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Characteristic effects of methylglyoxal and its degraded product formate on viability of human histiocytes: A possible detoxification pathway of methylglyoxal

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ABSTRACT

Methylglyoxal (MGO) is a toxic and highly reactive alpha-oxoaldehyde, elevated in the states of various diseases underlying enhanced oxidative stress. Furthermore, MGO has been reported to generate another aldehyde, formic acid (FA). In this sense, investigating the biological property of FA is crucially important. The present study examined the effects of MGO and FA on cell viability using the U937 human histiocytic cell line. FA showed a dose-dependent increase in cell viability at the concentrations of MGO in which cell viability decreased. The mechanism of the increase by FA involved the presence of endogenous hydrogen peroxide (H_2O_2) and tetrahydrofolate in the folate pathway, whereas that of the decrease in cell viability by MGO involved interaction with H_2O_2 and oxidative damage. These findings suggest that FA production by MGO degradation may play a role in attenuation of oxidative cellular injury caused by MGO. We hypothesize that FA generation pathway constitutes a detoxification system for MGO.

1. Introduction

Methylglyoxal (MGO) is a toxic and highly reactive alpha-oxoaldehyde that is generated in glycolysis [1–5]. Accumulating data have shown that MGO is involved in the pathological mechanisms for various disorders, including hypertension, chronic kidney disease (CKD), and diabetic nephropathy [6–12]. Aortic and plasma levels of MGO are reportedly increased in spontaneously hypertensive rats (SHR) [7] and administration of MGO causes insulin resistance and salt-sensitivity in SHR [6], and loss of endothelium and impaired vasodilation with elevated serum creatinine in Wistar rats [8]. MGO concentrations in the blood are elevated in advanced-stage CKD patients [10,11,13]. MGO levels in diabetic patients have been revealed to show a close relationship with progression of glomerular basement membrane, nephropathy, and carotid artery intima thickening [12,14]. Intracellular oxidative damage has been described as playing a central role in cytotoxic effects of MGO [9,15–17] and inducing apoptosis in various cells [16,18–22].

MGO is enzymatically metabolized by the glyoxalase system [4,23–25], reacting with the coenzyme glutathione to generate hemithioacetal, then lactoylglutathione, and finally D-lactate [1,20]. Loss of the detoxification capacity resulting from decreases in enzymes such as glyoxalase I and their functional loss under oxidative stress have been considered to contribute to elevated levels of MGO in the blood [26–28] or cells [29]. On the other hand, an aldehyde, MGO, has also been reported to generate another aldehyde, formic acid (FA), in the presence of hydrogen peroxide (H_2O_2) in vitro [30,31]. D-Lactate is reportedly related to FA production in vivo [32] and FA is identified as a product of an enzymatic oxidation of MGO in vitro [33]. Those strongly indicate that MGO is degraded by oxidative stress to generate FA.

FA plays an important physiological role with tetrahydrofolate (THF) in folate pathway in nucleic acid metabolism as a one-carbon donor [34–36] for atoms 2 and 8 of the purine ring. But is also a toxic factor that is elevated under conditions of methanol intoxication [37–40]. Pharmacological level of FA leads to prolonged metabolic acidosis [38,40], causing cytotoxicity in ocular and neuronal cells [41–45]. However, the effect of FA on cell viability below toxic levels has not been fully clarified yet.

The present study investigated the effects of MGO and its metabolite, FA, and influential factors on cell viability by using U937 human histiocytic cell line. The findings of the study may indicate a novel concept that FA generation pathway constitutes a detoxification system for MGO.

2. Materials

All materials used in the present study were purchased from Wako Pure Chemical Industries (Osaka, Japan); MGO (Sigma–Aldrich, WI, USA); FA (Wako Pure Chemical Industries); aminoguanidine (AG), an MGO scavenger (Sigma–Aldrich);

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pegylated catalase (peg-CAT), an endogenous H_2O_2 quencher (Sigma–Aldrich); methotrexate (MTX), an antifolate (Sigma–Aldrich); THF, a coenzyme in FA metabolism (MP Biomedicals, CA, USA); human histiocytic tumor cell line U937 (ECACC, Wiltshire, UK); penicillin (PC)/streptomycin (SM) (Wako Pure Chemical Industries); and calf fetal serum (CFS) (Invitrogen Corp., CA, USA).

3. Methods

3.1. Relationship between number of viable cells and ATP chemiluminescence

U937 cells were collected and cultured at 37 °C with 5% CO_2 in RPMI1640 culture medium supplemented with 10% FCS and PC/SM (RPMI(+)). After centrifugation at 1800 rpm for 5 min at 4 °C, collected cells were washed and resuspended in RPMI1640 (RPMI(−)), then adjusted to a concentration of 1.2–6.0 × 10^5 cells/ml. Aliquots of 100 μl of cell suspension were placed in a 96-well microplate. Viability of U937 cells was examined using a commercially available kit (Cell Titer-Glo Luminescent Cell viability assay; Promega Corp., WI, USA). Luciferin and luciferase in the reagents of the Cell Titer-Glo kit react with ATP released from lysed cells to produce oxyluciferin, generating chemiluminescence proportional to the ATP levels. Luminescence was measured using a chemiluminescence analyzer (GloMax 20.20n luminometer; Promega).

3.2. Measurement of U937 cell viability and intracellular reduced glutathione

Collected U937 cells were adjusted to concentrations of 1.2–6.0 × 10^5 cells/ml in RPMI(−) to test for viability or in phosphate-buffered saline (PBS) to test for reduced glutathione (GSH). Next, 50-μl aliquots of cell suspension were placed in a 96-well microplate, and 50 μl of test solution was added, containing final concentrations of MGO at 0.0–1.0 mM, or FA at 0.0–1.0 mM, and MGO at 0.0–1.0 mM/FA at 0.0–1.0 mM with AG at 5 mM, or MGO at 0.0–1.0 mM/FA at 0.0–1.0 mM with peg-CAT at 50 U/ml. Additionally, FA at 0.0–1.0 mM alone or with THF at 40 μM in PBS or FA with MTX at 100 nM in RPMI((+) with THF) to test for viability.

Viability of U937 cells was examined in RPMI(−) using the Cell Titer-Glo Luminescent cell viability assay, as mentioned above. GSH of U937 cells was examined in PBS using a GSH-Glo glutathione assay (Promega). Luciferin is generated from a luminogenic substrate catalyzed by GST in the presence of GSH, with resulting signal strength increasing with increasing GSH levels. After incubation, luminescence was measured using the chemiluminescence analyzer.

3.3. Analysis of apoptotic U937

Collected U937 cells, cultured and processed as mentioned above, were adjusted to a concentration of 1.2–6.0 × 10^5 cells/ml. As the next step, 50 μl of cell suspension was placed in each well of a 48-well microplate and 50 μl of test solution containing MGO or FA was then added. After a 4-h incubation period, cells were rinsed twice with PBS and resuspended in a binding buffer. Subsequently, 1 μl of annexin-V (FITC-labeled) (200 μg/ml) and 5 μl of propidium iodide (PI) (200 μg/ml) were added (annexin V-FITC kit; Beckman Coulter Inc., CA, USA) to a final volume of 100 μl and cells were incubated for 15 min in the dark. Cytofluorometric analysis was performed immediately after staining using a FACScan flowcytometer (BD Biosciences, CA, USA). More than 10,000 cells were analyzed in each sample. The percentage of positive events for cell markers in gated populations was determined using FlowJo software (Tree Star, Inc., OR, USA). Apoptosis was de-

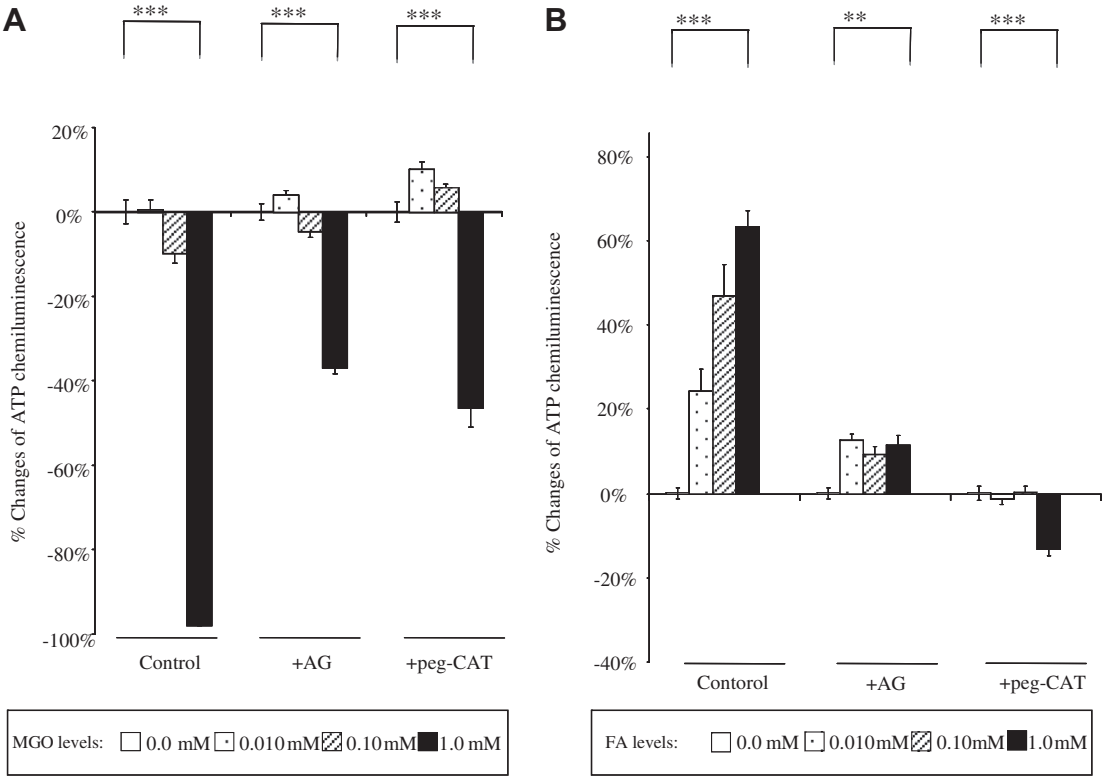

Fig. 1. Viability of U937 cells incubated in RPMI(−) was measured at 3 and 24 h: (A) MGO, MGO + AG (5.0 mM) and MGO + peg-CAT (50 U/ml); (B) FA, FA + AG (5.0 mM) and FA + peg-CAT (50 U/ml). (A) Percentage changes in viability at 24 h with MGO, MGO + AG (5.0 mM) and MGO + peg-CAT (50 U/ml) as compared to MGO 0.0 mM at 3 h are expressed as mean ± SEM (n = 5) (***p < 0.001). (B) Percentage changes in viability at 24 h with FA, FA + AG (5.0 mM) and FA + peg-CAT (50 U/ml) as compared to MGO 0.0 mM at 3 h are expressed as mean ± SEM (n = 5) (**p < 0.005; ***p < 0.001).