

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/280868379>

# Pathophysiological Insights of Methylglyoxal Induced Type-2 Diabetes

ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · AUGUST 2015

Impact Factor: 3.53 · DOI: 10.1021/acs.chemrestox.5b00171 · Source: PubMed

---

READS

54

## 5 AUTHORS, INCLUDING:



Sireesh Dornadula

SRM University

9 PUBLICATIONS 20 CITATIONS

[SEE PROFILE](#)



Ramkumar KM

53 PUBLICATIONS 652 CITATIONS

[SEE PROFILE](#)

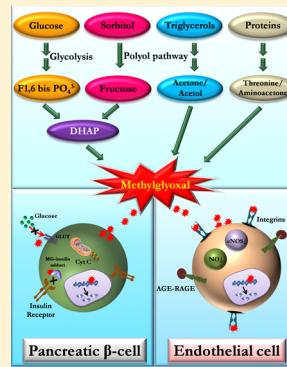
## Pathophysiological Insights of Methylglyoxal Induced Type-2 Diabetes

Sireesh Dornadula,<sup>†</sup> Bhakkiyalakshmi Elango,<sup>†</sup> Ponjayanthy Balashanmugam,<sup>†</sup> Rajaguru Palanisamy,<sup>‡</sup> and Ramkumar Kunka Mohanram<sup>\*,†</sup>

<sup>†</sup>SRM Research Institute, SRM University, Kattankulathur-603 203, Tamilnadu, India

<sup>‡</sup>Department of Biotechnology, Anna University-BIT Campus, Tiruchirappalli-620 024, Tamilnadu, India

**ABSTRACT:** Diabetes mellitus is a metabolic disorder constituting a major health problem whose prevalence has gradually increased worldwide over the past few decades. Type 2 diabetes mellitus (T2DM) remains more complex and heterogeneous and arises as a combination of insulin resistance and inadequate functional  $\beta$ -cell mass and comprises about 90% of all diabetic cases. Appropriate experimental animal models are essential for understanding the molecular basis, pathogenesis of complications, and the utility of therapeutic agents to abrogate this multifaceted disorder. Currently, animal models for T2DM are obtained as spontaneously developed diabetes or diabetes induced by chemicals or dietary manipulations or through surgical or genetic methods. The currently used diabetogenic agents have certain limitations. Recently, methylglyoxal (MG), a highly reactive compound derived mainly from glucose and fructose metabolism has been implicated in diabetic complications. MG is a major precursor of the advanced glycation end product (AGE) and promotes impaired functions of insulin signaling, GLUT transporters, anion channels, kinases, and endothelial cells and is finally involved in apoptosis. Recent array of literature also cited that higher concentrations of MG causes rapid depolarization, elevated intracellular  $\text{Ca}^{2+}$  concentration, and acidification in pancreatic  $\beta$ -cells. This review henceforth highlights the mechanism of action of MG and its implications in the pathophysiology of experimental diabetes.



### CONTENTS

Introduction	1666
Methylglyoxal	1667
Synthesis of MG	1667
MG Synthesis via Glycolysis	1668
MG Synthesis via the Polyol Pathway	1668
MG Formation via Threonine Catabolism	1668
Pathological Consequences of MG	1668
Perspectives on Diabetes	1668
Effects on Insulin	1668
Effects on Glucose Transporter	1668
Effects on the Anion Channel	1669
AGE Formation and Its Role in Oxidative Stress	1669
Effects on Transcription Factors	1669
Effects on Kinases	1669
Effects on Hemoglobin	1669
Effects on Endothelial Cells	1669
Triggering Apoptotic Pathway	1670
MG as a Suitable Model for T2DM	1670
Conclusions	1671
Author Information	1671
Corresponding Author	1671
Funding	1671
Notes	1671
Biographies	1671
Abbreviations	1672
References	1672

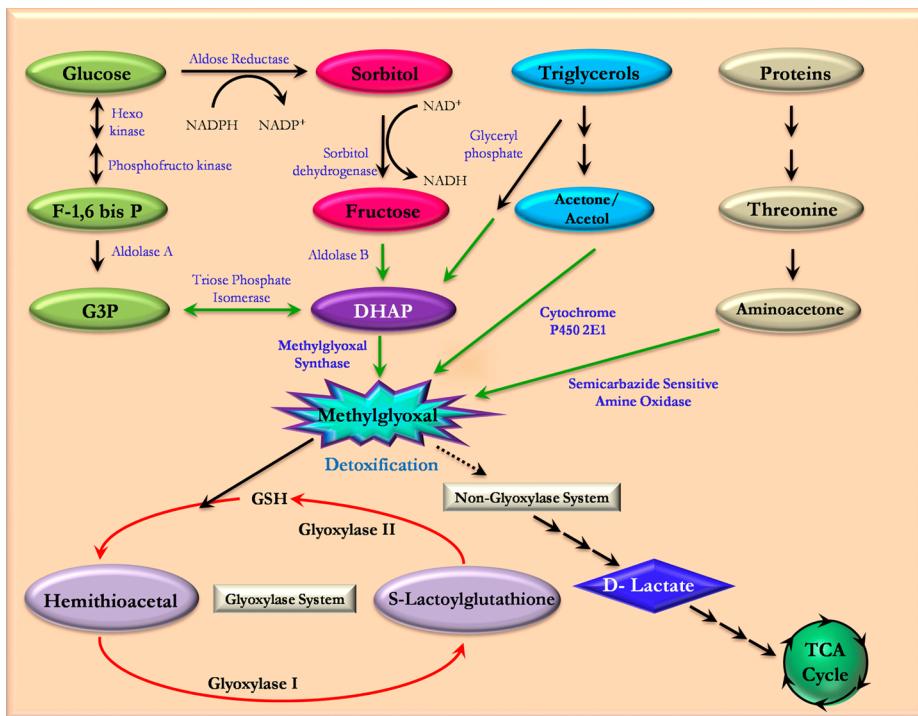
### INTRODUCTION

Diabetes is mainly characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.<sup>1</sup> Several pathogenic processes are involved in the development of diabetes which ranges from autoimmune destruction of the pancreatic  $\beta$ -cells with consequent insulin deficiency (T1DM) to abnormalities that result in resistance to insulin action (T2DM).<sup>2</sup> Globally, it is estimated that 387 million people suffer from diabetes for a prevalence of 8.3% and that the number of people with the disease is set to rise beyond 592 million in less than 25 years.<sup>3</sup> In particular, the number of people with T2DM is rapidly increasing in every country; the target organs develop resistance to insulin, though there is insulin producing functional  $\beta$ -cells in varying numbers, despite gradual and progressive apoptosis in the advanced stages.<sup>1,4,5</sup> Moreover, T2DM is influenced by various factors including genetic, environmental, and metabolic derangements.<sup>6</sup> Understanding of the disease remains more complex due to its multiple contributing factors and the lack of suitable animal models.

The development of promising experimental animal models for T2DM aids not only in understanding the pathophysiology of this disease but also in the development of drug candidates. The animal models that are being used to study T2DM widely differ in their physiological relevance. These models are developed based on chemically-induced diabetes, diet-induced diabetes,

Received: April 27, 2015

Published: August 6, 2015



**Figure 1.** Overview of major methylglyoxal synthesis and detoxification. Methylglyoxal is synthesized during carbohydrate, lipid, and amino acid metabolisms and involves both enzymatic and nonenzymatic processes. The enzymes that catalyze the reactions on methylglyoxal synthesis are methylglyoxal synthase, cytochrome P450 2E1, and semicarbazide sensitive amine oxidase. The nonenzymatic pathways include the spontaneous decomposition of DHAP, the Maillard reaction, the oxidation of acetol, and lipid peroxidation. Methylglyoxal detoxified via glyoxylase system (glyoxylase I and II) and nonglyoxylase system (methylglyoxal dehydrogenase, aldehyde reductase, aldehyde dehydrogenase, and carbonyl reductase), yields D-lactate and further enters the tricarboxylic acid cycle.

surgical procedures, or by genetic modifications.<sup>7,8</sup> The diabetogenic agents used so far to induce characteristic diabetic conditions include alloxan monohydrate, streptozotocin, ferric nitrilotriacetate, ditizona, and anti-insulin serum<sup>7,9</sup> and are reported to have several limitations. A high fat diet induces T2DM because free fatty acids interfere with the binding of insulin to its receptor, thereby developing insulin resistance. Yet this model requires a longer duration of time to mimic the conditions. Surgically developed diabetic animals are partially pancreatectomized and/or VMH lesioned,<sup>7,8,10</sup> where the developed animal models are intricate enough to handle it. Several genetically modified animal models for T2DM include leptin-deficient obese mice (*Lep<sup>ob</sup>*); leptin receptor-deficient mice (*LepR<sup>db/db</sup>*); Zucker diabetic fatty rats; insulin-dependent diabetic mice (*Ins2<sup>Akita</sup>*); and diabetic Goto-Kakizaki rats. Recently, generalized knockout and tissue-specific knockout mice models were also preferred in specific studies, which includes knockouts of IRS-1; IRS-2; GLUT-4; PTP-1B; PPAR- $\gamma$ ; glucokinase; GLUT 2; and  $\beta_3$ -receptor.<sup>7,8,10,11</sup> Genetic and surgical models are rarely preferred due to their requirement of high levels of technical skills and high mortality rates during the procedure. The use of different animal models depends on the purpose and type of the study, yet the selection of inappropriate animal models has been identified as one of the common issues in diabetic research.

Recently, MG (pyruvaldehyde or 2-oxopropanal), a highly reactive compound and strong advanced glycation end product (AGE) precursor, derived mainly from glucose and fructose metabolism, was found to induce insulin resistance and pancreatic  $\beta$ -cell dysfunction in animals.<sup>12</sup> Increasing evidence supports that the formation of MG, and related AGE is one of the

possible mechanisms that link dysfunction of  $\beta$ -cells and insulin resistance in diabetic patients. This review henceforth determinedly highlights the mechanism of action of MG and its implication in the pathophysiology of T2DM in experimental animals.

## METHYLGYOXAL

MG is a reduced derivative of pyruvic acid with an aldehyde and a ketone group.<sup>13</sup> It is formed as a byproduct of many metabolic pathways, especially glucose and fructose and is able to bind with DNA, proteins, and lipids. MG is a highly potent glycation agent with a specific reactivity of 20,000-fold higher than that of glucose<sup>14</sup> and generates MG-adducts such as 8-OH-dG (8-hydroxy-deoxyguanosine); CEdG (N2-(1-carboxyethyl)-deoxyguanosine); N $\delta$ -(S-hydro-4-imidazol-2-yl)ornithine (G-H1); fructosyl-lysine (FL); N $\epsilon$ -carboxymethyl-lysine (CML); and bis(lysyl) cross-links, MOLD (methylglyoxal lysine dimer).<sup>15</sup> The formation of these adducts causes deleterious effects in cellular functions.

## SYNTHESIS OF MG

In diabetes, the altered glucose metabolism causes increased respiration rates in mitochondria leading to oxidative stress, superoxide “leakage,” and activation of the nuclear enzyme, poly(ADP-ribose) polymerase-1 (PARP).<sup>16</sup> PARP activation depletes its substrate NAD $^+$ , slows the rate of glycolysis and electron transport, and inhibits the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Inhibition of GAPDH results in accumulation of glycolytic intermediates, thereby activating metabolic pathways (protein kinase C, polyol, and hexosamine) and perturbing cellular functions by reduced nitric

oxide levels, altered gene expression, and formation of AGEs.<sup>16–18</sup> These glycolytic intermediates act as direct precursors of MG which accumulates rapidly and causes imbalance in glucose homeostasis.

MG is synthesized mainly through three metabolic pathways: (i) glycolysis, (ii) polyol pathway, and (iii) threonine catabolism (Figure 1).

**MG Synthesis via Glycolysis.** Glucose is the prime energy source for various metabolic reactions. In cytosol, glucose is physiologically metabolized through a cascade of enzymatic reactions resulting in the synthesis of pyruvate via glycolysis. The intermediate metabolites of glycolysis mainly include glyceraldehyde-3-phosphate (G3P, a three carbon sugar carbohydrate) and its isomer dihydroxyacetone phosphate (DHAP),<sup>19</sup> which are the effective precursors of MG.<sup>20,21</sup> Increased flux of glucose leads to the activation of the MG pathway due to GAPDH inhibition and depletion of NAD. Methylglyoxal synthase (MGS) is highly specific for DHAP and catalyzes DHAP to MG and inorganic phosphate with enol pyruvaldehyde as intermediate.<sup>22</sup>

**MG Synthesis via the Polyol Pathway.** The polyol pathway is a two-step metabolic pathway where (i) glucose is converted to sorbitol utilizing NADPH, (ii) which is further converted to fructose using NAD.<sup>23</sup> Fructose undergoes a cascade of reactions and forms DHAP/G3P. This pathway switches to MG synthesis under conditions of unavailable nucleotides such as NAD and NADPH. Under hyperglycemia, the depletion of NAD leads to GAPDH inhibition and triggers the MG recruiting pathway resulting in enhanced production of MG. In addition, demand on NADPH depends on the GSH-dependent glyoxylase system<sup>19</sup> and ultimately leads to excessive formation of MG.

**MG Formation via Threonine Catabolism.** Aminoacetone (AA), the product of mitochondrial metabolism of threonine and glycine acts as one of the precursors of MG. Threonine dehydrogenase catalyzes the oxidation of threonine to glycine and acetyl-CoA using NAD<sup>+</sup>. In diabetes, the acetylCoA/CoASH ratio increases, resulting in excessive AA formation. Moreover, semicarbazide sensitive amine oxidase (SSAO), a copper- and quinone-dependent enzyme plays a key role in glucose transport and acts as an insulin mimic in adipose and vascular smooth muscle cells (VSMCs) via GLUT4 and GLUT1, respectively. Under hyperglycemic conditions, SSAO catalyzes oxidative deamination of primary amines to MG.<sup>24</sup> The augmented level of SSAO was reported in both insulin-dependent diabetes mellitus (IDDM) and noninsulin-dependent diabetes mellitus (NIDDM), and also in streptozotocin-induced diabetes.<sup>25</sup> Thus, the increased SSAO activity positively correlates insulin mimicry and diabetes complications.<sup>26</sup>

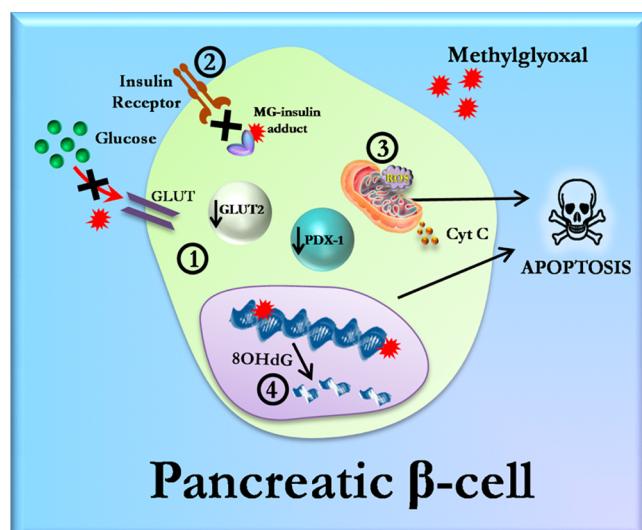
Thus, the formation of MG via various metabolic pathways provokes pathophysiological effects of diabetes, thereby mimicking the diseased condition.

## PATHOLOGICAL CONSEQUENCES OF MG

**Perspectives on Diabetes.** During diabetes, MG involved in the generation of free radicals, declined antioxidant status, glycation (of amino acids, proteins, and nucleic acids), and induction of AGE-RAGE interactions leading to detrimental conditions. Here, we summarize the pathological effects of MG and how it can act as a diabetogenic agent to mimic the conditions of T2DM.

**Effects on Insulin.** Insulin is a peptide hormone produced by  $\beta$ -cells of islets of Langerhans, which regulates glucose

homeostasis. Insulin resistance coupled with  $\beta$ -cell loss remains to be the hallmarks of T2DM. It has been reported that the highly reactive carbonyl MG alters the B-chain of human insulin, specifically at the N-terminus and arginine residue via Schiff base formation (Figure 2). The extent of modification was found to be



**Figure 2.** Pathological consequences of methylglyoxal in pancreatic  $\beta$ -cells. (1) Methylglyoxal reduces GLUT2 expression: methylglyoxal reduces basal glucose-stimulated insulin secretion in pancreatic  $\beta$ -cells due to significant decrease in GLUT2 and PDX-1 expressions. (2) Methylglyoxal-insulin adducts: the formation of methylglyoxal-insulin adducts leads to the reduction of insulin-mediated glucose uptake thereby impairing autocrine control of insulin release in pancreatic  $\beta$ -cells. (3) Release of cytochrome *c*: free radicals can be produced during both the formation and degradation of methylglyoxal, lead to cytochrome *c* release to the cytoplasm, thereby triggering the cascade of apoptosis. (4) DNA fragmentation: methylglyoxal induces DNA fragmentation and promotes  $\beta$ -cell apoptosis.

increased with a relative concentration of MG. In addition, the formation of MG-insulin adducts affects the insulin-mediated glucose uptake by its target cells or tissues, impairs autocrine control of insulin release from pancreatic  $\beta$ -cells, and decreases hepatic clearance of insulin from liver cells.<sup>27,28</sup> Acute exposure to MG induces the inhibition of insulin-stimulated phosphorylation of protein kinase B (PKB) at Ser473 and Thr308 and extracellular-regulated kinase 1/2 (ERK1-p44 and ERK2-p42)<sup>29</sup> with impaired insulin signaling in muscle and pancreatic  $\beta$ -cells.<sup>29</sup>

Liu and his co-workers reported a close correlation between insulin resistance and elevated MG accumulation in adipose and vascular smooth muscle tissues of experimental animals.<sup>30</sup> MG administration impaired insulin signaling as measured by decreased tyrosine phosphorylation of IRS-1 and kinase activity of PI3K.<sup>31</sup> These evidences indicate that MG impairs both insulin action and secretion, which resembles the conditions of T2DM.

**Effects on Glucose Transporter.** Extracellular and intracellular levels of glucose are mainly controlled by sodium ( $\text{Na}^+$ )-coupled glucose transporters (SGLT) and facilitative glucose transporters (GLUT).<sup>32</sup> Impaired functions of these transporters causes imbalance in the regulation of glucose metabolism leading to various diabetic complications. A total of 13 isoforms of glucose transporters found in humans are categorized as three subclasses: Class I (GLUTs 1–4) are glucose transporters; Class II (GLUTs 5, 7, 9 and 11) are fructose transporters; and Class III

(GLUTs 6, 8, 10, 12, and HMIT1) are structurally atypical members of the GLUT family.<sup>32,33</sup> GLUT1 is responsible for the basal uptake of glucose in many cell types, representing the foremost ubiquitously expressed isoform; GLUT4 is responsible for insulin-stimulated glucose uptake in peripheral tissues; however, its expression has also been reported in the brain, where glucose is the essential substrate for cerebral oxidative metabolism.<sup>32,33</sup>

Yoshida et al., reported that, administration of MG diminished the proficiency of glucose uptake and insulin-responsive glucose uptake by interfering with both GLUT1 and GLUT4.<sup>33</sup> Further studies reported that MG administration reduced glucose uptake due to dwindled levels of pancreatic glucose sensor GLUT2 in diabetic animals as well as isolated pancreatic islets.<sup>34,35</sup>

**Effects on the Anion Channel.** In pancreatic  $\beta$ -cells, which are sustained primarily by ATP, the ATP/ADP ratio determines  $K_{ATP}$  channel activity. Under normal conditions, the  $K_{ATP}$  channels in pancreatic  $\beta$ -cells are spontaneously active, allowing  $K^+$  to flow out of the cell. In the case of higher glucose metabolism and consequent increased levels of ATP, the  $K_{ATP}$  channels close, causing the membrane potential of the cell to depolarize, thus promoting insulin release.<sup>36</sup> MG is found to have no apparent effect on  $\beta$ -cell  $K^+$  channel activity but the metabolism of MG to D-lactate was reported to cause  $\beta$ -cell swelling and activation of the volume-sensitive anion channel, leading to depolarization.<sup>37</sup> Few other studies documented that AA and MG cause depolarization of the  $\beta$ -cell by the generation of an inward current ( $Ca^{2+}$ ,  $Na^+$ ) and accumulation of D-lactate.<sup>38</sup> Hence, these evidences delineate the role of MG on anion channels and  $\beta$  cell depolarization.

**AGE Formation and Its Role in Oxidative Stress.** AGEs play a major role in diabetic complications including retino-neuro-nephropathy.<sup>39</sup> MG is believed to contribute significantly to intracellular AGE formation, not only due to its higher reactivity but also due to its multiple origin<sup>40</sup> via glycolysis, lipolysis, and metabolism of amino acids like glycine and threonine.<sup>19,21,38</sup> Further, MG modifies free amino groups of lysine and arginine or thiol groups of cysteine moieties resulting in AGE formation. During diabetes, AGEs interact with soluble RAGE (sRAGE), increase oxidative stress, and subsequently evoke vascular inflammation, macrophage, and platelet activation and thrombosis, thereby playing an important role in the development and progression of vascular complications.<sup>41,42</sup> In addition, MG could modify nucleic acids by oxidation of guanine to form 8-hydroxy-2'-deoxyguanosine (8-OH-dG) and cause DNA fragmentation thereby inducing  $\beta$ -cell apoptosis.<sup>15</sup> MG also has the potential to cross-link DNA (dG) to proteins (Lys or Cys residues close to the DNA polymerase binding site), leading to reduced DNA replication and increased mutation rates.<sup>18</sup>

Further, MG increases the generation of ROS especially superoxide anions ( $O_2^-$ ) and  $H_2O_2$ , which dampens the mitochondrial antioxidant defense system and causes oxidative damage.<sup>43</sup> MG also disrupts the electron transport chain in mitochondria by limiting the activities of MnSOD (first-line enzyme in mitochondria to degrade superoxide) and complex III (transfers electrons from ubiquinone to cytochrome *c*), leading to the leakage of electrons and  $O_2^-$  formation.<sup>44</sup> These events are often considered to play key roles in diabetes-induced pathology.

**Effects on Transcription Factors.** PDX-1 and MafA are the major transcription factors promoting pancreatic development, maintenance of  $\beta$ -cell function, insulin gene transcription, insulin secretion, and  $\beta$ -cell survival.<sup>45,46</sup> MG pampers the expression of PDX-1 and MafA and causes impaired glucose stimulated insulin

secretion.<sup>34</sup> In normal conditions, NF- $\kappa$ B binds to its inhibitor I $\kappa$ B in the cytosol, and when the cell encounters any stress, cytokines, growth factors, and bacterial or viral antigens, NF- $\kappa$ B gets activated and translocates into the nucleus. Wu et al., reported that MG administration showed significant decrease in I $\kappa$ B $\alpha$  levels and increase in nuclear levels of NF- $\kappa$ B.<sup>47</sup>

**Effects on Kinases.** MG was reported to play a key role in cell proliferation and growth resulting in the modulation of growth factor signaling.<sup>48</sup> Increased accumulation of MG alters the platelet-derived growth factor (PDGF)-induced PDGFR $\beta$ -phosphorylation, ERK1/2-activation, and subsequent proliferation of mesenchymal cells (smooth muscle cells and skin fibroblasts). It also perturbs gp130/STAT3 signaling, a key regulator of cytokine-induced gene expression, and thereby increases cytotoxicity in neuroglial cells. Activation of PDGFR, recruits the mitogen-activated protein kinase (MAPK) and PI3 kinase pathways that aid in cell proliferation, development, differentiation, and growth.<sup>49</sup> MG was found to react with PDGFR and cause the suppression of PDGF signaling.<sup>48,50</sup> The upstream of MAPK includes ERK1/2 and serine/threonine-specific kinase Raf-1 that also helps in the regulation of cell survival, division, and differentiation. MG induces proteolytic degradation of Raf-1<sup>51</sup> and dampens insulin-stimulated ERK1/2 signaling by inhibiting the phosphorylation events of ERK.<sup>29</sup>

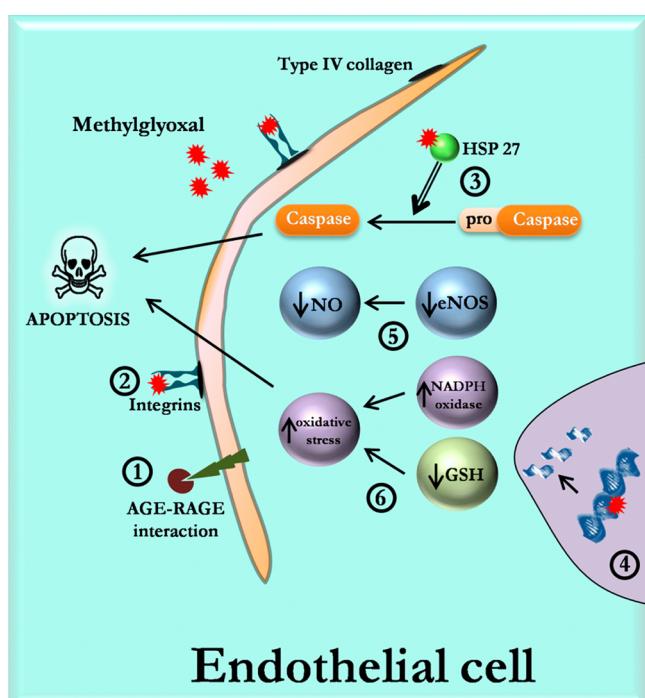
**Effects on Hemoglobin.** MG causes modification of structural and functional characteristics of hemoglobin (HbA<sub>0</sub>). In normoglycemic conditions, nonenzymatic fragmentation of triose phosphates is the major source of MG formation in red blood cells. During diabetes, MG synthesis was increased by stimulating the flux of glucose, fructose, dihydroxy-acetone, D-glyceraldehyde, acetone, and hydroxyacetone.<sup>52</sup> It has been demonstrated that MG modifies arginine residues (Arg-92 $\alpha$ , 141 $\alpha$ , and 104 $\beta$ ) of HbA<sub>0</sub> forming hydroimidazolones in MG-Hb adducts.<sup>53,54</sup> These arginine residues are reported to have higher rates of ligand and substrate recognition sites, which remains a potent target for MG binding and HbA<sub>0</sub> modification.<sup>55</sup>

The modified HbA<sub>0</sub> was found to lose its negative charge thereby increasing its  $\alpha$ -helical structure and release of iron.<sup>54</sup> Impairment of iron homeostasis leads to oxidative modification of proteins, lipids, and DNA leading to disturbances in their molecular functions.<sup>54</sup> Increase in the level of iron also promotes ROS generation, mainly OH<sup>-</sup> radicals that are synthesized through Fenton's reaction in the presence of Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub>.<sup>54</sup> Sen et al. observed that hemoglobin of streptozotocin-induced diabetic rats showed higher iron-dependent oxidative reactions.<sup>56</sup> An et al. also reported that free iron ions were released from the MG-modified Ferritin (iron storage protein), leading to pro-oxidative conditions, and dityrosine, a biomarker for protein modification by ROS formation was also detected in MG-modified ferritin.<sup>57</sup>

**Effects on Endothelial Cells.** Endothelial cells occupy a central role in maintaining vascular homeostasis. Impaired functions of these cells and resulting vascular defects are the major contributors of diabetic complications. The endothelium is highly susceptible to hyperglycemia-induced damage due to increased intracellular accumulation of glucose and its associated metabolites. AGE formation also has an important role in the development of diabetes related vascular complications. MG, the most reactive AGE precursor, has been shown to be a potent inducer of tyrosine phosphorylation and aggregation of a number of cellular proteins.<sup>58</sup> The surface sheath network of type 4-collagen in blood vessels binds integrins of vascular ECs thereby anchoring and sustaining the vascular endothelium. These

integrin binding sites of collagen are reported to be the potential targets for MG modification. During diabetes, MG disrupts integrin-dependent cell-matrix adhesion by binding to arginine residues in ECM, which leads to impairment of ECM attachment, viability, and angiogenic activity of ECs causing impairment of the vasculature.<sup>59</sup>

Increase in MG level and its high reactivity potential impairs endothelial functions in various ways (Figure 3): (i) increase in



**Figure 3.** Methylglyoxal-induced deleterious effects on endothelial cells. (1) AGE-RAGE interaction: methylglyoxal induces AGE formation, which induces receptor AGEs and causes upregulation of the transcription factor (NF- $\kappa$ B) and adhesion molecules (VCAM-1 and ICAM-1). (2) Endothelial cell dysfunction: methylglyoxal disrupts integrin-dependent cell-matrix adhesion by binding to arginine residues of the extracellular matrix (ECM) which leads to impairment of ECM association. (3) Glycation of Hsp 27: methylglyoxal-Hsp 27 adduct formation promotes caspase activation and triggers apoptosis. (4) DNA fragmentation: methylglyoxal induces DNA fragmentation leading to cell death through apoptosis. (5,6) Increased levels of methylglyoxal impairs endothelial functions in various ways such as (i) inhibition of eNOS, (ii) increase of NADPH, (iii) inhibition of glutathione, resulting in oxidative stress and further leading to apoptosis.

free radicals, formation of peroxynitrite ( $\text{ONOO}^-$ );<sup>58</sup> (ii) hexosamine pathway activity and condensed serine phosphorylation, inhibition of eNOS;<sup>60,61</sup> (iii) increased NADPH oxidase, overproduction of superoxide anion and oxidative stress;<sup>61</sup> (iv) reduced GSH and GSH-reductase levels, halts detoxification of MG by reacting with functional thiol groups of glutathione;<sup>61,62</sup> (v) progressive thickening of the vessel wall and endothelial pyknosis;<sup>63</sup> (vi) impairment of mitochondrial membrane potential and elevation of caspase-3;<sup>64</sup> (vii) activation of protein kinase C and NF- $\kappa$ B; (viii) AGE-RAGE interaction leading to the expression of cell adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1), and activation of NF- $\kappa$ B. Hence, these factors limit the functions of endothelial cells which ultimately lead to ED, triggering diabetic complications.

**Triggering Apoptotic Pathway.** Poly(ADP-ribose) polymerase (PARP) are a chromatin-associated enzymes that cause depletion of cellular NAD<sup>+</sup> and ATP.<sup>65</sup> Caspases are a family of cysteine proteases that regulate apoptosis by cleaving the cellular proteins and the formation of apoptotic bodies.<sup>66</sup> MG exposure was found to increase the cleavage of caspase-3, caspase-7, and PARP leading to insulin insensitivity thereby causing apoptosis.<sup>67</sup> In addition, MG also increased the phosphorylation of IRS-1 and IR, along with reduced phosphorylated Akt expression. Since the apoptotic pathway is closely related to PI3K/Akt activities,<sup>68</sup> insulin treatment ameliorates the degenerating apoptotic process by increasing phosphorylated Akt expression and subsequently inhibiting cleavage of PARP and caspases.<sup>67</sup> MG was reported to trigger the abnormal opening of mitochondrial transition pore by collapse of mitochondrial transmembrane potential, resulting in the rapid release of caspase activators.<sup>69</sup> However, the level of antiapoptotic protein, Bcl-2 was diminished upon MG treatment with improved expression of pro-apoptotic Bax and Caspase-3.<sup>64</sup> Further, MG administration exerts a detrimental effect on mitochondria, by increasing ROS (mitochondrial  $\text{H}_2\text{O}_2$ ) production and oxidative stress.<sup>43</sup>

Loreto et al. reported that MG mediated apoptosis is triggered via the extrinsic pathway and also by increasing proinflammatory cytokines (specifically TNF- $\alpha$ ) and caspase-8 regulators.<sup>70</sup> It mainly weakens the antioxidant system (inhibition of GSH-Px activity, impairment in catalase and SOD activities, and depletion of intracellular GSH) consequently leading to increased oxidative stress<sup>61,70</sup> and impairment of MG detoxification enzymes (glyoxalase I and II).<sup>71</sup> In addition, MG induced apoptosis also occurs via activation of p38 MAPK<sup>72</sup> and JNK pathways.<sup>73</sup> The modification of mitochondrial glutathione reductase (binding of SH groups at their active sites) by ROS generation also triggers apoptosis.<sup>43,74</sup>

Heat shock protein 27 (Hsp27) protects the cells against harmful stress, such as heat shock, heavy metal ions, and oxidative stress. It interrupts the activation of cytochrome *c* and procaspase-9 and thus inhibits the apoptotic cascade.<sup>18</sup> However, glycation of Hsp 27 by MG leads to MG-Hsp 27 adducts formation and modification of C-terminal arginine residues of Hsp27. These events encourage caspase activation and reduce the binding of Hsp27 to cytochrome *c*, thereby inducing apoptosis.<sup>18,75</sup>

## ■ MG AS A SUITABLE MODEL FOR T2DM

The most commonly used diabetogenic agents to induce diabetes, STZ, and alloxan are glucose analogues and cause acute killing of pancreatic  $\beta$ -cells but differ in their mode of action where STZ alkylates DNA and alloxan generates ROS.<sup>76</sup> Unlike these agents, MG causes gradual apoptosis of pancreatic  $\beta$ -cells and reduces its mass resulting in impaired insulin secretion. Collectively, the following critical points justify that the MG-induced diabetic model could resemble more closely the T2DM induced complications and are more suitable to study T2DM.

MG affects: (i) transcriptional factors PDX-1 and MafA interrupt insulin synthesis; (ii) glucose transport in  $\beta$ -cells; (iii) insulin action and secretion; and (iv) AGE-RAGE interactions; (v) antioxidant defense system, increases oxidative stress leading to ROS generation; (vi) mitochondrial membrane potential and intracellular  $\text{Ca}^{2+}$  concentration, and causes acidification in pancreatic  $\beta$ -cells and its mass.

## CONCLUSIONS

Many animal models that are used to mimic T2DM are obese either by genetic modification or dietary manipulation reflecting the human state where obesity is closely linked to the development of the disease. However, T2DM is a complicated metabolic disorder caused and influenced by multiple factors including genetics, the environment, and metabolic disarrangements. The morbidity and mortality rates of diabetes positively correlate with the development of both macro- and micro-vascular complications. Hence, it is highly desirable to use proper animal models to study the development of this disease and associated pathology especially vascular complications in diabetes which have been linked to dysfunction of endothelial cells. MG is a reactive glucose metabolite and a major precursor for AGEs. Multiple prospective studies have documented that the vascular tissue concentrations of MG increased 3- to 5-fold in clinical diabetes. During diabetes, increased levels of MG intensify the generation of ROS and AGE formation leading to vascular damage. MG administration is also found to affect insulin sensitivity and mimic most diabetic alterations that are being associated with the development of cardiovascular disease and the impairment of survival pathways *in vivo*. Few recent findings also suggest that hyperglycemia enhances MG production in diabetic subjects and impairs endothelial functions. Since endothelial dysfunction associated with insulin resistance appears to precede the development of hyperglycemia in patients with T2DM, MG-induced animal model with insulin resistance and endothelial dysfunction could be used for pharmacological testing, studies of genetics, and to gain a better understanding of disease mechanisms.

## AUTHOR INFORMATION

### Corresponding Author

\*Tel: +91-9940737854. Fax: +91-44-2745-23437. E-mail: [ramkumar.km@res.srmuniv.ac.in](mailto:ramkumar.km@res.srmuniv.ac.in).

### Funding

We are grateful for the support received from SRM University.

### Notes

The authors declare no competing financial interest.

### Biographies



**Mr. Sireesh Dornadula** obtained his Master's degree from Bangalore University, India in 2010. He worked as a Research Scholar at the University of Pittsburgh from 2011 to 2013. He is currently working as a Ph.D. student under the supervision of Dr. Ramkumar, and his research is centered on islet transplantation and management of diabetes.



**Mrs. Bhakkiyalaskhmi Elango** completed her Bachelor's and Master's degrees in Biotechnology from Anna University in 2011 where she worked on Disease Biology. Currently, she is working as a Senior Research Fellow at the Department of Biotechnology, SRM University. She has coauthored over 10 publications including those in *Curr. Med. Chem.*, *BPS-Brit. J. Pharmacol.*, *Pharm. Res.*, *Anal. Chem.*, *Cell Proliferation*, and *Food Chem Toxicol.* Her current research focuses on the identification of cell signaling modulators to prevent  $\beta$ -cells from apoptosis.



**Ms. B. Ponjyanthi** holds her Bachelor's and Master's degrees in Biotechnology from Anna University. She is currently working as a Ph.D. student under the supervision of Dr. Ramkumar, and her research work concentrates on high throughput screening of cell signaling modulators and their role in endothelial dysfunction in diabetes.



**Dr. Rajaguru Palanisamy** is a Professor and Head of Biotechnology Department, Anna University-BIT campus, Tiruchirappalli, India. He has over 40 publications in well reputed international journals including *Mut. Res.*, *Environ. Mol. Mutagen.*, *J. Hazard. Mater.*, *Cell Physiol. Biochem.*, and *Cell Biol. Toxicol.* The current research interests in Dr.

Rajaguru's lab are toxicogenomics and proteomics, and applications of antisense technology in cancer biology and diabetes.



**Dr. Ramkumar Kunka Mohanram** is an Assistant Professor in Life Sciences division at SRM Research Institute, SRM University, India, leading the Experimental Diabetes Research team. He has over 40 peer reviewed publications in reputed international journals including *Anal. Chem.*, *Chem. Res. Toxicol.*, *Am. J. Physiol. Renal. Physiol.*, *Curr. Drug Targets*, *Theranostics*, and *BPS-Brit. J. Pharmacol.* The current research interests in Dr. Ramkumar's group are (1) protective molecular pathways to improve pancreatic beta-cell dysfunction, (2) crosstalk mechanism between pancreatic islets and endothelial cells, and (3) development of a high throughput screening assay for cell signaling modulators.

## ■ ABBREVIATIONS

8-OH-dG, 8-hydroxy-deoxyguanosine; AA, aminoacetone; AGE, advanced glycation end product; DHAP, dihydroxyacetone phosphate; DM, diabetes mellitus; ERK, extracellular-regulated kinase; G3P, glyceraldehyde-3-phosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporters; Hsp27, heat shock protein 27; ICAM-1, intercellular cell adhesion molecule-1; IDDM, insulin-dependent diabetes mellitus; IRS-1, insulin receptor substrate 1; MAPK, mitogen-activated protein kinase; MG, methylglyoxal; MGS, methylglyoxal synthase; NIDDM, noninsulin-dependent diabetes mellitus; PARP, poly(ADP-ribose) polymerase-1; PDGF, platelet-derived growth factor; PDX-1, pancreatic and duodenal homeobox 1; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; PKC, protein kinase C; RAGE, receptor for advanced glycation endproducts; ROS, reactive oxygen species; SGLT, sodium ( $\text{Na}^+$ )-coupled glucose transporters; SSAO, semicarbazide sensitive amine oxidase; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; VCAM-1, vascular cell adhesion molecule-1; VSMCs, vascular smooth muscle cells

## ■ REFERENCES

- (1) American Diabetes Association (2009) Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 32, S62–S67.
- (2) Bhakkiyalakshmi, E., Sireesh, D., Rajaguru, P., Paulmurugan, R., and Ramkumar, K. M. (2015) The emerging role of redox-sensitive Nrf2-Keap1 pathway in diabetes. *Pharmacol. Res.* 91, 104–114.
- (3) Guariguata, L. (2013) Contribute data to the 6th edition of the IDF Diabetes Atlas. *Diabetes Res. Clin. Pract.* 100, 280–281.
- (4) Cernea, S., and Dobrea, M. (2013) Diabetes and beta cell function: from mechanisms to evaluation and clinical implications. *Biochem. Med.* 23, 266–280.
- (5) Anuradha, R., Saraswati, M., Kumar, K. G., and Rani, S. H. (2014) Apoptosis of beta cells in diabetes mellitus. *DNA Cell Biol.* 33, 743–748.
- (6) McFarlane, S. I., Shin, J. J., Rundek, T., and Bigger, J. T. (2003) Prevention of type 2 diabetes. *Curr. Diabetes Rep.* 3, 235–241.
- (7) Srinivasan, K., and Ramarao, P. (2007) Animal models in type 2 diabetes research: an overview. *Indian J. Med. Res.* 125, 451–472.
- (8) King, A. J. (2012) The use of animal models in diabetes research. *Br. J. Pharmacol.* 166, 877–894.
- (9) Rees, D. A., and Alcolado, J. C. (2005) Animal models of diabetes mellitus. *Diabetic Med.* 22, 359–370.
- (10) Chatzigeorgiou, A., Halapas, A., Kalafatakis, K., and Kamper, E. (2009) The use of animal models in the study of diabetes mellitus. *In Vivo* 23, 245–258.
- (11) Allen, T. J., Cooper, M. E., and Lan, H. Y. (2004) Use of genetic mouse models in the study of diabetic nephropathy. *Curr. Diabetes Rep.* 4, 435–440.
- (12) Matafome, P., Sena, C., and Seica, R. (2013) Methylglyoxal, obesity, and diabetes. *Endocrine* 43, 472–484.
- (13) Kalapos, M. P. (1999) Methylglyoxal in living organisms: chemistry, biochemistry, toxicology and biological implications. *Toxicol. Lett.* 110, 145–175.
- (14) Rabbani, N., and Thornalley, P. J. (2014) The critical role of methylglyoxal and glyoxalase 1 in diabetic nephropathy. *Diabetes* 63, 50–52.
- (15) Thornalley, P. J. (2008) Protein and nucleotide damage by glyoxal and methylglyoxal in physiological systems—role in ageing and disease. *Drug Metab. Drug Interact.* 23, 125–150.
- (16) Pacher, P., and Szabo, C. (2005) Role of poly(ADP-ribose) polymerase-1 activation in the pathogenesis of diabetic complications: endothelial dysfunction, as a common underlying theme. *Antioxid. Redox Signaling* 7, 1568–1580.
- (17) Rolo, A. P., and Palmeira, C. M. (2006) Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicol. Appl. Pharmacol.* 212, 167–178.
- (18) Price, C. L., and Knight, S. C. (2009) Methylglyoxal: possible link between hyperglycaemia and immune suppression? *Trends Endocrinol. Metab.* 20, 312–317.
- (19) Beisswenger, P. J., Howell, S. K., Smith, K., and Szwerdgold, B. S. (2003) Glyceraldehyde-3-phosphate dehydrogenase activity as an independent modifier of methylglyoxal levels in diabetes. *Biochim. Biophys. Acta, Mol. Basis Dis.* 1637, 98–106.
- (20) Rieder, S. V., and Rose, I. A. (1959) The mechanism of the triosephosphate isomerase reaction. *J. Biol. Chem.* 234, 1007–1010.
- (21) Phillips, S. A., and Thornalley, P. J. (1993) The formation of methylglyoxal from triose phosphates. Investigation using a specific assay for methylglyoxal. *Eur. J. Biochem.* 212, 101–105.
- (22) Saadat, D., and Harrison, D. H. (1999) The crystal structure of methylglyoxal synthase from Escherichia coli. *Structure* 7, 309–317.
- (23) Lindstad, R. I., and McKinley-McKee, J. S. (1993) Methylglyoxal and the polyol pathway. Three-carbon compounds are substrates for sheep liver sorbitol dehydrogenase. *FEBS Lett.* 330, 31–35.
- (24) Sartori, A., Mano, C. M., Mantovani, M. C., Dyszy, F. H., Massari, J., Tokikawa, R., Nascimento, O. R., Nantes, I. L., and Bechara, E. J. (2013) Ferricytochrome (c) directly oxidizes aminoacetone to methylglyoxal, a catabolite accumulated in carbonyl stress. *PLoS One* 8, e57790.
- (25) Meszaros, Z., Szombathy, T., Raimondi, L., Karadi, I., Romics, L., and Magyar, K. (1999) Elevated serum semicarbazide-sensitive amine oxidase activity in non-insulin-dependent diabetes mellitus: correlation with body mass index and serum triglyceride. *Metab., Clin. Exp.* 48, 113–117.
- (26) Stolen, C. M., Madanat, R., Marti, L., Kari, S., Yegutkin, G. G., Sariola, H., Zorzano, A., and Jalkanen, S. (2004) Semicarbazide sensitive amine oxidase overexpression has dual consequences: insulin mimicry and diabetes-like complications. *FASEB J.* 18, 702–704.
- (27) Jia, X., Olson, D. J., Ross, A. R., and Wu, L. (2006) Structural and functional changes in human insulin induced by methylglyoxal. *FASEB J.* 20, 1555–1557.
- (28) Oliveira, L. M., Lages, A., Gomes, R. A., Neves, H., Familia, C., Coelho, A. V., and Quintas, A. (2011) Insulin glycation by methylglyoxal

- results in native-like aggregation and inhibition of fibril formation. *BMC Biochem.* 12, 41.
- (29) Riboulet-Chavey, A., Pierron, A., Durand, I., Murdaca, J., Giudicelli, J., and Van Obberghen, E. (2006) Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. *Diabetes* 55, 1289–1299.
- (30) Liu, J., Desai, K., Wang, R., and Wu, L. (2013) Up-regulation of aldolase A and methylglyoxal production in adipocytes. *Br. J. Pharmacol.* 168, 1639–1646.
- (31) Guo, Q., Mori, T., Jiang, Y., Hu, C., Osaki, Y., Yoneki, Y., Sun, Y., Hosoya, T., Kawamata, A., Ogawa, S., Nakayama, M., Miyata, T., and Ito, S. (2009) Methylglyoxal contributes to the development of insulin resistance and salt sensitivity in Sprague-Dawley rats. *J. Hypertens.* 27, 1664–1671.
- (32) Rizzo, B., Zambonin, L., Angeloni, C., Leoncini, E., Dalla Sega, F. V., Prata, C., Fiorentini, D., and Hrelia, S. (2013) Steviol glycosides modulate glucose transport in different cell types. *Oxid. Med. Cell. Longevity* 2013, 348169.
- (33) Yoshida, A., Wei, D., Nomura, W., Izawa, S., and Inoue, Y. (2012) Reduction of glucose uptake through inhibition of hexose transporters and enhancement of their endocytosis by methylglyoxal in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 287, 701–711.
- (34) Dhar, A., Dhar, I., Jiang, B., Desai, K. M., and Wu, L. (2011) Chronic methylglyoxal infusion by minipump causes pancreatic beta-cell dysfunction and induces type 2 diabetes in Sprague-Dawley rats. *Diabetes* 60, 899–909.
- (35) Lee, B. H., Hsu, W. H., Chang, Y. Y., Kuo, H. F., Hsu, Y. W., and Pan, T. M. (2012) Ankaflavin: a natural novel PPAR $\gamma$  agonist upregulates Nrf2 to attenuate methylglyoxal-induced diabetes in vivo. *Free Radical Biol. Med.* 53, 2008–2016.
- (36) Craig, T. J., Ashcroft, F. M., and Proks, P. (2008) How ATP inhibits the open K(ATP) channel. *J. Gen. Physiol.* 132, 131–144.
- (37) Best, L., Miley, H. E., Brown, P. D., and Cook, L. J. (1999) Methylglyoxal causes swelling and activation of a volume-sensitive anion conductance in rat pancreatic beta-cells. *J. Membr. Biol.* 167, 65–71.
- (38) Sartori, A., Garay-Malpartida, H. M., Forni, M. F., Schumacher, R. I., Dutra, F., Sogayar, M. C., and Bechara, E. J. (2008) Aminoacetone, a putative endogenous source of methylglyoxal, causes oxidative stress and death to insulin-producing RINm5f cells. *Chem. Res. Toxicol.* 21, 1841–1850.
- (39) Singh, V. P., Bali, A., Singh, N., and Jaggi, A. S. (2014) Advanced glycation end products and diabetic complications. *Korean J. Physiol. Pharmacol.* 18, 1–14.
- (40) Wang, Y. H., Han, Y. P., Yu, H. T., Pu, X. P., and Du, G. H. (2014) Protocatechualdehyde prevents methylglyoxal-induced mitochondrial dysfunction and AGEs-RAGE axis activation in human lens epithelial cells. *Eur. J. Pharmacol.* 738, 374–383.
- (41) Ramasamy, R., Yan, S. F., and Schmidt, A. M. (2006) Methylglyoxal comes of AGE. *Cell* 124, 258–260.
- (42) Desai, K. M., Chang, T., Wang, H., Baniges, A., Dhar, A., Liu, J., Untereiner, A., and Wu, L. (2010) Oxidative stress and aging: is methylglyoxal the hidden enemy? *Can. J. Physiol. Pharmacol.* 88, 273–284.
- (43) Cardoso, S., Carvalho, C., Marinho, R., Simoes, A., Sena, C. M., Matafome, P., Santos, M. S., Seica, R. M., and Moreira, P. I. (2014) Effects of methylglyoxal and pyridoxamine in rat brain mitochondria bioenergetics and oxidative status. *J. Bioenerg. Biomembr.* 46, 347–355.
- (44) Wang, H., Liu, J., and Wu, L. (2009) Methylglyoxal-induced mitochondrial dysfunction in vascular smooth muscle cells. *Biochem. Pharmacol.* 77, 1709–1716.
- (45) Fujimoto, K., and Polonsky, K. S. (2009) Pdx1 and other factors that regulate pancreatic beta-cell survival. *Diabetes, Obes. Metab.* 11, 30–37.
- (46) Kaneto, H., Matsuoka, T. A., Kawashima, S., Yamamoto, K., Kato, K., Miyatsuka, T., Katakami, N., and Matsuhisa, M. (2009) Role of MafA in pancreatic beta-cells. *Adv. Drug Delivery Rev.* 61, 489–496.
- (47) Wu, L., and Juurlink, B. H. (2002) Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. *Hypertension* 39, 809–814.
- (48) Cantero, A. V., Portero-Otin, M., Ayala, V., Auge, N., Sanson, M., Elbaz, M., Thiers, J. C., Pamplona, R., Salvayre, R., and Negre-Salvayre, A. (2007) Methylglyoxal induces advanced glycation end product (AGES) formation and dysfunction of PDGF receptor-beta: implications for diabetic atherosclerosis. *FASEB J.* 21, 3096–3106.
- (49) Yesil, P., and Lammert, E. (2008) Islet dynamics: a glimpse at beta cell proliferation. *Histol. Histopathol.* 23, 883–895.
- (50) Nass, N., Vogel, K., Hofmann, B., Presek, P., Silber, R. E., and Simm, A. (2010) Glycation of PDGF results in decreased biological activity. *Int. J. Biochem. Cell Biol.* 42, 749–754.
- (51) Du, J., Zeng, J., Ou, X., Ren, X., and Cai, S. (2006) Methylglyoxal downregulates Raf-1 protein through a ubiquitination-mediated mechanism. *Int. J. Biochem. Cell Biol.* 38, 1084–1091.
- (52) Thornalley, P. J. (1996) Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification—a role in pathogenesis and antiproliferative chemotherapy. *Gen. Pharmacol.* 27, 565–573.
- (53) Gao, Y., and Wang, Y. (2006) Site-selective modifications of arginine residues in human hemoglobin induced by methylglyoxal. *Biochemistry* 45, 15654–15660.
- (54) Bose, T., Bhattacharjee, A., Banerjee, S., and Chakraborti, A. S. (2013) Methylglyoxal-induced modifications of hemoglobin: structural and functional characteristics. *Arch. Biochem. Biophys.* 529, 99–104.
- (55) Ahmed, N., and Thornalley, P. J. (2005) Peptide mapping of human serum albumin modified minimally by methylglyoxal in vitro and in vivo. *Ann. N. Y. Acad. Sci.* 1043, 260–266.
- (56) Sen, S., Roy, M., and Chakraborti, A. S. (2011) Ameliorative effects of glycyrrhizin on streptozotocin-induced diabetes in rats. *J. Pharm. Pharmacol.* 63, 287–296.
- (57) An, S. H., Lee, M. S., and Kang, J. H. (2012) Oxidative modification of ferritin induced by methylglyoxal. *BMB Rep.* 45, 147–152.
- (58) Nigro, C., Raciti, G. A., Leone, A., Fleming, T. H., Longo, M., Prevenzano, I., Fiory, F., Mirra, P., D'Esposito, V., Ulianich, L., Nawroth, P. P., Formisano, P., Beguinot, F., and Miele, C. (2014) Methylglyoxal impairs endothelial insulin sensitivity both in vitro and in vivo. *Diabetologia* 57, 1485–1494.
- (59) Dobler, D., Ahmed, N., Song, L., Eboigbodin, K. E., and Thornalley, P. J. (2006) Increased dicarbonyl metabolism in endothelial cells in hyperglycemia induces anoikis and impairs angiogenesis by RGD and GFOGER motif modification. *Diabetes* 55, 1961–1969.
- (60) Giacco, F., and Brownlee, M. (2010) Oxidative stress and diabetic complications. *Circ. Res.* 107, 1058–1070.
- (61) Dhar, A., Dhar, I., Desai, K. M., and Wu, L. (2010) Methylglyoxal scavengers attenuate endothelial dysfunction induced by methylglyoxal and high concentrations of glucose. *Br. J. Pharmacol.* 161, 1843–1856.
- (62) Okouchi, M., Okayama, N., and Aw, T. Y. (2009) Preservation of cellular glutathione status and mitochondrial membrane potential by N-acetylcysteine and insulin sensitizers prevent carbonyl stress-induced human brain endothelial cell apoptosis. *Curr. Neurovasc. Res.* 6, 267–278.
- (63) Berlanga, J., Cibrian, D., Guillen, I., Freyre, F., Alba, J. S., Lopez-Saura, P., Merino, N., Aldama, A., Quintela, A. M., Triana, M. E., Montequin, J. F., Ajamieh, H., Urquiza, D., Ahmed, N., and Thornalley, P. J. (2005) Methylglyoxal administration induces diabetes-like microvascular changes and perturbs the healing process of cutaneous wounds. *Clin. Sci.* 109, 83–95.
- (64) Tajes, M., Eraso-Pichot, A., Rubio-Moscardo, F., Guiuernau, B., Bosch-Morato, M., Valls-Comamala, V., and Munoz, F. J. (2014) Methylglyoxal reduces mitochondrial potential and activates Bax and caspase-3 in neurons: Implications for Alzheimer's disease. *Neurosci. Lett.* 580, 78–82.
- (65) Los, M., Mozoluk, M., Ferrari, D., Stepczynska, A., Stroh, C., Renz, A., Herceg, Z., Wang, Z.-Q., and Schulze-Osthoff, K. (2002) Activation and Caspase-mediated Inhibition of PARP: A Molecular Switch between Fibroblast Necrosis and Apoptosis in Death Receptor Signaling. *Mol. Biol. Cell* 13, 978–988.
- (66) Porter, A. G., and Janicke, R. U. (1999) Emerging roles of caspase-3 in apoptosis. *Cell Death Differ.* 6, 99–104.

- (67) Chu, J. M., Lee, D. K., Wong, D. P., Wong, R. N., Yung, K. K., Cheng, C. H., and Yue, K. K. (2014) Ginsenosides attenuate methylglyoxal-induced impairment of insulin signaling and subsequent apoptosis in primary astrocytes. *Neuropharmacology* 85, 215–223.
- (68) Franke, T. F., Hornik, C. P., Segev, L., Shostak, G. A., and Sugimoto, C. (2003) PI3K/Akt and apoptosis: size matters. *Oncogene* 22, 8983–8998.
- (69) Huang, S. M., Chuang, H. C., Wu, C. H., and Yen, G. C. (2008) Cytoprotective effects of phenolic acids on methylglyoxal-induced apoptosis in Neuro-2A cells. *Mol. Nutr. Food Res.* 52, 940–949.
- (70) Di Loreto, S., Zimmiotti, V., Sebastiani, P., Cervelli, C., Falone, S., and Amicarelli, F. (2008) Methylglyoxal causes strong weakening of detoxifying capacity and apoptotic cell death in rat hippocampal neurons. *Int. J. Biochem. Cell Biol.* 40, 245–257.
- (71) Dhar, A., Desai, K., Kazachmov, M., Yu, P., and Wu, L. (2008) Methylglyoxal production in vascular smooth muscle cells from different metabolic precursors. *Metab., Clin. Exp.* 57, 1211–1220.
- (72) Liu, B. F., Miyata, S., Hirota, Y., Higo, S., Miyazaki, H., Fukunaga, M., Hamada, Y., Ueyama, S., Muramoto, O., Uriuhara, A., and Kasuga, M. (2003) Methylglyoxal induces apoptosis through activation of p38 mitogen-activated protein kinase in rat mesangial cells. *Kidney Int.* 63, 947–957.
- (73) Chan, W. H., Wu, H. J., and Shiao, N. H. (2007) Apoptotic signaling in methylglyoxal-treated human osteoblasts involves oxidative stress, c-Jun N-terminal kinase, caspase-3, and p21-activated kinase 2. *J. Cell. Biochem.* 100, 1056–1069.
- (74) Amicarelli, F., Colafarina, S., Cattani, F., Cimini, A., Di Ilio, C., Ceru, M. P., and Miranda, M. (2003) Scavenging system efficiency is crucial for cell resistance to ROS-mediated methylglyoxal injury. *Free Radical Biol. Med.* 35, 856–871.
- (75) Sakamoto, H., Mashima, T., Yamamoto, K., and Tsuruo, T. (2002) Modulation of heat-shock protein 27 (Hsp27) anti-apoptotic activity by methylglyoxal modification. *J. Biol. Chem.* 277, 45770–45775.
- (76) Lenzen, S. (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 51, 216–226.