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Interaction of Methylglyoxal and Hydrogen Sulfide in Rat Vascular Smooth Muscle Cells

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Abstract

Hydrogen sulfide (H_2S) is a gasotransmitter with multifaceted physiological functions, including the regulation of glucose metabolism. Methylglyoxal (MG) is an intermediate of glucose metabolism and plays an important role in the pathogenesis of insulin resistance syndromes. In the present study, we investigated the effect of MG on H_2S synthesis and the interaction between these two endogenous substances. In cultured vascular smooth muscle cells (VSMCs), MG (10, 30, and 50 μM) significantly decreased cellular H_2S levels in a concentration-dependent manner, while H_2S donor, NaHS (30, 60, and 90 μM), significantly decreased cellular MG levels. The expression level and activity of H_2S-producing enzyme, cystathionine γ-lyase (CSE), were significantly decreased by MG treatment. NaHS (30–90 μM) significantly inhibited MG (10 or 30 μM)-induced ROS production. Cellular levels of GSH, cysteine, and homocysteine were also increased by MG or NaHS treatment. Furthermore, direct reaction of H_2S with MG in both concentration- and time-dependent manners were observed in *in vitro* incubations. In conclusion, MG regulates H_2S level in VSMCs by downregulating CSE protein expression and directly reacting with H_2S molecule. Interaction of MG with H_2S may be one of future directions for the studies on glucose metabolism and the development of insulin resistance syndromes. *Antioxid. Redox Signal.* 12, 1093–1100.

Introduction

HYDROGEN SULFIDE (H_2S) is the third gasotransmitter with multifaceted physiological functions (5, 19). Two pyridoxal-5′-phosphate-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), are responsible for the majority of endogenous H_2S production in mammalian tissues using L-cysteine as the substrate (1, 16). The expression of CSE and CBS is tissue specific. For instance, CBS is the major H_2S producing enzyme in the nervous system, whereas CSE is mainly expressed in vascular and nonvascular smooth muscle cells (5, 19, 29). Another less important endogenous source of H_2S is the nonenzymatic reduction of elemental sulfur to H_2S using reducing equivalents obtained from the oxidation of glucose (15).

H_2S exerts a host of biological effects on various types of cells and tissues. At micromolar concentrations, H_2S can have cytoprotective effects (26), while at millimolar concentrations it has been shown to be cytotoxic (6, 27, 28). Previous studies have also proved that H_2S upregulates the expression of anti-inflammatory and cytoprotective genes, including heme oxygenase-1 in pulmonary artery smooth muscle cells (14) and macrophages (13). The vascular relaxation effect of H_2S was proved largely due to the opening of K_{ATP} channels (26, 30). In line with its vasorelaxant effect, a H_2S donor was shown to induce a transient hypotensive response in animals (19, 30). In patients with coronary heart disease, plasma H_2S level was reduced from $\sim$50 to $\sim$25 μM (9). We recently showed that CSE deficiency and reduced endogenous H_2S production in vascular tissues resulted in the development of hypertension in CSE gene knockout mice (29).

Methylglyoxal (MG) is a metabolite of sugar, protein, and fatty acid, formed in virtually all mammalian cells, including vascular smooth muscle cells (VSMCs) (10). Increased MG production has been reported in human red blood cells, bovine endothelial cells, and VSMCs under hyperglycemic conditions or with increased availability of MG precursors such as fructose (18). We recently discovered that hypertension in spontaneously hypertensive rats was related to increased MG levels in plasma and vascular tissues in an age-dependent fashion (20, 21). It has been reported that an elevated MG level is associated with oxidative stress in vascular tissues (22, 25). MG can induce the production of reactive oxygen species (ROS), including peroxynitrite ($ONOO^-$), hydrogen peroxide (H_2O_2), and superoxide anion ($O_2^{\cdot-}$) in cultured VSMCs (2). Moreover, as a highly reactive dicarbonyl molecule, MG can interact with the side chains of arginine, lysine, and cysteine residues in proteins to yield different

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types of advanced glycation endproducts (3). In the present study, we investigated the interaction of MG and H_2S in VSMCs and the cellular effects of this interaction.

Materials and Methods

VSMC preparation

Rat thoracic aortic smooth muscle cell line (A-10 cells) was obtained from American Type Culture Collection (Manassas, VA) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37°C in a humidified atmosphere of 95% air and 5% CO_2, as described in our previous study (2). Cultured cells were grown to 60 ~ 80% of confluence before starved in serum-free DMEM for 24 h and then exposed to MG or H_2S treatments for 24 h. Treated and untreated cells were washed with ice-cold phosphate-buffered saline (PBS), and then harvested by trypsinization. For the determination of oxidized DCF production, cells were seeded in 96-well plates with equal amount of cells in each well ($\sim 4\times10^4$ cells) and treated as indicated above.

Measurement of cellular H_2S levels

H_2S was measured using a microelectrode as previously described (7, 25). Briefly, harvested cells were resuspended in 400 μl of cell lysis buffer (20 m*M* Tris-HCl at pH 7.5, 150 m*M* NaCl, 1 m*M* EDTA, 1 m*M* EGTA, 1% Triton X-100, 2.5 m*M* sodium pyrophosphate, 1 m*M* β-glycerolphosphate, 1 m*M* Na_3VO_4, 1 m*M* phenylmethylsulfonyl fluoride, and a proteinase inhibitor cocktail). H_2S in cell lysis (200 μl) was released by adding 5 ml of 80% sulfuric acid in a sealed filtering flask bubbled with N_2 gas for 15 min. Released H_2S was carried by N_2 gas to an absorber container (15 ml test tube), containing 1 ml of 1 *M* NaOH into which H_2S was absorbed. H_2S level in NaOH was measured with a microelectrode specific for sulfide (Lazar Research Laboratories Inc., Los Angeles, CA). H_2S concentration was calculated using a standard curve of NaHS at different concentrations and is expressed in nmol/mg protein.

Measurement of cellular MG level

Collected cells were lysed in a cell lysis buffer containing a proteinase inhibitor cocktail. MG in the supernatant of cell lysis was measured as previously reported (7,21). Briefly, samples were incubated with 10 m*M* *o*-phenylenediamine (*o*-PD, derivatizing agent) for 3 h at room temperature and protected from light. The quinoxaline formed between dicarbonyl compounds and *o*-PD, as well as the internal standard (5-methylquinoxaline) were measured using a Hitachi D-7000 high-performance liquid chromatography (HPLC) system (Hitachi Ltd., Mississauga, Ontario, Canada) (21). A Nova-Pak C18 column was used (Waters, MA). The mobile phase was composed of 8% (v/v) of 50 m*M* NaH_2PO_4 (pH 4.5), 17% (v/v) of HPLC grade acetonitrile, and 75% of water. Samples were measured in triplicates.

Measurement of CSE activity

CSE enzyme activity was determined by measuring the production rate of H_2S as reported (25). Briefly, collected cells were suspended in 400 μl of ice-cold potassium phosphate buffer (50 m*M*, pH 6.8) supplemented with proteinase inhibitor cocktail and lysed by sonication on ice. Supernatant (100 μl) was added to 1 ml of reaction mixture containing (m*M*): 100 potassium phosphate buffer (pH 7.4), 10 L-cysteine, and 2 pyridoxal-5′-phosphate. Cryovial test tubes (2 ml) were used as the center wells, each containing 0.5 ml 1% zinc acetate as trapping solution. Reaction was performed in a 25 ml Erlenmeyer flask. The flasks containing the reaction mixture and center wells were flushed with N_2 gas before being sealed with a double layer of parafilm. Reaction was initiated by transferring the flasks from ice to a 37°C shaking water bath. After incubating at 37°C for 90 min, 0.5 ml of 50% trichloroacetic acid was added to stop the reaction. The flasks were sealed again and incubated at 37°C for another 60 min to ensure a complete trapping of released H_2S gas from the mixture. Contents of the center wells were then transferred to test tubes, each containing 0.5 ml of water. Subsequently, 0.5 ml of 20 m*M* N,N-dimethyl-p-phenylenediamine sulfate in 7.2 *N* HCl was added immediately followed by addition of 0.5 ml 30 m*M* $FeCl_3$ in 1.2 *N* HCl. The mixture was kept at room temperature, protected from light, for 20 min followed by recording the absorbance at 670 nm with a spectrophotometer. H_2S concentration was calculated using a calibration curve of standard NaHS solutions.

RNA isolation and real-time quantitative PCR

Total RNA was isolated using RNeasy Mini Kit (QIAGEN, Mississauga, Ontario, Canada) according to the manufacturer's instructions. First strand cDNA was prepared from total RNA (5 μg) by reverse transcription using M-MLV reverse transcriptase (Invitrogen, Burlington, Ontario, Canada) and oligo(dT) primer. Real-time quantitative PCR was performed on iCycler iQ Real-time PCR Detection System (Bio-Rad, Hercules, CA). The primers of rat CSE were as following: forward 5′-GGACAAGAGCCGGAGCAATGGAGT-3′, reverse 5′- CCCCGAGGCGAAGGTCAAACAGT-3′. The primers for rat β-actin were: forward 5′-CGTTGACATCCGTAAAGAC-3′ and reverse 5′-TAGGAGCCAGGGCAGTA-3′. The PCR conditions were as following: denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 1 min, and extension at 72°C for 30 s. Specificity of the amplification was determined by melting curve analysis. Data were expressed as a ratio of the quantity of CSE mRNA to the quantity of β-actin mRNA.

Western blot analysis of CSE expression

Total proteins were extracted from harvested cells with 300 μl of cell lysis buffer as described above. Proteins (40 μg) were subject to Western blot analysis according to the procedure reported (4). The primary antibody dilutions were 1:500 for antibodies against CSE (Abnova, Taipei, Taiwan) and 1:5000 for β-actin. Western blots were digitized with Chemi Genius^2 Bio Imaging System (SynGene, Frederick, MD), quantified using software of GeneTools from SynGene and normalized against the quantity of loaded β-actin.

Measurement of ROS production

The formation of oxidized DCF was determined by a DCFH assay as described previously with minor modification (2). Briefly, starved cells were loaded with a membrane-

permeable and nonfluorescent probe DCFH-DA for 2 h at 37°C in phenol red-free DMEM, protected from light. Thereafter, the cells were washed three times with phenol red-free DMEM to remove the excess probe, followed by the treatment with or without MG or MG plus NaHS at desired concentrations for different time periods in phenol red-free DMEM. Once inside cells, DCFH-DA becomes the membrane-impermeable $DCFH_2$ in the presence of cytosolic esterases and further oxidized by H_2O_2 or $ONOO^-$ to form oxidized DCF with detectable fluorescence. Oxidized DCF was quantified by monitoring DCF fluorescence intensity with excitation at 485 nm and emission at 527 nm with a Fluoroskan Ascent plate reader (Thermo Labsystem, Beverly, MA) using Ascent software and expressed in arbitrary units.

Measurement of GSH, L-cysteine, and homocysteine levels

Levels of reduced glutathione (GSH), L-cysteine, and homocysteine in the supernatant of cell lysis were determined by derivation with 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) and reverse-phase HPLC using ultraviolet detection, as described in our previous study (20). Briefly, the reaction mixture for the analysis of free reduced sulfhydryl groups contained 250 μl 500 m*M* Tris–HCl buffer (pH 8.9), 65 μl sample or standard, 10 μl internal standard (400 μM D(-)-penicillamine in cold 5% sulfosalicylic acid containing 0.1 m*M* EDTA), and 175 μl 10 m*M* DTNB made up in 0.5 m*M* K_2HPO_4 (pH 7.2). After 5 min of derivatization, the mixture was acidified with 21.5 μl 7 *M* H_3PO_4, and 50 μl of the mixture was injected into the HPLC system. Chromatography was accomplished using isocratic elution on a Supelcosil LC-18-T column (150×4.6 mm, 3 μm) incubated at 37°C. The mobile phase consisted of 12.5% methanol (v/v) and 100 m*M* KH_2PO_4 (pH 3.85) at a flow rate of 0.9 ml/min. Sulfhydryl–DTNB derivatives were detected by ultraviolet absorbance at 345 nm. After 10 min of isocratic elution, the methanol concentration was increased to 40% and pumped for 8 min to elute excess DTNB reagent from the column. The methanol concentration was then decreased to 12.5% and pumped for 7 min before the next sample injection. For analyte quantification, standard curves were constructed by spiking the supernatant with various known amounts of GSH, L-cysteine, and homocysteine (Sigma, Oakville, Ontario, Canada). Samples were run in duplicate. Data were collected digitally with D-7000 HPLC System Manager software, Hitachi, Ltd., (Mississauga, Ontario, Canada) and peak areas were quantified.

Direct reaction of H_2S with MG

MG (10 μM) was mixed with H_2S at different concentrations (10, 50, and 100 μM) in cell-free PBS buffer and incubated at 37°C for 1 ~ 24 h. H_2S stock solution was prepared by bubbling H_2S in distilled H_2O for 30 min (30). After incubation, free MG was measured with HPLC as described above.

Chemicals and data analysis

MG and NaHS were obtained from Sigma-Aldrich (Oakville, Canada). The data are expressed as mean ± SEM from at least three independent experiments. Statistical analyses were performed using Student's *t* test or ANOVA. Statistical significance was considered at $p < 0.05$.

Results

MG treatment decreased H_2S level in VSMCs and vice versa

Cultured VSMCs were treated with MG at different concentrations for 24 h. After MG treatment, H_2S levels in cell lysates were significantly decreased in a MG concentration-dependent fashion (Fig. 1). In another group of experiments, the effect of H_2S treatment on MG level was studied. Cultured VSMCs were treated with H_2S donor, NaHS, at different concentrations for 24 h. After NaHS treatment, MG levels in cell lysates were significantly decreased in a NaHS concentration-dependent manner (Fig. 2).

MG-induced downregulation of CSE protein expression

In VSMCs, CSE is the major enzyme responsible for H_2S production. Thus, the CSE expression level in MG-treated cells was investigated. Quantitative-PCR results indicated that MG treatment did not significantly affect CSE mRNA levels (Fig. 3A). Western blot results showed that CSE protein level was significantly lower in 30 and 50 μM MG-treated cells, but not with 10 μM MG treatment (Fig. 3B and C). As shown in Fig. 3D, CSE activity in 30 and 50 μM MG-treated cells was significantly decreased by 14% and 29% in comparison to the untreated control. CSE activity in 10 μM MG-treated group was lower than the untreated control although the difference was not significant.

Effect of H_2S on MG-induced ROS production

ROS formation in VSMCs was significantly increased by MG (10, 30, and 50 μM) in both time- and concentration-dependent manners (Fig. 4A and B). Interestingly, 30 μM NaHS significantly decreased 10 μM MG-induced ROS production, but not 30 or 50 μM MG (Fig. 4A). The antioxidant effect of H_2S was observed within 8 h of application and continued thereafter (Fig. 4B). NaHS at 60 and 90 μM decreased 10 μM MG-induced ROS production (Fig. 4C). This effect of NaHS was more potent when the cells were treated with 30 μM MG

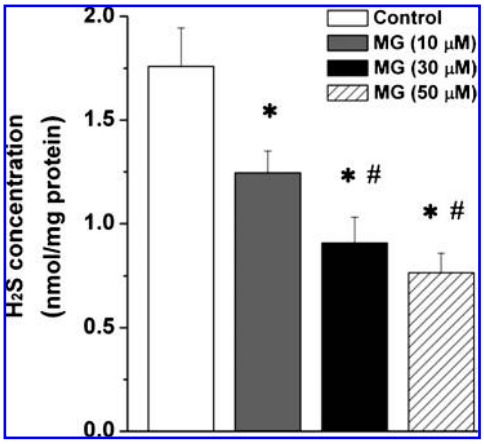

FIG. 1. H_2S level in MG-treated VSMCs. VSMCs were treated with MG at 10, 30, and 50 μM for 24 h, respectively. H_2S level in cell lysis was expressed in nmol/mg protein. $n = 4 \sim 6$; $^*p < 0.05$ *vs.* control; $^{\#}p < 0.05$ *vs.* MG (10 μM).

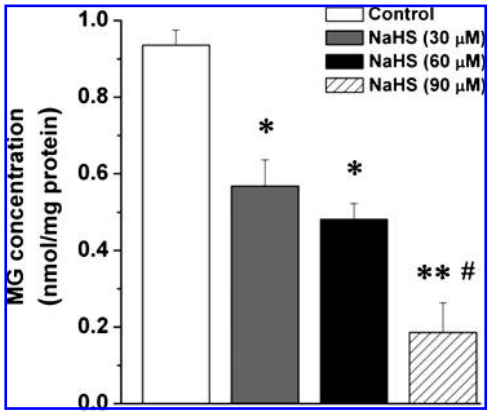

FIG. 2. MG level in NaHS-treated VSMCs. VSMCs were treated with H_2S donor NaHS at 30, 60, and 90 μM for 24 h, *respectively*. MG level in cell lysis was measured using HPLC as described in Materials and Methods; $n = 3$, $^{*}p < 0.05$ or $^{**}p < 0.01$ *vs.* control; $^{\#}p < 0.05$ *vs.* NaHS (30 or 60 μM).

(Fig. 4D). However, NaHS no longer offered the antioxidant effect against MG-induced ROS production once its concentration reached 120 μM (Fig. 4C and D).

GSH, cysteine, and homocysteine levels in MG- or NaHS-treated VSMCs

GSH is an important antioxidant agent that protects the cells from oxidative stress (31). After VSMCs were treated with MG for 24 h, cellular GSH level was significantly increased correspondingly to the concentrations of MG (10–50 μM) (Fig. 5A).Homocysteine is a precursor of cysteine synthesis, while cysteine is a precursor of GSH. MG treatment at 10 μM, but not 30 or 50 μM, significantly increased cysteine and homocysteine levels in cultured VSMCs (Fig. 5B and C).

The effects of H_2S on GSH, cysteine, and homocysteine levels were also investigated. Cellular GSH levels were significantly increased when cells were exposed to NaHS treatment as compared with control cells (Fig. 6A). However, GSH levels in 60 and 90 μM NaHS-treated cells were significantly lower than that in 30 μM NaHS-treated cells. L-cysteine levels were also significantly increased by NaHS treatments (30, 60, and 90 μM) (Fig. 6B).

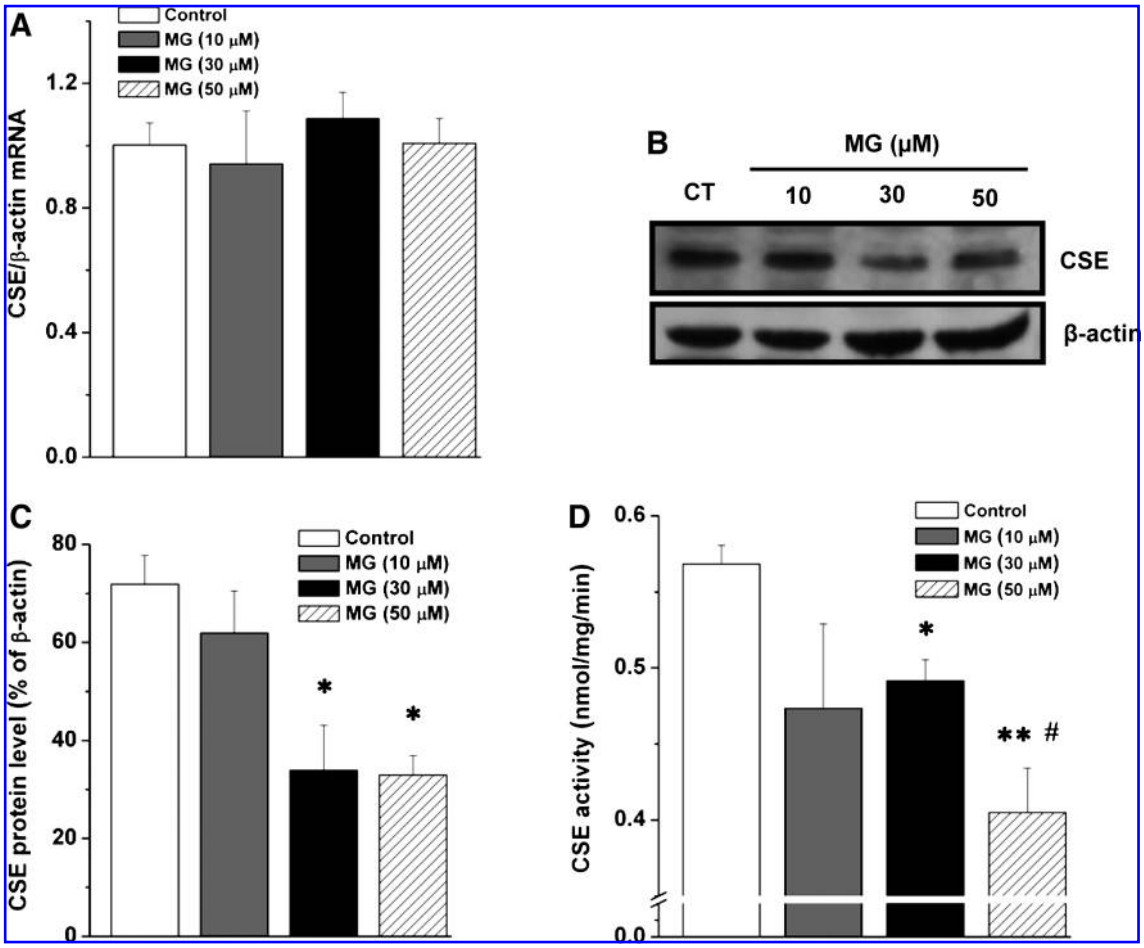

FIG. 3. Effects of MG treatment on CSE expression and activity. VSMCs were treated with MG at 10, 30, and 50 μM for 24 h, respectively. Treated cells were collected for RNA or protein extraction. (**A**) Real-time PCR results of CSE mRNA level in MG-treated cells; $n = 6$ for each group. (**B**) and (**C**) CSE protein level in MG-treated cells; $n = 4$ for each group, $^{*}p < 0.05$ *vs.* control. (**D**) CSE activity in MG-treated cells; $n = 6 \sim 9$, $^{*}p < 0.05$ or $^{**}p < 0.01$ *vs.* control; $^{\#}p < 0.05$ *vs.* MG (30 μM).

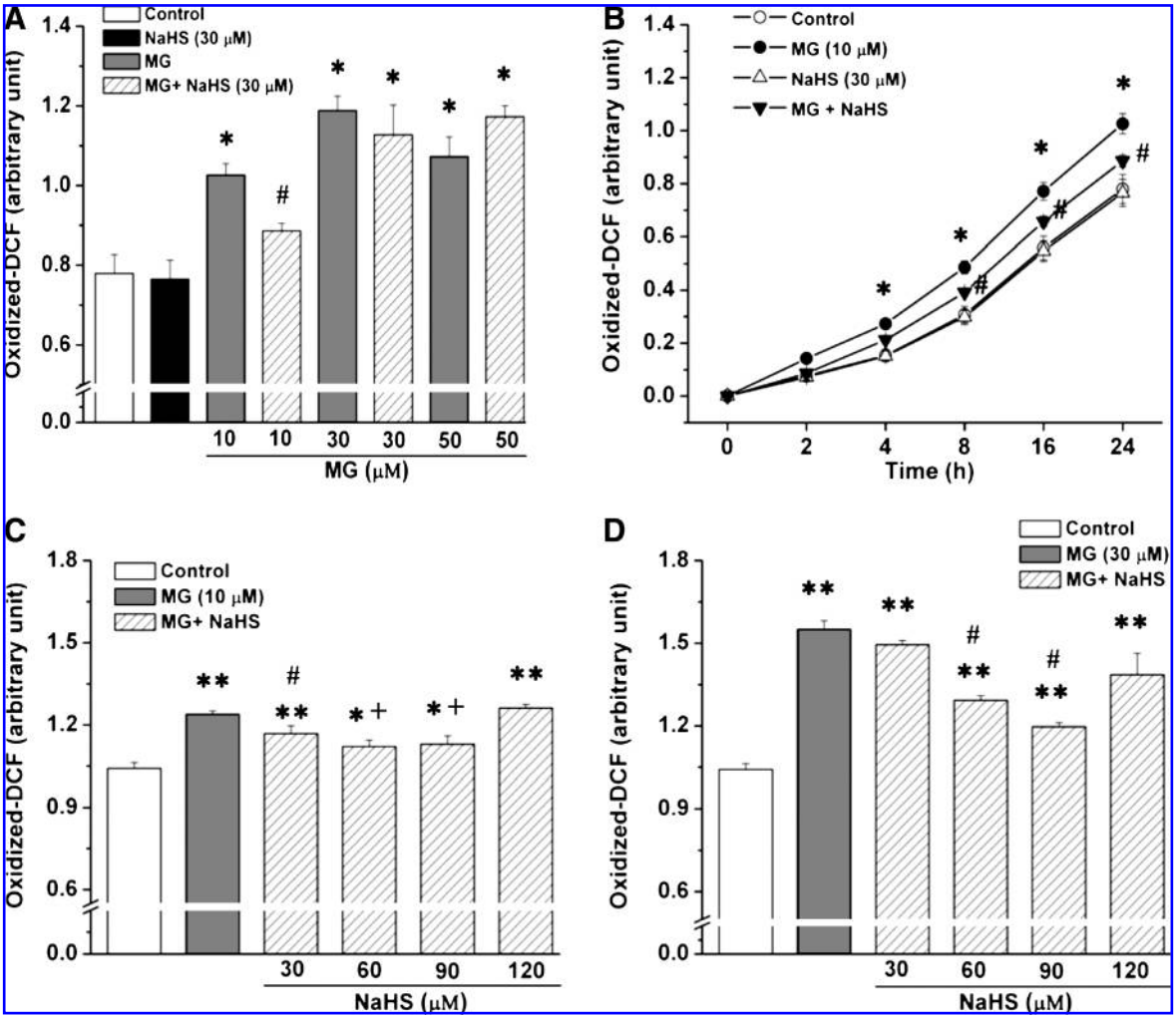

FIG. 4. Effect of NaHS on MG-induced ROS production in VSMCs. (**A**) Oxidized DCF level in cells treated with MG at different concentrations in the presence of NaHS (30 μM). (**B**) Time-dependent effect of NaHS (30 μM) on MG (10 μM)-induced ROS production. (**C**) and (**D**) Concentration-dependent effect of NaHS on MG (10 and 30 μM)-induced ROS production, $n = 8$ for each group, $^{*}p < 0.05$ or $^{**}p < 0.01$ *vs.* untreated control; $^{\#}p < 0.05$ *vs.* MG (10 μM) or *vs.* MG (30 μM) + NaHS (30 μM); $^{+}p < 0.05$ *vs.* MG (10 μM) + NaHS (30 μM).

Direct reaction of MG with H_2S

To understand the mechanism of H_2S and MG interaction, we tested whether MG directly reacts with H_2S molecule. For this purpose, MG (10 μM) was mixed with H_2S at different concentrations in cell-free PBS buffer and incubated at 37°C for 24 h. After incubation, free MG was measured with HPLC. H_2S at 50 and 100 μM, but not at 10 μM, significantly decreased MG levels (Fig. 7A). When the H_2S incubation time was <4 h, no change in MG level was observed. However, significant decreases in MG levels were detected after 8 h of incubation with the lowest level detected after 18 h incubation with H_2S (Fig. 7B).

Discussion

Under physiological condition, MG level is generally higher in vascular tissue than in other types of tissues (20). CSE is responsible for H_2S production in vascular tissue, endothelium, pancreas, and liver, while CBS produces H_2S mainly in brain and kidney (19, 25, 29). Obviously, MG and H_2S are co-produced in VSMCs. For instance, we found that the interaction of MG and H_2S lowers their respective cellular levels. We also found that CSE protein level was down-regulated by MG (30 and 50 μM) although no change of CSE mRNA level in MG-treated cells was observed. The above results indicate that MG treatment may decrease the translation of CSE mRNA or the stability of CSE proteins. However, the underlying mechanisms of MG treatment on CSE mRNA translation and the protein stability are not yet clear and will need further investigation. Consistent with decreased CSE protein levels after MG treatments, CSE enzyme activity was also decreased as indicated by a lower production rate of H_2S. Therefore, MG-induced decrease in CSE protein level could at least in part explain the decrease in H_2S level.

Whereas endogenous cellular H_2S level was significantly decreased by 10 μM MG treatment, CSE protein level or CSE activity was not significantly different from the untreated control. This phenomenon could be explained by a direct reaction between MG and H_2S, considering H_2S as a reducing agent and MG as a reactive dicarbonyl molecule. We found

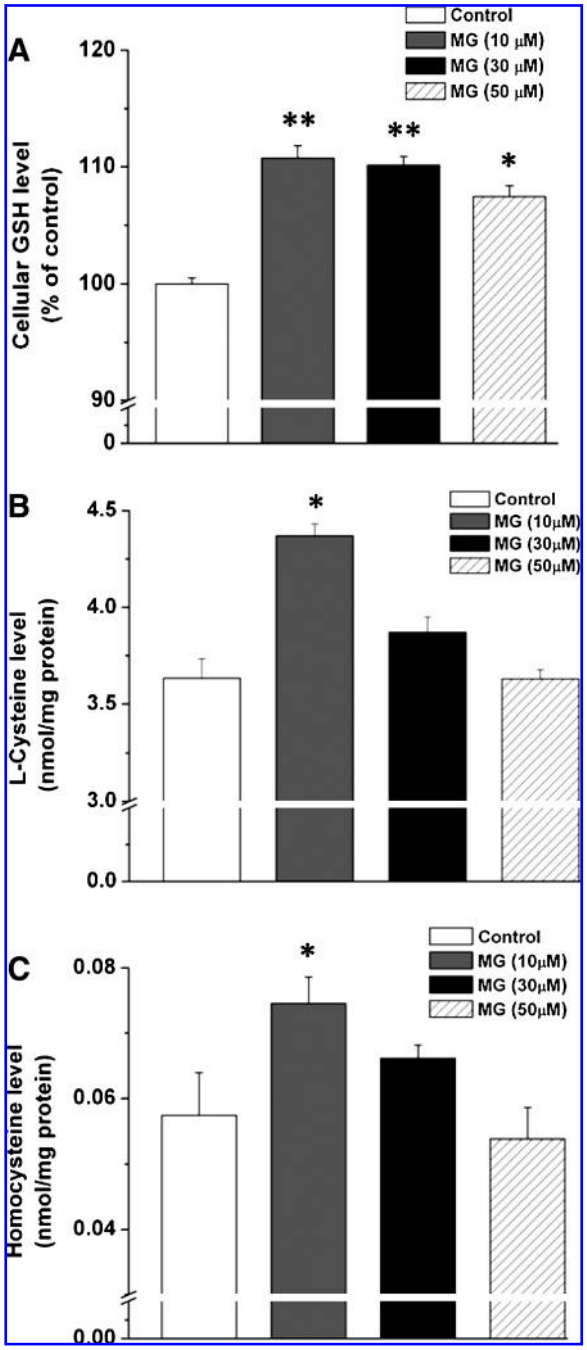

FIG. 5. GSH, cysteine, and homocysteine levels in MG treated VSMCs. Cells treated with MG at different concentrations were harvested after 24 h to determine cellular GSH (**A**), cysteine (**B**), and homocysteine (**C**) levels using HPLC method as described in Materials and Methods; $n = 4 \sim 7$ for each group; $^{*}p < 0.05$ or $^{**}p < 0.01$ *vs.* untreated control.

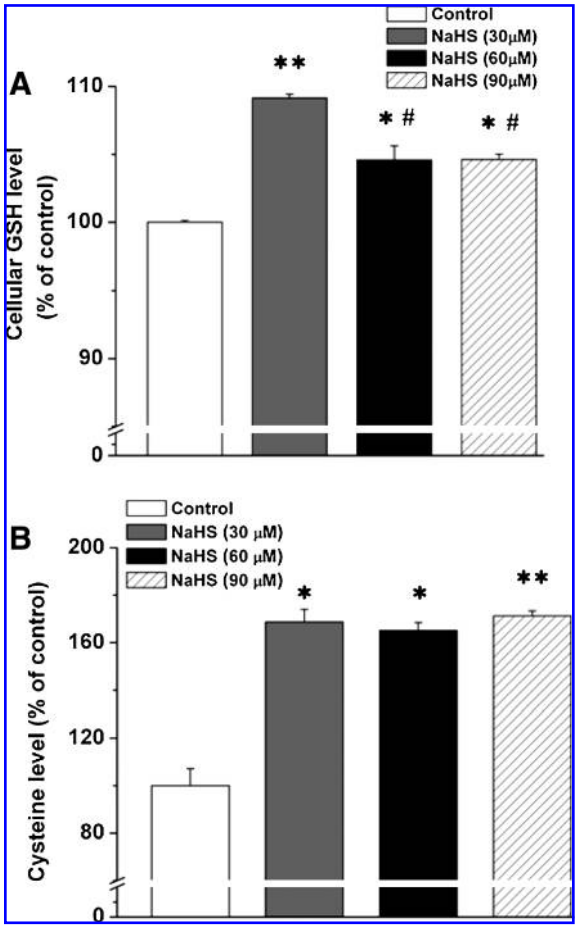

FIG. 6. GSH and cysteine levels in NaHS-treated VSMCs. Cells treated with NaHS at different concentrations were harvested after 24 h to determine cellular GSH (**A**) and cysteine (**B**) levels using HPLC method as described in Materials and Methods; $n = 4$ for each group; $^{*}p < 0.05$ or $^{**}p < 0.01$ *vs.* untreated control; $^{\#}p < 0.05$ *vs.* NaHS (30 μM).

that MG level in the cell-free MG/H_2S mixture was decreased 8 h after the incubation was started. This chemical reaction between MG with H_2S occurred in both time- and concentration-dependent manners. These data suggest that a direct reaction of MG with H_2S may be responsible for lower MG (10 μM)-induced decrease in H_2S level, while MG at 30–50 μM caused both a direct molecule-to-molecule reaction, as well as the downregulation of CSE protein expression. Consistently, the direct reaction of MG with H_2S may have caused the decreased MG level in H_2S donor-treated VSMCs.

In our previous study, we showed that MG increased ROS production in VSMCs by increasing $ONOO^-$, H_2O_2, and $O_2^{\bullet -}$ levels (2, 3). We also showed that H_2S protected VSMCs against homocysteine-induced oxidative stress (26). It was of interest to study the effect of H_2S on MG-induced ROS production. Our results support the notion that H_2S acts as an antioxidant (26). At the concentrations lower than 90 μM, H_2S decreased 10 and 30 μM MG-induced ROS production in a concentration-dependent manner, but had no effect on 50 μM MG-induced ROS generation. This could be due to the fact that MG-induced ROS formation at high concentrations overwhelms the antioxidant ability of H_2S. It is also important to note that H_2S at concentration higher than physiological related concentration, for example, 120 μM fails to inhibit low concentrations of MG (10 and 30 μM)-induced ROS production. This may be related to the toxicity and pro-apoptosis effect of H_2S at high concentrations (6, 27, 28).

One of the most important and abundant endogenous antioxidant is GSH, which is found at millimolar range in most

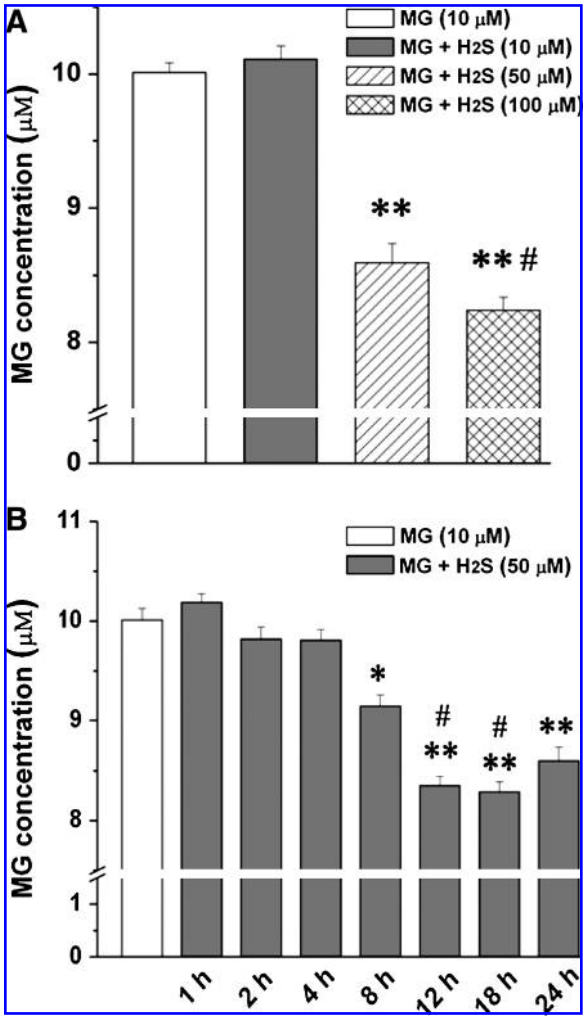

FIG. 7. Reaction of H_2S with MG. MG (10 μM) was mixed with H_2S at different concentrations (10, 50, and 100 μM) in PBS buffer and incubated at 37°C for 24 h. Free MG in the mixtures was measured with HPLC as described in Materials and Methods; $n = 3$, $^{*}p < 0.05$ or $^{**}p < 0.01$ *vs.* MG (10 μM); $^{\#}p < 0.05$ *vs.* MG (10 μM) + H_2S (50 μM) or MG (10 μM) + H_2S (50 μM) 8 h after incubation.

cells (31). GSH levels are significantly elevated when the cells are treated with MG (10, 30, and 50 μM) compared to that of the control group. Cysteine availability is the rate-limiting step in GSH synthesis, and homocysteine is the precursor to cysteine. We observed a corresponding increase of cysteine and homocysteine levels in cells treated with MG at the concentration of 10 μM, but not 30 or 50 μM. This may be due to the huge consumption of cysteine and homocysteine in order to maintain GSH at a certain level to compensate the higher ROS levels induced by MG (30 and 50 μM). On the other hand, H_2S treatment of VSMCs also increased GSH level, which may be attributed to H_2S-enhanced activity of γ-glutamylcysteine synthetase (11). Furthermore, H_2S may cause a feedback inhibition of CSE (12), which could lead to a decreased breakage of cysteine to produce H_2S. Consistently, the increased level of cysteine was observed after H_2S treatment. The consequent increased level of cysteine may inhibit the demethylation of methionine to produce homocysteine (17).

The physiological relevance for the interaction between MG and H_2S has not been previously investigated. Elevated MG level is linked with the development of hypertension and insulin resistance (3, 8, 23). In vascular tissue, elevated MG level is expected to lead to a decreased H_2S level based on the direct reaction of MG with H_2S and the downregulation of CSE expression by MG. One of the consequences of abnormally low H_2S level would decrease the opening of K_{ATP} channels and impair vascular relaxation, causing an increased peripheral circulation resistance and hypertension development or vascular complications of diabetes. In conclusion, MG can react with H_2S and cause a downregulation of the expression of CSE. MG may reduce H_2S production, whereas H_2S may limit the availability of free MG. As mentioned before, CSE is mainly expressed in vascular tissue, endothelium, pancreas, and liver, while MG is produced virtually all mammalian cells. Therefore, the interaction of MG with H_2S are expected to occur in VSMCs and possibly other types of tissues, which may provide one of future directions for the studies on glucose metabolism and the development of insulin resistance syndromes.

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Author Disclosure Statement

No competing financial interests exist.

References

1. Bukovska G, Kery V, and Kraus JP. Expression of human cystathionine beta-synthase in *Escherichia coli*: Purification and characterization. *Protein Expr Purif* 5: 442–448, 1994.
2. Chang T, Wang R, and Wu L. Methylglyoxal-induced nitric oxide and peroxynitrite production in vascular smooth muscle cells. *Free Radic Biol Med* 38: 286–293, 2005.
3. Chang T and Wu L. Methylglyoxal, oxidative stress, and hypertension. *Can J Physiol Pharmacol* 84: 1229–1238, 2006.
4. Chang T, Wu L, and Wang R. Inhibition of vascular smooth muscle cell proliferation by chronic hemin treatment. *Am J Physiol Heart Circ Physiol* 295: H999–H1007, 2008.
5. d'Emmanuele di Villa Bianca R, Sorrentino R, Maffia P, Mirone V, Imbimbo C, Fusco F, De Palma R, Ignarro LJ, and Cirino G. Hydrogen sulfide as a mediator of human corpus cavernosum smooth-muscle relaxation. *Proc Natl Acad Sci USA* 106: 4513–4518, 2009.
6. Deplancke B and Gaskins HR. Hydrogen sulfide induces serum-independent cell cycle entry in nontransformed rat intestinal epithelial cells. *FASEB J* 17: 1310–1312, 2003.
7. Dhar A, Desai K, Liu J, and Wu L. Methylglyoxal, protein binding and biological samples: Are we getting the true measure? *J Chromatogr B Analyt Technol Biomed Life Sci* 877: 1093–1100, 2009.
8. Jia X, Olson DJ, Ross AR, and Wu L. Structural and functional changes in human insulin induced by methylglyoxal. *FASEB J* 20: 1555–15557, 2006.
9. Jiang HL, Wu HC, Li ZL, Geng B, and Tang CS. [Changes of the new gaseous transmitter H_2S in patients with coronary heart disease]. *Di Yi Jun Yi Da Xue Xue Bao* 25: 951–954, 2005.
10. Kalapos MP. Methylglyoxal toxicity in mammals. *Toxicol Lett* 73: 3–24, 1994.

11. Kimura Y and Kimura H. Hydrogen sulfide protects neurons from oxidative stress. *FASEB J* 18: 1165–1167, 2004.
12. Kredich NM, Foote LJ, and Keenan BS. The stoichiometry and kinetics of the inducible cysteine desulfhydrase from *Salmonella typhimurium*. *J Biol Chem* 248: 6187–6196, 1973.
13. Oh GS, Pae HO, Lee BS, Kim BN, Kim JM, Kim HR, Jeon SB, Jeon WK, Chae HJ, and Chung HT. Hydrogen sulfide inhibits nitric oxide production and nuclear factor-kappaB via heme oxygenase-1 expression in RAW264.7 macrophages stimulated with lipopolysaccharide. *Free Radic Biol Med* 41: 106–119, 2006.
14. Qingyou Z, Junbao D, Weijin Z, Hui Y, Chaoshu T, and Chunyu Z. Impact of hydrogen sulfide on carbon monoxide/heme oxygenase pathway in the pathogenesis of hypoxic pulmonary hypertension. *Biochem Biophys Res Commun* 317: 30–37, 2004.
15. Searcy DG and Lee SH. Sulfur reduction by human erythrocytes. *J Exp Zool* 282: 310–322, 1998.
16. Stipanuk MH and Beck PW. Characterization of the enzymic capacity for cysteine desulphhydration in liver and kidney of the rat. *Biochem J* 206: 267–277, 1982.
17. Verhoef P, Steenge GR, Boelsma E, van Vliet T, Olthof MR, and Katan MB. Dietary serine and cystine attenuate the homocysteine-raising effect of dietary methionine: a randomized crossover trial in humans. *Am J Clin Nutr* 80: 674–679, 2004.
18. Wang H, Meng QH, Chang T, and Wu L. Fructose-induced peroxynitrite production is mediated by methylglyoxal in vascular smooth muscle cells. *Life Sci* 79: 2448–2454, 2006.
19. Wang R. Two's company, three's a crowd: Can H_2S be the third endogenous gaseous transmitter? *FASEB J* 16: 1792–1798, 2002.
20. Wang X, Desai K, Chang T, and Wu L. Vascular methylglyoxal metabolism and the development of hypertension. *J Hypertens* 23: 1565–1573, 2005.
21. Wang X, Desai K, Clausen JT, and Wu L. Increased methylglyoxal and advanced glycation end products in kidney from spontaneously hypertensive rats. *Kidney Int* 66: 2315–2321, 2004.
22. Wang X, Jia X, Chang T, Desai K, and Wu L. Attenuation of hypertension development by scavenging methylglyoxal in fructose-treated rats. *J Hypertens* 26: 765–772, 2008.
23. Wu L. Is methylglyoxal a causative factor for hypertension development? *Can J Physiol Pharmacol* 84: 129–139, 2006.
24. Wu L and Juurlink BH. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. *Hypertension* 39: 809–814, 2002.
25. Wu L, Yang W, Jia X, Yang G, Duridanova D, Cao K, and Wang R. Pancreatic islet overproduction of H_2S and suppressed insulin release in Zucker diabetic rats. *Lab Invest* 89: 59–67, 2009.
26. Yan SK, Chang T, Wang H, Wu L, Wang R, and Meng QH. Effects of hydrogen sulfide on homocysteine-induced oxidative stress in vascular smooth muscle cells. *Biochem Biophys Res Commun* 351: 485–491, 2006.
27. Yang G, Cao K, Wu L, and Wang R. Cystathionine gamma-lyase overexpression inhibits cell proliferation via a H_2S-dependent modulation of ERK1/2 phosphorylation and p21Cip/WAK-1. *J Biol Chem* 279: 49199–49205, 2004.
28. Yang G, Sun X, and Wang R. Hydrogen sulfide-induced apoptosis of human aorta smooth muscle cells via the activation of mitogen-activated protein kinases and caspase-3. *FASEB J* 18: 1782–1794, 2004.
29. Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, and Wang R. H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 322: 587–590, 2008.
30. Zhao W, Zhang J, Lu Y, and Wang R. The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. *EMBO J* 20: 6008–6016, 2001.
31. Zhao Y, Seefeldt T, Chen W, Wang X, Matthees D, Hu Y, and Guan X. Effects of glutathione reductase inhibition on cellular thiol redox state and related systems. *Arch Biochem Biophys* 485: 56–62, 2009.

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Abbreviations Used

CBS = cystathionine β-synthase
CSE = cystathionine γ-lyase
GSH = reduced glutathione
H_2S = hydrogen sulfide
MG = methylglyoxal
NaHS = sodium hydrosulfide
ROS = reactive oxygen species
VSMCs = vascular smooth muscle cells

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2. J. Liu, R. Wang, K. Desai, L. Wu. 2011. Upregulation of aldolase B and overproduction of methylglyoxal in vascular tissues from rats with metabolic syndrome. *Cardiovascular Research* . [CrossRef]
3. L. Zhang, G. Yang, G. Tang, L. Wu, R. Wang. 2011. Rat pancreatic level of cystathionine #-lyase is regulated by glucose level via specificity protein 1 (SP1) phosphorylation. *Diabetologia* . [CrossRef]
4. Neal D. Mathew, David I. Schlipalius, Paul R. Ebert. 2011. Sulfurous Gases As Biological Messengers and Toxins: Comparative Genetics of Their Metabolism in Model Organisms. *Journal of Toxicology* **2011**, 1-14. [CrossRef]
5. Kaushik M Desai, Tuanjie Chang, Ashley Untereiner, Lingyun Wu. 2011. Hydrogen sulfide and the metabolic syndrome. *Expert Review of Clinical Pharmacology* **4**:1, 63-73. [CrossRef]
6. Ming-Jie Wang, Wen-Jie Cai, Yi-Chun Zhu. 2010. Mechanisms of angiogenesis: Role of hydrogen sulphide. *Clinical and Experimental Pharmacology and Physiology* **37**:7, 764-771. [CrossRef]
7. Rui Wang . 2010. Hydrogen Sulfide: The Third Gasotransmitter in Biology and Medicine. *Antioxidants & Redox Signaling* **12**:9, 1061-1064. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]