# FREE RADICAL GENERATION BY METHYLGLYOXAL IN TISSUES

Kaushik M. Desai and Lingyun Wu*

*Department of Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon, Canada*

**SUMMARY**

Methylglyoxal (MG) is a reactive dicarbonyl intermediate of the glycolytic pathway. Increased oxidative stress is associated with conditions of increased MG, such as diabetes mellitus. Increased oxidative stress is due to an increase in highly reactive by-products of metabolic pathways, the so-called reactive oxygen species, such as superoxide anion, hydroxyl radical, hydrogen peroxide, nitric oxide and peroxynitrite. These reactive species react with a variety of proteins, enzymes, lipids, DNA and other molecules and disrupt their normal function. Oxidative stress causes many pathological changes that lead to vascular complications of diabetes mellitus, hypertension, neurodegenerative diseases and aging. In this review we summarize the correlation of elevated MG and various reactive oxygen species, and the enzymes that produce them or take part in their disposal, such as antioxidant enzymes and cofactors. The findings reported in various studies reviewed have started filling in gaps in our knowledge that will ultimately provide us with a clear picture of how the whole process that causes cellular dysfunction is initiated.

**KEY WORDS**

methylglyoxal, oxidative stress, MAPK, nuclear factor-κB, hydrogen peroxide, superoxide, peroxynitrite

* Author for correspondence:
Lingyun Wu
A120 Health Sciences Building
Department of Pharmacology
University of Saskatchewan
Saskatoon
SK S7N 5E5 Canada
e-mail: Lily.Wu@usask.ca

## INTRODUCTION

Methylglyoxal (MG) is a reactive dicarbonyl compound formed in the glycolytic pathway, mainly through spontaneous transformation of triose phosphates /1/. MG can also be formed from intermediates of protein metabolism (such as aminoacetone) and fatty acid metabolism (such as acetone). MG along with other reactive intermediates, such as glyoxal and 3-deoxyglucosone (3-DG), are also known as reactive carbonyl species (RCS). An excess of these dicarbonyl compounds causes carbonyl overload and stress /2,3/ in conditions such as diabetes mellitus /2/, hypertension /4/ and atherosclerosis /5,6/. In this review we discuss elevated levels of MG and oxidative stress.

## FREE RADICALS AND OXIDATIVE STRESS

Free radicals are atoms or molecules that have an unpaired electron, symbolized by $^{\bullet}$, in an orbit, and are more reactive than other atoms or molecules. Two free radicals can share their unpaired electrons by forming a covalent bond and at the same time forming a non-radical stable compound. On the other hand, a free radical can donate its unpaired electron to a molecule or get an electron from a molecule and the latter then becomes a free radical and a chain reaction begins.

The term 'reactive oxygen species' (ROS) includes oxygen-derived free radicals as well as highly reactive non-radicals which do not have an unpaired electron in their orbit, such as hydrogen peroxide ($H_2O_2$). An excess of free radicals and ROS in the body is known as oxidative stress and is associated with a number of disease conditions, such as atherosclerosis /7/, diabetes mellitus /8/, hypertension /4,9,10/, etc. ROS are generated through multiple sources in the cell as described below. ROS also act as second messengers in signal transduction and regulate gene transcription. When they are generated in response to cytokines, growth factors and certain hormones, they are involved in ion transport, transcription, neuromodulation and apoptosis /11,12/. ROS can activate transcription factors, such as nuclear erythroid 2 p45-related factor 2 (Nrf2), nuclear factor κB (NF-κB) and activator protein 1 (AP-1) /13/.

**Superoxide anion**

Superoxide ($O_2^{\bullet-}$) is a free radical that is formed by adding an electron to an oxygen molecule: $O_2 + e^- \rightarrow O_2^{\bullet-}$. Superoxide is produced in the body during mitochondrial electron transfer reactions /14,15/, by NADPH oxidase /16-18/, xanthine oxidase /19/, cytochrome P450 /20/, uncoupled endothelial nitric oxide synthase (eNOS) /21/ and by phagocytes /22/. The enzyme superoxide dismutase (SOD) /23/ removes superoxide formed in the body by catalyzing the reaction:

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (1)$$

The measurement of superoxide in biological samples is done with a widely used and sensitive lucigenin-enhanced chemiluminescence assay that uses the probe bis-*N*-methylacridinium nitrate (lucigenin) /24/. Superoxide is also measured with the fluorescent probe dihydroethidium (DHE). However, DHE oxidation yields at least two fluorescent products, 2-hydroxyethidium (EOH), known to be more specific for superoxide, and the less-specific product ethidium that may be due to peroxidase/$H_2O_2$ but not $H_2O_2$ alone /25/.

**Hydrogen peroxide**

Hydrogen peroxide ($H_2O_2$) is not considered as a free radical but it is also highly reactive and toxic to cells. $H_2O_2$ is formed by the reaction:

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (2)$$

that is catalyzed by SOD /23/. Other sources of $H_2O_2$ include xanthine oxidase /26/ and phagocytes /27/. $H_2O_2$ is especially believed to be one of the main ROS taking part in cell signalling /13/, including a possible role in insulin secretion /28/ and as an endothelium-derived hyperpolarizing factor /29/.

$H_2O_2$ is removed by the enzyme catalase. In addition, glutathione peroxidase (GSH-Px) can also convert $H_2O_2$ to water with the help of reduced glutathione (GSH) that is converted to oxidized glutathione (GSSG)

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O \quad (3)$$

$H_2O_2$ formation is usually measured by the formation of the fluorescent compound 2′,7′-dichlorofluorescein (DCF) from the probe 2′,7′-dichlorofluorescin diacetate (DCFH-DA). Once the probe enters the cells, DCFH-DA is converted into membrane-impermeable $DCFH_2$ and the latter is then instantly oxidized to form DCF in the cytosol /30/. This probe has been used as an overall index of oxidants and oxidative stress, but $H_2O_2$ may be the principal oxidant that converts it to the fluorescent DCF /31/. However, the DCFH assay is also sensitive to changes in peroxynitrite ($ONOO^-$) content /32,33/.

**Nitric oxide**

Nitric oxide (NO), produced by the nitric oxide synthases in the body, is another free radical with an unpaired electron and it reacts with superoxide /34/. NO has been established as an important signalling molecule in the body that plays a role in a variety of physiological as well as pathological processes /35/. It is mainly synthesized by three known isoforms of nitric oxide synthase (NOS), neuronal (nNOS), inducible (iNOS) and endothelial (eNOS) /35/. Two isoforms, nNOS and eNOS, are calcium-calmodulin-dependent, whereas iNOS is calcium-independent in terms of its activation /35/. NO is an endothelial vasodilator mediator as well as a neuro-transmitter in the central and enteric nervous systems /36/. It is also one of the cytotoxic components of phagocytic cells such as macrophages /37/.

Many methods have been used to measure NO production in cells including chemiluminescence in a reaction with ozone /38/. More recently, NO has been measured with the probe 4-amino-5-methyl-amino-2′,7′-difluorofluorescein (DAF-FM) /39,40/. The stable end products of NO, nitrite and nitrate, can be measured by the fluorescent probe 2,3-diaminonaphthalene /41/ that is about 50 times more sensitive than the commonly used Griess reaction assay /42/.

**Peroxynitrite**

NO rapidly reacts with superoxide to form the extremely strong and reactive oxidant peroxynitrite ($ONOO^-$), which can cross the cell membrane freely and accounts for most of the toxicity of excess NO /32,43,44/:

$$NO + O_2^{\bullet -} \rightarrow ONOO^- \quad (4)$$

Peroxynitrite can initiate cell signalling or cause oxidative injury, necrosis or apoptosis by interacting with lipids, DNA, and proteins /44/. *In vivo*, peroxynitrite is formed in many conditions, such as stroke, neurodegenerative disorders, diabetes mellitus, myocardial infarction, chronic inflammatory diseases and cancer /44/. Peroxynitrite was shown to cause nitration of the insulin molecule that may lead to its reduced receptor binding and insulin resistance /45/. Peroxynitrite also triggered apoptosis in cultured canine cerebral vascular smooth muscle cells (VSMCs) /46/. The formation of peroxynitrite also results in the inactivation of the biological function of NO.

As noted earlier, the DCFH assay, besides being the most common method for measuring $H_2O_2$, is also sensitive to changes in peroxynitrite content /32,33/. For this reason the NOS inhibitor $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME) can be used to differentiate peroxynitrite production from $H_2O_2$ in the DCFH assay /40/.

**Mitogen activated protein kinases**

Mitogen activated protein kinases (MAPKs) are serine/threonine kinases and consist of four subfamilies: the extracellular-signal-regulated kinases (ERKs), ERK5, JUN N-terminal kinases (JNKs) and the p38 kinases /47,48/. They activate a number of transcription factors and other protein kinases, such as MAPK-activated protein kinases (MAPKAPKs) /48/. MAPKs play a role in many processes, such as cell differentiation and apoptosis and the cell cycle. JNK and p38 are activated by oxidative stress, other environmental stress /49, 50/, pro-inflammatory stimuli /50/, and cytokines such as interleukin-1 (IL-1) /51/, transforming growth factor-β (TGF-β) /52/ and tumor necrosis factor-α (TNFα) /48/. ERK is activated by growth-promoting mitogenic stimuli. There are four isoforms of p38 which have some common substrates but different activities /53/. Activation of p38 leads to increased expression of mRNAs for cytokines and receptors involved in inflammation and immunity /54/.

**Nuclear factor-κB**

Nuclear factor-κB (NF-κB) is a family of transcription factors that consists of seven structurally related proteins. They are activated by

cytokines, ROS and other stimuli. They play a role in inflammation and regulate cardiovascular function and growth /55/. Generally, in resting cells a majority of NF-κB subunits are associated with a family of proteins called inhibitors of NF-κB (IκB). NF-κB is commonly activated by some bacteria and bacterial products, such as lipopolysaccharide, viruses, stress, mitogens and growth factors. NF-κB in turn activates targets such as the chemokines IL-1α and IFNγ, immune receptors, adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), stress response genes, such as angiotensin II and MnSOD, regulators of apoptosis, enzymes, some transcription factors, growth factors and cell cycle regulators /56/. NF-κB was the first transcription factor shown to be redox-regulated in Jurkat cells /57/.

## METHYLGLYOXAL AND OXIDATIVE STRESS

MG has been shown to increase oxidative stress in various *in vivo*, *in vitro* and cell culture studies. These studies typically measure some of the more common parameters of oxidative stress, such as superoxide anion, hydrogen peroxide, peroxynitrite and NF-κB. We summarize some of these findings and present a schematic overview of MG-induced increase in oxidative stress.

### Methylglyoxal and superoxide anion

Increased levels of superoxide have been found in association with increased MG levels *in vivo* in rats as well as after exposure of different cell types to exogenous MG, suggesting a direct link between MG and the formation of superoxide. Thus, increased superoxide production in the aorta of 13 week-old spontaneously hypertensive rats (SHR) was associated with elevated plasma and aortic MG levels and blood pressure, compared with age-matched Wistar Kyoto (WKY) rats /58/. Consequently, exposure of cultured rat aortic VSMCs /40/ to different concentrations (10-300 μM) of MG for 4 h resulted in a significantly increased superoxide production in a concentration- and time-dependent manner. This increased superoxide production was markedly reduced by the superoxide scavenger SOD or NAD(P)H oxidase inhibitor dipheynlene iodonium (DPI) /40/. Similarly, incubation of rat kidney mesangial cells with MG (100 μM for 2 h) increased

superoxide production /59/, which in turn increased mRNA levels for TGF-β1 and fibronectin, an effect prevented by SOD. Inhibition of MG-induced superoxide production with DPI indicated NADPH oxidase as its source. The authors suggested that MG-induced renal fibrosis may be linked to increased superoxide production /59/. Neutrophils are a common source of superoxide produced as a part of their bactericidal action. MG has been shown to increase superoxide production in neutrophils /60/. Incubation of neutrophils with 1 μM to 1 mM MG for 60 min at 37°C resulted in a concentration-dependent increase of basal as well as formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated production of superoxide. Moreover, this stimulant action of MG was seen in the absence of plasma proteins, indicating a direct action on the cells and not through modification of a plasma protein. The mechanism involves p38-MAPK-dependent exocytosis of granules that provide cytochrome $b_{558}$, a component of NADPH oxidase /60/, which produces superoxide. MG-induced formation of advanced glycation endproducts (AGEs) in mitochondrial proteins from streptozotocin-induced diabetic rat kidney cortex was associated with significantly increased production of superoxide and oxidative damage. Aminoguanidine improved mitochondrial respiration and decreased oxidative damage to mitochondrial proteins /61/.

Thus, activation of NADP oxidase and mitochondrial enzymes seem to be the main sources of MG-induced superoxide production. Future studies may well reveal other sources, such as cytochromes and xanthine oxidase.

**Methylglyoxal and hydrogen peroxide**

Similar to superoxide, significantly increased $H_2O_2$ production in the aorta of SHR was associated with elevated plasma and aortic MG levels and blood pressure, compared to WKY rats at 13 weeks of age /58,62/. In an earlier study, using the probe DCFH, we have shown that incubation of VSMCs with MG (50-500 μmol/l) for 24 h caused a significantly greater increase in oxidative stress in cells from SHR compared to those from WKY rats /63/. MG at a concentration of 100 μmol/l caused a two-fold increase in oxidative stress in VSMCs from SHR and a 1.57-fold increase in VSMCs from WKY rats. In a more recent study /40/, incubation of rat aortic VSMCs with MG increased the generation of $H_2O_2$ as measured by the DCFH-DA probe along with NOS inhibitor L-NAME. However, MG-induced oxidized DCF

was prevented by *N*-acetyl cysteine (NAC) and GSH, both scavengers of MG, but not by the $H_2O_2$ scavenger catalase. The failure of catalase to inhibit MG-induced oxidized DCF might be explained by the fact that it is cell membrane-impermeable. When the cells were treated with MG and catalase, MG-induced $H_2O_2$ might react with $DCFH_2$ to form oxidized DCF inside the cell before being scavenged by catalase, which is located in the extracellular space or medium /40/.

Leoncini and Poggi /64/ reported an accumulation of $H_2O_2$ in platelets after exposure to MG. Platelets from healthy human volunteers were incubated with increasing concentrations of MG (0.1-5.0 mM) for 2 min at 37°C. Subsequently, stimulation with thrombin, a platelet aggregating agent, significantly increased $H_2O_2$ that was measured by the DCFH-DA probe. Toxicity of MG was ruled out by the absence of release of L-lactic dehydrogenase /64/. The exact mechanism by which MG caused $H_2O_2$ production was not shown but a depletion of platelet GSH content and the consequent inhibition of glutathione peroxidase activity which can remove $H_2O_2$ /65/ was discussed as a possible mechanism /64/. Platelet activating agents, such as thrombin, arachidonic acid, and the $Ca^{2+}$ ionophore A23187, also cause an increase in $H_2O_2$ generation in platelets /65/. Thus, it is possible that agonists such as thrombin may be increasing MG production by enhancing the glycolytic pathway /66/ and MG in turn may be increasing $H_2O_2$ accumulation by depleting GSH /67/ and/or through some other mechanism.

MG has also been shown to increase $H_2O_2$ production in neutrophils /60/. Incubation of neutrophils with 1 μM to 1 mM MG in plasma for 60 min at 37°C resulted in a concentration-dependent increase of basal as well as phagocytosis of *Staphylococcus aureus*-induced production of $H_2O_2$. $H_2O_2$ production by 1 mM MG was blocked by pre-incubation with the MG scavenger aminoguanidine (5 mM). As with superoxide, MG-induced $H_2O_2$ was independent of the presence of plasma proteins, indicating a direct action of MG on the cells. Thus, MG activates p38-MAPK-dependent exocytosis of granules to provide cytochrome $b_{558}$ for NADPH oxidase, which is the source of $H_2O_2$ generation /60/.

In a study on myeloma cell line U266, MG (10 μM) + cisplatin (3 μg/ml) increased intracellular peroxides by 4-fold at 15 h, as measured by the DCFH-DA assay, when compared with cisplatin or MG alone. Pretreatment with NAC (10 mM) completely inhibited ROS accumu-

lation at 15 h induced by either cisplatin (3 μg/ml), MG (10 μM), or MG (10 μM) + cisplatin (3 μg/ml) in combination. Thus, MG and cisplatin worked synergistically to increase intracellular ROS generation /68/. The mechanism behind this synergistic effect is not clear.

**Methylglyoxal and nitric oxide/peroxynitrite**

The increase in blood pressure in the SHR, followed from 4 to 13 weeks of age, was associated with significantly increased NO production, increased iNOS expression and reduced eNOS expression in the aorta, along with elevated plasma and aortic MG levels, compared with age-matched WKY rats /62/. This increase was prevented by treatment of SHR with the MG scavenger aminoguanidine (1 g/l in drinking water) for 9 weeks starting at 4 weeks of age. *In vitro* treatment of rat aortic VSMCs with MG (0-100 μM) /40/ caused increased NO generation, as measured by DAF-FM. As described earlier, MG also increased production of superoxide that interacts with NO and leads to formation of peroxynitrite. The NOS inhibitor L-NAME was used to differentiate peroxynitrite production from $H_2O_2$ in the DCFH assay. MG-induced oxidized DCF was concentration- and time-dependently inhibited by L-NAME. This provides the evidence that MG can induce not only $H_2O_2$ but also peroxynitrite generation in rat VSMCs /40/. Increased peroxynitrite indicates increased NO and superoxide generation induced by MG, as described earlier. Immunocytochemical staining showed increased expression of iNOS in cells treated with MG (100 μM for 18 h) as compared to the untreated group. MG-induced iNOS expression was prevented by NAC (600 μM). Fructose (2.5-30 mM for 3-24 h) also causes increased peroxynitrite production in cultured rat aortic VSMCs that was mediated by elevated MG /69/. Thus, in all likelihood iNOS seems to be the source of increased NO formation in response to MG.

We have also found gender differences in the expression of nNOS in 24 week-old stroke-prone spontaneously hypertensive rats (SHRsp). Thus male Sprague-Dawley rats (SD) and SHRsp had decreased kidney eNOS levels compared to age-matched female SD and SHRsp, respectively. This was associated with increased kidney levels of iNOS and MG-induced AGEs, $N_\varepsilon$-carboxyethyl-lysine (CEL) and $N_\varepsilon$-carboxymethyl-lysine (CML), in male and female SHRsp compared to age-matched male and female SD rats /70/.

**Methylglyoxal and p38 MAPK**

Neutrophils in their normal state are quiescent but when primed by lipopolysaccharide and TNFα they become active to produce ROS and release proteolytic enzymes to kill bacteria. It has been shown that activation of p38 MAPK plays a role in the priming and activation of neutrophils /71,72/. MG activates p38 MAPK in neutrophils /60,71, 72/, human endothelial cells /73/ and rat kidney mesangial cells /74/. Inhibition of p38 MAPK prevented this effect of MG. Incubation of neutrophils with MG (1 mM for 15-60 min) significantly phosphorylated and activated p38 MAPK at 15 min and significantly increased plasma membrane expression of CD35 and CD66b at 45 and 60 min. CD35 and CD66b expression is indicative of exocytosis of secretory vesicles and specific granules /60/. This effect of MG was prevented by aminoguanidine (5 mM). The mechanism by which MG activates p38 MAPK may be through a direct or indirect action on the cell. The indirect action of MG may be through modification of a plasma protein, such as albumin, which activates p38 MAPK /75/, or by forming AGEs that bind to AGE receptors /1/ on neutrophils /76/ and activate them. The direct action of MG would be to enter the cells and directly stimulate the p38 MAPK signalling pathway. Since the stimulatory action of MG on neutrophils took place in protein-free buffer it indicates a direct action /60/. Reduced availability of GSH and changes in the redox state of a cell also activate p38 MAPK /77/, as observed in patients with uremia in whom the ratio of oxidized to reduced glutathione in plasma is increased /78/ along with a decrease in total glutathione concentration in neutrophils /79/.

**Methylglyoxal and activation of nuclear factor-κB**

As mentioned earlier, NF-κB plays a role in inflammation. Compared to VSMCs from WKY rats, in VSMCs from SHR /63/ the basal levels of nuclearly localized NF-κB p65 were high with an associated increased expression of ICAM-1, which were possibly responsible for inflammatory vascular responses /80/. An increased basal cytoplasmic level of IκBα (inhibitory protein for NF-κB) was also observed, which likely represents an adaptive response to enhanced activation of NF-κB in VSMCs from SHR because the IκBα gene has κB elements in its promoter region /81/. There were no differences in the cytoplasmic levels of IκBβ between SHR and WKY VSMCs. Activation of NF-κB

involves degradation of IκBα, followed by translocation of the p65/p50 heterodimer to the nucleus /82/. We found that within 3 h of treatment of SHR VSMCs with 300 μmol/l MG, there was a significant increase in the nuclear level of NF-κB p65 and a decrease in the cytoplasmic level of IκBα protein (but no change in the cytoplasmic levels of IκBβ), indicative of NF-κB activation. Although 300 μmol/l MG did cause a small but significant rise in cellular oxidative stress, as measured by DCFH oxidation and GSH-Red and GSH-Px inactivation in WKY VSMCs, it did not result in NF-κB activation, as no change was seen in nuclearly localized NF-κB p65 or in cytoplasmic IκBα. The activation of NF-κB in SHR VSMCs was associated with a large increase of ICAM-1 24 h after exposure to 100-300 μmol/l MG. A smaller but significant increase in ICAM-1 was also seen in WKY VSMCs 24 h after exposure to 300 μmol/l but not to 100 μmol/l MG. We have also found gender differences in the expression of NF-κB p65 in 24 week-old SHRsp. Thus, male SD rats and SHRsp had significantly increased kidney NF-κB p65 levels compared to age-matched female SD rats and SHRsp, respectively. Also, SHRsp had higher kidney NF-κB p65 levels compared to age-matched SD rats. This was associated with increased kidney levels of iNOS and MG-induced AGEs, CEL and CML, in male and female SHRsp compared to age-matched male and female SD rats /70/.

The activation of NF-κB by oxidants, such as superoxide and $H_2O_2$, has been observed in human endothelial cells and rat VSMCs /83,84/. We showed that $H_2O_2$ can induce degradation of IκBα protein in SHR VSMCs but not in WKY VSMCs. Our results also indicate that MG-induced activation of NF-κB is likely mediated through production of strong oxidants. Thus, MG caused a significant increase in cellular oxidative stress and increased GSSG content that was prevented by NAC. Also, MG caused significantly more activation of NF-κB in SHR VSMCs cells, which have a much higher level of endogenous oxidative stress, than those of WKY VSMCs. Moreover, NAC pretreatment decreased the extent of MG-induced activation of NF-κB in SHR VSMCs and decreased the extent of the associated induction of ICAM-1. This increased oxidative stress in turn activates NF-κB and this has also been observed in human endothelial cells /85, 86/. Activation of NF-κB produces downstream effects, such as expression of proinflammatory genes /87/. Increased activation of NF-

κB was also reported in macrophages from deoxycorticosterone acetate hypertensive rats /88/.

JNK, NF-κB, and peroxisome proliferator-activated receptor α (PPARα) are stress-response factors and their expression is regulated by redox cellular changes /89-93/. MG was shown to activate JNK and apoptosis signal-regulating kinase 1 (ASK1), associated with an increase in superoxide production, in Jurkat leukemia T cells /94,95/. MG did not activate NF-κB in SH-SY 5Y neuroblastoma cells /96/ in contrast to VSMCs /63/. However, MG induced increased expression of JNK and PPARα in SH-SY 5Y neuroblastoma cells, which have a defective antioxidant ability, making them more susceptible to MG-induced toxicity. Human glioblastoma ADF cells are able to scavenge MG and MG-induced ROS efficiently through antioxidants and hence are less susceptible to its toxic effects /96/.

MG has also been shown to increase oxidative stress through the induction of pro-inflammatory cytokines, IL-1β and IL-6, in cultured neural cells from rat hippocampus /97/ and neutrophils from non-diabetic humans /98/, respectively. This induction was prevented by pretreatment of neural cells with NAC /97/ or of neutrophils with GSH or metformin /98/.

Glycated proteins and AGEs /99,100/ also induce oxidative stress /101/, partly through induction of cytokines and growth factors /102, 103/. Furthermore, AGEs can interact with the receptor for AGEs (RAGE) and scavenger receptors, which are present in endothelial cells, VSMCs, and mononuclear phagocytes /104/. Activation of these receptors results in strong oxidant production /105/. The interaction of AGEs with RAGEs promotes expression of cell adhesion molecules, such as VCAM-1 and ICAM-1, on endothelial cells, and causes general vasculopathy /85,86/, which is likely mediated by NF-κB activation caused by the oxidative stress associated with RAGE activation.

**Methylyglyoxal and antioxidant enzymes: reduced glutathione**

MG can increase oxidative stress by inactivating antioxidant enzymes, such as GSH-Red /106/ and GSH-Px /107/, because of their glycation. MG is detoxified by the glyoxalase system that depends on the availability of GSH. MG has been shown to deplete GSH /108, 109/ so that the cell becomes more sensitive to oxidative stress /110/. This establishes a vicious cycle that leads to increased levels of MG.

For example, MG has been shown to reduce GSH content in platelets /67/, isolated hepatocytes /I11,112/ and hepatocytes from mice given MG /113/. MG (50-400 mg/kg, i.p.) reduced the liver glutathione content and increased lipid peroxidation in mice /114/. MG also induced depletion of GSH in spinal and cortical neurons /108,109/.

We have shown that incubation of VSMCs from SHR and WKY rats with MG (500 μmol/l) reduced the GSH content in these cells /63/. MG (300 μmol/l for 24 h) also increased the levels of GSSG that was inhibited by pretreatment of the cells with NAC (600 μmol/l for 30 min), indicating involvement of oxidative stress /63/. MG (100 μmol/l) decreased GSH-Px activity in both cell types but more so in SHR VSMCs. GSH-Px removes $H_2O_2$ with the help of GSH that is in turn oxidized to GGSG /22,115/. In VSMCs from SHR as well as WKY rats, MG (300-500 μmol/l) also reduced the activity of GSH-Red /63/, which acts as an antioxidant by converting GSSG to GSH /115/. *In vivo* in 13 week-old SHR, significantly reduced aortic and plasma GSH and GSH-Red and GSH-Px activity in the aorta were associated with elevated plasma and aortic MG levels and blood pressure, compared to age-matched WKY rats /58,62/.

In a study on mice /114/ intraperitoneal administration of different concentrations of MG (50-400 mg/kg) decreased activities of glutathione-*S*-transferase (GST), glyoxalase I and glyoxalase II in the liver. MG also affects the activity of antioxidant enzymes, such as SOD, catalase, GST, glyoxalase I and glyoxalase II, as determined *in vitro*. Thus, *in vitro* incubation of MG (0.1-10 mM) with the cytosolic fraction of liver cells resulted in inhibition of enzyme activity by 20-25% with the 10 mM concentration /114/. Glyoxalase I was affected the most by MG with 90% reduction of activity after 2 h incubation with 10 mM MG. A concentration of 1 mM MG for 15 min inhibited glyoxalase I activity by about 20% /114/. A concentration- and time-dependent inactivation of SOD was observed when the enzyme was incubated with MG (5 mM for 5 days) /116/. The inactivation of SOD was more pronounced with 30 mM MG for 24 h. Similarly, incubation of human Cu/Zn-SOD with MG led to the loss of enzymatic activity and release of copper ions from the protein /117/.

Thus, besides increasing oxidative stress mediators, such as superoxide, NO, peroxynitrite and $H_2O_2$, MG also inactivates antioxidant enzymes and cofactors which prevents disposal of MG and oxidants, and further aggravates the oxidative stress. A scheme of the proposed

cascade activated by elevated MG resulting in increased oxidative stress is presented in Figure 1.

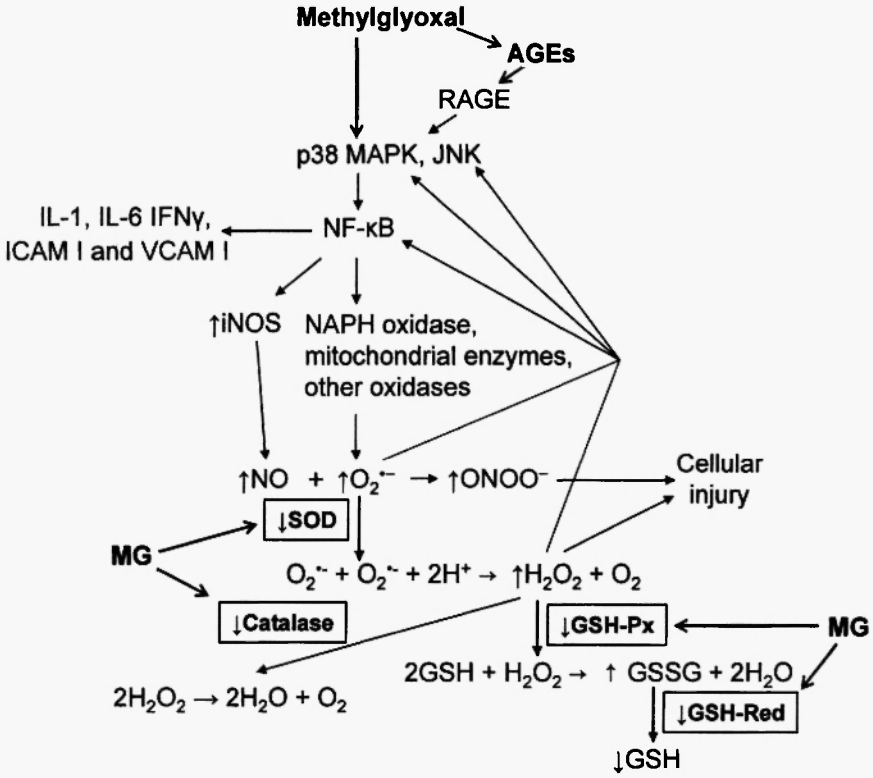


**Fig. 1:** Schematic diagram showing multiple effects of methylglyoxal that lead to increased oxidative stress in the cell. AGEs = advanced glycation end products; GSH-Px = glutathione peroxidase; GSH-Red = glutathione reductase; GSH = reduced glutathione; GSSG = oxidized glutathione; $H_2O_2$ = hydrogen peroxide; ICAM 1 = intercellular adhesion molecule 1; IFNγ = interferon γ; IL1α = interleukin 1α; JNK = JUN N-terminal kinase; MG = methylglyoxal; NF-κB = nuclear factor-kappaB; NO = nitric oxide; $O_2^{\bullet-}$ = superoxide anion; $ONOO^-$ = peroxynitrite; p38 MAPK = p38 mitogen activated protein kinase; RAGE = receptor for advanced glycation endproduct; SOD = superoxide dismutase; VCAM 1 = vascular cell adhesion molecule 1.

## CONCLUSIONS AND FUTURE DIRECTIONS

The concentrations of MG used in different studies on cultured cells range from 10 μM to 30 mM. The amount of MG that enters the cell from the culture medium may very well depend on the cell type. For example, in a recent study on cultured rat L6 muscle cells /118/ only 3% of the MG (2.5 mM) in the incubation medium was found inside the cells after 30 min. This concentration is on the high side. In our studies on cultured rat aortic VSMCs, we used concentrations from 10-300 μM and found significant effects on oxidative stress markers at 100 μM and increased NO production at 10 μM of MG. Systematic studies that establish optimum incubation concentrations of MG in different cell types and record effects on signalling molecules, oxidative stress markers and markers of apoptosis and toxicity are required to draw proper conclusions. Fortunately, a number of studies are being reported that are slowly filling in gaps in our knowledge. The image of MG as a purely toxic molecule looks likely to change with lower concentrations of MG being ascribed a signalling role.

In conclusion, MG has been convincingly shown by many studies to increase oxidative stress through its effects on p38 MAPK, NF-κB, iNOS, interleukins, NO, superoxide anion, peroxynitrite and hydrogen peroxide. Increased oxidative stress in turn may be inducing the pathological changes associated with conditions such as vascular complications of diabetes mellitus, hypertension and other conditions that have elevated MG levels.

## ACKNOWLEDGEMENTS

The authors thank the Heart and Stroke Foundation of Canada and the Canadian Institutes of Health Research for supporting this project.

## REFERENCES

1. Thornalley PJ. Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification–a role in pathogenesis and antiproliferative chemotherapy. Gen Pharmacol 1996; 27: 565-573.
2. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999; 48: 1-9.

3. Wu L. The pro-oxidant role of methylglyoxal in mesenteric artery smooth muscle cells. Can J Physiol Pharmacol 2005; 83: 63-68.
4. Chang T, Wu L. Methylglyoxal, oxidative stress and hypertension. Can J Physiol Pharmacol 2006; 84: 1229-1238.
5. Baynes JW, Thorpe SR. Glycoxidation and lipoxidation in atherogenesis. Free Radic Biol Med 2000; 28: 1708-1716.
6. Ahmed MU, Dunn JA, Walla MD, Thorpe SR, Baynes JW. Oxidative degradation of glucose adducts to protein. Formation of 3-($N^{\varepsilon}$-lysino)-lactic acid from model compounds and glycated proteins. J Biol Chem 1988; 263: 8816-8821.
7. Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004; 84: 1381-1478.
8. Ceriello A. Oxidative stress and diabetes-associated complications. Endocr Pract 2006; 12 (Suppl 1): 60-62.
9. de Champlain J, Wu R, Girouard H, Karas M, El Midaoui A, Laplante MA, Wu L. Oxidative stress in hypertension. Clin Exp Hypertens 2004; 26: 593-601.
10. Escobales N, Crespo MJ. Oxidative-nitrosative stress in hypertension. Curr Vasc Pharmacol 2005; 3: 231-246.
11. Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signaling, and cell injury. Free Radic Biol Med 2000; 28: 1456-1462.
12. Lander HM. An essential role for free radicals and derived species in signal transduction, FASEB J 1997; 11: 118-124.
13. Gloire G, Legrand-Poels S, Piette J. NF-kappaB activation by reactive oxygen species: fifteen years later. Biochem Pharmacol 2006; 72: 1493-1505.
14. Midaoui AE, Elimadi A, Wu L, Haddad PS, de Champlain J. Lipoic acid prevents hypertension, hyperglycemia, and the increase in heart mitochondrial superoxide production. Am J Hypertens 2003; 16: 173-179.
15. de Souza LF, Ritter C, Pens Gelain D, Andrades M, Bernard EA, Moreira JC, Dal-Pizzol F. Mitochondrial superoxide production is related to the control of cytokine release from peritoneal macrophage after antioxidant treatment in septic rats. J Surg Res 2007; 141: 252-256.
16. Wu R, Millette E, Wu L, de Champlain J. Enhanced superoxide anion formation in vascular tissues from spontaneously hypertensive and desoxycorticosterone acetate-salt hypertensive rats. J Hypertens 2001; 19: 741-748.
17. McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, Jo H, Harrison DG. Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. Am J Physiol 2003; 285: H2290-H2297.
18. Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest 1996; 97: 1916-1923.
19. White CR, Darley-Usmar V, Berrington WR, McAdams M, Gore JZ, Thompson JA, Parks, DA, Tarpey MM, Freeman BA. Circulating plasma

xanthine oxidase contributes to vascular dysfunction in hypercholesterolemic rabbits. Proc Natl Acad Sci USA 1996; 93: 8745-8749.
20. Fleming, I, Michaelis UR, Bredenkotter D, Fisslthaler B, Dehghani F, Brandes RP, Busse R. Endothelium-derived hyperpolarizing factor synthase (cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. Circ Res 2001; 88: 44-51.
21. Vasquez-Vivar J, Kalyanaraman B, Martasek P, Hogg N, Masters BS, Karoui H, Tordo, P, Pritchard KA Jr. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proc Natl Acad Sci USA 1998; 95: 9220-9225.
22. Darley-Usmar V, Halliwell B. Blood radicals: reactive nitrogen species, reactive oxygen species, transition metal ions, and the vascular system. Pharm Res 1996; 13: 649-662.
23. Fridovich I. Superoxide dismutases. Adv Enzymol 1974; 41: 35-48.
24. Münzel T, Afanas'ev IB, Kleschyov AL, Harrison DG. Detection of superoxide in vascular tissue. Arterioscel Thromb Vas Biol 2002; 22: 1761-1768.
25. Fernandes DC, Wosniak J Jr, Pescatore LA, Bertoline MA, Liberman M, Laurindo FR, Santos CX. Analysis of DHE-derived oxidation products by HPLC in the assessment of superoxide production and NADPH oxidase activity in vascular systems. Am J Physiol 2007; 292: C413-C422.
26. Zweier JL, Broderick R, Kuppusamy P, Thompson-Gorman S, Lutty GA. Determination of the mechanism of free radical generation in human aortic endothelial cells exposed to anoxia and reoxygenation. J Biol Chem 1994; 269: 24156-24162.
27. Carter WO, Narayanan PK, Robinson JP. Intracellular hydrogen peroxide and superoxide anion detected in endothelial cells. J Leukoc Biol 1994; 55: 253-258.
28. Pi J, Bai Y, Zhang Q, Wong V, Floering LM, Daniel K, Reece JM, Deeney JT, Andersen ME, Corkey BE, Collins S. Reactive oxygen species as a signal in glucose-stimulated insulin secretion. Diabetes 2007; 56: 1783-1791.
29. Matoba B, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. J Clin Invest 2000; 106: 1521-1530.
30. Royall JA, Ischiropoulos H. Evaluation of 2′,7′-dichlorofluorescin and dihydrorhodamine 123 as fluorescent probes for intracellular $H_2O_2$ in cultured endothelial cells. Arch Biochem Biophys 1993; 302: 348-355.
31. LeBel CP, Ischiropoulos H, Bondy SC. Evaluation of the probe 2′,7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol 1992; 5: 227-231.
32. Curtin JF, Donovan M, Cotter TG. Regulation and measurement of oxidative stress in apoptosis. J Immunol Meth 2002; 265: 49-72.
33. Kooy NW, Royall JA, Ischiropoulos H. Oxidation of 2′,7′-dichlorofluorescin by peroxynitrite. Free Radic Res 1997; 27: 245-254.
34. Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature 1986; 320: 454-456.

35. Moncada S. Nitric oxide: discovery and impact on clinical medicine. J R Soc Med 1999; 92: 164-169.
36. Zhang J, Snyder SH Nitric oxide in the nervous system. Annu Rev Pharmacol Toxicol 1995; 35: 213-233.
37. Green SJ, Scheller LF, Marletta MA, Seguin MC, Klotz FW, Slayter M, Nelson BJ, Nacy CA. Nitric oxide: cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. Immunol Lett 1994; 43: 87-94.
38. Leone AM, Rhodes P, Furst V, Moncada S. Techniques for the measurement of nitric oxide. Meth Mol Biol 1995; 41: 285-299.
39. Itoh Y, Ma FH, Hoshi H, Oka M, Noda K, Ukai Y, Kojima H, Nagano T, Toda N. Determination and bioimaging method for nitric oxide in biological specimens by diaminofluorescein fluorometry. Anal Biochem 2000; 287: 203-209.
40. Chang T, Wang R, Wu L. Methylglyoxal-induced nitric oxide and peroxynitrite production in vascular smooth muscle cells. Free Radic Biol Med 2005; 38: 286-293.
41. Andreas K Nussler AK, Glanemann M, Schirmeier A, Liu L, Nüssler NC. Fluorometric measurement of nitrite/nitrate by 2,3-diaminonaphthalene. Nat Protocols 2006; 1: 2223-2226.
42. Granger DL, Taintor RR, Boockvar KS, Hibbs JB Jr. Measurement of nitrate and nitrite in biological samples using nitrate reductase and Griess reaction. Meth Enzymol 1996; 268: 142-151.
43. Huie RE, Padmaja S. The reaction of NO with superoxide. Free Radic Res Commun 1993; 18: 195-199.
44. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev 2007; 87: 315-424.
45. Chi Q, Wang T, Huang K. Effect of insulin nitration by peroxynitrite on its biological activity. Biochem Biophys Res Commun 2005; 330: 791-796.
46. Li J, Su J, Li W, Liu W, Altura BT, Altura BM. Peroxynitrite induces apoptosis in canine cerebral vascular muscle cells: possible relation to neurodegenerative diseases and strokes. Neurosci Lett 2003; 350: 173-177.
47. Crews CM, Alessandrini AA, Erikson RL. Mouse ERK-1 gene product is a serine/threonine protein kinase that has the potential to phosphorylate tyrosine. Proc Natl Acad Sci USA 1991; 88: 8845-8849.
48. Ono K, Han J. The p38 signal transduction pathway: activation and function. Cell Signal 2000; 12: 1-13.
49. Rouse J, Cohen P, Trigon S, Morange M, Alonso-Llamazares A, Zamanillo D, Hunt T, Nebreda AR. A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. Cell 1994; 78: 1027-1037.
50. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, Davis RJ. Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. J Biol Chem 1995; 270: 7420-7426.

51. Freshney NW, Rawlinson L, Guesdon F, Jones E, Cowley S, Hsuan J, Saklatvala J. Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of Hsp27. Cell 1994; 78: 1039-1049.
52. Hanningan M, Zhan L, Ai Y, Huang CK. The role of p38 MAP kinase in TGF-β1-induced signal transduction in human neutrophils. Biochem Biophys Res Commun 1998; 246: 55-58.
53. Jiang Y, Chen C, Li Z, Guo W, Gegner JA, Lin S, Han J. Characterization of the structure and function of a new mitogen-activated protein kinase (p38β). J Biol Chem 1996; 271: 17920-17926.
54. Ashwell JD. The many paths to p38 mitogen-activated protein kinase activation in the immune system. Nat Rev Immunol 2006; 6: 532-540.
55. Brasier AR. The NF-kappaB regulatory network. Cardiovasc Toxicol 2006; 6: 111-130.
56. Tergaonkar V. NFkappaB pathway: a good signaling paradigm and therapeutic target. Int J Biochem Cell Biol 2006; 38: 1647-1653.
57. Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. EMBO J 1991; 10: 2247-2258.
58. Wang X, Desai K, Chang T, Wu L. Vascular methylglyoxal metabolism and the development of hypertension. J Hypertens 2005; 23: 1565-1573.
59. Ho C, Lee PH, Huang WJ, Hsu YC, Lin CL, Wang JY. Methylglyoxal-induced fibronectin gene expression through Ras-mediated NADPH oxidase activation in renal mesangial cells. Nephrology (Carlton) 2007; 12: 348-356.
60. Ward RA, McLeish KR. Methylgyoxal: a stimulus to neutrophil oxygen radical production in chronic renal failure? Nephrol Dial Transplant 2004; 19: 1702-1707.
61. Rosca MG, Mustata TG, Kinter MT, Ozdemir AM, Kern TS, Szweda LI, Brownlee M, Monnier VM, Weiss MF. Glycation of mitochondrial proteins from diabetic rat kidney is associated with excess superoxide formation. Am J Physiol 2005; 289: F420-F430.
62. Wang X, Chang T, Jiang B, Desai, K, Wu L. Attenuation of hypertension development by aminoguanidine in spontaneously hypertensive rats: role of methylglyoxal. Am J Hypertens 2007; 20: 629-636.
63. Wu L, Juurlink BH. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. Hypertension 2002; 39: 809-814.
64. Leoncini G, Poggi M. Effects of methylglyoxal on platelet hydrogen peroxide accumulation, aggregation and release reaction. Cell Biochem Funct 1996; 14: 89-95.
65. Maresca M, Colao C, Leoncini G. Generation of hydrogen peroxide in resting and activated platelets. Cell Biochem Funct 1992; 10: 79-85.
66. Leoncini G, Buzzi E. Thrombin induces *S*-D-lactoylglutathione accumulation by enhancing platelet glycolytic pathway. Int J Biochem 1994; 26: 661-665.
67. Leoncini G, Maresca M, Buzzi E. Inhibition of the glycolytic pathway by methylglyoxal in human platelets. Cell Biochem Funct 1989; 7: 65-70.

68. Godbout JP, Pesavento J, Hartman ME, Manson SR, Freund GG. Methylglyoxal enhances cisplatin-induced cytotoxicity by activating protein kinase Cδ. J Biol Chem 2002; 277: 2554-2561.
69. Wang H, Meng QH, Chang T, Wu L. Fructose-induced peroxynitrite production is mediated by methylglyoxal in vascular smooth muscle cells. Life Sci 2006; 79: 2448-2454.
70. Wang X, Desai K, Juurlink BH, de Champlain J, Wu L. Gender-related differences in advanced glycation endproducts, oxidative stress markers and nitric oxide synthases in rats. Kidney Int 2006; 69: 281-287.
71. McLeish KR, Knall C, Ward RA, Gerwins P, Coxon PY, Klein JB, Johnson GL. Activation of mitogen-activated protein kinase cascades during priming of human neutrophils by TNFα and GM-CSF. J Leukoc Biol 1998; 64: 537-545.
72. Ward RA, Nakamura M, McLeish KR. Priming of the neutrophil respiratory burst involves p38 mitogen-activated protein-kinase-dependent exocytosis of flavocytochrome $b_{558}$-containing granules. J Biol Chem 2000; 275: 36713-36719.
73. Akhand AA, Hossain K, Mitsui H, Kato M, Miyata T, Inagi R, Du J, Takeda K, Kawamoto Y, Suzuki H, Kurokawa K, Nakashima I. Glyoxal and methylglyoxal trigger distinct signals for MAP family kinases and caspase activation in human endothelial cells. Free Radic Biol Med 2001; 31: 20-30.
74. Liu BF, Miyata S, Hirota Y, Higo S, Miyazaki H, Fukunaga M, Hamada Y, Ueyama S, Muramoto O, Uriuhara A, Kasuga M. Methylglyoxal induces apoptosis through activation of p38 mitogen-activated protein kinase in rat mesangial cells. Kidney Int 2003; 63: 947-957.
75. Fan X, Subramaniam R, Weiss MF, Monnier VM. Methylglyoxal-bovine serum albumin stimulates tumor necrosis factor alpha secretion in RAW 264.7 cells through activation of mitogen-activating protein kinase, nuclear factor κB and intracellular reactive oxygen species formation. Arch Biochem Biophys 2003; 409: 274-286.
76. Collison KS, Parhar RS, Saleh SS, Meyer BF, Kwaasi AA, Hammami MM, Schmidt AM, Stern DM, Al-Mohanna FA. RAGE-mediated neutrophil dysfunction is evoked by advanced glycation end products (AGEs). J Leukoc Biol 2002; 71: 433-444.
77. Haddad JJ. The involvement of L-γ-glutamyl-L-cysteinyl-glycine (glutathione/GSH) in the mechanism of redox signaling mediating $MAPK^{p38}$-dependent regulation of pro-inflammatory cytokine production. Biochem Pharmacol 2002; 63: 305-320.
78. Himmelfarb J, McMenamin E, McMonagle E. Plasma aminothiol oxidation in chronic hemodialysis patients. Kidney Int 2002; 61: 705-716.
79. Schettler V, Wieland E, Methe H, Schuff-Werner P, Muller GA. Oxidative stress during dialysis: effect on free radical scavenging enzyme (FRSE) activities and glutathione (GSH) concentration in granulocytes. Nephrol Dial Transplant 1998; 13: 2588-2593.
80. De Martin R, Hoeth M, Hofer-Warbinek R, Schmid JA. The transcription factor NF-κB and the regulation of vascular cell function. Arterioscler Thromb Vasc Biol 2000; 20: E83-E88.

81. Chiao PJ, Miyamoto S, Verma IM. Autoregulation of IκBα activity. Proc Natl Acad Sci USA 1994; 91: 28-32.
82. Baeuerle PA. Pro-inflammatory signaling: last pieces in the NF-κB puzzle? Curr Biol 1998; 8: R19-R22.
83. Ogata N, Yamamoto H, Kugiyama K, Yasue H, Miyamoto E. Involvement of protein kinase C in superoxide anion-induced activation of nuclear factor-κB in human endothelial cells. Cardiovasc Res 2000; 45: 513-521.
84. Canty TG Jr, Boyle EM Jr, Farr A, Morgan EN, Verrier ED, Pohlman TH. Oxidative stress induces NF-κB nuclear translocation without degradation of IκBα. Circulation 1999; 100 (Suppl II): II-361–II-364.
85. Bierhaus A, Chevion S, Chevion M, Hofmann M, Quehenberger P, Illmer T, Luther T, Berentshtein E, Tritschler H, Muller M, Wahl P, Ziegler R, Nawroth PP. Advanced glycation end product-induced activation of NF-κB is suppressed by α-lipoic acid in cultured endothelial cells. Diabetes 1997; 46: 1481-1490.
86. Schmidt AM, Yan SD, Wautier JL, Stern D. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. Circ Res 1999; 84: 489-497.
87. Christman JW, Blackwell TS, Juurlink BHJ. Redox regulation of nuclear factor-κB: therapeutic potential for attenuating inflammatory responses. Brain Pathol 2000; 10: 153-162.
88. Beswick RA, Zhang H, Marable D, Catravas JD, Hill WD, Webb RC. Long-term antioxidant administration attenuates mineralocorticoid hypertension and renal inflammatory response. Hypertension 2001; 37: 781-786.
89. Poynter ME, Daynes RA. Peroxisome proliferator-activated receptor α activation modulates cellular redox status, represses nuclear factor-κB signalling, and reduces inflammatory cytokine production in aging. J Biol Chem 1998; 273: 32833-32841.
90. Inoue I, Noji S, Awata T, Takahashi K, Nakajima T, Sonoda M, Komoda T, Katayama S. Bezaflbrate has an antioxidant effect: peroxisome proliferator-activated receptor α is associated with $Cu^{2+}$,$Zn^{2+}$-superoxide dismutase in the liver. Life Sci 1998; 63: 135-144.
91. Dougherty CJ, Kubasiak LA, Prentice H, Andreka P, Bishopric NH, Webster KA. Activation of c-Jun N-terminal kinase promotes survival of cardiac myocytes after oxidative stress. Biochem J 2002: 362: 561-571.
92. Del Rio MJ, Velez-Pardo C. Monoamine neurotoxins-induced apoptosis in lymphocytes by a common oxidative stress mechanism: involvement of hydrogen peroxide ($H_2O_2$), caspase-3, and nuclear factor kappa-B (NF-κB), p53, c-Jun, transcription factors. Biochem Pharmacol 2002; 63: 677-688.
93. Li N, Karin M. Is NF-κB the sensor of oxidative stress? FASEB J 1999; 13: 1137-1143.
94. Du J, Suzuki H, Nagase F, Akhand AA, Yokoyama T, Miyata T, Kurokawa K, Nakashima I. Methylglyoxal induces apoptosis in Jurkat leukaemia T cells by activating c-jun N-terminal kinase. J Cell Biochem 2000; 77: 333-344.
95. Du J, Suzuki H, Nagase F, Akhand AA, Ma XY, Yokoyama T, Miyata T, Nakashima I. Superoxide-mediated early oxidation and activation of ASK1 are

important for initiating methylglyoxal-induced apoptosis process. Free Radic Biol Med 2001; 31: 469-478.

96. Amicarelli F, Colafarina S, Cattani F, Cimini A, Di Ilio C, Ceru MP, Miranda M. Scavenging system efficiency is crucial for cell resistance to ROS-mediated methylglyoxal injury. Free Radic Biol Med 2003; 35: 856-871.
97. Di Loreto S, Caracciolo V, Colafarina S, Sebastiani P, Gasbarri A, Amicarelli F. Methylglyoxal induces oxidative stress-dependent cell injury and up-regulation of interleukin-1β and nerve growth factor in cultured hippocampal neuronal cells. Brain Res 2004; 1006: 157-167.
98. Wang H, Meng QH, Gordon JR, Khandwala H, Wu L. Proinflammatory and proapoptotic effects of methylglyoxal on neutrophils from patients with type 2 diabetes mellitus. Clin Biochem 2007; 40: 1232-1239.
99. Lo TW, Westwood ME, McLellan AC, Seiwood T, Thornalley PJ. Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with *N*α-acetyl-arginine, *N*α-acetyl-cysteine and *N*α-acetyl-lysine, and bovine serum albumin. J Biol Chem 1994; 269: 32299-32302.
100. Oya T, Hattori N, Mizuno Y, Miyata S, Maeda S, Osawa T, Uchida K. Methylglyoxal modification of protein. Chemical and immunochemical characterization of methylglyoxal-arginine adducts. J Biol Chem 1999; 274: 18492-18502.
101. Kikuchi S, Shinpo K, Takeuchi M, Yamagishi S, Makita Z, Sasaki N, Tashiro K. Glycation - a sweet tempter for neuronal death. Brain Res Rev 2003; 41: 306-323.
102. Abordo EA, Westwood ME, Thornalley PJ. Synthesis and secretion of macrophage colony stimulating factor by mature human monocytes and human monocytic THP-1 cells induced by human serum albumin derivatives modified with methylglyoxal and glucose-derived advanced glycation endproducts. Immunol Lett 1996; 53: 7-13.
103. Westwood ME, Thornalley PJ. Induction of synthesis and secretion of interleukin 1β in the human monocytic leukemia THP-1 cells by human serum albumins modified with methylglyoxal and advanced glycation endproducts. Immunol Lett 1996; 50: 17-21.
104. Thornalley PJ. Cell activation by glycated proteins: AGE receptors, receptor recognition factors and functional classification of AGEs. Cell Mol Biol 1998; 44: 1013-1023.
105. Wautier MP, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. Am J Physiol 2001; 280: E685-E694.
106. Blakytny R, Harding JJ. Glycation (non-enzymic glycosylation) inactivates glutathione reductase. Biochem J 1992; 288: 303-307.
107. Paget C, Lecomte M, Ruggiero D, Wiernsperger N, Lagarde M. Modification of enzymatic antioxidants in retinal microvascular cells by glucose or advanced glycation end products. Free Radic Biol Med 1998; 25: 121-129.
108. Kikuchi S, Shinpo K, Moriwaka F, Makita Z, Miyata T, Tashiro K. Neurotoxicity of methylglyoxal and 3-deoxyglucosone on cultured cortical neurons:

synergism between glycation and oxidative stress, possibly involved in neurodegenerative diseases. J Neurosci Res 1999; 57: 280-289.
109. Shinpo K, Kikuchi S, Sasaki H, Ogata A, Moriwaka F, Tashiro K. Selective vulnerability of spinal motor neurons to reactive dicarbonyl compounds, intermediate products of glycation, in vitro: implication of inefficient glutathione system in spinal motor neurons. Brain Res 2000; 861: 151-159.
110. Meister A. Glutathione metabolism and its selective modification. J Biol Chem 1988; 263: 17205-17208.
111. Kalapos MP, Garzo T, Antoni F, Madl J. Effect of methylglyoxal on glucose formation, drug oxidation and glutathione content in isolated murine hepatocytes. Biochim Biophys Acta 1991; 1092: 284-290.
112. Kalapos MP, Garzo T, Antoni F, Mandl J. Accumulation of *S*-D-lactoylglutathione and transient decrease of glutathione level caused by methylglyoxal load in isolated hepatocytes. Biochim Biophys Acta 1992; 1135: 159-164.
113. Kalapos MP, Schaff Z, Garzo T, Antoni F, Mandl J. Accumulation of phenols in isolated hepatocytes after pretreatment with methylglyoxal. Toxicol Lett 1991; 58: 181-191.
114. Choudhary D, Chandra D, Kale RK. Influence of methylglyoxal on antioxidant enzymes and oxidative damage. Toxicol Lett 1997; 93: 141-152.
115. Juurlink BHJ. Management of oxidative stress in the CNS: the many roles of glutathione. Neurotox Res 1999; 1: 119-140.
116. Jabeen R, Mohammad AA, Elefano EC, Petersen JR, Saleemuddin M. Antibodies and Fab fragments protect Cu,Zn-SOD against methylglyoxal-induced inactivation. Biochim Biophys Acta. 2006; 1760: 1167-1174.
117. Kang JH. Modification and inactivation of human Cu,Zn-superoxide dismutase by methylglyoxal. Mol Cells 2003; 15: 194-199.
118. Riboulet-Chavey A, Pierron A, Durand I, Murdaca J, Giudicelli J, Van Obberghen E. Methylglyoxal impairs the insulin signaling pathways independently of the formation of intracellular reactive oxygen species. Diabetes 2006; 55: 1289-1299.