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Efficacy of Cilostazol a selective phosphodiesterase-3 inhibitor in rat model of Streptozotocin diabetes induced vascular dementia

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ABSTRACT

The present study has been designed to investigate the potential of Cilostazol a phosphodiesterase-3 (PDE-3) inhibitor in diabetes-induced vascular dementia (Vad) employing Wistar rats. A single dose of Streptozotocin (STZ) was used for the induction of diabetes and subsequent Vad in rats. Memory and learning abilities of rats were evaluated with Morris water maze (MWM) test. Serum glucose, body weight, vascular endothelial function, serum nitrite/nitrate levels, brain oxidative stress levels (viz. brain thiobarbituric acid reactive species and reduced glutathione levels), inflammatory markers (*viz.* brain myeloperoxidase activity and neutrophil infiltration in the brain hippocampal area) and brain acetylcholinesterase activity were also tested. Donepezil was used as positive control. Streptozotocin treated animals showed poor performance on MWM indicating impairment of learning and memory abilities with a significant reduction in body weight, vascular endothelial function, serum nitrite/nitrate levels, along with an increase in serum glucose, brain oxidative stress levels, inflammatory changes and brain acetylcholinesterase activity. Treatment with selective PDE-3 inhibitor, Cilostazol significantly attenuated, diabetes-induced impairment of learning and memory; endothelial dysfunction, and changes in various biochemical parameters. It is concluded that selective PDE-3 inhibitor, Cilostazol may be considered as the potential pharmacological agent for the management of diabetes-induced vascular dementia.

1. Introduction

Vascular dementia (Vad) is a type of dementia caused by decreased blood supply to brain regions which are involved in cognitive functions resulting from cerebrovascular disease and ischemic or hemorrhagic brain injury (Iemolo et al., 2009; Thal et al., 2012). Vascular dementia is also one of the most widespread causes of dementia after the Alzheimer disease having a high prevalence in elderly patients with an average age of 76.26 years (Montiel et al., 2014). Several studies have reported the association of risk factors like age, hypertension, diabetes, obesity, cigarette smoking, hyperlipidemia, coronary artery disease, cardiac arrhythmias and autoimmune disorders such as lupus or cerebral arteritis with vascular dementia (Schneck, 2008; Marshall, 2012; Peters, 2012). Diabetes associated dementia is most prominent among all other risk factors. A recent study reports that diabetic patients have 127% higher risk of vascular dementia than non-diabetics (Gudala et al., 2013). Diabetes mellitus is a highly complex disorder, therefore the exact mechanism behind the cognitive impairment is still unknown, but probably alterations in brain vasculature, disturbed cerebral insulin signaling, insulin resistance, glucose toxicity, oxidative stress, accumulation of advanced glycation end products, hypoglycemia and changes in amyloid metabolism may all of them be involved (Bornstein et al., in press). Cognitive decline in type 2 diabetic patients is associated with vascular endothelial dysfunction mediated by the decreased levels of nitric oxide (Singh et al., 2014). Consistent hyperglycemic conditions as in diabetes lead to the generation of reactive oxygen species (ROS) and suppression of endothelial nitric oxide synthase (eNOS) subsequently decreased levels of nitric oxide, which is the key factor for production of vascular endothelial dysfunction (Versari et al., 2009). Furthermore, patients predisposed to diabetes have been shown to display more complications after an acute of ischemic stroke than normoglycemic patients (Bornstein et al., in press). Although vascular dementia is a highly prevalent memory disorder after the Alzheimer disease, but no drug therapy has so far been approved for vascular dementia (Montiel et al., 2014). Management therapy for vascular dementia only treats the risk factors like hypertension, cardiovascular diseases, endothelial dysfunction, atherosclerosis and dyslipidemia. So there is a need to develop a specific drug therapy for vascular dementia.

The cyclic nucleotide phosphodiesterases are a family of enzymes that selectively hydrolyzes 3′ cyclic phosphate bonds of cAMP and cGMP thus generate inactive 5′-AMP and 5′-GMP forms (Mehats et al., 2002; Bender and Beavo, 2006; Francis et al., 2011). Eleven different families of mammalian phosphodiesterases (PDEs) have been identified which are derived from 21 genes with more than 50 different PDE proteins encoded by these genes with alternative splicing and/or with the use of multiple promoters (Mehats et al., 2002; Francis et al., 2011). PDE-3 subclasses of phosphodiesterases are also referred to as

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cGMP-inhibited phosphodiesterases, but they have a high affinity for both cAMP and cGMP (Meacci et al., 1992; Beavo, 1995). Transcripts of mRNA encoding PDE-3_A in humans are noted to be present in platelets, vascular smooth muscles, cardiac myocytes and oocytes, whereas PDE-3_B transcripts are found in adipose tissue, liver, pancreas and cardiovascular tissues (Shakur et al., 2001). Several studies document important role of PDE-3 in different pathological conditions like type 2 diabetes, heart failure, ischemic reperfusion injury, cerebral ischemia, atherosclerosis and Alzheimer disease (Kondo et al., 1999; Takahashi et al., 2002; Genovese et al., 2011; Park et al., 2011; Muhammed et al., 2012).

Selective PDE-3 inhibitors have been explored for their therapeutic benefits in number of above mentioned pathological conditions. Studies have also demonstrated the protective actions of PDE-3 inhibitors in atherosclerosis mediated by the suppression of vascular smooth muscle cell proliferation (Kondo et al., 1999). Selective PDE-3 inhibitors have also been reported to enhance nitric oxide and prostaglandin-I_2 production with additional anti-inflammatory, anti-apoptotic and anti-oxidant effects (Choi et al., 2002; Park et al., 2007; Ye et al., 2007; Genovese et al., 2011). Cilostazol is a quinolinone derivative and more specifically inhibits PDE-3_A isoform (Dindyal and Kyriakides, 2009; Dunkerley et al., 2002). Cilostazol is orally active and 95–98% protein bound especially to albumin (Dindyal and Kyriakides, 2009). Some recent reports have documented the potential of Cilostazol in Alzheimer disease; however utility of Cilostazol in vascular dementia is still unexplored (Hiramatsu et al., 2010; Park et al., 2011; Lee et al., in press).

A rat model of Streptozotocin (STZ) induced diabetes and diabetes-related complication is well reported. STZ (50 mg/kg, i.p.) in a single dose has been demonstrated to induce diabetes mellitus in rats with a stable hyperglycemia after 10 days. These diabetic rats have been further observed to exhibit significant endothelial dysfunction and marked memory loss after 7 weeks of STZ treatment (Leung et al., 2010, 2011; Singh et al., 2014; Raghavan et al., 2014; Taguchi et al., 2014).

Therefore, the present study was undertaken to investigate the efficacy of Cilostazol in a rat model of diabetes-induced vascular dementia.

2. Material and methods

2.1. Experimental animals

Male and female Wistar rats weighing 200–300 g were procured from Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, INDIA. Animals were freely provided with standard laboratory feed (Kisan Feeds Ltd, Chandigarh, India) and water. They were exposed to natural cycles of 12:00 h of light and dark. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) (107/99/CPCSEA/2013-09). The care of the animals was taken as per guidelines of the Committee for the Purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of Environment and Forest Government of India (Reg. No. 107/1999/CPCSEA).

2.2. Drugs and chemicals

Donepezil was obtained as a gift sample from Wokhardt Ltd, Baddi, Himachal Pradesh, India. Cilostazol was purchased from Cipla Limited. Folin–Ciocalteu's phenol and acetylthiocholine were purchased from Merck Limited, Mumbai, India. Streptozotocin was purchased from Sigma-Aldrich, USA. 5,5,dithiobis (2-nitro benzoic acid) (DTNB), reduced glutathione (GSH), bovine serum albumin (BSA), sulfanilamide, N-naphthylethylenediamine (NED) and thiobarbituric acid were purchased from Loba Chem, Mumbai, India. Sodium nitroprusside was purchased from SD fine chemicals limited, Mumbai, India. Phenylephrine was obtained from Aarti Industries, Dombivli (East), Maharashtra, India as a gift sample. Cilostazol was suspended in 0.5% *w/v* of Carboxymethylcellulose (CMC) whereas Donepezil was dissolved in saline. Cilostazol and Donepezil were administered orally via gavage and Streptozotocin was administered intraperitoneally in 0.1 M citrate buffer pH 4.5. The doses of Cilostazol selected on the basis of previous literature reports and partly our pilot studies (Wang et al., 2008; Hiramatsu et al., 2010; Park et al., 2011).

2.3. Experimental protocol

Experimental diabetes mellitus and associated dementia in rats were induced by a single dose of freshly prepared Streptozotocin (50 mg/kg *i.p.*) in 0.1 M citrate buffer (pH 4.5) (Rakieten et al., 1963; Brosky and Logothetopoulos, 1969). Serum glucose levels of the animals were measured with an interval of one week. These animals were subjected to behavioral and other assessments on the 52nd day after the Streptozotocin treatment (Sharma and Singh, 2010, 2011).

In total nine groups were employed in the present study and each group was comprised of minimum 5 animals.

2.3.1. Group I—Control ($n = 5$)

Rats were exposed to Morris water maze to acquisition trials from Day 1 to Day 4 and retrieval trials on 5th Day.

2.3.2. Group II—Vehicle (CMC) group ($n = 5$)

Rats were administered 0.5% CMC (10 ml/kg, *p.o.*) for 10 days followed by exposure to the Morris water maze. The treatment was continued during acquisition (from 11th to 14th day) and retrieval trials (on the 15th day) on the Morris water maze.

2.3.3. Group III—STZ treatment group ($n = 6$)

Rats were administered with a single dose of Streptozotocin (50 mg/kg, *i.p.*) and they were exposed to Morris water maze on the 52nd day of STZ administration.

2.3.4. Group IV—STZ and Donepezil treatment group ($n = 6$)

Donepezil (0.5 mg/kg, *p.o.*, daily) serving as positive control, was administered to the STZ (50 mg/kg, *i.p.*) treated rats, starting from the 42nd day of STZ treatment followed by exposure to Morris water maze on the 52nd day of STZ administration. The treatment was continued during acquisition (from 52nd to 55th day) and retrieval trials (on the 56th day) on the Morris water maze. The drug treatments were made 1 h prior to Morris water-maze exposure.

2.3.5. Group V—STZ and Cilostazol low dose ($n = 6$)

Cilostazol (15 mg/kg, *p.o.*, daily) was administered to the STZ (50 mg/kg, *i.p.*) treated rats, starting from the 42nd day of STZ treatment rest of the procedure was same as described in Group IV.

2.3.6. Group VI—STZ and Cilostazol medium dose ($n = 6$)

Cilostazol (30 mg/kg, *p.o.*, daily) was administered to the STZ (50 mg/kg, *i.p.*) treated rats, starting from the 42nd day of STZ treatment rest of the procedure was same as described in Group IV.

2.3.7. Group VII—STZ and Cilostazol high dose ($n = 6$)

Cilostazol (60 mg/kg, *p.o.*, daily) was administered to the STZ (50 mg/kg, *i.p.*) treated rats, starting from the 42nd day of STZ treatment rest of the procedure was same as described in Group IV.

2.3.8. Group VIII—Cilostazol per se ($n = 5$)

Rats were administered Cilostazol (60 mg/kg, *p.o.*, daily) for 10 days followed by exposure to the Morris water maze. The treatment was continued during acquisition (from 11th to 14th day) and retrieval trials (on the 15th day) on the Morris water maze.

2.3.9. Group IX—Donepezil per se ($n = 5$)

Rats were administered Donepezil (0.5 mg/kg, *p.o.*, daily) for 10 days followed by exposure to the Morris water maze. The treatment was

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