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RELATIONSHIP BETWEEN HYDROGEN SULFIDE LEVELS AND HDL-CHOLESTEROL, ADIPONECTIN, AND POTASSIUM LEVELS IN THE BLOOD OF HEALTHY SUBJECTS

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Abstract

Hydrogen sulfide (H₂S) is an important signaling molecule whose blood levels have been shown to be lower in certain disease states. Increasing evidence indicates that H₂S plays a potentially significant role in many biological processes and that malfunctioning of H₂S homeostasis may contribute to the pathogenesis of vascular inflammation and atherosclerosis. This study examined the fasting blood levels of H₂S, HDL-cholesterol, LDL-cholesterol, triglycerides, adiponectin, resistin, and potassium in 36 healthy adult volunteers. There was a significant positive correlation between blood levels of H₂S and HDL-cholesterol ($r=0.49$, $p=0.003$), adiponectin ($r=0.36$, $p=0.04$), and potassium ($r=0.34$, $p=0.047$), as well as a significant negative correlation with LDL/HDL levels ($r=-0.39$, $p=0.02$). This is the first demonstration of an association of circulating levels of H₂S with the HDL, LDL, and adiponectin homeostasis in the blood of healthy humans.

Keywords

H₂S; lipids; adiponectin

INTRODUCTION

Hydrogen sulfide is gaining acceptance as a signaling molecule and has been shown to elicit a variety of biological effects that may mediate the protection of cardiovascular pathophysiology (1-4). H₂S inhibits oxidative stress, promotes stimulation of K_{ATP} channels and relaxation and vasodilation in vascular smooth muscle cells (1-4). *In vivo*, H₂S has been shown to inhibit leukocyte endothelial cell interactions and ischemia-reperfusion injury in liver and heart in animal studies (1-4). Genetic deletion of the CSE enzyme in mice markedly reduces H₂S levels in the serum, heart, aorta and other tissues, and mice lacking CSE display pronounced hypertension and diminished endothelium dependent vasorelaxation (1). Furthermore, apolipoprotein E knock-out mice treated with an H₂S donor showed reduced atherosclerotic plaque size compared to controls (5). Several recent reviews

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Statement of Originality: There is no previous study that has examined relationship between blood levels of hydrogen sulfide and HDL-cholesterol and adiponectin levels in animal or human studies. This work has not been submitted before for publication or presentation.

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discuss the potential benefits of H₂S in biological systems and its role in the disease process, such as the physiological effects mediated by H₂S in the protection of mitochondrial function and cardiovascular pathophysiology (1-4). These studies suggest that H₂S is a physiological neuromodulator, vasodilator, and regulator of blood pressure. HDL-cholesterol and adiponectin are well known biomarkers whose elevated blood levels are known to have a beneficial effect in the prevention of cardiovascular disease in humans. This study examined the hypothesis that blood levels of HDL-cholesterol and adiponectin are positively related to H₂S levels in healthy human volunteers.

MATERIALS AND METHODS

Subject Enrollment

Informed written consent was obtained from all patients according to the protocol approved by the Louisiana State University Health Sciences Center Institutional Review Board (IRB). All patients included in this study were healthy adults (ages 21-65 years) with no history of any chronic illness. Those who were taking any supplemental vitamins or any herbal products were excluded from this study. Thirty six volunteers were enrolled in this study. All patients who gave written informed consent were invited to return to have blood drawn after fasting overnight. Women with a positive pregnancy test or those nursing infants were also excluded. Subjects who were taking any supplemental vitamins or herbal products were not included in this study. All participants who gave informed consent came to the clinic for the blood draw.

Blood Collection

Blood was drawn from patients after an overnight fast (8 hours). Following blood collection, serum tubes for chemistry profile and EDTA tubes for HbA_{1c} were promptly delivered to the LSUHSC clinical laboratories. Additional tubes of EDTA-blood were brought to the research laboratory. Clear plasma was separated via centrifugation at 3000 rpm (1500 x g) for 15 minutes.

Adiponectin, resistin, insulin, leptin, and H₂S assays

Adiponectin, resistin, insulin and leptin levels in the plasma were determined by the sandwich ELISA method using commercially available kits from Fisher Thermo Scientific Co. (Rockford, IL). All appropriate controls and standards as specified by the manufacturer's kit were used. In the cytokine assay, control samples were analyzed each time to check the variation from plate to plate on different days of analysis. Plasma hydrogen sulfide was determined using appropriate controls and standards as described previously (6), which is based upon the formation of Methylene Blue. Briefly, 200 μ L culture medium were mixed with 600 μ L 1% (w/v) zinc acetate to trap H₂S during formation of zinc sulfide, followed by the addition of 400 μ L N-dimethyl-*p*-phenylenediamine sulfate (20 mM in 7.2 M HCl) and 400 μ L FeCl₃ in (30 mM in 1.2 M HCl) to the test tube. After the reaction mixture was incubated in the dark at room temperature for 20 min, 250 μ L 10% (w/v) trichloroacetic acid was added to precipitate any protein that might be present in the culture media. Subsequently the reaction mixture was centrifuged at 10,000Xg for 10 min. The absorbance of the resulting solution was determined at 670 nm with a microplate reader. All samples were assayed in duplicate, and H₂S concentrations were calculated using standard Na₂S (0–62.5 μ M).

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise mentioned. Data were analyzed using ANOVA statistically with Sigma Stat. A *p* value of less than 0.05 for a statistical test was considered significant.

RESULTS AND DISCUSSION

Figure 1 illustrates the relationship between blood levels of H₂S and HDL-cholesterol, LDL/HDL-cholesterol, and triglyceride levels. There was a significant positive correlation between the blood levels of hydrogen sulfide and the levels of HDL-cholesterol (A), but a negative relationship with the levels of LDL/HDL cholesterol ratio (B). The relationship between H₂S and triglycerides was not statistically significant (C). Figure 2 illustrates the relationship between blood levels of H₂S and adiponectin (A), resistin (B), and potassium (C). There was a significant positive relationship between H₂S levels and both adiponectin and potassium levels. However, its relationship with resistin was not statistically significant. There was no relationship between blood levels of H₂S with body weight ($r=-0.08$, NS), BMI ($r=-0.09$, NS), insulin ($r=0.03$, NS), glucose ($r=-0.04$, NS), leptin ($r=0.01$, NS) or Non-HDL cholesterol ($r=-0.21$, NS).

H₂S is produced *in vivo* from L-cysteine by the action of two main enzymes, cystathionine β -synthase (CBS) and cystathionine- γ -lyase (CSE) (1-4). CSE is mainly expressed in the thoracic aorta, portal vein, ileum, heart, liver, kidney, and vascular smooth muscle, whereas CBS is highly expressed in the central and peripheral nervous systems (1). CSE is a major H₂S producing enzyme in the cardiovascular system (1). Human blood contains a significant amount (10-100 μ M) of H₂S (1, 4). This study demonstrates that a positive relationship exists between the blood levels of H₂S and HDL-cholesterol versus a negative relationship with the LDL/HDL ratio which indicates that circulating levels of H₂S is a regulator of HDL and LDL homeostasis in the blood. Studies in the literature demonstrate that garlic or onion, which increases H₂S in the blood, also increases HDL and decreases LDL (7). On the other hand, supplementation with exogenous hydrogen sulfide reduces the progression of atherosclerosis and plaque formation in apoE knock out mice (5). LDL brings cholesterol to cells for cellular repair and maintenance. HDL carries cholesterol away from the cells to the liver. Free radicals produced by macrophages, endothelial cells, or smooth cells can cause oxidation of LDL-cholesterol. Oxidized LDL is a major player in the development of atherogenesis. Oxidized LDL promotes endothelial cell injury, circulating monocyte adhesion, as well as their increased accumulation in plaques and formation of foam cells. Several studies demonstrate that H₂S is an antioxidant and regenerate GSH (4, 8). Antioxidant protection of LDL can prevent oxidative damage to LDL and thus the progression of atherosclerosis. *In vitro* studies have shown that exogenous supplementation with H₂S can inhibit oxidative damage of LDL (4). It appears that H₂S suppresses oxidation of LDL-cholesterol and HDL-cholesterol, which prevents endothelial dysfunction and progression of atherosclerosis.

The relationship between circulating H₂S and adiponectin is also interesting. A number of studies imply that adiponectin protects against atherosclerosis and inflammation (9). It has been suggested that adiponectin may increase levels of HDL-cholesterol by increasing reverse cholesterol transport and decreasing apoB100 and VLDL-TG secretion from the liver. Low adiponectin levels are associated with a high concentration of serum oxidized LDL in patients with type 2 diabetes mellitus and CAD [9]. Adiponectin is an anti-inflammatory that increases insulin sensitivity, which in turn can lower LDL levels and increase HDL levels in the blood. Whether H₂S has a direct effect on adipocyte biology is not known. The relationship between blood levels of H₂S and potassium is interesting. Animal studies suggest that H₂S promotes stimulation of K_{ATP} channels and relaxation and vasodilation in vascular smooth muscle cells (1, 4). This study did not determine blood pressure of study subjects. Previous studies do suggest a link between blood levels of H₂S, altered KATP channels and blood pressure in animal studies and that of blood levels of potassium with blood pressure in humans.

Cell culture studies using monocyte and adipocyte cell models demonstrate that H₂S or its precursor L-cysteine (LC) supplementation can lower oxidative stress and markers of vascular inflammation (10, 11). Dietary supplementation with sulfide rich extracts of garlic or onion or cysteine rich proteins (whey protein and α -lactoalbumin) or cysteine lowers the oxidative stress and inflammatory biomarkers in animal studies (7, 12). These studies indicate that compounds rich in sulfide or L-cysteine that may help lower inflammation by increasing circulating levels of hydrogen sulfide.

Recent studies report lower blood levels of H₂S in various disease states, such as, asthmatic patients, hypertensive patients and diabetic animals and patients, (11, 13-16). H₂S is emerging as an important signaling molecule. Increasing evidence has indicates that H₂S plays a potentially significant role in biological processes and that malfunction of H₂S homeostasis may contribute to the pathogenesis of vascular inflammation and atherosclerosis. This study demonstrates a positive relationship between levels of blood H₂S and the anti-inflammatory HDL-cholesterol and adiponectin. This provides the rationale for investigating whether supplementation with hydrogen sulfide compounds or its precursor LC, could be potentially beneficial in boosting circulating H₂S levels, which could reduce the occurrence of inflammatory biomarkers in a future clinical trial.

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Figure 1.

Relationship between plasma levels of hydrogen sulfide and levels of HDL-cholesterol (A), the LDL-HDL ratio (B), and triglyceride levels in healthy subjects (C). Note a significant positive relationship between hydrogen sulfide levels and HDL-cholesterol but a significant negative relation with the LDL/HDL-cholesterol ratio.

Figure 2. Relationship between plasma levels of hydrogen sulfide and levels of adiponectin (A), resistin (B), and potassium (C) in fasting blood from healthy subjects. Note a significant positive relationship between hydrogen sulfide levels and adiponectin and potassium levels.