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Hypothesis

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

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ABSTRACT

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_2 in the gastrointestinal tract. Acarbose, which is an α-glucosidase inhibitor, markedly increased H_2 production, with a weaker effect on methane production. Our hypothesis is based on our recent discovery that H_2 acts as a unique antioxidant, and that when inhaled or taken orally as H_2-dissolved water it ameliorates ischaemia–reperfusion injury and atherosclerosis development.

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 the α-glucosidase inhibitor acarbose was associated with a 25% reduction in the risk of progression to diabetes, a 34% reduction in the risk of developing *de novo* hypertension, and a 49% risk reduction for cardiovascular events [3]. Furthermore, a meta-analysis of seven long-term studies suggested that acarbose reduced the risk of myocardial infarction for patients with type 2 diabetes [4]. Such risk reduction for coronary heart disease events in patients with type 2 diabetes was not observed by the improved glycaemic control achieved with intensified treatment with insulin and glibenclamid [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_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, xanthione 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 the renal levels of ROS raise the blood pressure by influencing afferent arteriolar tone, tubulo-glomerular feedback response, and sodium reabsorption [9]. Increases in vascular ROS promote endothelial dysfunction, increased

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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 ·OH, which is the most reactive oxygen species. In comparison, O_2^{-}· and H_2O_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 (H_2) acts as an antioxidant with the following interesting properties: (i) H_2 permeates cell membranes and can target the cellular organelles, including the mitochondria and nuclei; and (ii) H_2 specifically quenches detrimental ROS, such as ·OH and peroxynitrite ($ONOO^-$), while maintaining the metabolic oxidation–reduction reaction and other less-potent ROS, such as O_2^{-}·, H_2O_2 and nitric oxide (NO·) [11]. We showed that inhalation of 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 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.

H_2 can also be administered orally in the form of H_2-dissolved water. Kajiyama et al. reported that supplementation with 900 ml/day (300 ml given three times a day) of 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 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 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 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 H_2-dissolved water is associated with a reduction of HNE immunoreactivity in the aorta. These results suggest that persistent intake of H_2 has the potential to reduce oxidative stress and may prevent cardiovascular disease.

3. Unexpected benefit of flatulence caused by α-glucosidase inhibitors

Is there any other way to supply H_2 to the body? H_2 is not produced endogenously in mammalian cells, since the hydrogenase activity responsible for the formation of H_2 gas may not be present [17]. Instead, spontaneous production of H_2 gas in the human body occurs *via* the fermentation of undigested carbohydrates by the resident enterobacterial flora. 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 α-glucosidase inhibitors [19]. Therefore, we examined whether the administration of α-glucosidase inhibitors increases the levels of 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 H_2 and methane (CH_4) were measured using the Breath Gas Analyzer Model TGA-2000 (TERAMECS, Kyoto, Japan). Acarbose treatment significantly increased the amount of exhaled 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 CH_4 production (Fig. 1). Acarbose treatment had no effect on H_2 or 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) H_2-dissolved water. After drinking 300 ml of H_2-dissolved water, the exhaled 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 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
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. Exhaled gas was collected in an aluminium bag at the point of mid-expiration at the indicated time-points (i.e., morning, before lunch, 2 h after lunch, before retiring), both before and after acarbose treatment. The exhaled gas samples were injected into the Breath Gas Analyzer to measure the H_2 and CH_4 concentrations.

Sex	Hydrogen								Methane							
	Before				After				Before				After			
	Morning	Before lunch	After lunch	Before retiring	Morning	Before lunch	After lunch	Before retiring	Morning	Before lunch	After lunch	Before retiring	Morning	before lunch	After lunch	Before retiring
M	1	2	11	10	34	21	74	90	0	3	2	2	8	3	9	9
M	8	6	3	1	17	25	48	19	4	4	2	2	4	4	6	3
M	46	14	20	20	76	32	52	56	5	2	2	3	7	4	5	6
M	3	6	3	8	85	44	58	91	23	34	20	19	11	9	8	12
M	43	31	25	10	64	62	62	45	4	32	2	2	8	6	6	5
M	8	3	9	13	20	24	40	41	1	1	3	5	4	4	5	7
M	37	15	17	11	30	53	46	38	5	2	1	1	5	5	3	5
F	10	30	32	29	54	15	14	30	2	8	7	8	11	7	6	7
M	15	2	5	6	26	20	33	11	5	1	1	2	7	6	8	4
M	52	44	56	42	38	54	31	49	18	15	18	17	10	16	12	16
M	5	5	1	21	3	5	21	70	11	22	14	44	29	27	38	50

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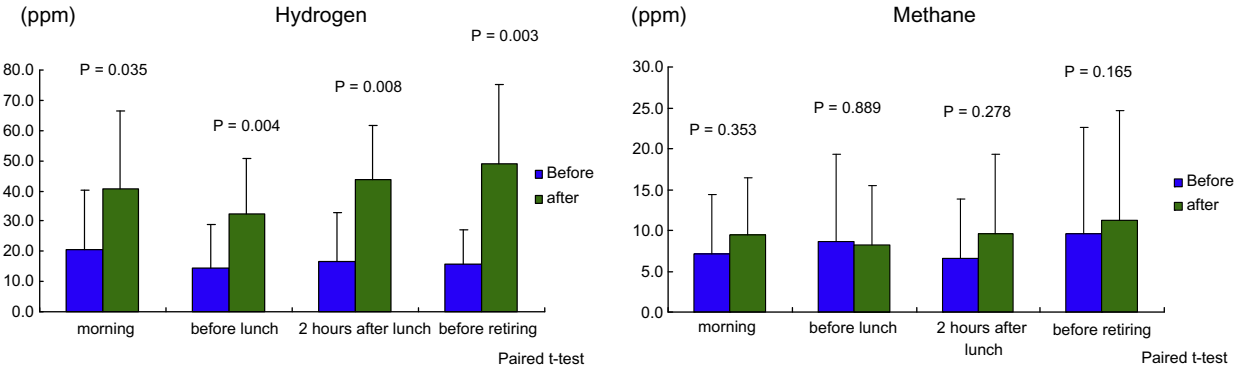

Fig. 1. Effects of acarbose on the levels of exhaled H_2 and CH_4. The values shown in the bar graphs are means ± S.D.

the same maximum levels of exhaled H_2 gas as compared to the consumption of 300 ml of H_2-dissolved water. Moreover, acarobose maintained this peak level continuously. It is noteworthy that the breath concentration of H_2 on a fasting morning remains high in people who take acarbose. These observations clearly indicate that the amounts of H_2 gas generated by acarbose in our current experiments are sufficient to reduce systemic oxidative stress. Oral administration of acarbose may be superior to drinking H_2-rich water in terms of maintenance of the appropriate H_2 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 H_2 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 H_2 gas in the gastrointestinal tract before absorption into the blood stream.

Conflict of interest statement

None declared.

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References

[1] Coutinho, M., Gerstein, H.C., Wang, Y. and Yusuf, S. (1999) The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care 22, 233–240.
[2] Barrett-Connor, E. and Ferrara, A. (1998) Isolated postchallenge hyperglycemia and the risk of fatal cardiovascular disease in older women and men. The rancho bernardo study. Diabetes Care 21, 1236–1239.
[3] Chiasson, J.L., Josse, R.G., Gomis, R., Hanefeld, M., Karasik, A. and Laakso, M. (2003) Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. JAMA 290, 486–494.
[4] Hanefeld, M., Cagatay, M., Petrowitsch, T., Neuser, D., Petzinna, D. and Rupp, M. (2004) Acarbose reduces the risk for myocardial infarction in type 2 diabetic patients: meta-analysis of seven long-term studies. Eur. Heart J. 25, 10–16.
[5] (1998) Intensive blood–glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 352, 837–853.
[6] Frantz, S., Calvillo, L., Tillmanns, J., Elbing, I., Dienesch, C., Bischoff, H., Ertl, G. and Bauersachs, J. (2005) Repetitive postprandial hyperglycemia increases cardiac ischemia/reperfusion injury: prevention by the α-glucosidase inhibitor acarbose. FASEB J. 19, 591–593.
[7] Madamanchi, N.R., Hakim, Z.S. and Runge, M.S. (2005) Oxidative stress in atherogenesis and arterial thrombosis: the disconnect between cellular studies and clinical outcomes. J. Thromb. Haemost. 3, 254–267.
[8] Touyz, R.M. (2004) Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? Hypertension 44, 248–252.
[9] Wilcox, C.S. (2003) Redox regulation of the afferent arteriole and tubuloglomerular feedback. Acta Physiol. Scand. 179, 217–223.
[10] Steinhubl, S.R. (2008) Why have antioxidants failed in clinical trials? Am. J. Cardiol. 101, 14D–19D.
[11] Ohsawa, I. et al. (2007) Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat. Med. 13, 688–694.
[12] Hayashida, K. et al. (2008) Inhalation of hydrogen gas reduces infarct size in the rat model of myocardial ischemia–reperfusion injury. Biochem. Biophys. Res. Commun. 373, 30–35.
[13] Kajiyama, S. et al. (2008) Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. Nutr. Res. 28, 137–143.
[14] Lusis, A.J. (2000) Atherosclerosis. Nature 407, 233–241.
[15] Berliner, J.A. and Watson, A.D. (2005) A role for oxidized phospholipids in atherosclerosis. N Engl. J. Med. 353, 9–11.
[16] Ohsawa, I., Nishimaki, K., Yamagata, K., Ishikawa, M. and Ohta, S. (2008) Consumption of hydrogen water prevents atherosclerosis in apolipoprotein E knockout mice. Biochem. Biophys. Res. Commun. 377, 1195–1198.
[17] Adams, M.W., Mortenson, L.E. and Chen, J.S. (1980) Hydrogenase. Biochim. Biophys. Acta 594, 105–176.
[18] Levitt, M.D. (1969) Production and excretion of hydrogen gas in man. N Engl. J. Med. 281, 122–127.
[19] Ladas, S.D., Frydas, A., Papadopoulos, A. and Raptis, S.A. (1992) Effects of α-glucosidase inhibitors on mouth to caecum transit time in humans. Gut 33, 1246–1248.

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