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Short communication

Atorvastatin affects the tissue concentration of hydrogen sulfide in mouse kidneys and other organs*

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Abstract:
Hydrogen sulfide (H_2S) is a crucial co-modulator of cardiovascular, nervous, digestive and excretory systems function. The pleiotropic action of atorvastatin exceeds simple 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibition and involves multiple biological mechanisms. This study assesses the influence of atorvastatin on the H_2S tissue concentration in mouse brain, liver, heart and kidney. Twenty-four female CBA strain mice received an intraperitoneal injection. The mice were given one of the following solutions: 0.1 mg atorvastatin (5 mg/kg of body weight (b.w.)/day – group D1, n = 8), 0.4 mg atorvastatin (20 mg/kg b.w./day – group D2, n = 8) or a saline physiological control (0.2 ml – group C, n = 8). A modified Siegel spectrophotometric method was used for the H_2S tissue concentration measurements. There was a remarkable rise in the H_2S concentration [μg/g] in the kidney (C: 5.26 ± 0.09, D1: 5.77 ± 0.11, p = 0.0003; D2: 7.48 ± 0.09, p < 0.0001). There were also slight H_2S tissue level changes in the brain (C: 1.61 ± 0.01, D1: 1.75 ± 0.03, p = 0.0001; D2: 1.78 ± 0.03, p < 0.0001), the heart (C: 4.54 ± 0.08, D1: 4.86 ± 0.10, p = 0.0027; D2: 4.56 ± 0.07, p = 0.6997) and the liver (C: 3.45 ± 0.03, D1: 3.27 ± 0.02, p = 0.0001; D2: 3.31 ± 0.02, p = 0.0003). Our study supports the influence of atorvastatin on H_2S tissue concentration in kidneys and other mouse organs.

Key words:
hydrogen sulfide, statins, HMG-CoA reductase inhibitors, kidney, mouse

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Abbreviations: ACE – angiotensin-converting enzyme, CO – carbon monoxide, HMG-CoA – 3-hydroxy-3-methylglutaryl-coenzyme A, H_2S – hydrogen sulfide, NO – nitric oxide

Introduction

Hydrogen sulfide (H_2S) is endogenously formed from L-cysteine by several enzymatic reactions and in non-enzymatic pathways in many tissues, especially in the nervous, cardiovascular, digestive and excretory systems. The enzymatic formation of H_2S is catalyzed by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST). H_2S acts as a 'gasotransmitter' and serves as a co-modulator of various physiological and pathophysiological processes. Thus, H_2S can affect the regulation of vascular tone, myocardial contractility, neurotransmission, insulin secretion, immune and inflammatory processes, gastric mucosal integrity, intestinal motility and perception [7, 17, 20]. Statins, which lower lipids by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, are widely administered in daily clinical practice. Although not fully understood, their action exceeds simple HMG-CoA reductase inhibition and involves numerous biological mechanisms [28]. The interaction between statins and endogenous H_2S is unknown. The aim of this study is to assess the influence of atorvastatin on the endogenous H_2S concentrations in mouse brain, heart, liver and kidney tissues.

Materials and Methods

Animals

Twenty-four CBA strain female mice weighing approximately 20 g were involved in the study. The animals were housed under standard laboratory conditions and had free access to water and food. They were kept at a temperature of 22–24°C with a 12 h light/dark cycle.

Study design

The study design consisted of intraperitoneal injections of 0.1 mg (5 mg/kg b.w. daily – group D1, n = 8) or 0.4 mg (20 mg/kg b.w. daily – group D2, n = 8) for 5 consecutive days at the same time (10:30 a.m.). The synthetic HMG-CoA inhibitor atorvastatin (Sortis, Parke-Davis/Pfizer, Germany) was dissolved in physiological saline (0.2 ml for each administration). The control population (n = 8) received physiological saline intraperitoneally in 2 ml portions. The animals were randomly assigned to each group. They tolerated the applied doses of atorvastatin well and remained in good condition until the end of the experiment. A modified Siegel method was utilized for the measurement of the H_2S tissue concentrations [22, 23].

This study has been performed in accordance with the guidelines for the care and use of laboratory animals accepted by the Bioethical Committee of the Jagiellonian University Medical College (Kraków, Poland).

Tissue sample preparation

Two hours after the last drug or physiological saline injection, the animals were killed by cervical dislocation. The brain, heart, liver and kidney tissues of each animal were quickly removed, homogenized with 0.01 M sodium hydroxide (NaOH) and frozen. Each tissue was combined with NaOH in different proportions (brain: 1 to 4, liver and kidney: 1 to 5 and heart: 1 to 10). Then, 50% trichloroacetic acid (TCA) was added to the samples. The TCA solution (0.5 ml) was added to 2 g of brain or liver samples in tight 3 ml capsules, and 0.25 ml was added to 1 g of heart or kidney sample in tight 2 ml capsules. These suspensions were shaken, and the resultant mixture was centrifuged. Subsequently, 1.5 ml brain or liver and 0.75 ml heart or kidney supernatant samples were moved to 2 ml tight capsules with 0.15 ml or 0.075 ml of 0.02 M N,N-dimethyl-p-phenyldiamine sulfate in 7.2 M hydrochloric acid (HCl). Then 0.15 ml or 0.075 ml portions of 0.03 M iron(III) chloride ($FeCl_3$) in 1.2 M HCl were added, respectively. After 20 min in the dark, the contents were shaken for 1 min with 1 ml of chloroform.

H_2S tissue concentration measurements

The absorbance was measured at 650 nm with a Varian Cary 100 spectrophotometer. A standard curve was

prepared with an iodometrically determined 0.0001 M sodium sulfide (Na_2S) solution. Four concurrent analyses of every analyzed tissue type were performed for each group of animals.

Statistical analysis

Statistical analysis was performed within the R environment by the Student's *t*-test. Data were considered statistically significant when $p < 0.05$.

Results and Discussion

There was a significant rise in the H_2S concentration in the kidney, brain and heart tissues of group D1. Both doses of atorvastatin decreased the level of H_2S in the liver tissue (Tab. 1). Our study has shown that the biological action of atorvastatin interferes with the complex system of sulfur-containing compounds. The H_2S tissue concentration changes reflect the altered release of H_2S from the respective organs.

There are at least two forms of sulfur storage in the brain and other organs: acid labile sulfur and bound sulfane sulfur. Cytoplasmatic bound sulfur is postulated to absorb and store exogenously applied and endogenously produced H_2S. Endogenous H_2S is released from the bound sulfur pool in the presence of physiological concentrations of glutathione and cysteine in slightly alkaline conditions. Acid-labile sulfur is found in the iron–sulfur clusters of non-heme iron sulfur proteins, which are involved in oxidative phosphorylation localized mainly in the mitochondria [10]. The method applied in our experimental protocol determines the free H_2S concentration in the examined tissues.

A number of recent studies have revealed the significant biological role of H_2S, which is lipophilic and freely permeates plasma membranes. Its action encompasses numerous intracellular mechanisms, including the stimulation of adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP}), the maintenance of protein thiol groups in their reduced state, the reaction with reactive oxygen and nitrogen species (ROS and RNS) – protection of proteins and lipids from ROS/RNS-mediated damage, the stimulation of adenylate cyclases, cysteine transport to the cell and reduced glutathione (GSH) synthesis, influence on extracellular signal-regulated protein kinases (ERKs), the inhibition of L-type calcium channels, the stimulation of transient receptor potential vanilloid receptor channel type 1 (TRPV1 channel) and the reduction of NF-κB complex activation [11, 12, 16–18, 26, 30]. H_2S participates in the relaxation of vessels through the opening of ATP-sensitive potassium channels and the metabolic inhibition in the vascular smooth muscle cells. It inhibits their proliferation *via* the mitogen-activated protein kinase signaling pathway and induces their apoptosis [5, 6, 9, 13, 34–37]. H_2S interacts with the carbon monoxide (CO) and nitric oxide (NO) systems in a complex manner that affects their synthesis and the biological responses elicited within target tissues and organs. All three gases bind to hemoglobin and temper mitochondrial oxidative phosphorylation by inhibiting cytochrome c oxidase [15].

HMG-CoA reductase inhibitors impede the production of mevalonate, which not only decreases cholesterol synthesis but also diminishes the generation of isoprenoids. These compounds normally attach post-translationally to intracellular signaling proteins that control diverse cellular function. Not surprisingly, the statins exerted additional effects beyond lipid lowering. Indeed, numerous studies have shown that HMG-CoA reductase inhibitors have a broad array of anti-

Tab. 1. Hydrogen sulfide (H_2S) concentration in mouse brain, heart, liver and kidney tissues following the administration of 5 mg/kg b.w. and 20 mg/kg b.w. atorvastatin (groups D1 and D2, respectively)

H_2S tissue concentration (μg/g)	Control group (n = 8)	D1 (n = 8)	p (control *vs.* D1)	D2 (n = 8)	p (control *vs.* D2)
Brain	1.61 ± 0.01	1.75 ± 0.03	0.0001	1.78 ± 0.03	< 0.0001
Heart	4.54 ± 0.08	4.86 ± 0.10	0.0027	4.56 ± 0.07	0.6997
Liver	3.45 ± 0.03	3.27 ± 0.02	0.0001	3.31 ± 0.02	0.0003
Kidney	5.26 ± 0.09	5.77 ± 0.11	0.0003	7.48 ± 0.09	< 0.0001

inflammatory, antiproliferative and immunomodulatory actions that are commonly described as pleiotropic effects [4, 28]. Statins have recently achieved a well-established status in medicine due to the results of clinical trials showing that these drugs significantly diminish the risk of cardiovascular morbidity and mortality in patients with cardiovascular diseases and in certain groups with chronic kidney disease [27].

Each organ has different metabolism, paracrine regulation and specific transmitter interactions. Our observation that atorvastatin increases the H_2S concentration in brain, kidney and the low-dose heart tissue group but decreases the H_2S in liver tissue only confirms this complexity and heterogeneity. The relevancy of H_2S tissue level changes and the activity of sulfur containing compounds is a field for future research. The mechanisms of H_2S generation and alteration remain obscure but are likely to involve NO and CO, which are released by statins [28]. Preexisting clinical data encourage the exploration of H_2S involvement in Alzheimer's disease; lower risk of symptoms and reduced neuropathologic changes have been observed during statin treatment [3, 21, 24]. HMG-CoA inhibitors also have effects in acute coronary syndromes and hypotensive properties [19, 29].

The most pronounced H_2S concentration change following atorvastatin administration was observed in the kidney tissue. Hydrogen sulfide has been recognized as an important participant in renal function control. It affects both vascular and tubular actions by increasing the glomerular filtration rate (GFR), urinary sodium and potassium excretion, and fractional excretion of sodium and potassium [33]. Moreover, H_2S has been identified as an inhibitor of angiotensin-converting enzyme (ACE); it complexes with the zinc atom at or close to the enzyme active site [14]. Statins appear to protect kidneys *via* cholesterol reduction and unclear mechanisms not mediated by cholesterol. Subgroup analyses of major clinical studies and meta-analyses of smaller trials indicate that statin therapy slows the decline of the glomerular filtration rate and reduces proteinuria in patients with chronic kidney disease [1]. These observations prompt further research into the role of H_2S in the physiology and pathology of kidney.

Atorvastatin affects H_2S biology by its many pleiotropic actions. H_2S generation is altered by aspirin, other non-steroidal anti-inflammatory drugs (NSAIDs) and the ACE inhibitor ramipril. It is postulated that H_2S might participate in some of the effects of these drugs [2, 8, 25, 31, 32]. This poses another argument for further studies on the role of H_2S, hydrosulfide ion (HS^-) donors and agents releasing endogenous H_2S.

In conclusion, atorvastatin has a broad impact on the endogenous sulfur metabolism in different mouse organs. This effect is reflected by H_2S tissue concentration changes in mouse kidney, brain, liver and heart upon atorvastatin administration

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Conflicts of interest:
None declared.

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