Glyoxal Damages Human Aortic Endothelial Cells by Perturbing the Glutathione, Mitochondrial Membrane Potential, and Mitogen-Activated Protein Kinase Pathways

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Research Article

Keywords: Cardiovascular diseases, Human aortic endothelial cells, Mitogen activated protein kinase pathways, Glyoxal, Laboratory indexes

Posted Date: March 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-270505/v1>

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Version of Record: A version of this preprint was published at BMC Cardiovascular Disorders on December 1st, 2021. See the published version at <https://doi.org/10.1186/s12872-021-02418-3>.

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3 **Kinase Pathways**

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33 **Running title:** Aortic endothelial cell damage by glyoxal

34 **word count:** 4948, **figures:** 5

35

36 **Abstract**

37

38 **Background:** Exposure to glyoxal, the smallest dialdehyde, is associated with several
39 diseases; humans are routinely exposed to glyoxal because of its ubiquitous presence
40 in foods and the environment. The aim of this study was to examine the damage
41 caused by glyoxal in human aortic endothelial cells.

42 **Methods:** Cell survival assays and quantitative fluorescence assays were performed
43 to measure DNA damage; oxidative stress was detected by colorimetric assays and
44 quantitative fluorescence, and the mitogen-activated protein kinase pathways were

assessed using western blotting.

Results: Exposure to glyoxal was found to be linked to abnormal glutathione activity, the collapse of mitochondrial membrane potential, and the activation of mitogen-activated protein kinase pathways. However, DNA damage and thioredoxin oxidation were not induced by dialdehydes.

Conclusions: Intracellular glutathione, members of the mitogen-activated protein kinase pathways, and the mitochondrial membrane potential are all critical targets of glyoxal. These findings provide novel insights into the molecular mechanisms perturbed by glyoxal and may facilitate the development of new therapeutics and diagnostic markers for cardiovascular diseases.

Keywords: Cardiovascular diseases, Human aortic endothelial cells, Mitogen-activated protein kinase pathways, Glyoxal, Laboratory indexes

Introduction

Glyoxal (GX), the smallest dialdehyde, is the most widely used retarding agent in the synthesis of chemicals, such as novel bio-adhesives for wood [1-3], or those from the environment [4]. In humans, a high dietary glycemic load leading to insulin resistance causes alterations in glucose and lipid metabolism, leading to production of excess aldehydes, including GX and methylglyoxal, which are associated with the progression of certain diseases, including cardiovascular disorders [5, 6]. With respect to tissue dysfunction, GX has been implicated in the progression of several degenerative conditions, such as Alzheimer's disease and diabetes mellitus, in which increased serotonin release from cells might stimulate serotonin-mediated intestinal

motility [7]. Furthermore, the consumption of high-carbohydrate diets might also induce the endogenous formation of GX [6]. Regarding DNA damage and tumor growth, DNA duplexes can undergo intra- and inter-cross-linking damage through the formation of GX–guanine adducts [8, 9]. Exposure to 0.1% GX was found to cause a significant increase in tumor size in the small intestines of male and female mice [10]. GX can also trigger changes to erythrocytes. Specifically, it induces the modification of arginine residues of HbA0-forming G-H1, which decreases free iron-mediated oxidative reactions. GX-derived G-H1-mediated changes in the structural and functional properties of the heme protein, observed *in vivo*, may be of clinical significance [11]. Erythrocytes take up exogenous GX and convert it primarily to glycolate, and approximately 1% is converted to oxalate. This pathway of oxalate formation may be enhanced in diabetes and other diseases associated with increased oxidative stress [12]. Glutathione (GSH), a reactive Cys residue containing a tripeptide, is the major soluble antioxidant molecule in cells due to its abundance in the cytosol, nucleus, and mitochondrion [13-16]. Thioredoxin-1(Trx1) mainly acts by cleaving the disulfide bonds of oxidized proteins, thus affecting intracellular scavenging of oxidative stress. This action relies on cell survival, cell growth, and gene transcription. [17]. Finally, the enzymes involved in glycolysis are heavily modified by GX, which can significantly inhibit the activity of GAPDH, which in turn may contribute to the pathological processes by impairing glycolytic processes [18].

The toxic effects of aldehydes include protein damage caused by the exposure of cells to endogenous and exogenous aldehydes, which leads to the formation of covalent adducts with proteins, causing dysfunction. In addition, DNA damage such as DNA double strand breaks (DSBs) [19], DNA–protein crosslinks (DPCs), or DNA interstrand crosslinks (ICLs), [20] occurs when a protein or DNA base in one strand undergoes further reactions with aldehyde–DNA base adducts in the opposite strand [21]. The FANC pathway, which includes the complementation group A (FANCA), is

the major mechanism underlying the repair of ICLs induced by aldehydes [19].

Previous studies have shown that aldehydes [17-19] are independent risk factors for cardiovascular disease. For example, GX is associated with critical targets in the human aortic endothelial cells (HAECs), which are implicated in several cardiovascular diseases. GX is also involved in the development of abnormal structures and functions of the arteries, such as atherosclerosis [5-6]. The main objective of this study was to examine the damage-inducing effects of GX on HAECs and to provide novel insights into the molecular mechanisms that are perturbed by GX, which may, in turn, facilitate the development of new therapeutics and diagnostic markers for cardiovascular diseases.

Materials and Methods

2.1. Chemicals

Buthionine sulfoximine (BSO), iodoacetic acid (IAA), iodoacetamide (IAM), auranofin (Auro), and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) were purchased from Solarbio (Beijing, China); CCCP and Auro were solubilized in dimethyl sulfoxide (DMSO) before use. GX (40%, v/v) was obtained from Xiya Chemical Industry (Jinan, China) and was dissolved in MilliQ water (Massachusetts, USA) before use. HAECs were purchased from Bena Culture Collection Co. (Beijing, China). Fanconi anemia complementation group A (FANCA) siRNA and siRNA-mate were purchased from GenePharma Co., Ltd. (Shanghai,

China). The GSH peroxidase assay kit was obtained from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Trx1, phospho extracellular signal regulated kinase (P-ERK), phospho c-Jun N-terminal kinase (P-JNK), and phospho p38 kinase (P-P38) antibodies were purchased from Abcam (Cambridge, UK), and Enhanced chemiluminescence (ECL) western blotting substrate was obtained from Biosharp (Hefei, China). The mitochondrial membrane potential kit was purchased from Abbkine (California, USA), fetal bovine serum was obtained from Tianhang (Zhejiang, China)

2.2. siRNA Knockdown of FANCA

FANCA siRNA and siRNA-mate were mixed for 10 min at room temperature to form siRNA–liposome complexes and were transfected into HAECs to inhibit the expression of FANCA. HAECs were maintained in Dulbecco's modified Eagle's medium with 10% inactivated fetal bovine serum (FBS) in a 5% CO₂ atmosphere at 37°C in a humidified incubator for 48–96 h, followed by harvesting of lysates for western blot analyses.

2.3 Cell Culture and Survival Assays

HAECs were cultured as described previously. For the survival assays with GX, 12 h after plating of the cells, the medium was replaced with fresh medium containing a

range of indicated concentrations of GX (0, 50, 100, 150, and 200 μ M) every day, and cells were incubated for 8 days to form colonies. For survival assays, BSO, which can specifically inhibit γ -glutamylcysteine synthetase, was used to deplete the intracellular GSH concentration. HAECs were incubated in culture medium with 1 mM BSO for up to 72 h. Auro is an inhibitor of thioredoxin reductase, which specifically interferes with the reduction of oxidized Trx1 and was applied at a concentration of 0–6 μ M for 24 h, while HAEC membrane permeability to protons was augmented with CCCP treatment (0–800 μ M for 2 h).

HAECs were rinsed twice with PBS after treatment; then, the cells were incubated in fresh medium for 8 days to form colonies. Only colonies with more than 50 cells were counted. Drug lethal dose (LD₉₀) values were determined based on the GX dose that produced 90% cell damage, as confirmed from survival curves.

2.4. Measurement of GSH Levels

To determine the critical targets of GX, we assessed the changes in the intracellular GSH level. HAECs were treated with GX (LD₉₀). Untreated cells were used as the negative control group. Using a GSH detection kit, GSH was measured immediately after treatment.

The biochemical principle of this protocol is that dithionitrobenzoic acid reacts with sulfhydryl compounds, resulting in a yellowish coloration of the compounds, a process which is catalyzed by GSH. Briefly, HAECs (1×10^6) were suspended and lysed by ultrasound after treatment with GX (LD₉₀, 0.12 mM, for 8 days) or BSO. The

162 samples were mixed with GSH (1 mM) and reagent 1 and incubated at 37°C for 5 min.
163 This was followed by adding a reaction mixture of reagents 2-5 to the samples and
164 incubating at 37°C for 15 min. Results were measured using an absorbance microplate
165 reader (420 nm; Molecular Devices; California, USA).

167 2.5. Measurement of the Redox State of Trx1

168 The samples with reduced and oxidized cysteine thiol groups were treated with GX,
169 Trx1 proteins bearing different charges were separated by urea-polyacrylamide gel
170 electrophoresis (PAGE) and selectively detected by western blotting [22]

172 Preparation of Electrophoretic Migration Markers: All cysteine residues were
173 reduced by incubating HAECs with 3.5 mM dithiothreitol (DTT) in urea buffer (8 M
174 urea, 100 mM tris-HCl [pH 8.2], and 1 mM EDTA) for 30 min at 37°C.
175 Electrophoretic migration markers were treated with 15 mM IAA + 15 mM IAM for
176 alkylation at 37°C for 15 min.

177 Preparation of the Trx1 Proteins with an Unknown Redox State. After treatment
178 with GX (LD₉₀, 0.12 mM, for 8 days) or Auro, the Trx1 proteins were alkylated with
179 200 µL of urea buffer (30 mM IAA) for 15 min at 37°C. Two milliliters of acetone–1
180 N HCl (4°C, 98:2 v/v) was used to precipitate proteins, and unreacted IAA was
181 removed by microcentrifugation for 5 min at 11,000 g at 4°C. The pellet was
182 resuspended and washed in acetone–1 N HCl–H₂O (4°C, 98:2:10 v/v/v) three times
183 by microcentrifugation for 5 min at 11,000 g at 4°C. TRX1 proteins were then

reduced by incubating them with 200 μ L of urea buffer containing 3.5 mM DTT for 30 min at 37°C. Trx1 proteins were mixed with 10 mM IAM for 15 min at 37°C.

Detection of the Redox State of Trx1. The treated Trx1 samples and the electrophoretic migration markers were analyzed with an 8 M urea-PAGE gel, using a 2.5% stacking gel and 12% separating gel run at a constant current of 5 mA for 3.5 h. Proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Darmstadt, DE) after electrophoresis and incubated at 4°C for 24 h in TBST (20 mM tris-HCl [pH 7.6], 140 mM NaCl and 0.1% tween). The membranes were probed with the relevant primary antibody (ABCAM, ab109385, 1:20000) at 4°C overnight, followed by three rinses with TBST. They were then incubated with the enzyme-labeled goat anti-rabbit IgG (1:5000) at 37°C for 2 h. The membrane was incubated with the ECL western blotting substrate after washing with TBST. Chemiluminescence was quantified on a ChemiDoc XRS + system (Bio-Rad (California, USA), and results were determined using ImageJ 1.47v.

2.6. Measurement of the Mitochondrial Membrane Potential

To determine critical GX targets, we determined the effect of GX on mitochondrial function. HAECs were treated with GX (LD₉₀, 0.12 mM, for 8 days) or without GX (control) and subsequently assessed using a mitochondrial membrane potential assay kit. The measurements were taken immediately after treatment. Briefly, cells were washed and seeded onto a 96-well plate with 100 μ L buffer/well. After

treatment with GX (LD₉₀) or CCCP, a working solution of the carbocyanine dye JC-1 (100 µL/well) was added, followed by incubation in the dark at 37°C for 10 min. Results were measured on a Hitachi F-2500 fluorescence spectrophotometer (Tokyo, JPN) at λ_{ex} = 529 nm and λ_{em} = 590 nm.

2.7 Western Blotting

HAECs were washed with PBS and then harvested for treatment with the protein inhibitor (Hefei, China); 30 µg of proteins were subjected to separation on a 10% resolving sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel. The separated proteins were then transferred onto PVDF membranes using a blotting instrument (Bio-Rad (California, USA)). The samples were incubated with the relevant primary antibody at 4°C overnight, after which they were blocked with 5% bovine serum albumin (20 mM tris-HCl [pH 7.6], 140 mM NaCl and 0.1% Tween) for 2 h at 37°C. The steps thereafter were the same as those mentioned in section 2.5.3 after incubation with the primary antibody.

2.8 Measurement of DPCs

HAECs were treated with GX (LD₉₀, 0.12 mM, for 8 days) and without GX (control) to measure DPCs [23]. Briefly, cells were processed with sarkosyl, mixed with 9.3 g of CsCl (Wako,JPN) and sedimented at 500 000×g at 20°C for 4 h (CsCl

density gradient centrifugation) to isolate chromosomal DNA. Fluorescein isothiocyanate was dissolved in dimethylformamide to a final concentration of 0.1 mM to label DPCs. DPCs were measured with a Hitachi F-2500 fluorescence spectrophotometer(Hitachi, JPN) (λ ex = 490 nm, λ em = 520 nm).

2.9 Measurement of DSBs

HAECs were irradiated with X-rays (marker), with GX (LD₉₀, 0.12 mM, for 8 days) or no GX (control), after which DSBs [19] were detected by static-field gel electrophoresis. Cells (10⁴) were loaded on plug molds, and the plugs were slid into 0.6% SeaKem gold agarose gels; electrophoresis was performed for 36 h at a field strength of 0.6 V cm in 0.5× TBE buffer to isolate DSBs. The samples were detected with a UV transilluminator and captured using a digital camera (Bio-Rad (California, USA)). The band intensity was subsequently determined using ImageJ software (version 1.47).

2.10 Statistical Analysis

Data are presented as mean $\pm$ standard deviation of results from three to five repetitions. Statistical analysis was performed using the SPSS 22 package. *T*-test was used to evaluate the statistical differences for paired data.

Results

3.1 DNA Repair is Not Important in Attenuating GX Toxicity

ICLs are amongst the most serious types of DNA damage induced by aldehydes [24, 25]. To determine whether GX generates ICLs in HAECs, cells were exposed to GX in the culture medium to determine the tolerance mechanism to DNA damage. HAECs deficient in the FANC pathway ($LD_{90} = 0.15$ mM) were insensitive to GX in comparison with HAECs that were confirmed to be positive for the FANC pathway ($LD_{90} = 0.12$ mM) (Fig. 1A). Upon treating HAECs with GX (LD_{90} , 0.12 mM, for 8 days), the DPC (Fig. 1C) and DSB (Fig. 1B) results were similar to those in the negative control group ($p > 0.05$). Thus, we concluded that DPCs, ICLs, and DSBs were not the primary reasons for DNA damage (Fig. 1B and 1C).

3.2. GX Partially Inactivates HAECs Via Disruption of GSH

Intracellular GSH levels decreased after treatment with GX (55% relative to the negative control group), as well as BSO (62% relative to untreated control cells; Fig. 2A). BSO induced moderate damage (60% survival) as observed by evaluation with clonogenic assays (Fig. 2B). Thus, our results show that depletion of GSH occurs in the presence of GX, which contributes to cytotoxicity in HAECs.

3.3 Depletion of Mitochondrial Membrane Potential Causes Moderate Cytotoxicity

After treatment with CCCP, HAEC membrane permeability was augmented with respect to protons; the cells showed decreased mitochondrial membrane potential (58% relative to that in negative control cells) compared to that with GX treatment (55% relative to that in the untreated control cells; Fig. 3A). CCCP induced HAEC damage (84% survival), as observed by the clonogenic assay (Fig. 3B). Thus, GX-induced disruption of the mitochondrial membrane potential is an important reason for cell damage.

3.4 Oxidation of Trx1 Causes Moderate Cytotoxicity

Trx1 is a thiol protein and critical regulator of the redox state balance. To determine critical GX targets, we assessed the effect of GX on the oxidation of Trx1. HAECs were treated with GX (LD₉₀) and without GX (control) to induce different charges, which were measured immediately. The resulting modified proteins were separated by urea-PAGE and measured by western blotting. According to the different redox states, several distinct bands were separated (Fig. 4A). An upper shift of bands was observed immediately after treatment with GX (LD₉₀; GX band = 1.87 relative to that in the untreated control cells Fig. 4A). Auro is an inhibitor of thioredoxin reductase, which interferes with the reduction of oxidized Trx1, thus resulting in an upper shift of bands (Auro band = 3.29 relative to that in untreated control cells). Thus, the rates of oxidized Trx1 with GX treatment changed compared to those in the untreated control cells. The results showed a dramatic decrease in cell survival (32% survival) after the

treatment with Auro when analyzed by the clonogenic assay (Fig. 4B). Thus, oxidized Trx1 that was subjected to interference by GX showed moderate cytotoxicity.

3.5 Activation of Mitogen-activated Protein (MAP) Kinase Pathways, including P-ERK, P-JNK, and P-P38

HAECs were incubated with GX in culture medium to determine its effect on the MAP kinase pathways. HAECs were treated with GX (LD₉₀) and without GX (control), and then effects were measured immediately. Results showed that activation levels of the MAP kinase pathway members increased immediately after treatment with GX, including P-ERK (105%), P-JNK (314%), and P-P38 (159%) in comparison to control levels (Fig. 5).

Discussion

As an important pollutant, endogenous GX can be produced by the metabolism of carbohydrates and oxidation of lipids or nucleic acids, whereas exogenous GX is present in foods and is released to the environment through activities such as smoking [26, 27]. Aldehydes differ in their reactivity and damaging effects in HAECs due to their different chemical structures [24, 28]. However, to our knowledge, no study has shown the targets of critical damage in HAECs or the mechanism of protein damage resulting from GX cytotoxicity in conjunction with that of DNA damage, because concurrent DNA damage by dialdehydes yields similar results [24, 29].

ICLs constitute the most serious of the DNA damage types induced by aldehydes. The primary mechanism of ICL damage repair induced by aldehydes is the FANC pathway [24, 25]. Previous research has shown that ICLs, but not DPCs and DSBs, cause the critical cytotoxicity mediated by monoaldehydes [24, 25]. This study explored the effects of DNA damage-induced cytotoxicity by a dialdehyde (GX). We found that the FANC repair-deficient pathway is not essential for cell survival by mitigating the cytotoxicity of GX. The DPCs, ICLs, and DSBs were also not the primary reasons underlying cytotoxicity. ICLs induced by GX in human leukocytes were confirmed in a previous study, which correlated these with type 2 diabetes mellitus [8]. The differences in results from those of HAECs could be attributed to the use of different cell types. Our results differ from the results of studies on monoaldehydes as dialdehydes and monoaldehydes are completely different in their chemical structures [24, 30]. To the best of our knowledge, this is the first study to report such a relationship between DNA damage and the pathologically significant induction of cardiovascular disease by dialdehydes.

GSH is an important antioxidant that has medical applications for anti-decrepitude and in liver protection, free radical scavenging, cancer treatment, and incretion maladjustment in clinic [13-15]. We therefore sought to determine the effect of intracellular GSH depletion and the collapse of mitochondrial membrane potential. Our results showed that the major cytotoxic mechanism by which GX induced cardiovascular disease includes the depletion of intracellular GSH and the collapse of mitochondrial membrane potential. Previous research has shown that GX induces

oxidative stress in different diseases, such as diabetic mellitus [31]. These results are also similar to the results of studies performed on monoaldehydes [24, 29, 31].

Endogenous antioxidant pathways, such as Trx1, protect from an increase in oxidative stress. Trx1 has been reported as a cornerstone in maintaining the cellular redox status and regulation of signaling mechanisms [17, 32]. We also identified the role of oxidized Trx1 in inducing cardiovascular disease mediated by GX, by using a modified western blot analysis. GX-mediated reduction of oxidized Trx1 resulted in partial HAEC cytotoxicity. To the best of our knowledge, this is the first report to directly assess the reduction of oxidized Trx1 in HAECs using the modified western blot analysis. Similar results have indicated that dialdehydes (methylglyoxal) can assist glyoxalase and Trx1 at low concentrations but have an opposite effect at high concentrations [33]. Chemical modification of Trx1 by monoaldehydes can contribute to the development of cardiovascular disease by interfering with the redox signaling functions of Trx1 [24, 33]. However, the underlying reason needs to be elucidated in further research.

We also determined the effect of GX on the activation of MAP kinase pathways using western blot analysis and determined that they indeed contain critical targets, including P-ERK, P-JNK, and P-P38 MAPK, in HAECs. Previous studies have shown that P-JNK and P-ERK induce human cell apoptosis [34, 35]. To the best of our knowledge, this is the first study to directly assess the relationship between HAEC apoptosis and the activation of MAP kinase pathways induced by GX.

In short, the critical effects of GX include the depletion of intracellular GSH,

activation of MAP kinase pathways, and collapse of the mitochondrial membrane potential. Oxidative stress induces the depletion of intracellular GSH, and suppression of the p38 MAPK signaling pathway can alleviate oxidative stress [36]. P-P38 MAPK can induce oxidative stress and apoptosis in human lens epithelial cells [37]. Thus, previous studies have shown that these critical targets affected each other to damage HAECs.

Summary and Conclusions

GX is a serious pollutant that humans are extensively exposed to, and it is subsequently a major factor underlying cardiovascular disorders. However, thus far, the molecular mechanisms underlying this action of GX have remained unknown. This study shows that critical targets include the depletion of intracellular GSH, activation of MAP kinase pathways and collapse of mitochondrial membrane potential, also showing that DNA repair is not important in attenuating GX toxicity. The present findings provide novel insights into the understanding of these molecular mechanisms and may help in the development of new therapeutic targets and diagnostic markers in cardiovascular disease, as well as to provide a reference for the progression of precision medicine.

Declaration

Acknowledgements

We would like to thank Editage for English language editing.

Ethics Approval

N/A

Consent to Participate

N/A

Consent to publication

N/A

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

L-Y J, M-Z X, and G-A Z conceived and designed the study.

M-Z X, C-G, J-Z, J-Q D, G-J L performed the experiments.

M-Z X and C-G wrote the paper.

M-Z X, C-G, L-Y J and G-A Z reviewed and edited the manuscript.

L-Y J, M-Z X, G-A Z, C-G, J-Z, J-Q D, G-J L, K-T S, L-W, D-Y B: (1) made substantial contributions to the conception or design of the work or to the acquisition,

analysis, or interpretation of data for the work; (2) participated in drafting the work or revising it critically for important intellectual content; (3) approved the final version of the manuscript; and (4) agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

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445 **Disclosure Statement**

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447 The authors have no conflicts of interest to report.

448

449 **Funding**

450

451 This study was supported by key scientific and technological research projects of

Henan Province Foundation (2018020346), key scientific and technological research projects of Henan Province Foundation (LHGJ20190456), first affiliated hospital of Xinxiang Medical University youth science fund project (QN-2019-B02), technology breakthrough plan of Science and Technology Department of Xinxiang (GG2019037)

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Fig 1. Analysis of DNA Damage Induced by Glyoxal (GX) in Human Aortic Endothelial Cells (HAECs) Deficient in the FANC Pathway.

Fig 1. Analysis of DNA damage induced by glyoxal (GX) based on human aortic endothelial cells (HAECs) deficient in the FANC pathway. (A) HAECs were treated with a range of concentrations (50, 100, 150, 200 μ M) of GX for 8 days, and their survival was determined. Drug lethal dose (LD₉₀) values were confirmed from survival curves and are indicated by an arrow. (B) Double strand break (DSB) induction. DSBs were detected using static-field gel electrophoresis after treatment with the fraction of DNA released relative to the total DNA. (C) Analysis of DNA–protein crosslinks (DPCs). $p < 0.05$ indicates statistical significance.

A:

B:

Fig. 2. Effect of Glyoxal (GX) on Intracellular Glutathione (GSH).

Fig. 2. Effect of glyoxal (GX) on intracellular glutathione (GSH). (A) Data are the means $\pm$ standard deviations of three separate tests. Statistically significant differences are indicated by an asterisk, $p < 0.05$ indicates statistical significance. (B) Buthionine sulfoximine (BSO; 1 mM) was used to treat human aortic endothelial cells (HAECs) for different times, and cell survival was subsequently determined.

A:

B:

Fig. 3. Effect of Glyoxal (GX) on the Mitochondrial Membrane Potential.

Fig. 3. Effect of Glyoxal (GX) on the mitochondrial membrane potential. (A) Human aortic endothelial cells (HAECs) were treated with GX. Statistically significant differences are indicated by an asterisk, $p < 0.05$ indicates statistical significance. . (B) Survival of cyanide *m*-chlorophenylhydrazine (CCCP)-treated HAECs.

A:

B:

Fig. 4. Redox state of Trx1. (A) Effects of glyoxal (GX) on the redox state of Trx1. Trx1 was either in a completely reduced state (iodoacetic acid (IAA) band) or completely oxidized state (iodoacetamide (IAM) band). Statistical differences are indicated by an asterisk, $p < 0.05$ indicates statistical significance. (B) Survival of human aortic endothelial cells (HAECs).

Fig. 5 Effect of Glyoxal (GX) on MAP Kinase Pathways.

Fig. 5. Effect of glyoxal (GX) on MAP kinase pathways. Statistical differences are indicated by an asterisk $p < 0.05$ indicates statistical significance.

Figures

A:

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Figure 1

Analysis of DNA Damage Induced by Glyoxal (GX) in Human Aortic Endothelial Cells (HAECs) Deficient in the FANCA Pathway. Analysis of DNA damage induced by glyoxal (GX) based on human aortic endothelial cells (HAECs) deficient in the FANCA pathway. (A) HAECs were treated with a range of concentrations (50,

100, 150, 200 μ M) of GX for 8 days, and their survival was determined. Drug lethal dose (LD 90 values were confirmed from survival curves and are indicated by an arrow. (B) Double strand break (DSB) induction. DSBs were detected using static field gel electrophoresis after treatment with the fraction of DNA released relative to the total DNA. (C) Analysis of DNA protein crosslinks (DPCs). $p < 0.05$ indicates statistical significance.

A:

B:

Figure 2

Effect of Glyoxal (GX) on Intracellular Glutathione (GSH). Effect of glyoxal (GX) on intracellular glutathione (GSH). (A) Data are the means $\pm$ standard deviations of three separate tests. Statistically significant differences are indicated by an asterisk, $p < 0.05$ indicates statistical significance. (B) Buthionine sulfoximine (BSO; 1 mM) was used to treat human aortic endothelial cells (HAECs) for different times, and cell survival was subsequently determined.

A:

B:

Figure 3

Effect of Glyoxal (GX) on the Mitochondrial Membrane Potential. Effect of Glyoxal (GX) on the mitochondrial membrane potential. (A) Human aortic endothelial cells (HAECs) were treated with GX. Statistically significant differences are indicated by an asterisk, $p < 0.05$ indicates statistical significance. (B) Survival of cyanide m chlorophenylhydrazine (CCCP) treated HAECs.

A:

B:

Figure 4

Redox state of Trx1. (A) Effects of glyoxal (GX) on the redox state of Trx1. Trx1 was either in a completely reduced state (iodoacetic acid (IAA) band) or completely oxidized state (iodoacetamide (IAM) band). Statistical differences are indicated by an asterisk, $p < 0.05$ indicates statistical significance. (B) Survival of human aortic endothelial cells (HAECs).

Figure 5

Effect of glyoxal (GX) on MAP kinase pathways. Statistical differences are indicated by an asterisk $p < 0.05$ indicates statistical significance.