Brain Research Bulletin 133 (2017) 31–41

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

Possible role of endothelin receptor against hyperhomocysteinemia and β-amyloid induced AD type of vascular dementia in rats

Major Singh [a], Atish Prakash [a,b,*]

[a] Department of Pharmacology, ISF College of Pharmacy, Ghal Kalan, Moga 142-001, Punjab, India
[b] Division of Pediatric Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

ARTICLE INFO

Article history:
Received 15 April 2016
Received in revised form 25 January 2017
Accepted 28 February 2017
Available online 6 March 2017

Keywords:
Alzheimer's disease
Vascular dementia
Endothelial dysfunction
Mitochondrial dysfunction
Oxidative stress

ABSTRACT

Vascular dementia (VaD) is considered as the second commonest form of dementia after Alzheimer's disease (AD). The study was designed to investigate the effect of endothelin receptor against β-amyloid induced AD type of vascular dementia. This disease was induced by combine administration of single ICV (intracerebroventricle) infusion of β-amyloid (Aβ) once and chronic oral administration of L-Methionine for 21 days. Bosentan (dual endothelin receptor antagonist) was administered for 21 days. Behavioral alterations were observed during different time interval of the study. Animals were killed immediately following the last behavior session. Oxidative parameters, acetylcholinesterase activity, neuro-inflammatory markers, amyloid beta levels were determined in hippocampus and cortex while serum homocysteine, serum nitrite carotid artery superoxide anion level were also determined. Endothelial function was measured on isolated carotid artery using myograph instrument. Aβ + L-Methionine showed more significant development of cognitive and vascular endothelial deficits, manifested in terms of increase in serum homocysteine level, endothelial dysfunction, impairment of learning and memory, enhanced brain acetylcholinesterase activity, marked mito-oxidative damage in rats. We have observed that L-Methionine and combination of Aβ + L-Methionine significantly enhanced Aβ level both in cortex as well as hippocampus. Treatment of bosentan attenuated Aβ + L-Methionine induced impairment of learning and memory, enhanced Aβ level, mitochondrial and endothelial dysfunction. The results of present study concluded that bosentan offers protection against β-amyloid-induced vascular dementia in rats. Endothelin receptor may be considered as a potential pharmacological target for the management of AD type of vascular dementia.

1. Introduction

Vascular dementia (VaD) is the second leading cause of dementia (Sharma and Singh, 2010, 2011) and having a strong correlation with various vascular complications such as diabetes, hyperhomocysteinemia (HHcy), hyperlipidemia (HL) etc. (Daviglus et al., 2011; Gao et al., 2012). HHcy risk has been found to be associated with Alzheimer's disease (AD) and other AD related disorders (Ravaglia et al., 2005; Obeid and Herrmann, 2006). The actual molecular relationship between high concentration of homocysteine (Hcy) and AD pathogenesis is yet to understand, because it could reveal novel targets for drug discovery in AD research. Recently, it has been suggested that homocysteine accelerates the βA deposition, Tau phosphorylation and cognitive deficit in Hcy induced animal model of AD (Li et al., 2014). Endothelial dysfunction is one of the earliest symptom considered to occur during cerebrovascular dysfunction associated with AD or other forms of dementia. Endothelial cells produce endogenous mediators i.e. Endothelin (ET) and nitric oxide (NO) for maintenance of hemodynamic responses (Bohm and Pernow, 2007).

Recently, it has been suggested that cerebrovascular dysfunction in AD is due to indirect actions of β-A_{1-42} deposition and increased production of ET_1, causing chronic reduction in CBF. It has been reported that ECEs has Aβ degrading property, therefore ET receptor antagonists may be beneficial in the treatment of AD

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, amyloid-β; BBB, blood brain barrier; BSA, bovine serum albumin; CBF, cerebral blood flow; ECE, endothelin converting enzyme; ELT, escape latency time; eNOS, endothelial nitric oxide synthase; ET, endothelin; HHcy, hyperhomocysteinemia; HL, hyperlipidemia; NO, nitric oxide; ROS, reactive oxygen species; SDH, succinate dehydrogenase; TBARS, thiobarbituric acid reactive substances; TSTQ, time spent in target quadrant; VaD, vascular dementia.

* Corresponding author at: Division of Pediatric Infectious Diseases, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Ross Building, Room 1109, Baltimore, MD 21205, USA.
E-mail addresses: aprakas6@jhmi.edu, atishprakash@gmail.com (A. Prakash).

http://dx.doi.org/10.1016/j.brainresbull.2017.02.012
0361-9230/

(Palmer and Love, 2011). Many reports have indicated that ET-1 is upregulated by astrocytes in a number of brain pathologies, including stroke, traumatic brain injury, AD, cancer, and multiple sclerosis (Hostenbach et al., 2016). Astrocyte and glial cell can contribute to the development of secondary brain damage by activating ET receptors in both an autocrine and paracrine manner.

L-Methionine plays an important role in dementia by inducing cerebrovascular endothelium dependent oxidative stress (Carluccio et al., 2007). Although ET receptor antagonists have found to be effective in various cerebrovascular disorders such as ischemic stroke (Kaundal et al., 2012) but the exact function in vascular dementia still unclear. Role of endothelin receptors (ET_A and ET_B) in dementia is yet to be discovered and endothelin receptor antagonists are potentially remained to be unexplored in vascular dementia. Aβ, a peptide derived from the amyloid precursor protein (APP) found in cerebral plaques and plasma leads to cerebral amyloid angiopathy, which is associated with an increased risk of stroke. Several experimental studies have demonstrated that Aβ may induce endothelial dysfunction of both cerebral and systemic vessels (Lee et al., 2003).

The present study was designed to investigate the possible role of endothelin receptors underlying the neuroprotective effect bosentan against combined effect of Aβ and L-Methionine induced AD type of vascular dementia.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 180–250 g (3–4 months), were used in the present study. Animals were obtained from Central Animal House facility of I.S.F. College of Pharmacy, Moga, Punjab, India. They were housed in-group of three, under standard laboratory conditions of temperature (22 ± 1 °C), relative humidity (60%) and light/dark cycle with food and water ad libitum. Animals were acclimatized to laboratory conditions before the experiment. All the behavioral assessments were carried between 9:00 and 17:00 h. The experimental protocol no. 137 dated 18.01.2014 was approved by the Institutional Animal Ethics Committee (IAEC) of I.S.F. College of Pharmacy, Moga and was carried out in accordance with the guidelines of the Indian National Science Academy (INSA) for the use and care of the experimental animals.

2.2. Intracerebroventricular administration of β-A_{1-42}

Rats were anesthetized with ketamine hydrochloride (80 mg/kg) and diazepam (3 mg/kg, i.p.) positioned in a stereotaxic apparatus (Stoelting Co. USA. MODEL NO: 53311). The head was positioned in a frame and a midline sagittal incision was made in the scalp. Two holes were drilled in the skull for ICV injection of β-A_{1-42} oligomers. Co-ordinates for the ICV Aβ infusion were 0.8 mm posterior to bregma, 1.8 mm lateral to the sagittal suture and 3.6 mm beneath the cortical surface. Each Rat was infused ICV with amyloid-β_{1-42} oligomers once (3 nmol/3 μL), 1.5 μL through each hole by using Hamilton micro syringe (Maurice et al., 1996). The scalp was then closed with a suture. After surgery, all animals received gentamicin (5 mg/kg, i.p.) to prevent sepsis. The surgery was identical except for drilling of holes in the sham-operated rats. To promote the diffusion, the micro syringe was left in place for a period of 2 min following injection. Special care of the animals was taken during the post-operative period.

2.3. Drugs and treatment schedule

Bosentan (CIPLA LTD. Goa INDIA), Aβ (GenxBio Delhi INDIA), L-Methionine (HIMEDIA), Donepezil (Alkem Laboratories Ltd Mumbai, India) were used in the experiment. Doses are selected based on previous studies and those reported in the literature. ICV β-A_{1-42} (3 nmol/3 μL) for once (Prakash and Kumar, 2014) and L-Methionine (1.7 mg/kg) (Koladiya et al., 2009), Bosentan (50 and 100 mg/kg) (Abdelsaid et al., 2014; Singh et al., 2014) and donepezil (1 mg/kg) (Gawel et al., 2014) were administered by oral gavage for 21 days. Chemicals and all the reagents used in the present study were of analytical grade. Animals were selected randomly based on their body weights into seven groups were employed in and each group was comprised of six animals (Table 1). Study was performed in multiple phases as per shown in experimental protocol in Fig. 1.

Table 1
Classification of treatment group.

Group	Treatment
I	Sham control
II	L-Methionine (1.7 g/kg)
III	β-A_{1-42} treatment group (3 nmol/3 μL) ICV
IV	β-A_{1-42} + L-Methionine treatment group
V	β-A_{1-42} + L-Methionine + bosentan (50 mg/kg)
VI	β-A_{1-42} + L-Methionine + bosentan (100 mg/kg)
VII	β-A_{1-42} + L-Methionine + donepezil (1 mg/kg)

2.4. Behavioral parameters

2.4.1. Assessment of spatial learning and memory by Morris water maze

Morris water-maze apparatus (MWM) is most commonly used model to test memory (Morris, 1984). The MWM procedure is based on the principle that animals hate swimming and hence when placed in a large pool of water tank its normal tendency is to escape it by searching for a safe zone platform. MWM consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1 °C). The water was made opaque with non-toxic white colored dye. The tank was divided into four equal quadrants. A submerged platform (10 cm × 10 cm), painted white was placed in the middle of the target quadrant of this pool, 1 cm beneath surface of water. The position of platform was kept unchanged all through the training session. The water maze task was carried out for four consecutive days from day 10–13. The rats received four consecutive daily training sessions in the following 4 days, with each trial having a ceiling time of 120 s. Escape latency time (ELT) to locate the hidden platform in MWM was noted as index of acquisition or learning. Probe test was carried out on day 14 and 21, platform was removed and each animal was allowed to explore in the pool for 120 s. The time spent by the animal in target quadrant in search for the hidden platform is noted as index of retrieval (Kumar et al., 2012).

2.4.2. Novel object recognition

The object recognition test was performed as described by Hattiangady and Shetty (2012) with minor modifications. The test involved three successive trials for each rat with an inter-trial interval of 60 min. The first two trials comprised placing the rat in the center of an empty open field box and allowing the rat to freely explore the empty box for 2 min (first trial, the habituation phase), and placing the rat in the center of the open field box having two identical objects (FO1 and FO2) on opposite sides of the box and allowing the rat to freely explore the objects for 2 min (second trial). The third trial (the objection recognition memory testing phase) commenced 60 min after the second trial where the rat was allowed to explore objects for 2 min in the same open field box comprising one object used in the trial 2 (i.e. the FO1, familiar object 1) and a new object replacing the second object used in trial 2 (i.e. the NO, novel object). A rat is considered to be exploring an object when its nose is within 2 cm of the object. All objects used in this study were