Activation of Macrophages and Microglia by Interferon–γ and Lipopolysaccharide Increases Methylglyoxal Production: A New Mechanism in the Development of Vascular Complications and Cognitive Decline in Type 2 Diabetes Mellitus?

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Abstract. Methylglyoxal (MGO), a dicarbonyl compound derived from glucose, is elevated in diabetes mellitus and contributes to vascular complications by crosslinking collagen and increasing arterial stiffness. It is known that MGO contributes to inflammation as it forms advanced glycation end products (AGEs), which activate macrophages via the receptor RAGE. The aim of study was to investigate whether inflammatory activation can increase MGO levels, thereby completing a vicious cycle. In order to validate this, macrophage (RAW264.7, J774A.1) and microglial (N11) cells were stimulated with IFN-γ and LPS (5 + 5 and 10 + 10 IFN-γ U/ml or μg/ml LPS), and extracellular MGO concentration was determined after derivatization with 5,6-Diamino-2,4-dihydroxypyrimidine sulfate by HPLC. MGO levels in activated macrophage cells (RAW264.7) peaked at 48 h, increasing 2.86-fold (3.14 ± 0.4 μM) at 5 U/ml IFN-γ + 5 μg/ml LPS, and 4.74-fold (5.46 ± 0.30 μM) at 10 U/ml IFN-γ+10 μg/ml LPS compared to the non-activated controls (1.15 ± 0.02 μM). The other two cell lines, J774A.1 macrophages and N11 microglia, showed a similar response. We suggest that inflammation increases MGO production, possibly exacerbating arterial stiffness, cardiovascular complications, and diabetes-related cognitive decline.

Keywords: Cognitive decline, inflammation, methylglyoxal, type 2 diabetes mellitus, vascular complications

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia. Epidemiological and clinical studies have shown that patients with T2DM are at an increased risk for developing vascular dementia (2.3-fold) [1–4] and Alzheimer’s disease (AD)-type dementia (1.8-fold) [5, 6]. A longitudinal cohort study has demonstrated that patients with T2DM are also at a 65% increased risk for developing AD [6]. The mechanistic pathways underlying the development of dementia in T2DM are unclear, but it is likely to involve neurodegenerative [7, 8], cerebrovascular, and inflammatory
mechanisms [9]. Since T2DM is characterized by hyperglycemia, the association of cognitive decline can be explained by the pathological consequences caused by excess levels of glucose in body. In general, the process of storing surplus glucose as glycogen in insulin sensitive tissues, such as skeletal muscle, adipose tissue, or liver [10], is disturbed in T2DM, leading to hyperglycemia and resulting diabetic complications [11, 12].

Due to the failure of the glucose uptake mechanism (in insulin sensitive tissues) in hyperglycemic situations [13], circulating glucose molecules can react with specific amino acids of tissue proteins to form Schiff’s bases (an unstable product), which undergo rearrangement to produce Maillard products called advanced glycation end products (AGEs) [14, 15]. Pathological consequences of AGEs formation involve the formation of highly reactive α-dicarbonyl compounds or AGE precursors such as glyoxal (GO), methylglyoxal (MGO), and 3-deoxyglucosone (3-DG) [16]. Among them MGO can also be generated either via changes in glycolysis and the pentose phosphate pathway [17] or by lipid and amino acid oxidation [18, 19]. In turn, in vivo, highly reactive α-dicarbonyl compounds (e.g., MGO) can also react with amino acids (e.g., lysine) to form AGEs (e.g., methylglyoxal-lysine dimer) [20, 21]. In addition, AGEs are also derived from dietary components such as red meat, cheese, sugar-sweetened fruit drinks, sports drinks, mixed fruit juices, confectionery (snacks), dried fruits, cakes, and cereals [22]. Consumption of an AGE-rich meal (3-fold higher), in comparison to a normal diet, increases the level of AGEs 1.5-fold in serum [23]. Studies have shown that the rate of formation and the levels of AGEs are increased in T2DM compared to non-diabetics [24, 25].

It has been suggested that AGEs contribute to the complications of T2DM, including retinopathy [26–28], neuropathy [15], nephropathy [29], and cognitive impairment [30–33] by different modes of action—physical and via receptor (RAGE) mediated effects [32].

Chronic interaction of circulating AGEs or glucose (due to hyperglycemia) with long lived proteins, such as collagen, laminin, or elastin, within the extracellular matrix of the vascular basement membrane increases the risk of cross linking leading to arterial stiffening [34–36]. Arterial stiffening causes vascular smooth muscle cell dysfunction followed by impaired vasoilation, increased vasoconstriction, proliferation, and migration [36–39]. Simultaneously AGEs cross linking causes the quenching of nitric oxide (NO) [40, 41], an endothelium derived relaxing factor released due to physical stimuli [42–44]. This, in turn, stimulates vascular endothelial growth factor [45–47] to activate monocytes and endothelial nitric oxide synthase in response to decreased endothelial NO. Activated monocytes infiltrate and differentiate into macrophages at the arterial wall [48–50]. In due course, this traps oxidized lipids, forming foam cells [51–53]. Foam cells stimulate the proliferation of macrophages, which, in turn, attract T-lymphocytes to induce smooth muscle cell proliferation (angiogenesis) in the arterial walls [54, 55], followed by collagen accumulation [56, 57]. Finally, these events result in the formation of a lipid-rich atherosclerotic lesion with a fibrous cap [58–60].

On the other hand, circulating AGEs bind to RAGE [61] and activate various downstream signaling pathways, including the activation of nuclear factor (NF)-κB in addition to oxidative stress [24, 62]. Translocation of NF-κB into the nucleus mediates the expression of pro-inflammatory genes, including intercellular adhesion molecule (ICAM)-1, which facilitate monocyte adhesion to vascular endothelial cells [63–67]. Monocytes develop into macrophages in the tunica intima, which recruits and accumulates lipids on the artery wall, resulting in an enhanced fatty streak formation [68, 69]. Over time, they develop into advanced lesions, characterized by smooth muscle cell accumulation, necrotic core formation, and lipid accumulation. Some advanced lesions in the arteries, in due course, form atherosclerotic plaques which can cause blood flow impedance or become unstable, leading to rupture or stenosis [70, 71].

Either of these events, arterial stiffening or atherosclerotic blockage of arteries (e.g., internal carotid artery), may lead to stroke in T2DM [72–74] due to elevated levels of AGEs and its precursors. Post-traumatic changes in brain due to stroke results in various events, such as excitotoxicity through glutamate accumulation [75], acidotoxicity (toxicity due to lactic acidosis) and ionic imbalance [76], peri-infarct depolarization [77], oxidative and nitrative stress [78, 79], inflammation and apoptosis [80]. These molecular level changes lead to neuronal cell loss and might play a significant role in the development of cognitive decline in patients with T2DM [81–85].

Thus, a number of studies have correlated the levels of AGEs [31, 86, 87] and MGO [86, 88] with cognitive decline in T2DM patients. Simultaneously, the elevated levels of MGO have been correlated
with increased pro-inflammatory mediators in T2DM patients with diabetic nephropathy [89], supporting a link between AGEs and inflammation. However, it is not clear if inflammation in turn might lead to the production of increased levels of MGO, leading to higher AGE production, creating a vicious cycle between AGEs and inflammation. To solve this question, this study investigates if pro-inflammatory activation (e.g., with lipopolysaccharide [LPS] and interferon [IFN]-γ) of macrophages and microglial cells might lead to increased MGO production (leading to more AGE formation), completing this vicious cycle.

MATERIALS AND METHODS

Materials

RAW 264.7, J774A.1 cells, LPS (Escherichia coli serotype 0127:B8), resazurin sodium, trypan blue, 5,6-diamino-2,4-dihydroxypyrimidine sulfate (DDP) and diethylenetriaminepentaacetic acid (DTPA) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). N11 cells were a gift from Birgit Kraus, University of Regensburg, Germany. Cell culture reagents, including Dulbecco’s modified eagle’s medium (DMEM), penicillin, streptomycin, fetal bovine serum (FBS), and L-glutamine were purchased from Merck, (Australia). Murine IFN-γ was purchased from Peprotech (Lonza Australia Pty Ltd, Vic, Australia). HPLC grade methanol, disodium hydrogen phosphate (Na2HPO4), and sodium dihydrogen phosphate (NaH2PO4) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). N11 cells were a gift from Birgit Kraus, University of Regensburg, Germany. Cell culture reagents, including Dulbecco’s modified eagle’s medium (DMEM), penicillin, streptomycin, fetal bovine serum (FBS), and L-glutamine were purchased from Merck, (Australia). Murine IFN-γ was purchased from Peprotech (Lonza Australia Pty Ltd, Vic, Australia). HPLC grade methanol, disodium hydrogen phosphate (Na2HPO4), and sodium dihydrogen phosphate (NaH2PO4) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). N11 cells were a gift from Birgit Kraus, University of Regensburg, Germany. Cell culture reagents, including Dulbecco’s modified eagle’s medium (DMEM), penicillin, streptomycin, fetal bovine serum (FBS), and L-glutamine were purchased from Merck, (Australia). Murine IFN-γ was purchased from Peprotech (Lonza Australia Pty Ltd, Vic, Australia). HPLC grade methanol, disodium hydrogen phosphate (Na2HPO4), and sodium dihydrogen phosphate (NaH2PO4) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). N11 cells were a gift from Birgit Kraus, University of Regensburg, Germany. Cell culture reagents, including Dulbecco’s modified eagle’s medium (DMEM), penicillin, streptomycin, fetal bovine serum (FBS), and L-glutamine were purchased from Merck, (Australia).

Activation of cells and collection of supernatants

The cell lines (RAW 264.7, J774A.1, and N11) were maintained in 10% FBS in DMEM supplemented with penicillin (200 U/mL) and streptomycin (200 µg/mL) in a humidified atmosphere at 37°C with 5% CO2, as described previously [90–92]. Briefly, the cells were cultured in T75cm² flasks and passaged at a confluency of 80%. For the activation experiments, 60,000 cells/well were plated into the inner 80 wells (Greiner plate) or all wells (Eppendorf plate) of a 96-well microtiter plate and incubated overnight at 37°C with 5% CO2. After 24 h, the medium was removed and replaced with fresh 0.1% FBS in DMEM. The cell lines were stimulated with LPS and IFN-γ at a final concentration of 5U IFN-γ/ml plus 5 µg/ml LPS and 10 U IFN-γ/ml plus 10 µg/ml LPS. Medium was collected at 0, 12, 24, 36, 48, 60, and 72 h.

Determination of MGO level

A modified protocol of the derivatization methodology previously established by Espinosa-Mansilla et al. [93] was used. A pre-column derivatization technique was used, due to the low absorbance of MGO, which included the conversion of MGO to methylllumazine (a highly fluorescent pteridine derivative) using DDP. For precipitation and release of MGO from proteins, 20 µL of 55% trichloroacetic acid was added to 180 µL of the collected cell culture medium. The sample was vortexed and incubated at room temperature for 20 min to allow for MGO release and protein precipitation to occur. The sample was centrifuged at room temperature for 20 min at 15000 rpm in an Eppendorf mini centrifuge. Following centrifuging, 150 µL of the supernatant was transferred into a labelled thermo-resistant plastic vial containing 70 µL of 2.5 mM sodium phosphate buffer (pH 9.5) and 35 µL of 5 mM DTPA chelator. Finally, 5 µL of 3 mM DDP was added and incubated at 60°C for 2 h. After incubation, 14.5 µL of HCl (37%) was added to adjust the pH to 4.0 and 50 µL of the sample was injected into the chromatographic system. A Dionex™ HPLC system equipped with a P680 HPLC pump, ASI-100 automated sample injector, column oven (thermostated column compartment TCC-100) equipped with a phenyl-hexyl column at 40°C, PDA UV/Visible detector, and a RF-2000 fluorescent detector were used. The Chromleone 6.7 chromatography data system from Dionex™ was used to control the instrument (pump, column oven, and detectors), acquire data, and quantify the peak areas. A composition of formic acid (0.01%)/water (solvent A) and methanol (solvent B) with gradient elution system at a flow rate of 0.75 ml/min was used as a mobile phase. Methylllumazine was quantified via fluorescence intensity (excitation/emission 330/460 nm) using a standard curve (external calibration: slope, m = 19.69; Intercept, C = 0.06).

Determination of cell viability

Cell viability was assessed using a colorimetric assay involving the cellular reduction of resazurin to resorufin. Resazurin was dissolved in PBS to
give a concentration of 0.001% (w/v). To determine cell viability, incubation media was aspirated from wells and replaced with 100 μL of resazurin solution and incubated at 37°C for 1 h. After 1 h incubation, the fluorescence of formed resorufin was measured with excitation at 530 nm and emission at 590 nm using a POLARstar Omega microplate reader (BMG Labtech, Mornington, Australia) [91, 94].

**Measurement of nitrite levels by the Griess assay**

Nitric oxide release was determined by the Griess reagent quantification of nitrite (NO\(^-\)) as described previously [95]. The Griess reagent was made up of equal volumes of 1% sulphanilamide and 0.1% napthyethylene-diamine in 5% HCl. From each well, 50 μL of cell culture medium was transferred to a fresh 96-well plate and mixed with 50 μL of Griess reagent. This reagent forms a violet color in the presence of nitrite [95]. The absorbance was read at 540 nm using a POLARstar Omega microplate reader (BMG Labtech, Mornington, Australia).

**Data calculation**

All experiments were carried out in triplicate with the results being expressed as mean ± standard deviation (SD). Statistical analysis was performed using the software Graph Pad Prism 5. A one-way analysis of variance (one way ANOVA) was used for quantitative data when comparing differences between at least three independent groups. If significant, a post hoc analysis using Dunnett’s test was performed.

**RESULTS**

**Effect of inflammatory activation of macrophages and microglia on MGO production**

The purpose of this study was to investigate if inflammatory activation increases the production of MGO. Macrophages (RAW264.7 and J774A.1) and microglia (N11) were activated with two different concentrations of LPS and IFN-γ (5 + 5 or 10 + 10U IFN-γ/ml and 5 μg/ml LPS). Following activation, cell culture medium was collected at specific time intervals (0, 12, 24, 36, 48, 60, and 72h), and the concentration of MGO (and NO as a measure of inflammatory activation) and cell viability was determined.

Inflammatory activation of the cells was confirmed by monitoring the production of NO (measured as nitrite) (Fig. 1, for e.g., only results of the RAW 264.7 cells is shown). Nitrite levels in the medium increased steadily over 72 h in all three cell lines activated with 5U IFN-γ/ml plus 5 μg/ml LPS and 10U IFN-γ/ml plus 10 μg/ml LPS). Cell viability remained greater than 80%, up to 72 h, for all three cell lines (Fig. 2, for e.g., only results of the RAW 264.7 cells is shown).

A background concentration of MGO in fresh cell culture media was found to be 0.26 ± 0.01 μM (Fig. 3a-c). In the following time-course experiments, the levels of MGO in the non-activated cells were also determined. MGO levels rose from 0.26 μM at 0 h to no more than 1.6 μM in all three cell lines over 72 h (Fig. 3a-c).

In cells activated with 5U IFN-γ /ml plus 5 μg/ml LPS, the production of MGO rapidly increased in the first 48 h of the experiment, reaching 3.14 ± 0.4 μM in RAW 264.7 cells, 2.72 ± 0.57 μM in J774A.1 cells, and 2.68 ± 0.06 μM in N11 cells at 48 h. In samples activated with 10U IFN-γ/ml plus 10 μg/ml LPS, the production of MGO showed larger increase, with levels reaching 5.46 ± 0.3 μM in RAW 264.7 cells, 3.78 ± 0.1 μM in J774A.1 cells, and 4.03 ± 0.34 μM in N11 cells at 48 h (Fig. 3a-c).
DISCUSSION

In this study, we show that pro-inflammatory activation of macrophages and microglia using a mixture of LPS and IFN-\(\gamma\) increases the production of MGO in a time- and dose-dependent manner. As approximately 70–80% of the cells were viable after 36–48 h (Fig. 2), and MGO is cell membrane permeable [96, 97], it is likely that MGO was released continuously from cells during inflammatory activation, and not because of necrosis and/or cell wall rupture.

To understand which pathways are involved in inflammatory activation, and to offer a therapeutic target for intervention which might be useful to reduce MGO levels, we describe briefly the signaling pathways for our inflammatory assay. LPS and IFN-\(\gamma\) elicit their effects via distinct receptors such as the toll like receptor-4 (TLR-4) [98, 99] and type 2 IFN [100, 101] receptor, respectively. These receptors are expressed on the cell surface of the macrophage and microglial cells [102–104]. In general, LPS binds to LBP (LPS-Binding Protein), which delivers LPS to CD (cluster of differentiation)-14, a high-affinity LPS receptor to form CD14-LPS complex [105, 106]. CD14 chaperones LPS to the ectodomain area of the TLR-4 containing a protein called myeloid differentiation factor 2 (MD2) [98, 107, 108]. This leads to receptor dimerization with its lateral TLR4-MD2-LPS complex and activates an intracellular signaling cascade via myeloid differentiation primary response protein 88 (MYD88) and/or TIR domain-containing adaptor protein inducing IFN-\(\beta\) (TRIF) pathways [98, 104]. MYD88 activation leads to the production of cytokines and chemokines in addition to cell survival, whereas the TRIF pathway
leads with inflammasome activation and type I interferon production [98, 100].

MYD88 pathway regulates the phosphorylation of IKK (inhibitor of κB kinase) via activation of various downstream proteins, such as IRAK (interleukin-1 receptor associated- kinase), TRAF6 (tumor necrosis factor receptor associated factor-6), and TAK1 (TGF-β activated kinase-1) [109]. This leads to phosphorylation of the I-kappaBα:p50:p65 complex and releases the transcription factor, p50:p65 domains called nuclear factor-kappaB (NF-κB). NF-κB translocates from the cytoplasm into the nucleus, where it interacts with the DNA and triggers transcription of the inducible nitric oxide synthase (iNOS) [110, 111]. In addition TAK1, with its adaptor proteins TAB1 and TAB2, activates mitogen-activated protein kinase kinases [112, 113], resulting in production of CREB and AP1 [114, 115]. CREB and AP1 translocate into the nucleus to regulate the expression of various pro-inflammatory cytokines and chemokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, etc. [115].

IFN-γ binds to type 2 IFN receptors (IFNGR1 and 2). IFN-γ induced oligomerization of IFNGR1 and 2 phosphorylate and activate Jak1, Jak2, and STAT1 proteins bound to the cytoplasmic domains of the IFN-γ receptor subunits [116]. The STAT1 homodimers translocate to the nucleus and bind to gamma interferon activated site elements in the DNA [117]. They synthesize the mRNA for the transcription factor IRF1 (interferon response factor-1) and are released in to the cytoplasm. IRF1, in turn, translocates into the nucleus and stimulates the transcription of iNOS expression [118]. Studies also suggest that IRF1 participates in the STAT1-mediated transcription via binding to the ISRE (interferon sensitive response element) [119, 120].

iNOS expression (and therefore NO production) is greatly increased due to the synergistic effect of IFN-γ plus LPS via IRF1 interaction at the response elements in the DNA with NF-κB by synergistic activation of the iNOS promoter [121, 122].

In our study, it is highly evident that activation of macrophages with LPS/IFN-γ increases nitric oxide production (Fig. 1). We believe that NO might be directly involved in the increase of MGO levels by inactivating enzymes of the glycolytic pathway, such as triose phosphate isomerase (TPI) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH), via nitration of the tyrosine residues, leading to increase in levels of dihydroxyacetone phosphate (DHAP) and, as this compound degrades to MGO, thereby higher MGO levels [123].

In glycolysis [124, 125], fructose-bisphosphate aldolase [126] catalyzes the breakdown of fructose 1,6-bisphosphate to generate glyceraldehyde 3-phosphate (G3P) and DHAP. However, DHAP in the presence of TPI reverts rapidly to G3P [127]. G3P in turn is converted into 1, 3-biphosphoglycerate (1, 3-BPG) in the presence of G3PDH [128] and so on. Unfortunately, inactivation of TPI by NO blocks the conversion of DHAP to G3P [129, 130]. Simultaneously, inactivation of G3PDH inhibits the conversion of G3P to 1, 3-BPG. Overall, this leads to the shutdown of the process of glycolysis and accumulation of DHAP. However, DHAP is an unstable molecule which undergoes auto-oxidation and β-elimination to generate MGO and phosphate [131].

Another possible reason for the increase in MGO levels upon inflammatory activation could be a depletion of reduced glutathione (GSH). MGO is detoxified by the glyoxalase (GLO) system by a GSH-dependent reaction [132]. GSH is a rate limiting co-factor for the detoxification of MGO by glyoxalase-I (GLO-I) and converts it into an intermediate S-D-lactoylglutathione, which undergoes further breakdown to form D-lactate and glutathione (which is replenished and re-entered into further co-factor activity) by glyoxalase-II (GLO-II) [133].

GSH is also involved in the detoxification of hydrogen peroxide by GSH peroxidase and thus protects cells from oxidative stress [134]. Studies have shown that in diabetes, nicotinamide adenine dinucleotide phosphate (NADPH), used by glutathione reductase for the reduction of oxidized glutathione (GSSG) to GSH, is also used by aldose reductase for the reduction of glucose to sorbitol through the polyol pathway [135–137]. The competition for NADPH could be responsible for the decreased GSH levels found in T2DM [138]. The decrease in GSH leads to a diminished activity of GLO-I, which leads to accumulation of MGO [139]. Thus, accumulated MGO leads to the production of MGO derived AGEs, causing receptor dependent and independent effects [29, 35, 140, 141], which again enters into a vicious cycle of events and so on (Fig. 4).

Furthermore, our experimental results can be correlated with the effects (at a molecular level) of clinical conditions such as obesity, insulin resistance, and hyperglycemia, the major causes for the development of T2DM [142–145], which can explain the completion of the vicious cycle. Obesity and insulin resistance are the primary reason for increased
oxidative stress [142–144], whereas chronic hyperglycemia leads to elevated levels of AGEs which induces oxidative stress via receptor RAGE [140, 145, 146]. Oxidative stress in turn activates NF-κB [147–150], expression of iNOS, pro-inflammatory cytokines [151, 152], and attenuates the GSH stores in host cells and tissues [153–155]. In addition, expression of inflammatory cytokines also increases the production of NO via activation of iNOS [156–158].

As stated above, increased NO leads to elevated levels of DHAP which may increase the level of MGO and MGO derived AGEs. A supportive study [159] also states that MGO levels are elevated in parallel with increased oxidative stress and AGEs in spontaneously hypertensive rats. Overall studies suggest that one cause for high levels of MGO is the subsequent decrease in GSH levels during oxidative stress in T2DM.
As a consequence, “cross-linking of AGEs at the collagen of vascular basement membrane of large arteries increases the risk of cardiovascular problems followed by stroke, which may lead to brain ischemia and results in cognitive decline in T2DM?”

A number of clinical observations in patients with T2DM might be in line with our cell-based observations, that MGO is linked to arterial stiffness, cardiovascular complication, and possible cognitive decline.

A 5-y prospective follow-up study [160] correlated the baseline plasma levels of MGO with clinical parameters such as intima-media thickness (IMT), systolic blood pressure (SBP), etc., and linked it to diabetic macroangiopathy or microangiopathy. The values of IMT, SBP, etc., all increased in comparison to baseline levels (233 ± 26.5nmol/l) of MGO during the 5-y period, and the levels of MGO correlated significantly with the percentage of changes of IMT (the coefficient of determination, R² = 0.3932; co-relation co-efficient, r= 0.627; p>0.01). They suggest MGO as a predictor in T2DM for intima-media thickening, vascular stiffening, and elevation of SBP and its clinical usefulness as a biomarker for diabetic macroangiopathy.

AGEs such as N-ε-carboxymethyllysine (CML) were found to be dominant in the serum of diabetic and non-diabetics. Kilhovd et al. [161] have suggested that non-CML AGEs may also play a significant role in the development of macrovascular disease in T2DM. The levels of AGEs and CML (values were expressed as median [5th-95th percentile]) were significantly increased in elderly patients with T2DM (AGE – 7.4 [4.4–10.9] U/ml; CML – 15.6 [5.6–29.9] mU/ml) compared with non-diabetic control subjects (AGE – 4.2 [1.6–6.4] U/ml; 8.6 [4.4–25.9] U/ml) and levels of AGEs (8.1 [6.4–10.9] U/ml), but not CML (7.1 [3.5–9.8] mU/ml), were significantly higher in patients with T2DM and coronary artery disease (CAD) than in patients without T2DM.

A number of clinical studies have correlated the occurrence of cognitive decline due to CAD, heart failure (2–8 fold rise), and stroke (2–4 fold rise) with high levels of cytokines [162–166] and AGEs [86, 87].

A cross-sectional study [31] has shown that the elevated levels of AGEs (3.54 ± 1.27 U/mL) in diabetes impairs cognitive functioning when compared with non-mild cognitive impairment controls (2.71 ± 1.18 U/mL). A population study [87] with 276 diabetic elders exhibited mild cognitive impairment (analyzed using Montreal Cognitive Assessment score) with high serum AGEs levels (2.19 ± 1.12 ng/ml) compared with non-diabetic controls (1.04 ± 0.82).

As our results suggest a link between inflammation and MGO production, one could also speculate that increased formation of AGEs in areas of microglial activation may also contribute to crosslinking of amyloid plaques in AD [13, 167, 168].

Hence, we conclude inflammation may elevate the levels of MGO in T2DM and contribute to exacerbation of diabetic complications and cognitive decline. Future research should be directed toward analyzing the levels of MGO in patients with T2DM and correlating it with inflammatory markers such as TNF-α, IL-6, or IL-1B may provide potential information regarding the role of inflammation in solving the diabetes-related complications. From a pharmacotherapeutic perspective, reducing the inflammation in T2DM may decrease the level of MGO-derived AGEs and could block the vicious cycle. In addition, a combination therapy with a MGO scavenger, such as carnosine and tenilsetam [169–171], could potentially decrease the risk of AGEs cross linking in the arteries and prevent the severity of cognitive decline in T2DM.

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