Neurobiology of Learning and Memory 104 (2013) 82–91

Contents lists available at SciVerse ScienceDirect

Neurobiology of Learning and Memory

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

Hydrogen sulfide slows down progression of experimental Alzheimer's disease by targeting multiple pathophysiological mechanisms

Daniela Giuliani [a,*], Alessandra Ottani [a], Davide Zaffe [b], Maria Galantucci [a], Flavio Strinati [c], Renzo Lodi [d], Salvatore Guarini [a,*]

[a] Department of Biomedical, Metabolic and Neural Sciences, Section of Pharmacology and Molecular Medicine, University of Modena and Reggio Emilia, Modena, Italy
[b] Department of Biomedical, Metabolic and Neural Sciences, Section of Human Morphology, University of Modena and Reggio Emilia, Modena, Italy
[c] Past Health Director of Tabiano's Spa, Tabiano, Italy
[d] Department of General Surgery and Surgical Specialties, University of Modena and Reggio Emilia, Modena, Italy

ARTICLE INFO

Article history:
Received 25 March 2013
Revised 8 May 2013
Accepted 22 May 2013
Available online 31 May 2013

Keywords:
Alzheimer's disease
Pathophysiological pathways
Learning and memory
Hydrogen sulfide
Spa-waters
Neuroprotection

ABSTRACT

It has been previously reported that brain hydrogen sulfide (H_2S) synthesis is severely decreased in Alzheimer's disease (AD) patients, and plasma H_2S levels are negatively correlated with the severity of AD. Here we extensively investigated whether treatment with a H_2S donor and spa-waters rich in H_2S induces neuroprotection and slows down progression of AD. Studies with sodium hydrosulfide (a H_2S donor) and Tabiano's spa-water were carried out in three experimental models of AD. Short-term and long-term treatments with sodium hydrosulfide and/or Tabiano's spa-water significantly protected against impairment in learning and memory in rat models of AD induced by brain injection of β-amyloid$_{1-40}$ (Aβ) or streptozotocin, and in an AD mouse model harboring human transgenes APP_{Swe}, $PS1_{M146V}$ and tau_{P301L} (3xTg-AD mice). The improvement in behavioral performance was associated with hippocampus was size of Aβ plaques and preservation of the morphological picture, as found in AD rats. Further, lowered concentration/phosphorylation levels of proteins thought to be the central events in AD pathophysiology, namely amyloid precursor protein, presenilin-1, $Aβ_{1-42}$ and tau phosphorylated at Thr181, Ser396 and Ser202, were detected in 3xTg-AD mice treated with spa-water. The excitotoxicity-triggered oxidative and nitrosative stress was counteracted in 3xTg-AD mice, as indicated by the decreased levels of malondialdehyde and nitrites in the cerebral cortex. Hippocampus reduced activity of c-jun N-terminal kinases, extracellular signal-regulated kinases and p38, which have an established role not only in phosphorylation of tau protein but also in inflammation and apoptosis, was also found. Consistently, decrease in tumor necrosis factor-α level, up-regulation of Bcl-2, and down-regulation of BAX and the downstream executioner caspase-3, also occurred in the hippocampus of 3xTg-AD mice after treatment with Tabiano's spa-water, thus suggesting that it is also able to modulate inflammation and apoptosis. Our findings indicate that appropriate treatments with H_2S donors and Tabiano's spa-waters, and may be other spa-waters rich in H_2S content, might represent an innovative approach to slow down AD progression in humans by targeting multiple pathophysiological mechanisms.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia. Generally, this progressive neurodegenerative and incurable disease is diagnosed in people over 65 years of age (sporadic AD), although the less prevalent early-onset Alzheimer's (familial or genetic Alzheimer's) can occur much earlier (Iqbal & Grundke-Iqbal, 2010, 2011; Ittner & Götz, 2011; Tayeb, Yang, Price, & Tarazi, 2012). In developed countries, AD is one of the most economically costly diseases to society (Gustavsson et al., 2011): indeed, an estimated 26.6 million people worldwide had AD in 2006, and this number may quadruple by 2050.

Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; APP, amyloid precursor protein; ERK1/2, extracellular signal-regulated kinases; H_2S, hydrogen sulfide; JNK, c-jun N-terminal kinases; MAPK, mitogen-activated protein kinases; MDA, malondialdehyde; NaHS, sodium hydrosulfide; NO, nitric oxide; PS1, presenilin-1; PS2, presenilin-2; 3xTg-AD, triple-transgenic AD; STZ, streptozotocin; TNF-α, tumor necrosis factor-α.

* Corresponding authors. Address: Department of Biomedical, Metabolic and Neural Sciences, Section of Pharmacology and Molecular Medicine, University of Modena and Reggio Emilia, via G. Campi 287, 41125 Modena, Italy. Fax: +39 59 2055376.

E-mail addresses: daniela.giuliani@unimore.it (D. Giuliani), salvatore.guarini@unimore.it (S. Guarini).

1074-7427/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nlm.2013.05.006

In AD brain, presenilin-1 (PS1) and presenilin-2 (PS2) (which are part of the γ-secretase complex), and β-secretases process the amyloid precursor protein (APP) to generate amyloid β (Aβ) peptides: as a matter of fact, clinical features of AD are manifested morphologically by brain excessive accumulation of extracellular aggregation of Aβ peptides in the form of amyloid plaques, and intracellular neurofibrillary tangles composed of hyperphosphorilated tau protein, mainly in the hippocampus and cerebral cortex (Cole & Vassar, 2007; Iqbal & Grundke-Iqbal, 2010, 2011; Ittner & Götz, 2011; Sivanandam & Thakur, 2012; Sperling, Karlawish, & Johnson, 2013). Experimental evidence indicates that Aβ accumulation and tau hyperphosphorylation trigger an excitotoxic and inflammatory response, leading to synaptic dysfunction, neurodegeneration and apoptotic death associated with marked neuronal loss. In these pathophysiological pathways, an important role is played by free radicals, nitric oxide (NO), glutamate, several cytokines, mitogen-activated protein kinases (MAPK), Bcl-2 family members and caspases (Bagheri, Joghataei, Mohseni, & Roghani, 2011; Cotella et al., 2012; Friedlander, 2003; Gao, Zhou, & Hong, 2012; Ittner & Götz, 2011; Munoz & Ammit, 2010; Sanchez et al., 2012; Sivanandam & Thakur, 2012; Tayeb et al., 2012; Yu et al., 2009). A combination of impaired cholinergic transmission and high glutamate activity underlies the main symptomatology of AD, namely memory loss and severe cognitive decline (Galimberti, Ghezzi, & Scarpini, 2013; Tayeb et al., 2012).

Hydrogen sulfide (H_2S) is a colorless, flammable, water-soluble gas and Tabiano's spa-waters (Italy) are particularly rich in H_2S (strong sulfydrometric degree, that is, more than 100 mg/l) (Artusi, Marenghi, & Pisaneschi, 1982; Coruzzi, Adami, Pozzoli, Solenghi, & Grandi, 2010). H_2S is increasingly being recognized as an important signaling molecule in various body systems, and accumulating evidence demonstrates that H_2S donor compounds exert significant beneficial effects in several animal models of inflammation and ischemia/reperfusion injury (Lowicka & Beltowski, 2007; Martelli et al., 2012; Nicholson & Calvert, 2010; Szabo, 2007). H_2S is endogenously produced also in the brain, probably with a neuromodulatory role. It has been previously reported that brain H_2S synthesis is severely decreased in AD patients (Eto, Asada, Arima, Makifuchi, & Kimura, 2002; Kamoun, 2004), and plasma H_2S levels are negatively correlated with the severity of AD (Liu, Liu, Jiang, Huang, & Yan, 2008). Recent data showed that the H_2S donor sodium hydrosulfide reduces Aβ generation in cultured cells, and Aβ-induced cognitive impairment in rats, as detected in a short-term study (Xuan et al., 2012; Zhang et al., 2011). Further, inhaled H_2S resulted able to prevent neurodegeneration in a mouse model of Parkinson's disease, another incurable, chronic neurodegenerative disorder (Kida et al., 2011).

Aim of the present study was to extensively evaluate the possible ability of a short- and long-term treatment with a H_2S donor and Tabiano's spa-water to counteract the progression of AD. To this end, in three different animal models of AD we investigated learning and memory, brain morphological alterations, amyloid/tau cascade, excitotoxic, inflammatory and apoptotic responses.

2. Materials and methods

2.1. Animals

All animal procedures were in strict accordance with the European Community regulations on the use and care of animals for scientific purposes (CEE Council 89/609; Italian D.L. 22-1-92 No. 116). Animals were kept in air-conditioned colony rooms (temperature 21 ± 1 °C, humidity 60%) on a natural light/dark cycle, with food in pellets and tap water available ad libitum. Throughout the study, body weight was recorded, and rectal temperature was maintained close to 37 °C by means of heating lamps. Animal sacrifice at the end of the study was performed under general anesthesia with sodium pentobarbital intraperitoneally (i.p.) (Sigma–Aldrich, Milan, Italy). Experimental procedures were approved by the Committee on Animal Health and Care of Modena and Reggio Emilia University.

2.2. Rat models of AD

Adult male wistar rats weighing, at the start of the study, 260–280 g b.w. (8 week-old) were used (Charles River Laboratories, Calco, Italy). Under i.p. sodium pentobarbital (50 mg/kg) anesthesia, rats were placed in a stereotaxic apparatus and, after skull drilling, injection cannulae were implanted intracerebroventricularly (i.c.v.): in order to induce AD, these rats (termed streptozotocin rats) received a bilateral i.c.v injection of streptozotocin (1.5 mg/kg at day 1 plus 1.5 mg/kg at day 3, in 4 μl of artificial cerebrospinal fluid) (Grünblatt, Hoyer, & Riederer, 2004; Salkovic-Petrisic et al., 2011). Other groups of rats (termed Aβ rats) were placed under anesthesia in the stereotaxic apparatus and, after skull drilling, received into the hippocampus a single bilateral injection of $A\beta_{1-40}$ (10 μg in 1 μl saline) (Miguel-Hidalgo, Alvarez, Cacabelos, & Quack, 2002; Xuan et al., 2012). Sham Aβ and sham streptozotocin animals received the same surgical procedure, except that saline and artificial cerebrospinal fluid instead of Aβ and streptozotocin were into the hippocampus or i.c.v. injected, respectively.

2.3. Transgenic mouse model of AD

Male 12 week-old (at the start of the study) 3xTg-AD mice and their wild-type littermates (Jackson Laboratories, Bar Harbor, Maine) were employed. These mice harbor human transgenes APP_{Swe}, $PS1_{M146V}$ and tau_{P301L} (that is, they co-express mutant human APP, PS1 and tau protein, respectively) (Oddo et al., 2003; Vale et al., 2010; Wang et al., 2010).

2.4. Sodium hydrosulfide, spa-water, and treatment schedules

Investigations with sodium hydrosulfide (a H_2S donor; Sigma–Aldrich, St. Louis, MO), and spa-water with high H_2S content (kindly provided by Terme di Salsomaggiore e di Tabiano, Tabiano, Italy), were carried out. Spa-water originates from Tabiano's Fonte Pergoli with the following characteristics: H_2S level 129 mg/l (HS^- 50 mg/l and not ionized H_2S 79 mg/l), pH 6.4, osmolarity 41 mOsm/l, SO_4^{--} 1360 mg/l, Ca^{++} 673 mg/l, Mg^{++} 59.5 mg/l, HCO_3^- 602 mg/l, Cl^- 90 mg/l, Na^+ 69.3 mg/l, Li^+ 0.035 mg/l, K^+ 4.6 mg/l, Fe^{++} < 0.005 mg/l, NH_4^+ 0.6 mg/l, SiO_2 25.4 mg/l, F^- 0.55 mg/l. In some assessments, both sodium hydrosulfide and spa-water were tested, and in other assessments only spa-water, as follows. *Experimental group A* (Aβ rats): dose–response study with sodium hydrosulfide, 0.25, 0.5 and 1 mg/kg dissolved in 1 ml/kg saline, i.p. administered once daily for 15 days starting 3 h after Aβ, and sacrificed at day 15; *experimental group B* (Aβ rats): dose–response study with spa-water, 3, 6 and 12 ml/kg i.p. administered for 15 days starting 3 h after Aβ, and sacrificed at day 15; *experimental group C* (streptozotocin rats): sodium hydrosulfide, 0.5 mg/kg i.p. administered once daily on alternate weeks for 5 months starting 3 h after the first administration of streptozotocin, and sacrificed at the end of the fifth month; *experimental group D* (streptozotocin rats): spa-water, 12 ml/kg i.p. administered once daily on alternate weeks for 5 months starting 3 h after the first administration of streptozotocin, and sacrificed at the end of the fifth month; *experimental group E* (3xTg-AD mice): spa-water, 12 ml/kg i.p. administered once daily for 12 weeks starting at 12 weeks of age, and sacrificed at 24 weeks. Control animals (naïve rats, Aβ rats, sham Aβ rats, streptozotocin rats, sham streptozotocin rats, 3xTg-AD