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IMARS
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The first review article of this issue is written by Prof. Kei Fukami. Prof. Fukami at Kurume University School of Medicine, Kurume, Japan, focused on the link between glucagon-like peptide-1 (GLP-1) and AGEs-RAGE axis in diabetic nephropathy. GLP-1 is of current interest as a gut hormone lowering the blood glucose. He summarized the novel beneficial effect of GLP-1 on diabetic nephropathy by lowering the activation of AGEs-RAGE axis.

The second article is a memorial article by Dr. Naoto Koyama at Institute for Innovation, Ajinomoto Co., Inc., a winner of Young Investigator Award of the 22nd Japanese Maillard Reaction Society (JMARS) meeting held on December 21-22 in Tokyo last year. He demonstrated in vitro and in vivo vascular effects (vasodilative activity) of L-Arg-derived AGE, imidazolone such as MG-H1.

Further, this issue includes the article entitled “Dietary advanced glycation end products as an environmental contributor to type 1 diabetes” by Dr. Danielle J Borg at Mater Medical Research Institute, Queensland, Australia. This article focused on the impact of dietary AGEs on development of type 1 diabetes.

From this issue, Dr. Yukio Fujiwara, a new contributing editor, compiles “Papers of Editors’ Choice” in the section of Highlights of the glycation literature. This new effort will give you updated and valuable information.

The IMARS Highlights editors look forward to submission of your articles related to glycation in the field of food and medical sciences as always. Please contact us!

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Glucagon-Like Peptide-1-based therapies improve diabetic nephropathy by blocking AGEs-RAGE system

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AGEs-RAGE axis in diabetic nephropathy

Diabetic nephropathy is the most common cause of end-stage renal disease in the world, and accounts for significant increases in morbidity and mortality in patients with diabetes. According to the World Health Organization, it is expected that the number of patients with diabetes will rise to 370 million by 2030 in the world (1). It has also been reported that about 25-40% of type-1 or type-2 diabetic patients develop diabetic nephropathy within 20-25 year of the onset of diabetes (2), therefore development of the prevention therapy for diabetic nephropathy are urgently needed. From large clinical trials including UK Prospective Diabetes Study (UKPDS) (3) and Diabetes Control and Complication Trial (DCCT) (4), intensive control of blood pressure or glucose reduced development and progression of diabetic nephropathy, however, number of patients with end-stage renal failure induced by diabetes is still increasing despite of strict blood pressure or glycemic control.

Recently, advanced glycation end products (AGEs) have been focused on the pathogenesis of diabetic nephropathy due to their biochemical properties. AGEs accumulate in the glomeruli and tubulointerstitium as a result of hyperglycemia, aging, or uremia in patients with diabetes, and then alter the structure of kidney including glomerulosclerosis or interstitial fibrosis (5). There is a growing body of evidence that AGEs and receptor for AGEs (RAGE) interaction stimulates oxidative stress generation and subsequently evokes inflammatory reactions, thereby causing progressive alteration in renal architecture and loss of renal function in diabetes (5; 6). RAGE-overexpressing diabetic mice have been found to have progressive glomerulosclerosis with renal dysfunction, compared with diabetic littermates lacking the RAGE transgene (7). Diabetic homozygous RAGE null mice failed to develop significantly increased mesangial matrix expansion or thickening of the glomerular basement membrane (8). Furthermore, deletion of RAGE is also reported to prevent diabetic nephropathy in the OVE26 type-1 diabetic mouse, a model of progressive glomerulosclerosis, and decline of renal function (9). It is also suggested that generation of reactive oxygen species (ROS) are increased in diabetes and
subsequently progress diabetic renal damages. We have previously reported that AGEs stimulate intracellular ROS generation and subsequently activate renin-angiotensin system (RAS)-transforming growth factor-β (TGF-β) pathway through RAGE in rat mesangial cells (6). Numerous data have demonstrated the possible involvement of ROS and AGEs as early initiating events occurring long before morphological changes are obvious. ROS has also been known as an important source of AGEs such as pentosidine and Nε-carboxymethyllysine (CML) and accumulate in the kidney. Accumulation of AGEs not only plays an anatomical structural alteration, but also induces ROS-mediated mesangial cell apoptosis or hypertrophy via the RAGE, thereby contributing to in part to glomerular hyperfiltration, an early renal dysfunction in diabetes (10; 11).

**GLP-1-based therapies in diabetic nephropathy**

Glucagon-like peptide-1 (GLP-1) is one of the incretins, a gut hormone secreted from L cells, open-type interstitial epithelial endocrine cells located mainly in the distal ileum and colon, in response to food intake (12). GLP-1 is produced from the transcription product of the proglucagon gene expressed in the pancreas, intestine and brain. The active forms of circulating GLP-1 are GLP-1 (7-36) and GLP-1 (7-37), which result from the selective cleavage of the proglucagon molecule (13). Since GLP-1 not only augments glucose-induced insulin release from pancreatic β-cells, but also suppresses glucagon secretion and slows gastric emptying (12), and plasma intact GLP-1 levels are decreased after meal loading in type-2 diabetic patients compared with healthy subjects (14), it has been proposed as a potential therapeutic target for the treatment of patients with type 2 diabetes. However, native GLP-1 is rapidly degraded by the proteolytic enzyme, dipeptidyl peptidase-4 (DPP-4), and has a very short half-life (12). Therefore, DPP-4-resistant GLP-1 receptor (GLP-1R) agonists and DPP-4 inhibitors are clinically used as a GLP-1-based medicine for the treatment of diabetes. The biological actions of GLP-1 on pancreatic cells are mainly mediated by high-affinity GLP-1R (15). In addition, GLP-1R is shown to exist in extra-pancreatic tissues, including brain, peripheral nervous system, kidney, heart and vasculature (16; 17). These observations suggest that GLP-1 could act on extra-pancreatic tissues as well to elicit diverse biological reactions in diabetic complications. In the kidney, it has been reported that long-term treatment of GLP-1 analog exendin-4 ameliorates albumin excretion rate, glomerular hypertrophy, extracellular matrix accumulation (ECM) and increased pro-fibrotic cytokines such as TGF-β and type IV collagen expression seen in diabetic nephropathy through improving metabolic anomalies in db/db mice (18). Further, Kodera et al. have shown that administration of exendin-4 ameliorates renal injury through its anti-inflammatory and anti-profibrotic actions such as intercellular adhesion molecule-1 (ICAM-1) and type IV collagen, as well as decreasing oxidative stress and nuclear factor-κB (NF-κB) activation in kidney tissue without lowering blood glucose levels in a rat model of type-1 diabetes (19). Recent evidence suggests that renal protective effects of GLP-1 are mediated via the inhibition of angiotensin II (Ang II) actions on cRaf(Ser259) and diminished by diabetes because of protein kinase C-β (PKC-β) activation and the increased degradation of GLP-1R in the glomerular endothelial cells (20). Therefore, GLP-1 and GLP-1R are therapeutic targets for the pathogenesis of diabetic nephropathy.
Role of GLP-1 and AGEs-RAGE axis in diabetic nephropathy

Mesangial cells occupy a central anatomical position in the glomerulus, playing crucial roles in maintaining structure and function of glomerular capillary tufts (21). They actually provide structural support for capillary loops and modulate glomerular filtration by its smooth muscle activity (21-23). Increased MCP-1 expression associated with monocyte infiltration in mesangium has been observed in the early phase of diabetic nephropathy (24). Further, deficiency of MCP-1 was shown to markedly decrease albuminuria, renal injury and fibrosis in streptozotocin-induced diabetic mice (25). It has also been reported that AGEs-induced mesangial apoptosis and dysfunction may contribute in part to glomerular hyperfiltration, an early renal dysfunction in diabetes. Indeed, we have previously showed that AGEs induce apoptosis and overexpression of MCP-1 in cultured human mesangial cells (10). These observations suggest that AGEs accumulation in the glomeruli could be implicated in inflammatory and fibrogenic reactions in diabetic nephropathy as well via promoting the secretion of MCP-1 by mesangial cells.

GLP-1R is expressed in mesangial cells and proximal tubular cells (26; 27). We have recently found that administration of GLP-1 reduces RAGE mRNA and protein levels and subsequently inhibits the AGEs-induced ROS generation and MCP-1 expression in human cultured mesangial cells. Small interfering RNAs (siRNAs) raised against GLP-1R reduced GLP-1R level and blocked the GLP-1-induced down-regulation of RAGE mRNA level in mesangial cells (26). In addition, an analogue of cAMP, 8-bromo-cAMP mimicked the effects of GLP-1 on RAGE gene expression, ROS generation and MCP-1 mRNA level in mesangial cells. These findings suggest that GLP-1 could inhibit the harmful effects of AGEs-RAGE axis on mesangial cells via GLP-1R-mediated cAMP elevation (Fig.1). AGEs induce RAGE and MCP-1 expression in mesangial cells via NADPH oxidase-mediated ROS generation (28; 29). Therefore, it is conceivable that the AGEs-RAGE-induced ROS generation further augment RAGE expression in mesangial cells, making a vicious cycle and being involved in diabetic nephropathy. So, the positive feedback loop between RAGE and NADPH oxidase-mediated ROS generation may be a molecular target of GLP-1-GLP-1R-cAMP axis in mesangial cells (Fig. 1).

Nitric oxide (NO) is a multifunctional molecule critical to a number of physiologic and pathologic processes in humans (30; 31). NO not only inhibits inflammatory proliferative reactions in vascular wall cells but also exerts antithrombogenic and endothelial cell protective properties in vivo (30; 31). Therefore, impaired production and/or bioavailability of NO are considered to play a role in vascular complications in diabetes, such as diabetic nephropathy and cardiovascular disease (32). Indeed, circulating level of asymmetric dimethylarginine (ADMA), an endogenous NO synthase inhibitor, is increased in early diabetic nephropathy in type-1 diabetes and associated with future cardiovascular events in these individuals (33). Furthermore, we have previously found that serum levels of AGEs are positively associated with soluble form of RAGE and ADMA in patients with chronic kidney disease, thus suggesting the active involvement of the AGEs-RAGE system in the elevated levels of ADMA (34). Recently, we demonstrated that GLP-1 inhibited AGEs-induced RAGE gene expression, ROS generation, and protein arginine methyltransferase-1 (PRMT-1) mRNA levels, responsible enzymes that mainly
generate ADMA, and subsequently decreased ADMA level in cultured human proximal tubular cells. In addition, RAGE-Ab or an antioxidant N-acetylcysteine (NAC) was found to inhibit the AGEs-induced tubular cell gene expression of PRMT-1, a rate-limiting enzyme for ADMA generation (35). We also found that continuous intraperitoneal infusion of the GLP-1 analog exendin-4 inhibited renal RAGE gene expression, reduced urinary excretion level of 8-OHdG (an oxidative stress marker), improved histologic changes, and decreased PRMT-1, MCP-1 and ICAM-1 mRNA levels and ADMA generation. There is accumulating evidence that ADMA, an endogenous inhibitor of NO synthase, plays a role in endothelial dysfunction, cardiovascular remodeling, proteinuria and progression of renal damage in high-risk patients for cardiovascular disease, such as those complicated with diabetes and chronic kidney disease (32; 36; 37). Therefore, suppression of the AGEs-RAGE-induced ADMA generation by GLP-1 may be a novel therapeutic target for nephropathy and other vascular complications in diabetes.
References


Vascular Effects of Imidazolones, L-Arginine–derived Advanced Glycation End-products (AGEs)

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INTRODUCTION & AIM OF THIS STUDY

Because Maillard reaction promotes formation of various biologically active compounds, it could be utilized to add health-benefiting properties to foods. However, due to its chemical complexity, it is quite difficult to attribute a particular health effect to a single chemically-defined Maillard reaction product (MRP). Therefore, health implications of dietary MRPs are still controversial.

While there are many reports which relate advanced glycation end-products (AGEs) to a cardiovascular risk, Schmitt et.al. recently reported an endothelial nitric oxide synthase (eNOS) enhancing activity by a chemically-defined model MRP derived from maltol and propylamine, HMPP (3-hydroxy-2-methyl-1-propyl-4(1H)-pyridone)(¹). Because it is well established that NO produced by the eNOS is cardioprotective, their result may suggest that some MRPs are beneficial for prevention of cardiovascular diseases; however, its mode of action and in vivo effects are not known.

L-Arginine (Arg) is the sole substrate for NOS, and also a major target of glycation by carbohydrate-derived Maillard reaction intermediates, such as methylglyoxal (MG), to form a variety of Arg-derived AGEs including MG-derived hydromimidazolone (MG-H1) which is one of the major products(²). Although hydromimidazolone free adducts have been detected in some selected beverages and foods(³), health effect of the ingested compounds is scarcely investigated.

The aim of this study was to examine in vitro and in vivo vascular effects of Arg-derived imidazolones.
METHODS

L-Arg-derived imidazolones were synthesized by heating L-Arg (Ajinomoto, Tokyo, Japan) in the presence of D-glucose or dicarbonyls, such as methylglyoxal (MG; Sigma, St Louis, MO, USA) and 3-deoxyglucosone (3DG; Wako Pure Chemical Industries, Osaka, Japan), and purified with RP-HPLC-UV and cation exchange chromatography (CEC) as shown in Figure 1. For large-scale preparation of MG-H1 [1], L-Arg (0.5M) was incubated with MG (final conc. 0.05M) for 1hr at 30°C (pH13) and followed by the RP-HPLC purification. Structure and purity of purified imidazolones were determined with ESI-MS and 1H-NMR. A single peak in RP-HPLC-UV at 210nm was also confirmed for each material.

Vascular effects of the imidazolones on rat thoracic aortic rings were examined by isometric tension recording and eNOS phosphorylation assay. Effect of intravenously-, or orally-administered MG-derived hydroimidazolone isomer1 (MG-H1) on blood pressure was assessed in phenylephrine (PE; Sigma)-induced hypertensive rats with a polygraph system.

![Figure 1. Schematic overview of imidazolone preparation.](image)

RESULTS

A total of seven 5-hydro-, or 5-methylimidazolones ([1]-[7]) were structurally identified (Table 1). [1] and [7] were identical to the known imidazolones frequently abbreviated as ‘MG-H1’ (methylglyoxal-derived hydroimidazolone isomer 1) and ‘3DG-H1’ (3-deoxyglucosone-derived hydroimidazolone isomer 1), respectively. When subjected to in vitro isometric tension measurement, all of them relaxed rat thoracic aortic rings (Table 2). The vasodilation induced by these compounds occurred rapidly; usually started within a few minutes upon addition of test samples to the organ bath although the kinetics depended on sample concentration. Neither D-glucose, L-Arg nor the amadori rearrangement compound, fructosylarginine, were active in this setting (Table 2).
Table 1. L-Arg-derived imidazolones separated and identified in this study and their structures.

<table>
<thead>
<tr>
<th>ID</th>
<th>Chemical Name</th>
<th>MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>N6-(5-hydro-5-methyl-4-imidazolon-2-yl)-L-ornithine (methylglyoxal-derived hydroimidazolone isomer1; MG-H1)</td>
<td>228</td>
</tr>
<tr>
<td>[2]</td>
<td>N6-(5-methyl-5-hydroxymethyl-4-imidazol-2-yl)-L-ornithine</td>
<td>258</td>
</tr>
<tr>
<td>[3]</td>
<td>N6-[5-hydro-5-(2-hydroxyethyl)-4-imidazol-2-yl]-L-ornithine</td>
<td>258</td>
</tr>
<tr>
<td>[4]</td>
<td>N6-[5-methyl-5-(1,2-dihydroxyethyl)-4-imidazol-2-yl]-L-ornithine</td>
<td>288</td>
</tr>
<tr>
<td>[5]</td>
<td>N6-[5-hydro-5-(2,3-dihydroxypropyl)-4-imidazol-2-yl]-L-ornithine</td>
<td>288</td>
</tr>
<tr>
<td>[6]</td>
<td>N6-[5-methyl-5-(1,2,3-trihydroxypropyl)-4-imidazol-2-yl]-L-ornithine</td>
<td>318</td>
</tr>
<tr>
<td>[7]</td>
<td>N6-[5-hydro-5-(2,3,4-trihydroxybutyl)-4-imidazol-2-yl]-L-ornithine</td>
<td>318</td>
</tr>
<tr>
<td></td>
<td>(3-deoxyglucosone-derived hydro-imidazolone isomer1; 3DG-H1)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. *In vitro* vasodilative activity of the imidazolones and related compounds.

<table>
<thead>
<tr>
<th>Test samples</th>
<th>% Maximal relaxation at 100μM</th>
<th>EC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidazolones:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1] ‘MG-H1’</td>
<td>95 ± 2</td>
<td>13</td>
</tr>
<tr>
<td>[2]</td>
<td>92 ± 2</td>
<td>nt</td>
</tr>
<tr>
<td>[3]</td>
<td>62 ± 8</td>
<td>71</td>
</tr>
<tr>
<td>[4]</td>
<td>49 ± 3</td>
<td>nt</td>
</tr>
<tr>
<td>[5]</td>
<td>91 ± 3</td>
<td>nt</td>
</tr>
<tr>
<td>[6]</td>
<td>93 ± 3</td>
<td>nt</td>
</tr>
<tr>
<td>[7] ‘3DG-H1’</td>
<td>90 ± 5</td>
<td>25</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Amadori products:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructosylarginine</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Vasodilative activity of test samples was examined by isometric tension measurement of rat thoracic aortic rings (2-3 mm in length) pre-contracted with 1μM phenylephrine (PE). Results were expressed as percentage of the maximal relaxation at indicated concentration or as EC50 value.

The activities of the tested imidazolones were almost completely abrogated by endothelium-denudation, an eNOS inhibitor (L-NAME), a soluble guanylate cyclase
inhibitor (ODQ) and a NO scavenger (cPTIO), but not by a cyclooxygenase inhibitor, indomethacin (Table 3). The imidazolones [2], [3], [7] also induced phosphorylation of eNOS-Ser1177 and Akt-Ser473 in a concentration-dependent manner, which was inhibited by a PI3K inhibitor, wortmannin (Figure 2).

Table 3. Effects of endothelium removal and various inhibitors on the vasodilative activity of the imidazolones

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Intact (control)</td>
<td>93 ± 3</td>
<td>62 ± 8</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>−Endothelium</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+L-NAME (100μM)</td>
<td>0</td>
<td>2 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>+cPTIO (300μM)</td>
<td>10 ± 4</td>
<td>13 ± 6</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>+ODQ (1μM)</td>
<td>4 ± 2</td>
<td>1 ± 1</td>
<td>2 ± 0</td>
</tr>
<tr>
<td>+Indomethacin (10μM)</td>
<td>95 ± 3</td>
<td>65 ± 9</td>
<td>93 ± 2</td>
</tr>
</tbody>
</table>

L-NAME; Nω-nitro-L-arginine methyl ester, cPTIO; 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, ODQ; 1H[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one

Rat thoracic aortic rings (10-15 mm in length) were pre-incubated with or without of a PI3K inhibitor, wortmannin (Wo; 200 nM), for 20 min in Hanks solution, then treated with purified imidazolones ([2], [3], and [7]) for 5 min. The lumen of each ring was gently washed on ice with 40 μL of 1% triton X-100 lysis buffer supplemented with protease and phosphatase inhibitor cocktails to obtain endothelium-rich lysate. The lysate was subjected to western blot analysis for (phospho-)eNOS and (phospho-)Akt.

Orally-administered MG-H1 (100 mg/kg) was rapidly absorbed and appeared in circulation of mice (Figure 3). Time to peak plasma concentration (T_{max}) and the peak plasma concentration (C_{max}) were ≤15 min and ≥174 μM, respectively.
MG-H1 [1] aqueous solution was orally administered to each male ICR mouse at 100 mg/kg body weight. Blood was drawn from inferior vena cava periodically for 6 hours and MG-H1 in the plasma was measured by LC-MS.

Intravenously-, or orally-administered MG-H1 significantly decreased blood pressure (BP) in PE-induced hypertensive rats (Figure 4). To assess whether there is an additive or synergistic effect of MG-H1 and L-Arg (substrate for NOS) on PE-induced hypertension, MG-H1 was orally administered under infusion with L-Arg via femoral vein. Reduction in BP with MG-H1 co-administered with L-Arg was greater than with each compound alone (Figure 5).

BP in Male Wistar rats (9-10 wk) was monitored via the right carotid artery cannulated and connected to a pressure transducer coupled with polygraph system. PE was infused via jugular vein at a rate of 75 mg/kg/min to raise mean arterial pressure to approx. 160 mmHg. After BP stabilization, MG-H1 (40 mg/kg) was infused into femoral vein. MG-H1 (1 g/kg) was also administered orally by gastric intubation.
DISCUSSION

Because, to a greater or lesser extent, all the imidazolones but L-Arg, D-glucose and fructosylarginine relaxed aortic rings, it was suggested that Maillard reaction generated vasodilative products and the imidazolone structure contributed to the effect. Imidazolone [1] and [7] were identical to the known imidazolones frequently abbreviated as ‘MG-H1’ (methylglyoxal-derived hydroimidazolone isomer 1) and ‘3DG-H1’ (3-deoxyglucosone-derived hydroimidazolone isomer 1), respectively, while the others were CAS-unregistered minor imidazolones. Although we did not analyze the reaction mechanism to form these minor imidazolones in this study, these compounds might be the derivatives of α-oxoaldehydes formed from D-glucose as is the case with MG-H1 and 3DG-H1. To the best of our knowledge, this is the first report to demonstrate vasodilative activity of structurally-defined arginine-derived AGE, imidazolones.

We are not sure which structure of the imidazolones is critical for the activity, since differences in the biological activity among these compounds were relatively small. A methyl group in the 5-position of imidazolone ring and structures of sugar- or dicarbonyl-derived moieties did not seem to affect the activity strongly. Taking into account stereoisomers and possible keto-enol tautomerization, precise studies of structure/activity relationships are warranted to determine structure critical for the vascular effect.

In vitro vasodilative activity of the imidazolones is possibly due to an indirect enhancement of eNOS activity via stimulation of PI3K/Akt signaling pathway (Figure 6), because the activity was shown to be endothelium-dependent, inhibited by NO-cGMP axis inhibitors (Table 3), accompanied by eNOS-Ser1177 phosphorylation,
and PI3K inhibitor-sensitive (Figure 2), while not affected by a cyclooxygenase inhibitor, indomethacin (Table 3). The possibility that L-Arg released by decomposition of imidazolones was provided as a substrate for eNOS is unlikely, because L-Arg itself did not relax aortic rings in this experimental setting.

Interaction between eNOS and MG-H1 has been investigated by Lai et.al. They found that MG-H1 inhibited all of three NOS isoforms (eNOS, nNOS, and iNOS) in a cell-free enzymatic study. However, IC$_{50}$ value of MG-H1 was 1280 M which was much higher concentration than that required to induce vasorelaxation in our study (EC$_{50}$; 13 µM). The authors concluded in their literature that it is unlikely that glycated L-arginine derivative, MG-H1, competitively inhibits the enzymatic activities of the three isoforms of NOS in physiological system because the IC$_{50}$ values are too high relative to MG-H1 concentration in plasma (0.11 – 5.5 µM). On the other hand, a lack of eNOS enhancing activity in their cell-free system indicates that a direct activation of eNOS by imidazolones is unlikely and is consistent with our idea that the imidazolones enhance eNOS activity indirectly.

At this point, cellular molecule(s) which mediate(s) the imidazolone-induced PI3K/Akt activation is unknown. The hydroimidazolones has been considered to be the most likely candidate epitope for specific binding of AGE-modified proteins to AGE receptors; however, to date, whether or not low-molecular-weight imidazolone free adducts can bind to and activate AGE receptors is obscure. Although we have evaluated binding activity of MG-H1 to some selected receptors which are known to be involved in vascular tonus control and/or eNOS activation, a good candidate for its receptor has yet to be found (data not shown). A more comprehensive evaluation of molecules including AGE receptors might be necessary.

![Figure 6. Putative eNOS activation by imidazolones.](image)

AA; arachidonic acid, ACase; adenylate cyclase, COX; cyclooxygenase, eNOS; endothelial nitric oxide synthase, Indo; indomethacin, sGCase; soluble guanylate cyclase, Wo; wortmannin
MG-H1 was quickly absorbed into the peripheral circulation of mice and gradually returned to the basal level by 6 hours when it was administered orally (Figure 3). To assess in vivo effects of MG-H1, we employed the PE-induced hypertensive rat model to mimic the in vitro isometric tension measurement system. Rats were dosed either intravenously or orally with purified MG-H1, and significant reduction of BP was observed in both cases. Slower and long-lasting BP reduction was seen in orally-administered rats whereas rapid and transient reduction was seen in i.v.-administered rats (Figure 4). This was presumably a reflection of difference in pharmacokinetics of MG-H1 in each administration setting. The BP-lowering effects in rats may be a consequence of enhancement of eNOS activity by MG-H1. If MG-H1 indirectly stimulate eNOS activity, additive or synergistic effects could be seen when co-administered with eNOS substrate, L-Arg. Greater reduction in BP with MG-H1 co-administered with L-Arg (Figure 5) may support the above notion; however, further studies are required to obtain more direct and convincing evidences for the possible eNOS stimulation in vivo, such as an increased level of NOx and/or cGMP in blood or tissue.

Although both the eNOS enhancing and anti-hypertensive effects are generally recognized as favorable features for functional foods and medicines, actual health relevance of the effects of imidazolones remains elusive because our animal model is artificially produced by PE infusion. In addition, we only examined acute effects of MG-H1 while chronic effects of AGEs are matters of concern. Therefore, impact of imidazolones on health should be evaluated carefully in other disease model animals such as spontaneous hypertensive animals with impaired endothelial function in longer term experiments.

Ahmed et al. reported that MG-H1 free adduct present in raw bovine milk was approximately 50 nM (3). Reported free MG-H1 concentration in normal human plasma (110 nM (6), 583 nM (7)) may suggest the limited impact of regular food-derived MG-H1 on human plasma MG-H1 concentration. However, MG-H1 is not the only imidazolone formed by Maillard reaction as we have shown here. Further studies to illustrate total imidazolones profile in thermally-processed foods are needed to evaluate health impact of regular intake of food-derived imidazolones.

In conclusion, our study is the first to demonstrate in vitro and in vivo vascular effects of chemically-defined L-Arg-derived AGE, imidazolones. These results could be of help to elucidate health relevance of both endogenous and food-derived imidazolones and also to develop strategies for designing food functionality by utilizing Maillard reaction.
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Dietary advanced glycation end products as an environmental contributor to type 1 diabetes

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Type 1 diabetes (T1D) is defined by the autoimmune destruction of the insulin-producing beta cells, within the pancreatic islets of Langerhans. The growing prevalence of T1D in the last three decades suggests a change in environmental stimuli rather than variations in genetic susceptibility [1]. Several putative non-genetic factors resulting in likely risk for T1D have been suggested, including increased maternal age at delivery, maternal enteroviral infections and caesarean section. Recently, focus has been on early dietary changes as a potential environmental contributor to T1D [2]. We are particularly interested in advanced glycation end products as a causative trigger for T1D.

Advanced glycation end products (AGEs) result from the late stages of the Maillard reaction whereby reactive carbonyls produced by the degradation of Amadori products, react with amino groups of proteins [3]. Recognised sources of AGEs include endogenous production within the body, and that which are consumed in the modern diet. Of particular note, previous studies have shown the correlation between dietary AGEs and circulating AGE levels within humans [4] and rodents [5]. Changes associated with pancreatic beta cell function and survival have been supported by reports demonstrating an imbalance of homeostatic levels of AGEs. In the mouse model of autoimmune T1D, the non-obese diabetic (NODShiLt) mouse, when treated with an AGE-restricted diet, fewer NODShiLt mice developed autoimmune diabetes, compared to NODShiLt mice fed with standard chow; a diet rich in AGEs [6]. Moreover, our group confirmed lowering circulating AGEs by alagebrium chloride, an AGE lowering agent, decreased the incidence in autoimmune diabetes in NODShiLt mice [7]. These studies also demonstrated severe beta cell dysfunction in rodents fed with high AGE diets or injected with glycated albumin, including a decline in first phase insulin secretion [7] and high plasma glucose with lower fasting plasma insulin [6, 7]. Recent in vitro data suggests a direct association between the impairment of insulin secretion and pathogenic levels of AGEs. High levels of AGE were shown to induce inducible nitric oxide synthase dependent pathways affecting beta cell membrane depolarisation and thus insulin release [8]. Evidence of modifications to the insulin gene promoter, ultimately resulting in the suppression of insulin gene transcription, have also been reported [9].

Early islet inflammation leading to the infiltration of islets by mononuclear cells is known as insulitis and it is a common hallmark of T1D. Insulitis likely results from the cross-talk between the immune cells and pancreatic beta cells, mutual cytokine secretion and continued release of beta cell self-antigen, leading to the activated cell death of beta cells and subsequent autoimmunity [10]. Surprising results from our recent study demonstrated in healthy rats with no predisposition to
autoimmune diabetes, chronic injection of glycated rat serum albumin led to substantial insulitis compared to rats injected with non-glycated albumin [7]. Similar mononuclear islet infiltration was seen in high AGE fed NODShiLt offspring bred from dams who to were fed a high AGE diet compared to NODShiLt offspring given reduced AGE diets [6]. Taken together, these findings highlight the unfavourable effect of imbalanced AGE levels on pancreatic beta cells, regardless of T1D genetic susceptibility.

Several critical questions still remain to be answered for future research. Firstly, how do the combination of AGES and their receptors impact epigenetically on T1D resistance and susceptibility genes? How much protection is given once metabolic dysfunction is corrected in combination with an autoimmune setting? Finally, the level of interaction between circulating AGE levels and other environmental factors which may contribute to T1D, like gut microbiota [2], requires further investigation.

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Glyoxalase Centennial: 100 Years of Glyoxalase Research and Emergence of Dicarbonyl Stress 27—29 November 2013, University of Warwick (UK)

The Biochemical Society Focused Meeting “Glyoxalase Centennial: 100 years of Glyoxalase Research and Emergence of Dicarbonyl Stress” will be held at University of Warwick (Coventry, UK) on November 27-29, 2013.

Meeting background
This year is the centenary of the Glyoxalase System. It provides protection against damaging modification of proteins and nucleotides by methylglyoxal. Methylglyoxal accumulation - “dicarbonyl stress” – may provide the basis for improved understanding of mechanisms of disease and health decline in ageing, and suggest new strategies for therapeutics and functional food development. Please join us.

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