Beneficial Effect of Protein Tyrosine Phosphatase Inhibitor and Phytoestrogen in Dyslipidemia-Induced Vascular Dementia in Ovariectomized Rats

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Background: Estrogen deficiency and increase in protein tyrosine phosphatase (PTPase) activity may be a key mechanism in postmenopausal dyslipidemia–induced vascular dysfunction and dementia. Thus, the present study has been designed to investigate the effect of biochanin A (BCA, a phytoestrogen) and sodium orthovanadate (SOV), an inhibitor of PTPase in dyslipidemia-induced vascular dementia in ovariectomized rats. *Methods:* Female Wistar rats were ovariectomized and fed on high fat diet for 4 weeks to produce dyslipidemia. Dyslipidemia was assessed by estimation of serum lipid levels including total cholesterol, triglyceride, HDL, and LDL levels. Dementia was assessed in terms of increase in brain acetylcholinesterase (AChE) activity and attenuation of learning ability (escape latency time) and memory retention (time spent in target quadrant) using Morris water maze. Vascular dysfunction was assessed in terms of attenuation of acetylcholine-induced endothelium-dependent relaxation (isolated carotid ring preparation), mRNA expression of endothelial nitric oxide synthase, and increase in serum thiobarbituric acid reactive species, superoxide anion level. Neurodegeneration was assessed in hippocampus by hematoxylin and eosin staining. BCA (2.5 and 5 mg/kg) and SOV (5 and 10 mg/kg) were administered alone and in low-dose combination to ovariectomized dyslipidemic rats. *Results:* BCA (2.5 and 5 mg/kg), SOV (5 and 10 mg/kg), and donepezil (1 mg/kg) significantly improves vascular function, and learning and memory ability and decreases the neuronal cell death, oxidative stress, and AChE in ovariectomized dyslipidemic rats. *Conclusions:* Thus, it may be concluded that BCA and SOV attenuate vascular dysfunction and dementia in dyslipidemic ovariectomized rats. **Key Words:** Vascular dementia—ovariectomy—dyslipidemia—vascular endothelium dysfunction.

Vascular dementia (VaD) is a neurological disorder that occurs when the brain's supply of oxygenated blood is repeatedly disrupted by strokes or cerebrovascular pathology, leading to significant accumulated damage to brain tissue and function.[1] Postmenopausal estrogen deficiency and concurrent dyslipidemia may also lead to VaD.[2] Ovarian hormone estrogen is an important vasoprotective and neuroprotective molecule with marked effects on both vasculature and neuronal functions. Estrogen deficiency associated with menopause is the major risk factor for VaD. Dyslipidemia causes cerebrovascular dysfunction consequent on reduced perfusion and VaD. Further decrease in estrogen level after menopause is also clinically associated with dyslipidemia. Dyslipidemia and its manifestations such as increased low-density lipoprotein (LDL), cholesterol, and triglyceride (TG) level makes postmenopausal women more prone to vascular abnormalities that cause cerebral hypoperfusion and

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Received January 17, 2015; revision received February 14, 2015; accepted February 24, 2015.
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1052-3057/$ - see front matter

http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2015.02.019

resultant dementia. Clinically no current therapeutic intervention can both effectively control neuronal function and prevent/arrest microvascular complications leading to dementia.[2]

Protein tyrosine phosphatase has been reported to dephosphorylate phosphoinositide-3 kinase (PI3K)/Akt pathway, enhance proapoptotic genes such as caspase, BAD, and BCL-2, and also decrease the number and viability of endothelial progenitor cells, thus reducing postnatal blood vessel repair.[3] Estrogen rapidly activates endothelial nitric oxide synthase (eNOS) via a PI3K-dependent pathway.[4] Upregulation of endothelial nitric oxide (NO) production plays an important role in the vasoprotective effects of estrogen.[5,6] The cerebral vasculature is a significant target tissue for this hormone, and in vivo exposure to estrogen increases NO-mediated vasodilation in rodent cerebral arteries.[7–9] Thus, the present study has been designed to investigate the effect of biochanin A (BCA, a phytoestrogen) and sodium orthovanadate (SOV, a PTPase inhibitor) on VaD in dyslipidemic ovariectomized rats.

Material and Methods

Animals

The experimental protocol was approved by the Institutional Animal Ethical Committee. Age-matched female Wistar rats weighing 150-200 g were employed in the present study. The animals were acclimatized to the laboratory condition before experiments and were exposed to normal day and light cycle. All the experiments were carried out in accordance with the guidelines of the Indian National Science Academy for the use and care of experimental animals.

Drugs and Chemicals

BCA and SOV were obtained from Sigma-Aldrich Ltd., New Delhi, India. Donepezil (DNZ) was obtained as a gift sample from Alkem Laboratories Ltd (Mumbai, India). All other reagents and chemicals used in the present study were of analytical grade.

Experimental Protocol

Female Wistar rats were ovariectomized and fed on high-fat diet. Forty-eight rats were randomly divided into 8 groups (6 in each group): group 1, normal control; group 2, ovariectomized dyslipidemic control; group 3, ovariectomized dyslipidemic rats with BCA (2.5 mg/kg, per os [p.o.]) treatment for last 2 weeks; group 4, ovariectomized dyslipidemic rats with BCA (5 mg/kg, p.o.) treatment for last 2 weeks; group 5, ovariectomized dyslipidemic rats with SOV (5 mg/kg, p.o.) treatment for last 2 weeks; group 6, ovariectomized dyslipidemic rats with SOV (10 mg/kg, p.o.) treatment for last 2 weeks; group 7, ovariectomized dyslipidemic rats with SOV and BCA (2.5 mg/kg, p.o. + 5 mg/kg, p.o.) treatment for last 2 weeks; group 8, ovariectomized dyslipidemic rats with DNZ (1 mg/kg, p.o.) treatment for last 2 weeks. Treatment schedule is shown in Figure 1.

Induction and Assessment of VaD

Rats were ovariectomized and subsequently fed high-fat diet (powdered normal pellet diet [NPD] [365 g/kg], lard [310 g/kg], casein [250 g/kg], cholesterol [10 g/kg], vitamin and mineral mix [60 g/kg], Dexter/Laveus [DL]-methionine [03 g/kg], yeast powder [01 g/kg], sodium chloride [01 g/kg]) to produce dyslipidemia-induced endothelial dysfunction.[10] After 4 weeks, rats were subjected to Morris water maze (MWM) test for the evaluation of their learning and memory status. The high-fat diet and drug treatment were continued during acquisition trials on MWM. Blood samples for biochemical estimation were collected just before killing the rats. The blood was kept at room temperature for 30 minutes and then centrifuged at 3000 rpm for 20 minutes, aliquoted, and stored at −20°C until analysis was carried out.

Wire Myograph Assessment of Vascular Endothelial Dysfunction

Acetylcholine-Induced Endothelium-Dependent and Sodium Nitroprusside–Induced Endothelium-Independent Relaxation on Isolated Rat Carotid Artery Ring Preparation

The rats were killed by cervical dislocation, followed by decapitation. The carotid artery was exposed and carefully dissected out. The carotid artery was placed in ice cold aerated Krebs–Henseleit solution (NaCl, 119 mM; KCl, 4.7 mM; $NaHCO_3$, 25 mM; $MgSO_4$, 1.0 mM; glucose, 11.1 mM; KH_2PO_4, 1.2 mM, and $CaCl_2$, 2.5 mM), and the connective tissue was removed. Isolated carotid artery was cut into 1.5- to 2-mm-wide rings and 1 ring was

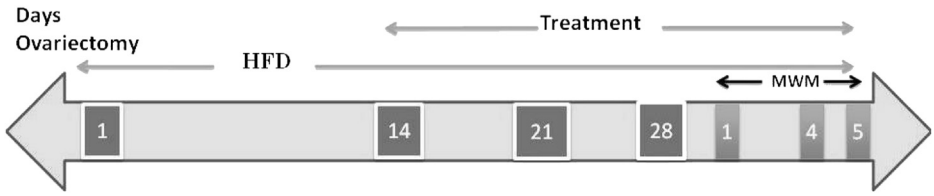

Figure 1. *Treatment schedule.*