53*, 2008–2016.

(21) Lee, B. H.; Hsu, W. H.; Liao, T. H.; Pan, T. M. The *Monascus* metabolite monascin against TNF- $\alpha$ -induced insulin resistance via suppressing PPAR- $\gamma$  phosphorylation in C2C12 myotubes. *Food Chem. Toxicol.* **2011**, *49*, 2609–2617.

(22) Wagner, A. E.; Boesch-Saadatmandi, C.; Dose, J.; Schultheiss, G.; Rimbach, G. Anti-inflammatory potential of allyl-isothiocyanate: role of Nrf2, NF-( $\kappa$ )B and microRNA-155. *J. Cell. Mol. Med.* **2012**, *16*, 836–843.

(23) Okulicz, M. Multidirectional time-dependent effect of sinigrin and allyl isothiocyanate on metabolic parameters in rats. *Plant Foods Hum. Nutr.* **2010**, *65*, 217–224.

(24) Lee, B. H.; Huang, Y. Y.; Wu, S. C. Hepatoprotective activity of fresh *Polygonum multiflorum* against Hep G2 hepatocarcinoma cell proliferation. *J. Food Drug Anal.* **2011**, *19*, 26–32.

(25) Lee, C. C.; Hsu, W. H.; Shen, S. R.; Cheng, Y. H.; Wu, S. C. *Fagopyrum tataricum* (buckwheat) improved high-glucose-induced insulin resistance in mouse hepatocytes and diabetes in fructose-rich diet-induced mice. *Exp. Diabetes Res.* **2012**, DOI: 10.1155/2012/375673.

(26) Hirai, S.; Takahashi, N.; Tsuyosh Ackerman, W. E.; Zhang, X. L.; Rovin, B. H.; Kniss, D. A. Modulation of cytokine-induced cyclooxygenase 2 expression by PPAR $\gamma$  ligands through NF $\kappa$ B signal disruption in human WISH and amnion cells. *Biol. Reprod.* **2005**, *73*, S27–S35.

(27) Matschinsky, F.; Liang, Y.; Kesavan, P.; Wang, L.; Froguel, P.; Velho, G.; Cohen, D.; Permutt, M. A.; Tanizawa, Y.; Jetton, T. L.; Niswender, K.; Magnuson, M. A. Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. *J. Clin. Invest.* **1993**, *92*, 2092–2098.

(28) Kim, H. I.; Cha, J. Y.; Kim, S. Y.; Kim, J. W.; Roh, K. J.; Seong, J. K.; Lee, N. T.; Choi, K. Y.; Kim, K. S.; Ahn, Y. H. Peroxisomal

proliferator-activated receptor- $\gamma$  upregulates glucokinase gene expression in beta-cells. *Diabetes* **2002**, *51*, 676–685.

(29) Kim, H. S.; Noh, J. H.; Hong, S. H.; Hwang, Y. C.; Yang, T. Y.; Lee, M. S.; Kim, K. W.; Lee, M. K. Rosiglitazone stimulates the release and synthesis of insulin by enhancing GLUT-2, glucokinase and BETA2/NeuroD expression. *Biochem. Biophys. Res. Commun.* **2008**, *367*, 623–629.

(30) Lee, B. H.; Hsu, W. H.; Huang, T.; Chang, Y. Y.; Hsu, Y. W.; Pan, T. M. Effects of monascin on anti-inflammation mediated by Nrf2 activation in advanced glycation end product-treated THP-1 monocytes and methylglyoxal-treated Wistar rats. *J. Agric. Food Chem.* **2013**, *61*, 1288–1298.

(31) Choi, J. H.; Banks, A. S.; Estall, J. L.; Kajimura, S.; Bostrom, P.; Laznik, D.; Ruas, J. L.; Chalmers, M. J.; Kamenicka, T. M.; Bluhner, M.; Griffin, P. R.; Spiegelman, B. M. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPAR $\gamma$  by CDK5. *Nat. Med.* **2010**, *466*, 451–457.

(32) Burns, K. A.; Vanden Heuvel, J. P. Modulation of PPAR activity via phosphorylation. *Biochim. Biophys. Acta* **2007**, *1771*, 952–960.

(33) Ewaschuk, J. B.; Naylor, J. M.; Zello, G. A. D-Lactate in human and ruminant metabolism. *J. Nutr.* **2005**, *135*, 1619–1625.

(34) Inoue, Y.; Maeta, K.; Nomura, W. Glyoxalase system in yeasts: Structure, function, and physiology. *Semin. Cell Dev. Biol.* **2011**, *22*, 278–284.

(35) Dubois, M.; Pattou, F.; Kerr-Conte, J.; Gmyr, V.; Vandewalle, B.; Desreumaux, P.; Auwerx, J.; Schoonjans, K.; Lefebvre, J. Expression of peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ) in normal human pancreatic islet cells. *Diabetologia* **2000**, *43*, 1165–1169.

(36) Lin, C. Y.; Gurlo, T.; Haataja, L. O.; Hsueh, W. A.; Butler, P. C. Activation of peroxisome proliferator-activated receptor- $\gamma$  by rosiglitazone protects human islet cells against human islet amyloid polypeptide toxicity by a phosphatidylinositol 3'-kinase-dependent pathway. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 6678–6686.

(37) Xie, W.; Zhao, Y.; Gu, D.; Du, L.; Cai, G.; Zhang, Y. Scorpion in combination with gypsum: novel antidiabetic activities in streptozotocin-induced diabetic mice by up-regulating pancreatic PPAR $\gamma$  and PDX-1 expression. *J. Evidence-Based Complementary Altern. Med.* **2011**, DOI: 10.1093/ecam/nej031.

(38) Yang, C.; Chang, T. J.; Chang, J. C.; Liu, M. W.; Tai, T. Y.; Hsu, H. W.; Chuang, L. M. Rosiglitazone (BRL 49653) enhances insulin secretory response via phosphatidylinositol-3-kinase pathway. *Diabetes* **2001**, *50*, 2598–2602.

(39) Kim, H. I.; Kim, J. W.; Kim, S. H.; Cha, J. Y.; Kim, K. S.; Ahn, Y. H. Identification and functional characterization of the peroxisomal proliferator response element in rat GLUT2 promoter. *Diabetes* **2000**, *49*, 1517–1524.

(40) Diradourian, C.; Girard, J.; Pegorier, J. P. Phosphorylation of PPARs: from molecular characterization to physiological relevance. *Biochimie* **2005**, *87*, 33–38.

(41) Vander-Jagt, D.; Hunsaker, L. Methylglyoxal metabolism and diabetic complications: roles of aldose reductase, glyxalase-I, betaine aldehyde dehydrogenase and oxoaldehyde dehydrogenase. *Chem. Biol. Interact.* **2003**, *143/144*, 341–351.

(42) Guha, M.; Bai, W.; Nadler, J. L.; Natarajan, R. Molecular mechanisms of tumor necrosis factor alpha gene expression in monocytic cells via hyperglycemia-induced oxidant stress-dependent and -independent pathways. *J. Biol. Chem.* **2000**, *275*, 17728–17739.

(43) Jia, X.; Olson, D. J.; Ross, A. R.; Wu, L. Structural and functional changes in human insulin induced by methylglyoxal. *FASEB J.* **2006**, *20*, 1555–1557.

(44) Xue, M.; Rabbani, N.; Momiji, H.; Imbasi, P.; Anwar-Maqsud, M.; Kitteringham, N.; Park-Kevin, B.; Souma, T.; Moriguchi, T.; Yamamoto, M.; Thornalley, P. J. Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. *Biochem. J.* **2012**, *443*, 213–222.

(45) Lee, M. W.; Chun, S. W.; Kim, S. H.; Lee, Y.; Kang, E. S.; Cha, B. S.; Lee, H. C. Lithospermic acid B protects beta-cells from cytokine-induced apoptosis by alleviating apoptotic pathways and activating



anti-apoptotic pathways of Nrf2-Ho-1 and Sirt1. *Toxicol. Appl. Pharmacol.* **2011**, 252, 47–54.

(46) Song, M. Y.; Kim, E. K.; Moon, W. S.; Park, J. W.; Km, H. J.; So, H. S.; Park, R.; Kwon, K. B.; Park, B. H. Sulforaphane protects against cytokine- and streptozotocin-induced beta-cell damage by suppressing the NF-kappa B pathway. *Toxicol. Appl. Pharmacol.* **2009**, 235, 57–67.

(47) Pugazhenth, S.; Akhoy, L.; Selvaraj, G.; Wang, M.; Alam, J. Regulation of heme oxygenase-1 expression by demethoxy curcuminoids through Nrf2 by a PI3-kinase/Akt-mediated pathway in mouse beta-cells. *Am. J. Physiol. Endocrinol. Metab.* **2007**, 293, E645–E655.

(48) Aleksunes, L. M.; Reisman, S. A.; Yeager, R. L.; Goedken, M. J.; Klaassen, C. D. Nuclear factor erythroid 2-related factor 2 deletion impairs glucose tolerance and exacerbates hyperglycemia in type 1 diabetic mice. *J. Pharmacol. Exp. Ther.* **2010**, 333, 140–151.

(49) Cho, H. Y.; Gladwell, W.; Wang, X.; Chorley, B.; Bell, D.; Reddy, S. P.; Kleeberger, S. R. Nrf2-regulated PPARgamma expression is critical to protection against acute lung injury in mice. *Am. J. Respir. Crit. Care Med.* **2010**, 182, 170–182.

(50) Cho, H. Y.; Reddy, S. P.; Debiase, A.; Yamamoto, M.; Kleeberger, S. R. Gene expression profiling of Nrf2-mediated protection against oxidative injury. *Free Radical Biol. Med.* **2005**, 38, 325–343.

(51) Reddy, R. C.; Standiford, T. J. Nrf2 and PPARgamma: PPARgamma against oxidant-induced lung injury. *Am. J. Respir. Crit. Care Med.* **2010**, 182, 134–135.

(52) Coughlan, M. T.; Yap, F. Y. T.; Yong, D. C. K.; Andrikopoulos, S.; Gasser, A.; Thallas-Bonke, V.; Webster, D. E.; Miyazaki, J. I.; Kay, T. W.; Slattery, R. M.; Kaye, D. M.; Drew, B. G.; Kingwell, B. A.; Fourlanos, S.; Groop, P. H.; Harrison, L. C.; Knip, M.; Forbes, J. M. Advanced glycation end products are direct modulators of beta-cell function. *Diabetes* **2011**, 60, 2523–2532.

(53) Shu, T.; Zhu, Y.; Wang, H.; Lin, Y.; Ma, Z.; Han, X. AGEs decrease insulin synthesis in pancreatic beta-cell by repressing PDX-1 protein expression at the post-translational level. *PLoS One* **2011**, 6, Article ID e18782.

(54) Zhao, Z.; Zhao, C.; Zhang, X. H.; Zheng, F.; Cai, W.; Vlassara, H.; Ma, Z. A. Advanced glycation end products inhibit glucose-stimulated insulin secretion through nitric oxide-dependent inhibition of cytochrome c oxidase and adenosine triphosphate synthesis. *Endocrinology* **2009**, 150, 2569–2576.

(55) Puddu, A.; Sanguinetti, R.; Durante, A.; Viviani, G. L. Pioglitazone attenuates the detrimental effects of advanced glycation end-products in the pancreatic beta cell line HIT-T15. *Regul. Pept.* **2012**, 177, 79–84.

(56) Kaneto, H.; Miyatsuka, T.; Kawamori, D. PDX-1 and MafA play a crucial role in pancreatic beta-cell differentiation and maintenance of mature beta-cell function. *Endocr. J.* **2008**, 55, 235–252.

(57) Hsu, W. H.; Lee, B. H.; Liao, T. H.; Hsu, Y. W.; Pan, T. M. Monascus-fermented metabolite monascin suppresses inflammation via PPARgamma regulation and JNK inactivation in THP-1 monocytes. *Food Chem. Toxicol.* **2012**, 50, 1178–1186.

(58) Hsu, W. H.; Lee, B. H.; Lu, I. J.; Pan, T. M. Ankaflavin and monascin regulate endothelial adhesion molecules and endothelial NO synthase (eNOS) expression induced by tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in human umbilical vein endothelial cells (HUVECs). *J. Agric. Food Chem.* **2012**, 60, 1666–1672.

(59) Shi, Y. C.; Liao, V. H. C.; Pan, T. M. Monascin from red mold dioscorea as a novel antidiabetic and antioxidative stress agent in rats and *Caenorhabditis elegans*. *Free Radical Biol. Med.* **2012**, 52, 109–117.