Pharmacological Research 103 (2016) 177–179

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Letter to the Editor

Is melanin a source of bioactive molecular hydrogen?

Molecular hydrogen (dihydrogen; H_2) has traditionally been described as a biologically inactive gas, with low capacity to react with most biomolecules. However, in the past two decades hydrogen emerged as a potent therapeutic agent, with antioxidant, anti-inflammatory and anti-apoptotic effects demonstrated in a plethora of animal disease models and human studies. Prominent effects of supplemental H_2 in clinical environment are observed especially in oxidative stress-mediated disorders, including neurodegenerative, metabolic, inflammatory and skin diseases [1]. Hydrogen can reach and react with cytotoxic reactive oxygen species (ROS) at the site of cellular damage, and protect tissues against acute and chronic oxidative injuries [2]. In addition, treatment with H_2 affected signal transduction and blood buffering capacity [3], suggesting that scavenging ROS might not be a unique mechanism of its action *in vivo*. Supplemental hydrogen has been involved in very promising results so far, yet several enigmas remain to be resolved regarding its role in health and disease. In particular, no answer has been provided why large quantities of gut-derived endogenous hydrogen have no systemic effects, while supplemental H_2 demonstrates a prominent effect in much less amounts than that produced by intestinal bacteria. In this paper we discuss an alternative sites for endogenous H_2 production in the human body that might be responsible for systemic effects of hydrogen, and its possible role in the pathogenesis of oxidative stress-related disorders.

It is well known that the endogenous H_2 is produced by enteral bacteria as a byproduct of anaerobic metabolism. Approximately 150 mL of hydrogen gas per day occurs in the gut *via* the fermentation of undigested carbohydrates by resident enterobacterial flora [4]. Hydrogen gas is further transferred to the portal circulation, and excreted through the breath in significant amounts. Its bioavailability and metabolic pathways are not fully understood. Few studies investigated effects of gut-derived hydrogen as an antioxidant, with systemic effects of bacteria-driven H_2 were found to be absent or negligible [5]. It seems that the endogenous H_2 originating from intestinal bacteria mainly behaves as an inert gas. Although traditionally referenced as a unique site for hydrogen production, the gut might not be the only source of endogenous H_2 in the human body. A recent paper hypothesized an alternative mechanism for endogenous H_2 production in the brain [6], with hydrogen released from newly recognized site (or sites) possibly having a potential to produce systemic effects.

It appears that melanin, a dark brown to black natural pigment, could split water molecules and generate H_2 as demonstrated by some *in vitro* studies [7,8]. Due to its photo-electrochemical properties and polymeric structure, melanin quenches photons and through semiconductor photocatalysis (also known as switching) dissociates water into H_2 and oxygen [9]. Serban and Nissenbaum

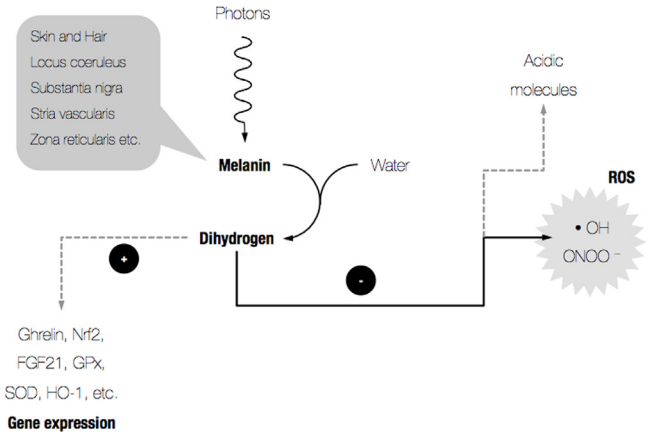

Fig. 1. An alternative mechanism for endogenous synthesis of molecular hydrogen (dihydrogen). Photochemical reaction of melanin with water releases dihydrogen to the cell in melanin-rich tissues. Molecular hydrogen could stimulate (+) or inhibit (−) several cellular processes, and bioactive molecules (for detailed review see Ohta [1]). Solid line indicates direct reduction of reactive oxygen species (ROS) with dihydrogen. Dashed lines indicate indirect up-regulation of gene expression of pro-inflammatory cytokines and hormones by dihydrogen, and reduction of organic acids. *Abbreviations:* Nrf2—nuclear factor erythroid-related factor 2; FGF21—fibroblast growth factor 21; SOD—superoxide dismutase; GPx—glutathione peroxidase; HO-1—heme oxygenase-1; •OH—hydroxyl radical; $ONOO^-$—peroxynitrate.

[7] reported a light-induced production of H_2 from water by catalysis with melanoidin, a derivative of melanin, with tracer experiments indicate that H_2 evolved originates from water. After a lag time of 10–20 min, H_2 production rate (during 4 h irradiation) averaged 0.14 μmol H_2 per minute in the solution containing ~4 mg of melanin analog. It seems that artificial or natural light (406 nm with bandwidth ~12 nm) activates melanin-mediated photo-reactivity in aqueous media of human retinal pigment epithelium cells [10], with the difference in switching potential of melanin is attributed to the pigment content in different melanosomes of human hair [11]. However, above preclinical studies with human cells did not evaluate the rate of H_2 production by melanin. Once endogenous hydrogen is obtained, it might react with ROS and provide protection against oxidative stress (Fig. 1). In fact, endogenous hydrogen may critically contribute to the balanced antioxidant defense in the cell since H_2 selectively scavenges the hydroxyl radical, the most cytotoxic of ROS, while preserving other ROS (*e.g.*, nitric oxide radical, hydrogen peroxide) important in cell physiology and homeostasis [2]. Due to its small molecular weight, H_2 can rapidly penetrate biomembranes and reach subcellular components which are the main site of ROS generation. In addition, melanin-driven molecular hydrogen might also display other biological effects by up- or down-regulation of gene expression of various pro-inflammatory cytokines and hormones [1].

http://dx.doi.org/10.1016/j.phrs.2015.12.002

Since melanin is found in several areas of the human body (*e.g.*, epidermis, hair, iris, stria vascularis, adrenal gland, locus coeruleus), melanin-driven production of endogenous H_2 might be possible in great quantity. This might emphasize the importance of systematic melanin-driven production of endogenous H_2 as a delicate antioxidant and protective component. On the other hand, failure of melanin to produce molecular hydrogen might compromise cellular protection from acute and chronic oxidative injury, and induce cellular damage. It has been suggested that Parkinson's disease (PD) might be due to insufficient production of endogenous H_2 in substantia nigra, a brain structure rich in melanin, to protect the brain from oxidative stress [6]. Furthermore, a reduction or absence of skin melanin has been identified as a risk factor for oxidative stress-related skin disorders [12], in which insufficient production of endogenous H_2 might play a role [13]. Having this in mind, restoring disturbances in the natural production and pooling of H_2 by melanin, or using supplemental hydrogen might be considered as a novel treatment approaches for oxidative stress-induced diseases. So far, very few studies evaluated the possible therapeutic effects of molecular hydrogen in diseases affecting melanin-rich tissues where oxidative stress plays a major role. Fujita et al. [14] reported beneficial effects of H_2 in Parkinson's disease, with hydrogen-containing water (0.08–1.15 ppm of H_2) significantly reducing oxidative stress and loss of dopaminergic neurons in PD model mice. A pilot clinical study of hydrogen therapy in Japanese patients with levodopa-medicated PD [15] found a significant improvement in total Unified Parkinson's Disease Rating Scale (UPDRS) scores in participants supplemented with 1000 mL/day of hydrogen rich-water. Although preliminary research reported encouraging results of hydrogen provision, no study evaluated pre-intervention levels of melanin-driven production of H_2 and its tissue dynamics. In addition, due to the fact that switching was only seen when melanin was hydrated [8], it seems reasonable to count the cell hydration as a relevant factor of melanin-driven H_2 production. Therefore, cellular hypohydration or dehydration may negatively affect melanin-driven production of endogenous hydrogen, and jeopardize its role in antioxidant defense, which warrants further investigation. The next logical step would be to measure melanin-driven H_2 production in relevant organs (such as skin and brain), and to determine its kinetics and possible roles for antioxidant defense in health and disease. However, molecular hydrogen is a dissipative, penetrative and short-lived molecule that tends to rapidly react with oxidizing species, making it difficult to assess its concentration in the human body. Availability of sensitive assays intended for immediate analysis of hydrogen level in blood and/or tissue homogenates may help to acknowledge melanin-driven production of molecular hydrogen as one of the critical factors in molecular medicine of oxidative stress.

It is well known that mammalian cells do not have their own dehydrogenase which metabolizes molecular hydrogen [16], implying that H_2 does not seem to be produced from any substrates through enzyme-mediated reactions in mammals. However, H_2 could be produced from melanin by a non-enzymatic photochemical reaction as postulated here, and then show beneficial effects. Although only a limited number of *in vitro* studies are currently available on the hydrogen-generating properties of melanin [7,8], this might be considered as an alternative source of bioactive H_2 at least in small quantities [17]. *In vivo* studies are highly warranted to decipher the possible production of H_2 by melanin-rich tissues. Nevertheless, more mechanistic information is needed to confirm and further define possible bioactivity of melanin-driven H_2 in health and disease.

In conclusion, a possible systematic production of endogenous molecular hydrogen by melanin has been suggested as a newly identified component of cellular defense in oxidative stress. Supplemental hydrogen might be beneficial for disturbances in melanin-driven production of hydrogen. *In vivo* studies are needed to fully describe its production, kinetics and utilization in the human body.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgment

This work was supported by the Serbian Ministry of Science (Grant No. 175037).

References

[1] S. Ohta, Molecular hydrogen as a preventive and therapeutic medical gas: initiation, development and potential of hydrogen medicine, Pharmacol. Ther. 144 (2014) 1–11.
[2] I. Ohsawa, M. Ishikawa, K. Takahashi, M. Watanabe, K. Nishimaki, K. Yamagata, K. Katsura, Y. Katayama, S. Asoh, S. Ohta, Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals, Nat. Med. 13 (2007) 688–694.
[3] S.M. Ostojic, Serum alkalization and hydrogen-rich water in healthy men, Mayo Clin. Proc. 87 (2012) 501–502.
[4] X.F. Zheng, H.J. Sun, Z.F. Xia, Hydrogen resuscitation, a new cytoprotective approach, Clin. Exp. Pharmacol. Physiol. 38 (2011) 155–163.
[5] M. Ito, M. Hirayama, K. Yamai, S. Goto, M. Ito, M. Ichihara, K. Ohno, Drinking hydrogen water and intermittent hydrogen gas exposure, but not lactulose or continuous hydrogen gas exposure, prevent 6-hydorxydopamine-induced Parkinson's disease in rats, Med. Gas Res. 2 (2012) 15.
[6] S. Brenner, Parkinson's disease may be due to failure of melanin in the substantia nigra to produce molecular hydrogen from dissociation of water, to protect the brain from oxidative stress, Med. Hypotheses 82 (2014) 503.
[7] A. Serban, A. Nissenbaum, Light induced production of hydrogen from water by catalysis with ruthenium melanoidins, Int. J. Hydrogen Energy 25 (2000) 733–737.
[8] A. Solis, M.E. Lara, L.E. Rendon, Photoelectrochemical properties of melanin, Nat. Precedings (2007), Available from Nature at: http://precedings.nature.com/documents/1312/version/1.
[9] A. Braun, F. Boudoire, D.K. Bora, G. Faccio, Y. Hu, A. Kroll, B.S. Mun, S.T. Wilson, Biological components and bioelectronic interfaces of water splitting photoelectrodes for solar hydrogen production, Chemistry 21 (2015) 4188–4199.
[10] M. Rózanowska, A. Bober, J.M. Burke, T. Sarna, The role of retinal pigment epithelium melanin in photoinduced oxidation of ascorbate, Photochem. Photobiol. 65 (1997) 472–479.
[11] A. Samokhvalov, L. Hong, Y. Liu, J. Garguilo, R.J. Nemanich, G.S. Edwards, J.D. Simon, Oxidation potentials of human eumelanosomes and pheomelanosomes, Photochem. Photobiol. 81 (2005) 145–148.
[12] A. Godic, B. Poljsak, M. Adamic, R. Dahmane, The role of antioxidants in skin cancer prevention and treatment, Oxid. Med. Cell. Longev. 2014 (2014), 860479.
[13] S.M. Ostojic, Eumelanin-driven production of molecular hydrogen: a novel element of skin defense? Med. Hypotheses 85 (2015) 237–238.
[14] K. Fujita, T. Seike, N. Yutsudo, M. Ohno, H. Yamada, H. Yamaguchi, K. Sakumi, Y. Yamakawa, M.A. Kido, A. Takaki, T. Katafuchi, Y. Tanaka, Y. Nakabeppu, M. Noda, Hydrogen in drinking water reduces dopaminergic neuronal loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease, PLoS One 4 (2009) e7247.
[15] A. Yoritaka, M. Takanashi, M. Hirayama, T. Nakahara, S. Ohta, N. Hattori, Pilot study of H_2 therapy in Parkinson's disease: a randomized double-blind placebo-controlled trial, Mov. Disord. 28 (2013) 836–839.
[16] P.M. Vignais, B. Billoud, Occurrence, classification, and biological function of hydrogenases: an overview, Chem. Rev. 107 (2007) 4206–4272.
[17] T. Sarna, Properties and function of the ocular melanin—a photobiophysical view, J. Photochem. Photobiol. B 2 (1992) 215–258.

Sergej M. Ostojic[a,b,*]
[a]
Faculty of Sport and PE, University of Novi Sad, Serbia
[b]
University of Belgrade School of Medicine, Belgrade, Serbia

Aleksandra Vojvodic-Ostojic

Faculty of Technology and Metallurgy, University of Belgrade, Serbia

* Corresponding author at: Biomedical Sciences Department, Faculty of Sport and PE, University of Novi Sad, Lovcenska 16, Novi Sad 21000, Serbia. Fax: +381 21 450 199.
E-mail address: sergej.ostojic@chess.edu.rs (S.M. Ostojic)

19 October 2015

30 November 2015

1 December 2015
Available online 3 December 2015