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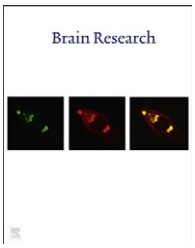

Research Report

Effects of resveratrol on blood homocysteine level, on homocysteine induced oxidative stress, apoptosis and cognitive dysfunctions in rats

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ABSTRACT

We aimed to examine the protective effects of resveratrol against homocysteine induced oxidative stress, apoptosis and cognitive impairment. Rats were randomly divided into three groups. Control group received standard rat food; homocysteine group (Hcy group) received daily methionine at a dose of 1 g/kg-body weight dissolved in drinking water for thirty days; third group (Hcy+Res group) received same amount of methionine plus 20 mg/kg/day resveratrol intraperitoneally for thirty days. Cognitive performances of the animals were tested by Morris water maze test. Then all animals were sacrificed to study lipid peroxidation (LPO), DNA fragmentation and p53 mRNA expression in the rat brain. The aortas of the sacrificed rats were processed for histopathological examination. Apoptosis in the aortas was assessed by TUNEL staining. Resveratrol significantly decreased serum levels of homocysteine, reversed Hcy induced LPO increase, decreased DNA fragmentation and p53 mRNA expression in the rat brains, and improved homocysteine induced impairment of long term spatial memory. Resveratrol could inhibit homocysteine induced apoptosis and histopathological deterioration in the rat aortic sections. In conclusion, resveratrol is effective in preventing homocysteine induced vascular and neural defects. In hyperhomocysteinemic rat model, our findings consequently warrant in future studies to reveal the true improvement mechanism of resveratrol.

Abbreviations: Hcy, homocysteine; NO, nitric oxide; LPO, lipid peroxidation; MDA, malondialdehyde; 4-HDA, 4-hydroxyalkenals; PAR, proteinase activated receptor; MPP, 1-methyl-4-phenyl pyridinium; AMPK, AMP-activated kinase; ROS, reactive oxygen species; NF-κB, nuclear factor-kappa B; SIRT1, sirtuin 1; NMDA, N-methyl-D-aspartate; SAH, S-adenosyl homocysteine; DTT, dithiothreitol; PMSF, phenylmethylsulfonylfluoride; TUNEL, Terminal deoxynucleotidyl transferase mediated dUTP digoxigenin nick end-labeling

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1. Introduction

Resveratrol (3,5,4′-trihydroxystilbene) is a compound that is considered as a phytoalexin. It is synthesized in limited number of plants and produces resistance to fungal infections in the plants. Synthesis of resveratrol in plants is stimulated by stress, infection, injury and UV-irradiation (Soleas et al., 1997). It has drawn attention because of its potential benefits against cancer, cardiovascular and neurological diseases.

Cardiovascular and neurological diseases are active fields of research for resveratrol. Prevention of oxidative injury and improvement of antioxidant capacity of tissues (Mokni et al., 2007a; Robb et al., 2008), increase in tissue nitric oxide (NO) level (Tsai et al., 2007), inhibition of nuclear factor-kappa B (NF-κB), activation of which results in increase of inflammatory mediators (Kumar and Sharma, 2010), alteration of neurotransmission (Schmatz et al., 2009), promotion of neuronal survival via a deacetylase, sirtuin 1 (SIRT1) (Kim et al., 2007) and prevention of apoptosis (Moriya et al., 2011) are mechanisms reported to underlie its' beneficial effects on neural and vascular tissues. These effects of resveratrol has been shown in the context of middle cerebral artery occlusion stroke (Sinha et al., 2002), ischemia/reperfusion injury (Mokni et al., 2007b), focal cerebral ischemia injury (Tsai et al., 2007), traumatic brain injury (Ates et al., 2007), kainic acid induced neurotoxicity (Wang et al., 2004), Alzheimer's disease (Karuppagounder et al., 2009), 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinsonism (Lu et al., 2008) 6-hydroxydopamine-induced model of Parkinson's disease (Khan et al., 2010), and 3-nitropropionic acid-evoked peripheral neuropathy (Binienda et al., 2010) models in rats.

It has been reported that resveratrol increases endothelial NO synthetase and NO level (Wallerath et al., 2002), inhibits platelet aggregation, activation (Shen et al., 2007; Yang et al., 2008) and synthesis of endotelin 1, an injurious substance to the vasculature (Nicholson et al., 2010; Ruef et al., 2001). It has beneficial effects on lipid profile (Berrougui et al., 2009). Number of smooth muscle cells in response to injury was reduced and intimal hyperplasia of injured vessel was effectively inhibited by resveratrol (Zou et al., 2000). Akar et al. (2011) have reported that resveratrol protects endothelial integrity, which was demonstrated by electron microscopy. It has also improved flow-mediated dilation, a measure of endothelial function, in hypercholesterolemic rabbits (Zou et al., 2003). Blood pressure, an important risk factor for vascular diseases, could be lowered by resveratrol in obese rats (Rivera et al., 2009).

Homocysteine (Hcy) is an amino acid derived from the metabolism of methionine. It has drawn attention because of its deleterious effects on vascular and neural tissues. Its' oxidative character for living tissues is quite well documented. Homocysteine is also accused of causing abnormality in brain maturation (McCall et al., 1996, Koz et al., 2011), apoptosis and impairment of neural plasticity (Streck et al., 2003; Baydas et al., 2005a). Deleterious effects of Hcy on developing brain in prenatal period have also been shown (Baydas et al., 2007; Koz et al., 2010).

Thrombin and proteinase activated receptors (PARs) may have important role in Hcy and resveratrol mediated actions. There are four types of PARs expressed in vascular, immune cells, astrocytes and neurons. Thrombin is a potent activator of PAR-1, PAR-3 and PAR-4 (Soh et al., 2010). Hcy can induce production of reactive oxygen species (ROS) by increasing NADPH and decreasing thioredoxin expression through activation of PAR-4 (Tyagi et al., 2005). On the other hand, it has been shown that oxidative stress can activate PAR-2 (Aman et al., 2010). Hcy augments thrombin induced platelet aggregation, and resveratrol can reduce Hcy induced superoxide anion production and platelet aggregation in vitro (Malinowska and Olas, 2011). Resveratrol can inhibit thrombin signaling in platelets and endothelial cells (Kaneider et al., 2004). It has been shown that, resveratrol and thrombin react spontaneously in the test tube and this reaction results in alteration of thrombin conformation and improvement of stability of resveratrol (Zhang et al., 2011a, 2011b).

There are some in vitro studies suggesting that resveratrol inhibits homocysteine induced defects. In vivo studies testing resveratrol in the context of homocysteine induced oxidative stress, apoptosis, and cognitive dysfunction are lacking. We aimed to test resveratrol against hyperhomocysteinemia in a rat model and see whether it prevents homocysteine induced oxidative stress, apoptosis and cognitive impairment or not.

2. Results

Mean plasma Hcy concentration of the Hcy group was significantly higher than that of the control rats (Control: 5,21 μM/L and Hcy group: 19,16 μM/L; $P<0.001$). Treatment with resveratrol reduced Hcy levels significantly in Hcy+Res group compared to Hcy group (11,19 μM/L vs. 19,16 μM/L, $P<0.01$).

Lipid peroxidase levels in hippocampi of rats were quantified in three subcellular fractions (mitochondria, nuclei, and cytosol) and results are shown in Fig. 1. The group treated with methionine alone showed a significant increase in the level of LPO in mitochondrial and nuclear subfractions of hippocampal regions of brain homogenates compared to that of control group. Most striking increase was seen in mitochondrial subfraction ($p<0.001$ compared to control group). Concomitant administration of resveratrol and methionine, significantly decreased the level of LPO in mitochondrial ($p<0.01$) and nuclear fraction compared to Hcy group ($p<0.05$). There was no significant change in the level of LPO in cytosolic fractions by the treatment of resveratrol.

Genomic DNA was isolated from the hippocampi and DNA fragmentation was assessed by agarose gel electrophoresis. DNA fragmentation, a hallmark of apoptosis, was observed in hippocampi of Hcy group. Administration of resveratrol effectively inhibited DNA fragmentation induced by Hcy (Fig. 2).

Mitochondrial RNA levels of p53 were measured with semi-quantitative PCR and β-actin was used as an internal control. Hyperhomocysteinemia increased hippocampal p53 mRNA above the control value ($P<0.01$) that was reduced by the treatment with resveratrol ($p<0.05$; Fig. 3).

Terminal deoxynucleotidyl transferase mediated dUTP digoxigenin nick end-labeling (TUNEL) positivity, assessed under light