Hypothesis

Are the effects of α-glucosidase inhibitors on cardiovascular events related to elevated levels of hydrogen gas in the gastrointestinal tract?

Yoshihiko Suzuki\textsuperscript{a}, Motoaki Sano\textsuperscript{c,*}, Kentaro Hayashida\textsuperscript{c}, Ikuroh Ohsawa\textsuperscript{a,b}, Shigeo Ohta\textsuperscript{a}, Keiichi Fukuda\textsuperscript{c}

\textsuperscript{a}Department of Biochemistry and Cell Biology, Institute of Development and Aging Science, Graduate School of Medicine, Nippon Medical School, Kawasaki City 211-8533, Japan
\textsuperscript{b}The Center of Molecular Hydrogen Medicine, Institute of Development and Aging Science, Graduate School of Medicine, Nippon Medical School, Kawasaki City 211-8533, Japan
\textsuperscript{c}Department of Regenerative Medicine and Advanced Cardiac Therapeutics, Keio University School of Medicine, Tokyo 160-8582, Japan

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A B S T R A C T

The major side-effect of treatment with α-glucosidase inhibitors, flatulence, occurs when undigested carbohydrates are fermented by colonic bacteria, resulting in gas formation. We propose that the cardiovascular benefits of α-glucosidase inhibitors are partly attributable to their ability to neutralise oxidative stress via increased production of H\textsubscript{2} in the gastrointestinal tract. Acarbose, which is an α-glucosidase inhibitor, markedly increased H\textsubscript{2} production, with a weaker effect on methane production. Our hypothesis is based on our recent discovery that H\textsubscript{2} acts as a unique antioxidant, and that when inhaled or taken orally as H\textsubscript{2}-dissolved water it ameliorates ischaemia–reperfusion injury and atherosclerosis development.

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1. Introduction

A growing body of evidence supports the notion that postprandial hyperglycaemia plays an important role in the development of cardiovascular disease. Large epidemiological studies have shown that the serum glucose concentration 2 h after an oral glucose challenge is a powerful predictor of cardiovascular risk [1,2].

α-Glucosidase inhibitors are pharmacological agents that specifically reduce postprandial hyperglycaemia through retardation of disaccharide digestion, thereby reducing glucose absorption by the small intestine. The STOP-NIDDM trial demonstrated that the treatment of patients who had impaired glucose tolerance with α-glucosidase inhibitors and glibenclamide [5]. Inhibition of postprandial hyperglycaemia by α-glucosidase inhibitors alleviates cardiac ischaemia–reperfusion injury in mice [6]. These findings suggest that α-glucosidase inhibitors interfere with the development of macrovascular diseases through additional mechanisms distinct from the expected modulation of postprandial hyperglycaemia.

2. Molecular hydrogen (H\textsubscript{2}) acts as a novel antioxidant

Clinical evidence and experimental results strongly implicate reactive oxygen species (ROS) as the leading etiologic agent of cardiovascular diseases, including hypertension, atherosclerosis, angina pectoris, myocardial infarction, and heart failure [7,8]. The mechanisms for ROS production are diverse, and include increases in the activities of NAD(P)H-oxidase, xanthine oxidase, cyclooxygenase, and lipoxygenase, as well as uncoupling of nitric oxide synthase, dysfunction of the mitochondrial respiratory chain, and decreased bioavailability of antioxidants, all of which contribute to increased oxidative stress. An increase in ROS production reduces the bioavailability of nitric oxide (NO), synergistically advancing the pathogenesis of cardiovascular disease, since NO plays important roles in the regulation of vascular tone, inhibition of platelet aggregation, and suppression of smooth muscle cell (SMC) proliferation. Increases in renal levels of ROS raise the blood pressure by influencing afferent arteriolar tone, tubuloglomerular feedback response, and sodium reabsorption [9]. Increases in vascular ROS promote endothelial dysfunction, increased

* Corresponding author. Address: Department of Regenerative Medicine and Advanced Cardiac Therapeutics, Keio University School of Medicine, 35 Shinanomachi Shinjuku-ku, Tokyo 160-8582, Japan. Fax: +81 3 5363 3875.
E-mail address: msano@sc.itc.keio.ac.jp (M. Sano).
contractility, monocyte invasion, VSMC proliferation, and increased deposition of extracellular matrix proteins, all of which contribute to the pathogenesis of hypertension, atherosclerosis, and plaque rupture. In the brain, increased production of ROS mediates hypertension by increasing sympathetic outflow. Various antioxidants have been tested for their abilities to reduce the risk of cardiovascular disease. However, these trials have not verified the importance of antioxidants in the prevention of cardiovascular disease [10]. These outcomes can be partially explained by the dual roles of ROS. Most of the detrimental effects of ROS are attributed to \( \cdot \)OH, which is the most reactive oxygen species. In comparison, \( \mathbf{O}_2^- \) and \( \mathbf{H}_2\mathbf{O}_2 \) have lower oxidative energies and, paradoxically, are implicated as crucial signalling components in the establishment of favourable tolerance to oxidative stress. Consequently, the inhibition of both these pathways (e.g., by antioxidants) can have a deleterious outcome.

Recently, we discovered that molecular hydrogen (\( \mathbf{H}_2 \)) acts as an antioxidant with the following interesting properties: (i) \( \mathbf{H}_2 \) permeates cell membranes and can target the cellular organelles, including the mitochondria and nuclei; and (ii) \( \mathbf{H}_2 \) specifically quenches detrimental ROS, such as \( \cdot \)OH and peroxy radicals (\( \mathbf{ONOO}^- \)), while maintaining the metabolic oxidation–reduction reaction and other less-potent ROS, such as \( \mathbf{O}_2^- \), \( \mathbf{H}_2\mathbf{O}_2 \) and nitric oxide (\( \mathbf{NO}^\cdot \)) [11]. We showed that inhalation of \( \mathbf{H}_2 \) gas, given at an incombustible level, limited the extent of myocardial infarction resulting from myocardial ischaemia–reperfusion injury, thereby preventing deleterious left ventricular remodelling in the rat [12]. Importantly, the inhaled \( \mathbf{H}_2 \) gas was transported rapidly in the circulation and reached the ‘at-risk’ ischaemic myocardium before the coronary blood flow of the occluded infarct-related artery was re-established.

\( \mathbf{H}_2 \) can also be administered orally in the form of \( \mathbf{H}_2 \)-dissolved water. Kajiyama et al. reported that supplementation with 900 ml/day (300 ml given three times a day) of \( \mathbf{H}_2 \)-dissolved water for 8 weeks reduced the levels of several biomarkers of oxidative stress, such as plasma oxidized low-density lipoprotein (LDL) cholesterol and urinary 8-isoprostanes, and improved glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance [13]. Furthermore, supplementation with \( \mathbf{H}_2 \)-dissolved water normalized the oral glucose tolerance test in four out of six patients with impaired glucose tolerance. The reduction in the expression of biomarkers associated with systemic oxidative stress can be ascribed to the reductive property of \( \mathbf{H}_2 \) gas. The formation of 4-hydroxynonenal (HNE) through lipoprotein oxidation plays an etiologic role in atherosclerotic lesion progression [14,15]. Oxidized LDL is taken up by macrophages through scavenger receptors, to form foam cells. Foam cells secrete growth factors that induce SMC migration from the media into the neointima. We demonstrate that the ingestion of \( \mathbf{H}_2 \)-dissolved water ad libitum for 6 months prevents the development of atherosclerosis in apolipoprotein E-knockout mice, which represent a model of spontaneously developing atherosclerosis [16]. This anti-atherogenic effect of \( \mathbf{H}_2 \)-dissolved water is associated with a reduction of HNE immunoreactivity in the aorta. These results suggest that persistent intake of \( \mathbf{H}_2 \) has the potential to reduce oxidative stress and may prevent cardiovascular disease.

### 3. Unexpected benefit of flatulence caused by \( \alpha \)-glucosidase inhibitors

Is there any other way to supply \( \mathbf{H}_2 \) to the body? \( \mathbf{H}_2 \) is not produced endogenously in mammalian cells, since the hydrogenase activity responsible for the formation of \( \mathbf{H}_2 \) gas may not be present [17]. Instead, spontaneous production of \( \mathbf{H}_2 \) gas in the human body occurs via the fermentation of undigested carbohydrates by the resident enterobacterial flora. \( \mathbf{H}_2 \) is transferred to the portal circulation and excreted through the breath in significant amounts [18]. Flatulence is regarded as the major side-effect of treatment with \( \alpha \)-glucosidase inhibitors [19]. Therefore, we examined whether the administration of \( \alpha \)-glucosidase inhibitors increases the levels of \( \mathbf{H}_2 \) production in the gastrointestinal tract. Eleven healthy volunteers (10 males and 1 female) were administered acarbose at a dosage of 300 mg/day (100 mg three times a day) for 4 days under free-feeding conditions (Table 1). On Day 4 of the experiment, the levels of exhaled \( \mathbf{H}_2 \) and methane (\( \mathbf{CH}_4 \)) were measured using the Breath Gas Analyzer Model TGA-2000 (TERAMECS, Kyoto, Japan). Acarbose treatment significantly increased the amount of exhaled \( \mathbf{H}_2 \) at every time-point examined (n = 11, P < 0.05, paired t-test, as compared to before treatment with acarbose), whereas it had modest effects on \( \mathbf{CH}_4 \) production (Fig. 1). Acarbose treatment had no effect on \( \mathbf{H}_2 \) or \( \mathbf{CH}_4 \) production in 2/11 volunteers.

Kajiyama treated patients with type 2 diabetes or impaired glucose tolerance with 900 ml/day (300 ml three times a day) of \( \mathbf{H}_2 \)-dissolved water. After drinking 300 ml of \( \mathbf{H}_2 \)-dissolved water, the exhaled \( \mathbf{H}_2 \) gas concentration reached a maximum of 56 ± 27.8 ppm at 15 min, and returned to the baseline level at 150 min. This peak level of \( \mathbf{H}_2 \) gas reduced the levels of oxidative stress biomarkers and improved glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance [13]. In the present study, we show that oral administration of acarbose at a dosage of 300 mg/day (100 mg given three times a day) can reach

### Table 1

<table>
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<tr>
<th>Sex</th>
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<th>Hydrogen After</th>
<th>Methane Before</th>
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the same maximum levels of exhaled H2 gas as compared to the consumption of 300 ml of H2-dissolved water. Moreover, acarbose maintained this peak level continuously. It is noteworthy that the breath concentration of H2 on a fasting morning remains high in people who take acarbose. These observations clearly indicate that the amounts of H2 gas generated by acarbose in our current experiments are sufficient to reduce systemic oxidative stress. Oral administration of acarbose may be superior to drinking H2-rich water in terms of maintenance of the appropriate H2 gas levels in the body.

4. Conclusion

Based on these observations and experimental results, we propose that α-glucosidase inhibitors reduce the risk of cardiovascular disease in patients with impaired glucose tolerance or type 2 diabetes, and that these benefits can be attributed at least in part to the abilities of these drugs to neutralise oxidative stress by increasing the production of H2 in the gastrointestinal tract. To investigate the relationship between the cardiovascular benefits of α-glucosidase inhibitors and H gas production by the gut microbiota, we should examine whether the cardiovascular benefits afforded by these drugs are diminished by scavenging H2 gas in the gastrointestinal tract before absorption into the blood stream.

Conflict of interest statement

None declared.

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