Acta Histochemica 115 (2013) 418–424

Contents lists available at SciVerse ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Silicic acid in drinking water prevents age-related alterations in the endothelium-dependent vascular relaxation modulating eNOS and AQP1 expression in experimental mice: An immunohistochemical study

Barbara Buffoli[a,1], Eleonora Foglio[a,1], Elisa Borsani[a], Christopher Exley[b], Rita Rezzani[a], Luigi Fabrizio Rodella[a,*]

[a] Human Anatomy, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[b] The Birchall Centre, Lennard-Jones Laboratories, Keele University, Newcastle-under-Lyme, Staffordshire, UK

ARTICLE INFO

Article history:
Received 9 August 2012
Received in revised form 4 October 2012
Accepted 9 October 2012

Keywords:
Silicon
Aorta
Kidney vessels
eNOS
AQP-1
Mice

ABSTRACT

The maintenance of endothelial integrity is of great importance in coping with age-related vascular alterations. Endothelium-derived nitric oxide is one of the various vasoactive substances able to regulate vascular tone and homeostasis, and whose decrease is known to be related with senescence in endothelial cells. There are reports on the efficacy of silicon, especially as silicic acid, in protecting vascular integrity during age-related vascular diseases. The aim of this study was to evaluate the ability of supplementation of silicic acid in drinking water in the maintenance of vascular health in a mouse model of early physiological aging. In particular, we evaluated the relationship between Si supplementation and endothelial nitric oxide synthase (eNOS) expression, taking into account also the aquaporin-1 (AQP-1) isoform that, as recently reported, seems to be involved in nitric oxide transport across cell membranes. Our results showed that silicic acid supplementation increased both eNOS and AQP-1 expression, suggesting that silicic acid modulation of endothelial nitric oxide synthase and aquaporin-1 could represent a potential strategy against age-related vascular senescence.

Introduction

It is well-established that structural changes in the vasculature occur during aging, resulting in increased vascular or arterial stiffness. In particular, the reduction of elastic properties of vessels wall with aging is determined, besides the remodeling of its structural main components, also by damaged vascular tone, which, in turn, is determined by a deregulation of the endothelium-mediated balance between vasoconstriction and vasorelaxation (Cernadas et al., 1998; Gaballa et al., 1998). For this reason, endothelial dysfunction is recently emerging among the key components in the physiopathology of vascular senescence (Van der Loo et al., 2000; Chen et al., 2002).

Vascular endothelium is able to regulate vascular tone and homeostasis by generating a number of vasoactive molecules, such as vasoconstrictors (Pasyk and Jakobczak, 2004) and vasodilators (Harris et al., 1999) including nitric oxide (NO). NO is a free radical in the form of a highly diffusible gas (Hayashi et al., 2008) that is produced by endothelial NO synthase (eNOS) through oxidative conversion of L-arginine to L-citrulline. NO formed by the vascular endothelial cells (ECs) diffuses to the adjacent cells, such as vascular smooth muscle cells (VSMCs), platelets and leucocytes, where it performs many of its beneficial effects, such as vasodilation, antithrombotic, anti-inflammatory and antiproliferative effects (Cernadas et al., 1998; Sawada and Liao, 2009). Endothelium-derived NO is known to be particularly important to maintain normal vascular tone and homeostasis (Ignarro et al., 1987; Palmer et al., 1987) and to prevent the progression of age-related vascular disorders (Ignarro and Napoli, 2004). In particular, senescent ECs are characterized by decreased production of endothelium-derived NO, due to a decreased eNOS activity (Chen et al., 2002; Hayashi et al., 2005; White et al., 2006) that could be attributable to a reduction in eNOS protein expression as well as reduced eNOS phosphorylation (Hoffmann et al., 2001; Matsushita et al., 2001).

There are several reported studies regarding the use of different molecules able to maintain the structural and physiological properties of the vessel wall. Among them, silicon (Si) seems to be a protective element against the development of age-related vascular diseases (Najda et al., 1991; Trincă et al., 1999). Si is a major component in our environment and is a major trace element in animal diets (Pennington, 1991; Exley et al., 1997; Exley,

Abbreviations: AQP-1, aquaporin-1; DAB, diaminobenzidine tetrahydrochloride; ECs, endothelial cells; eNOS, endothelial nitric oxide synthase; IOD, integrated optical density; NO, nitric oxide; Si, silicon; VSMCs, vascular smooth muscle cells.

* Corresponding author at: Human Anatomy, Department of Clinical and Experimental Sciences, University of Brescia, V.le Europa 11, 25123 Brescia, Italy.
E-mail address: rodella@med.unibs.it (L.F. Rodella).

[1] Equally share the first authorship.

0065-1281/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.acthis.2012.10.002

2009a; Jugdaohsingh et al., 2002; Powell et al., 2005). It is maintained in trace concentrations (serum levels of about 0.5 mg/L in the soluble form) in the human body (Carlisle, 1984; Lai et al., 2002; Jugdaohsingh et al., 2008; Exley, 2009b), similarly to that of other physiologically important elements, such as iron, copper and zinc (Dobbie and Smith, 1982). Soluble silica is the most available source of Si for human use and, unlike crystalline silica, has no associated toxicity (Exley et al., 1997; Jugdaohsingh et al., 2000; Reffitt et al., 2003; Maehira et al., 2011). Nowadays, there is considerable interest in the effects of soluble silica on human health in contrast to earlier researches, which focused solely on the toxic effects of inhaled crystalline silica, such as silicosis (Martin, 2007; Lacasse et al., 2009). In particular, some published data indicate the role of Si in maintaining the integrity, the stability and the elastic properties of arterial walls (Loeper and Lemaire, 1966; Schwarz et al., 1977). In support of these data, it has been also demonstrated that Si content of the healthy human aorta decreases considerably with age (Dawson et al., 1978; Schwarz, 1978). An epidemiological study confirmed a strong positive correlation between Si dietary supplementation and reduced mortality due to age-related vascular diseases (Schwarz et al., 1977).

Accordingly, given that physiological aging is associated with an increased incidence of vascular diseases (Barton, 2005) and with an attenuated NO-mediated endothelium dependent relaxation (Barton et al., 1997) and in light of the documented efficacy of Si in protecting vascular integrity and function during age-related vascular diseases (Nakayama et al., 2009), the aim of the present study was to evaluate the ability of silicic acid supplementation in drinking water in the maintenance of vascular health (in thoracic aorta and kidney vessels) using an animal model of early physiological aging. We used a mouse model of early physiological aging (C57BL6/J mice aged 19 months at the end of the experiments), considering previous data showing that unlike aged humans, old mice fed a regular diet develop vascular aging hallmarks (such as NO-dependent endothelial dysfunction and stiffness), without any other cardiovascular risk factors (as for instance atherosclerotic lesions, *Diabetes mellitus* or hypertension) (Bulckaen et al., 2008; Stämpfli et al., 2010).

Moreover, some recent data have suggested the involvement of Aquaporin-1 (AQP-1), the first identified member of aquaporins, in NO transport across cell membranes (Agre et al., 1993, 1995; Herrera and Garvin, 2007) and in the hydration of the extracellular matrix (Bondy et al., 1993) which occur during the repair process after vascular damage (Majesky et al., 1992). In particular, AQP-1 expression in VSMCs and ECs at aortic levels was reported (Shanahan et al., 1999) and it was suggested to be implicated in NO transport out of ECs and into VSMCs (Herrera and Garvin, 2007). Accordingly, on the basis of this relationship between NO and AQP-1, a further aim of this study was to evaluate the alterations of AQP-1 expression in the same experimental model.

Materials and methods

Animal groups

Thirty male C57BL/6 mice (Harlan Laboratories, San Pietro al Natisone (UD), Italy), 5 weeks old at the beginning of the experimental protocol (weighing 21.05 ± 1.65 g) were used in the study. They were housed in a controlled environment, with a regular 12 h light/dark cycle in a temperature controlled room (20 ± 1 °C). Food and water were provided ad libitum. For each group, body weight and daily water consumption of the mice were monitored three times a week. Body weight and water consumption measurements for each group at the end of the experiments (18 months) were recorded.

Table 1
Silicic acid content and pH of the mineral waters used in this study, conducted by A2A Ciclo Idrico, an Italian company for water chemical analyses for tap water[1], by the University of Pavia, Department of General Chemistry (30th June 2008) for the silicic acid-poor drinking water[2] and by the University "Federico II" of Napoli, Department of Preventive Medicine, Section of Hygiene (22 June 2008) for the silicic acid-rich drinking water[3].

	Tap water	Silicic acid-poor drinking water	Silicic acid-rich drinking water
Silica	20 mg/L[1]	10 mg/L[2]	86 mg/L[3]
pH	7.5	7.4	6.1

Experimental design

The animals were randomly divided in 3 groups ($n = 10$ per group) and treated for 18 months. Group 1 were control mice treated with tap water; Group 2, were mice treated with the silicic acid-poor drinking water; Group 3, were mice treated with silicic acid-rich drinking water.

The length of treatment was chosen in light of fact that C57BL6/J mice have a life expectancy of roughly 2 years. Aged mice used in this study were approximately 76 weeks old, which is estimated as corresponding more or less to 60–65 years for a human (Bulckaen et al., 2008; Stämpfli et al., 2010).

In treated groups, instead of tap water, two different kinds of bottled mineral water were used as they are commercially available and given to the mice as their source of drinking water: (1) silicic acid-poor drinking water, which contains low Si levels (SiO_2 10 mg/L, Si < 5 mg/L); (2) silicic acid-rich drinking water, which contains high Si levels (SiO_2 86 mg/L, Si < 43 mg/L). The silicic acid content and pH values of the waters used in this study are described in Table 1. Water bottles were replaced three times a week.

At the end of the treatment period, all the animals were killed by cervical dislocation and the thoracic portion of aorta and the kidneys were removed. Every effort was made to minimize animal suffering and the studies were performed according to Italian Law on the protection of laboratory animals. All the experimental procedures were approved by the Italian Ministry of Health.

Tissue processing

The organs (thoracic portion of aorta and kidney) were fixed in 10% neutral buffered formalin for 48 h and then embedded in paraffin wax (Sigma–Aldrich, St. Louis, MO, USA) using a standard protocol. Serial transverse sections (7 μm thick) of each sample were cut using a microtome and then used for the immunohistochemical analyses.

eNOS and AQP-1 immunohistochemistry

Five randomly selected thoracic aorta and kidney sections per animal have been used for both eNOS and AQP-1 immunohistochemistry. Briefly, the sections were deparaffinized in xylene, rehydrated in descending concentration of ethanol solutions and subjected to antigen retrieval boiling tissue sections (98 °C) in 10 mM citrate buffer, pH 6.0, for 10 min, followed by cooling at room temperature for 20 min (Yamashita, 2007). Endogenous peroxidase activity was blocked by incubation with a solution of 3% hydrogen peroxide for 10 min The sections were then incubated with appropriate normal serum (Vector Labs., Burlingame, CA, USA) for 1 h and successively with rabbit polyclonal antibody against eNOS (diluted 1:70; AnaSpec, San Jose, CA, USA) or rabbit polyclonal antibody against AQP-1 (diluted 1:500; Alpha Diagnostic Int., San Antonio, TX, USA) for 1 h at room temperature and overnight at +4 °C. After incubation in primary antibodies, the sections were sequentially incubated in appropriate biotinylated immunoglobulins and