Biochemical and Biophysical Research Communications 372 (2008) 254–259

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Dietary resveratrol administration increases MnSOD expression and activity in mouse brain

Ellen L. Robb [a], Lieke Winkelmolen [c], Naomi Visanji [d], Jonathan Brotchie [b], Jeffrey A. Stuart [a,*]

[a] Department of Biological Sciences, Brock University, 500 Glenridge Road, St. Catharines, Ont., Canada
[b] Toronto Western Research Institute, Toronto Western Hospital, Toronto, Ont., Canada
[c] Department of Pharmaceutical Sciences, University Utrecht, Utrecht, The Netherlands
[d] Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Ont., Canada

ARTICLE INFO

Article history:
Received 6 May 2008
Available online 16 May 2008

Keywords:
MnSOD
Superoxide dismutase
Resveratrol
Antioxidant enzyme
Brain
Heart
Liver
Mitochondria
Reactive oxygen species

ABSTRACT

trans-Resveratrol (3,4′,5-trihydroxystilbene; RES) is of interest for its reported protective effects in a variety of pathologies, including neurodegeneration. Many of these protective properties have been attributed to the ability of RES to reduce oxidative stress. *In vitro* studies have shown an increase in antioxidant enzyme activities following exposure to RES, including upregulation of mitochondrial superoxide dismutase, an enzyme that is capable of reducing both oxidative stress and cell death. We sought to determine if a similar increase in endogenous antioxidant enzymes is observed with RES treatment *in vivo*. Three separate modes of RES delivery were utilized; in a standard diet, a high fat diet and through a subcutaneous osmotic minipump. RES given in a high fat diet proved to be effective in elevating antioxidant capacity in brain resulting in an increase in both MnSOD protein level (140%) and activity (75%). The increase in MnSOD was not due to a substantial proliferation of mitochondria, as RES treatment induced a 10% increase in mitochondrial abundance (Citrate Synthase activity). The potential neuroprotective properties of MnSOD have been well established, and we demonstrate that a dietary delivery of RES is able to increase the expression and activity of this enzyme *in vivo*.

trans-Resveratrol (3,4′,5-trihydroxystilbene; RES), a bioactive component of red wine, has become well known for its reported ability to extend lifespan in model organisms ranging from yeast to vertebrates [1–4]. In addition to lifespan extension, RES has also shown putative protective actions against neurodegeneration, cancer, cardiovascular disease, diabetes, and the detrimental effects associated with high fat diets [5,6]. Oxidative stress is a shared observation in many of these pathologies and resistance to oxidative stress is a strong correlate of lifespan potential [7]. A RES induced decrease in cellular oxidative stress may provide a mechanism by which this polyphenol is able to exert a wide range of beneficial effects. Although RES has antioxidant properties related to the presence of its phenolic hydroxyl groups, low bioavailability, and a weak ability to directly scavenge reactive oxygen species, (ROS), makes cytoprotection via direct chemical reactions unlikely [8,9]. A more plausible hypothesis is that RES initiates a cascade of intracellular events that lead to an upregulation of cellular defense systems, which in turn protect against oxidative stress.

Interactions between RES and intracellular signalling molecules including sirtuins and the fork head family of transcription factors have been reported both *in vivo* and *in vitro* [6,10,11]. Recent studies suggest that activation of SIRT1, and its target PGC-1α, by RES in mice leads to changes in mitochondrial number and function [11]. As the primary source of ROS production in most cell types [7], mitochondria are important components of responses aimed at decreasing oxidative stress. The mitochondrial isoform of superoxide dismutase, MnSOD, is therefore a downstream target of many signalling pathways proposed to mediate cellular stress resistance [12]. Previous work by our laboratory has shown that RES is able to induce MnSOD in a human lung fibroblast cell line (MRC-5) [13]. An elevation of MnSOD *in vivo* in response to RES would be a significant finding given the enzyme's importance in various models of disease [14]. Many of RESs reported *in vivo* effects are consistent with an increase in mitochondrial ROS metabolism; however, observation of antioxidant enzyme activities, including MnSOD, in normal mice following chronic RES treatment has not yet been reported. We hypothesize that an important action of RES may be to reduce intracellular oxidative stress by increasing mitochondrial ROS metabolism.

The aim of this study was to examine antioxidant enzymes in the brain, heart, and liver of mice administered RES for 4

* Corresponding author. Fax: +1 905 688 1855.
E-mail address: jstuart@brocku.ca (J.A. Stuart).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.05.028

consecutive weeks. To assess the influence of delivery method three routes of RES administration were tested: incorporation into a standard laboratory diet, incorporation into a high fat diet or delivery via a subcutaneous osmotic minipump. Here, we report that both dietary and subcutaneous RES delivery methods are capable of altering the activities of key antioxidant enzymes GPx, CAT, and MnSOD, and increasing mitochondrial content in heart, brain and liver.

Materials and methods

Materials. C57 BL6 mice were obtained from Charles River Laboratories (Charles River, Canada). Alzet 2004 minipumps were purchased from Alzet (Cupertino, USA). *trans*-Resveratrol (purity >95%) was purchased from ChromaDex Inc. (Irvine, USA). Mouse chow, AIN-93G and AIN-93G modified (60% calories from fat), was purchased from DYETS (Bethlehem, USA). Chemicals and materials used in Western blotting were as in Robb et al. [13].

Animal care conditions. C57 BL6 mice (Charles River, Canada) were housed in groups of three in a temperature and humidity controlled environment subject to a 12 h light/dark cycle. Standard mouse chow and water were available ad libitum to the minipump treatment groups. Dietary delivery groups were given controlled access to food, water was available ad libitum. All treatment protocols adhered to CCAC guidelines.

Resveratrol treatment. At 5 weeks, mice were introduced to RES by one of three delivery methods; in a high fat diet, a standard laboratory diet or through a subcutaneous osmotic minipump (MP). RES was incorporated into mouse chow of both standard and high fat diets at a concentration of 0.1% (w/w), such that mice obtained a RES intake of approximately 200 mg/kg/day. Alzet 2004 minipumps were preloaded with 50% degassed DMSO or 1.825 M RES prepared in 50% degassed DMSO. The minipumps were implanted subcutaneously under isoflorane anesthesia and released vehicle or RES at a flow rate of 0.25 μL/h to give a dosage of 100 mg/kg/day. The standard diet (SD) group contained 8 control mice and 8 RES-treated mice. The high fat diet (HFD) group contained 6 control mice and 8 RES-treated mice. The osmotic minipump group contained 5 control mice and 8 RES-treated mice.

Tissue harvesting. Animals were sacrificed by cervical dislocation at the end of 4 weeks of treatment. Brain, liver, and heart were removed and immediately frozen on dry ice.

Tissue homogenization. Frozen tissues were homogenized in two volumes of ice-cold buffer containing 10 mM KH_2PO_4 (pH 7.3), 30 mM KCl, 20 μM EDTA, and 0.1% Triton X-100 using a polytron homogenizer. The homogenates were centrifuged at 500 g for 10 min. The resulting supernatant was collected and protein concentration was determined by the Bradford method.

Enzyme activities. Citrate synthase, catalase, and glutathione peroxidase activities were assayed as in Robb et al. [13]. The activities of MnSOD and CuZnSOD were measured using an in-gel assay

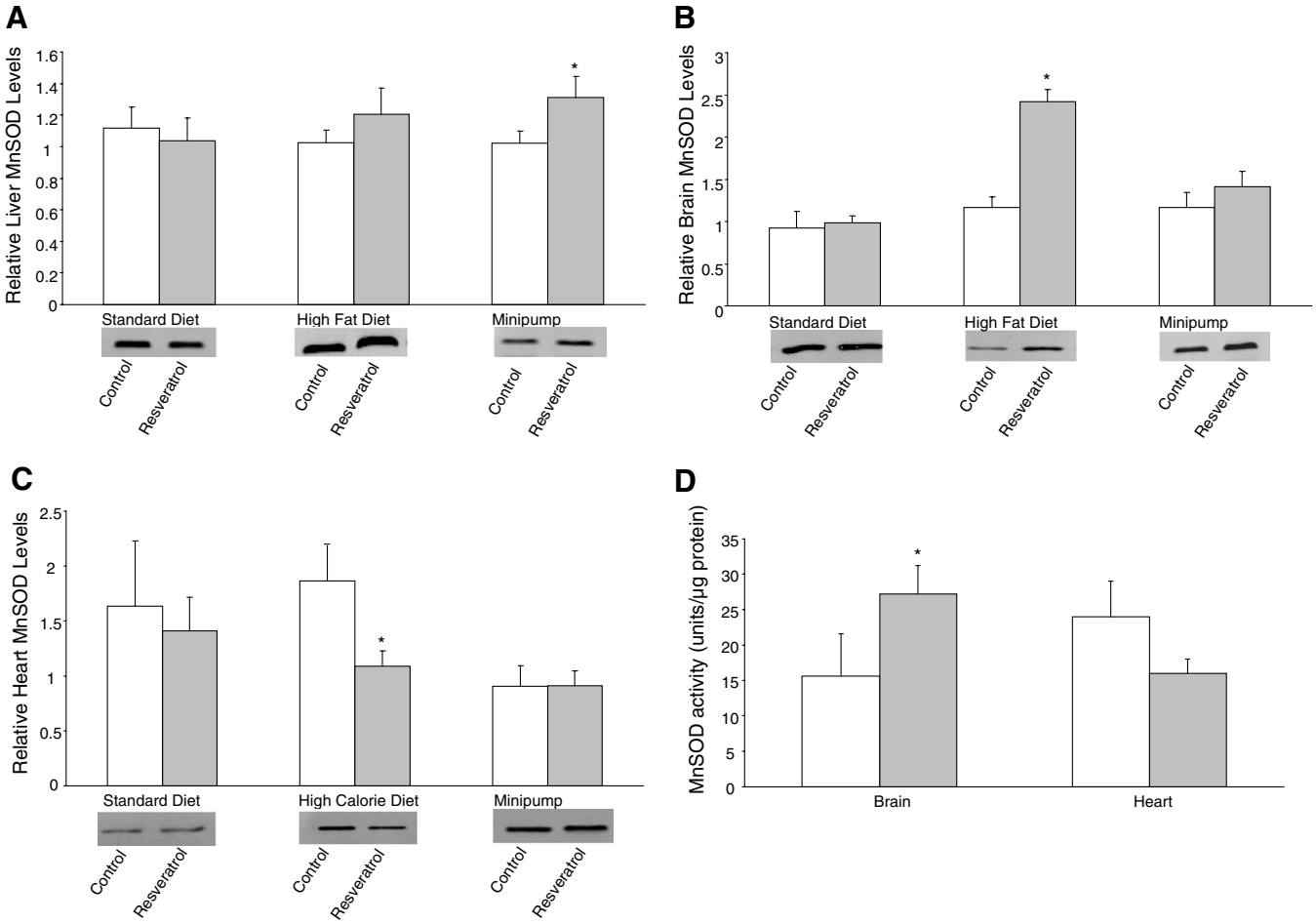

Fig. 1. MnSOD protein level and activity in brain, heart, and liver tissue of control (open bars) and resveratrol (solid bars) groups of three treatment methods ($n = 5–8$). Relative changes in MnSOD protein level in tissue homogenates and representative Western blots showing MnSOD protein band in (A) liver homogenates (B) brain homogenates and (C) heart homogenates. Relative change was measured using an internal standard as a reference and values were interpolated form a standard curve. Values shown are means ± SEM of duplicate Western blots for each homogenate. (D) Activity of MnSOD in brain and heart of high fat diet group. Values shown are means ± SEM of duplicate measurements of four homogenates per group. *Significantly different from control group ($P < 0.05$).