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A novel natural Nrf2 activator with PPARγ-agonist (monascin) attenuates the toxicity of methylglyoxal and hyperglycemia

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ABSTRACT

Methylglyoxal (MG) is a toxic-glucose metabolite and a major precursor of advanced glycation endproducts (AGEs). MG has been reported to result in inflammation by activating receptor for AGEs (RAGE). We recently found that *Monascus*-fermented metabolite monascin acts as a novel natural peroxisome proliferator-activated receptor-γ (PPARγ) agonist that improves insulin sensitivity. We investigated the metabolic, biochemical, and molecular abnormalities characteristic of type 2 diabetes in MG-treated Wistar rats treated with oral administration of monascin or rosiglitazone. Monascin (a novel PPARγ agonist) activated nuclear factor-erythroid 2-related factor 2 (Nrf2) and down-regulated hyperinsulinmia in oral glucose tolerance test (OGTT). Monascin was able to elevate glyoxalase-1 expression via activation of hepatic Nrf2, hence, resulting in MG metabolism to D-lactic acid and protected from AGEs production in MG-treated rats. Rosiglitazone did not activate Nrf2 nor glyoxalase expression to lower serum and hepatic AGEs levels. Monascin acts as a novel natural Nrf2 activator with PPARγ-agonist activity were confirmed by Nrf2 and PPARγ reporter assays in Hep G2 cells. These findings suggest that monascin acts as an anti-diabetic and anti-oxidative stress agent to a greater degree than rosiglitazone and thus may have therapeutic potential for the prevention of diabetes.

Introduction

In diabetic patients, there is a positive correlation between high methylglyoxal (MG) concentration in the blood and hyperglycemia (Dhar et al., 2011). Hyperglycemia facilitates the formation of advanced glycation end-products (AGEs) in type-2 diabetes. Recent studies have shown that MG administration results in inflammation (Wu et al., 2011), insulin resistance (Dhar et al., 2010), oxidative damage (Cohen, 2003), cardiovascular disease (Rabbani et al., 2011), suppression of insulin production, and secretions that are caused by damage to the pancreas (Dhar et al., 2011). MG is a highly reactive dicarbonyl compound (a metabolic product of glucose) (Thornalley, 1996) and several lines of clinical evidence suggest that MG reacts with protein, resulting in the irreversible formation of AGEs in patients with hyperglycemia and diabetes (Negre-Salvayre et al., 2009; Reddy and Standiford, 2010; Wu et al., 2011).

MG also shows cytotoxic effect (Koizumi et al., 2011) and results in hepatic damage (Dong et al., 2010). In vitro studies suggest that MG impairs insulin-mediated glucose uptake in adipocytes (Jia et al., 2006) and muscle cells (Riboulet-Chavey et al., 2006). Moreover, MG suppresses insulin secretion and production in INS-1E pancreatic islet β-cells (Dhar et al., 2011). MG also impairs pancreatic function, elevates fasting plasma glucose, and reduces insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation in adipocytes (Dhar et al., 2010, 2011). We have found that nuclear factor-erythroid 2-related factor 2 (Nrf2) activation attenuates MG toxic effect by metabolizing MG to D-lactic acid (Lee et al., 2012). Recently, Nrf2 has been activated by resveratrol to attenuate Hep G2 liver cells injury induced by MG (Cheng et al., 2012).

Nrf2 is essential for heme oxygenase-1 (HO-1) and glyoxalase-1 expressions (Keum et al., 2003; Kobayashi and Yamamoto, 2005; Vander-Jagt and Hunsaker, 2003). Glyoxalase-1 catalyzes the conversion of MG to D-lactic acid, thereby limiting the generation of AGEs (Vander-Jagt and Hunsaker, 2003). Some antioxidants have been found to attenuate oxidative damage by activating Nrf2 (Weng et al., 2011; Yeh and Yen, 2006).

Monascin is obtained from *Monascus*-fermented products, which is an antioxidant to attenuate streptozotocin-induced pancreatic damage (Shi et al., 2012). Monascin is reported to improve insulin sensitivity in tumor necrosis factor-α-induced C2C12 cells by upregulating peroxisome proliferator-activated receptor-γ (PPARγ) (Lee et al., 2011). In addition to improving insulin sensitivity, PPARγ agonists are reported to bind to PPAR response element (PPRE) binding sites in both glucose transporter 2 (GLUT2) and glucokinase (GCK) promoter regions, thereby up-regulating the expression of these genes (Higuchi et al., 2011; Irwin et al., 2011; Kim and Ahn, 1998). The aim of this study is to investigate the mechanism by which monascin regulates PPARγ and

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eliminates inflammation and diabetes that was induced by MG in vitro and in vivo. Our data indicated that monascin may elevate Nrf2 activity to attenuate MG-resulted in hyperglycemia.

Methods and materials

PPARγ agonist assay. The PPARγ agonist activity of monascin was confirmed by LanthaScreen™ TR-FRET PPARγ coactivator assay kit (Invitrogen, Carlsbad, CA, USA). The assay was carried out according to the normative manual.

Nrf2 and PPARγ luciferase assay. Hep G2 cell was obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). Hep G2 cells were maintained in Minimum Essential Medium supplemented with 10% FBS, streptomycin (100 mg/mL), and penicillin (100 U/mL), in a 5% CO_2 incubator at 37 °C. PPARγ reporter assay was carried out by the kit (SA Bioscience; Frederick, MD, USA) in Hep G2 cells. In addition, a DNA fragment containing three copies of the ARE4 from glutamate-cysteine ligase (GCL) gene was used; this DNA fragment was subcloned into a pGL3-promoter vector to construct pGL3-ARE4-Luc. Hep G2 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). After treatment with samples for various times, 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. Male Wistar rats (4 weeks old) were obtained from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Animals were acclimatized for 1 week prior to use, they were 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 7 groups, including (a) control (saline administration; rats = 6), (b) MG administered to Wistar rats (600 mg/kg bw by oral administration; rats = 6), (c) MG + monascin (10 mg/kg bw by oral administration; rats = 6), (d) MG + monascin + GW9662 (10 mg/kg bw by intraperitoneal injection; rats = 6), (e) MG + rosiglitazone (10 mg/kg bw by oral administration; rats = 6), (f) MG + rosiglitazone + GW9662 (rats = 6), and (g) MG + monascin + PPARγ siRNA (Santa Cruz, Burlingame, CA, USA) (rats = 3). For hepatic PPARγ silencing, total PPARγ siRNA (800 μg/rat) was administered by tail vein-injection using the hydrodynamic technique reagent (Mirus, Madison, WI, USA) at week-1 and week-3. MG, monascin, and rosiglitazone were administered for 28 days, and GW9662 was administered for 3 times/week.

Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). OGTT and ITT were performed at day-24 and day-26 by the method of Dhar et al. (2010). OGTT and ITT were carried out according to Supplemental Methods and Materials.

The AGEs, D-lactic acid, MG, TNF-α, and IL-1β measurements. The AGEs and MG ELISA kits (Biolabs, Inc., Beverly, MA, USA), TNF-α ELISA kit (eBioscience, San Diego, CA, USA), and IL-1β ELISA kit (Peprotech, Rocky Hill, NJ, USA), D-lactic acid kit (MyBiosource, San Diego, CA, USA) were purchased to measure the levels.

Real-time PCR. Real-time PCR method is provided in Supplemental Methods and Materials.

Western immunoblotting. Western blot method is provided in Supplemental Methods and Materials.

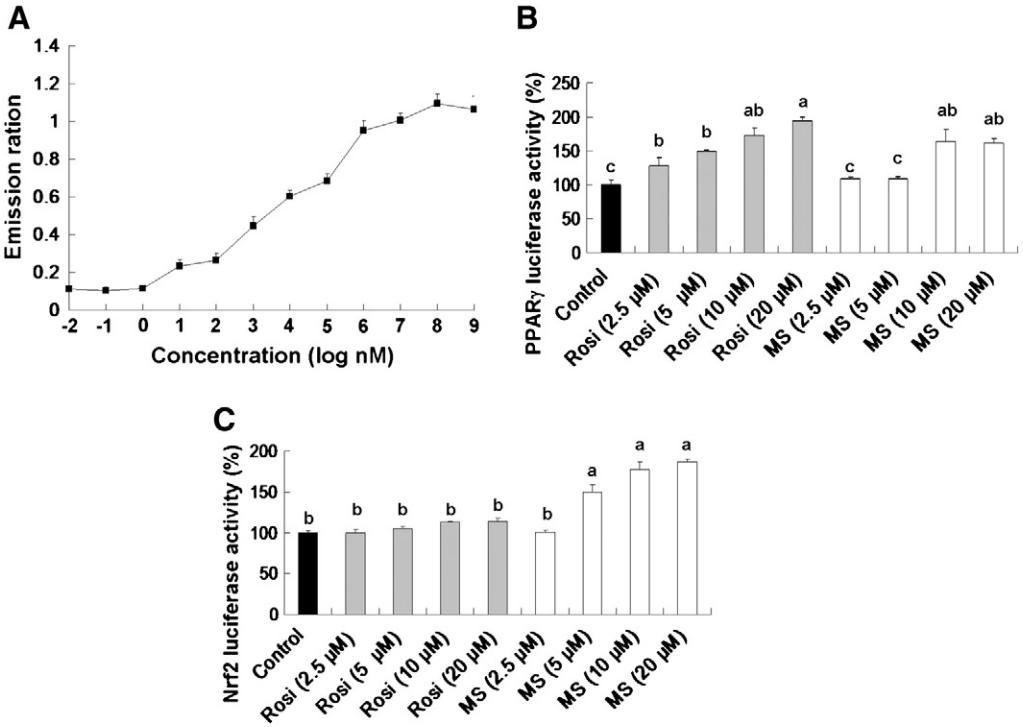

Fig. 1. (A) Activity of monascin acting as a PPARγ agonist. (B) PPARγ activation in Hep G2 cells by monascin (2.5–20 μM) and rosiglitazone (2.5–20 μM) treatment for 24 h. (C) Nrf2 activation in Hep G2 cells by monascin (2.5–20 μM) and rosiglitazone (2.5–20 μM) treatment for 24 h. Data were shown as mean ± SEM. [a,b,c] Values with consecutive superscripted letters are significantly different from each other ($p < 0.05$). All experiments were performed in triplicate, and the values expressed represent the average of three separate experiments.