Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 89 (2008) 535–545
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Exploitation of HIV protease inhibitor Indinavir as a memory restorative agent in experimental dementia

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Received 31 July 2007; received in revised form 29 January 2008; accepted 4 February 2008
Available online 12 February 2008

Abstract

The present study was undertaken to investigate the beneficial effect of HIV protease inhibitor Indinavir on memory deficits associated with experimental dementia of Alzheimer disease's (AD) type. Dementia was induced in Swiss albino mice by administration of Celecoxib (100 mg kg^{-1} orally, daily for 9 days) or Streptozotocin (3 mg kg^{-1} administered intracerebroventricularly on 1st and 3rd day) and the cognitive behaviors of Swiss albino mice were assessed using Morris water maze test. Brain acetyl cholinesterase (AChE) activity was measured by Ell Mann's method. Brain thiobarbituric acid reactive species (TBARS) levels and reduced glutathione (GSH) levels were measured by Ohokawa's and Beutler's method respectively to assess total oxidative stress. Donepezil (0.1 mg kg^{-1} i.p.) served as positive control in the present investigation. Celecoxib as well as Streptozotocin (STZ) produced a significant loss of learning and memory. Indinavir (100 and 200 mg kg^{-1} orally) successfully attenuated Celecoxib as well as STZ induced cognitive deficits. Higher levels of brain AChE activity, TBARS and lower levels of GSH were observed in Celecoxib as well as STZ treated animals, which were significantly attenuated by Donepezil and Indinavir. Study highlights the potential of Indinavir in memory dysfunctions associated with dementia of AD.

Keywords: Indinavir; Donepezil; Dementia; Oxidative stress; Beta-amyloid

1. Introduction

Dementia is a mental disorder characterized by impairment of memory and loss of intellectual ability, sufficiently severe as to interfere with one's occupational or social activities. Prevalence rate for dementia increases exponentially with advancing age (Kawas et al., 2000; Vas et al., 2001). In USA around 10% people aged above 65 years suffer from mild to moderate dementia. The dementing condition, that has gained much attention in the recent years is Alzheimer's disease (AD), which is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas. Over 50% cases of memory impairment accounts for AD (Torre et al., 2004). It is typically characterized by progressive loss of memory followed by dementia (Suh and Kim, 2004). Histological hallmarks of AD are senile plaques and neurofibrillary tangles composed of paired helical filaments (Howlett et al., 2000; Klein et al., 2001). Senile plaques are extra-neuronal deposits of insoluble aggregates of beta-amyloid (1–40; 1–42) peptides, degradation products of the larger amyloid precursor protein (APP) catalyzed by beta-amyloid converting enzyme i.e. beta-secretase and gamma-secretase (Selkoe, 2001; Abramov et al., 2004, Kim et al., 2007). Neuroscientists all over the world are trying to develop a remedy for Alzheimer's disease and related dementia. Current therapeutic strategies for AD are mainly focused on improvement of memory i.e. only providing symptomatic relief without any effect on neuronal loss. Hence there is a great need to develop an agent, which may improve memory as well as block neurodegenerative changes in AD brain. Structurally beta-amyloid converting enzyme (BACE) shows a close resemblance with HIV protease enzyme (Hong et al., 2000), and the inhibitors of HIV protease are expected to modulate BACE activity. So, Indinavir a HIV protease inhibitor, originally used for management of AIDS may also be considered to modulate BACE activity. Therefore, in the present study an

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doi:10.1016/j.pbb.2008.02.012

attempt has been made to investigate the beneficial effect of Indinavir a HIV protease inhibitor in memory deficits associated with dementia of AD in mice, employing Celecoxib (a selective cox-2 inhibitor) induced dementia (Sharma et al., accepted for publication and in press) and Streptozotocin induced dementia (Lannert and Hoyer, 1998; Sharma and Gupta, 2001) as separate animal models. Donepezil a well known clinically used acetyl cholinesterase inhibitor for the management of AD served as positive control in this investigation.

2. Materials and methods

2.1. Animals

Swiss albino mice (20–30 g) of either sex (procured from IVR, Izatnagar, Bareilly) were employed in the present study. They were housed in departmental animal house with free access to water and standard laboratory pellet chow diet (Kisan Feeds Ltd, Mumbai, India). The mice were exposed to12 h light and 12 h dark cycle. The experiments were conducted between 9.00 and 17.00 h in a semi sound-proof laborotary. The animals were acclimatized to the laboratory condition 5 days prior to behavioural study. The protocol of study was duly approved by institutional animal ethical committee of the department and care of the animals was carried out as per the guidelines of 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 reagents

All the drug solutions were freshly prepared before use. Celecoxib was obtained as gift from IPCA Laboratories Ltd., Bombay. Donepezil HCl was a gift from Wokhardt Ltd., Badi, India. Indinavir was a gift from Zydus Research Center, Ahmedabad, India. Streptozotocin and 1,1,3,3 tetra-methoxy propane were purchased from Sigma Aldrich, USA. 5,5′-dithiobis (2-nitro benzoic acid) DTNB, Bovine serum albumin (BSA), and Glutathione reduced (GSH) standard were purchased from Sisco Research Laboratories Pvt Ltd., Mumbai, India. Thiobarbituric acid was purchased from Loba Chemie, Mumbai. Celecoxib was suspended in 1% w/v of sodium carboxy methyl cellulose (CMC). Donepezil and Indinavir were dissolved in distilled water, and Streptozotocin was dissolved in artificial cerebro spinal fluid (CSF) prepared according to the method as described by Sakurada et al. (1999). Celecoxib, CMC and Indinavir were administered orally with the help of an oral tube (cannula), Donepezil was administered intraperitoneally and Streptozotocin and artificial CSF were delivered intracerebroventricularly.

2.3. Intracerebroventricular administration of Streptozotocin (STZ i.c.v.)

Mice were anesthetised with anesthetic ether (Haley and McCormick, 1957). Intracerebroventricular (i.c.v.) injections were made with hypodermic needle of 0.4 mm external diameter attached to a 10 μl Hamilton microlitre syringe (Top Syringe, Mumbai, India). The needle was covered with a polypropylene tube except 3 mm of the tip region so as to insert this much portion of the needle perpendicularly through the skull into the brain of mouse.

The injection site was 1 mm to right or left midpoint on the line drawn through to the anterior base of the ears. Injections were performed into right or left ventricle randomly. Two doses of STZ (3 mg kg^{-1}) were administered by i.c.v. injection bilaterally. The second dose was administered after 48 h of first dose. The concentration was adjusted so as to deliver 10 μl in an injection. The injection was made at two places as it is difficult to administer 10 μl at a single site. Control group mice were given i.c.v. injection of artifical cerebro spinal fluid (CSF) in similar manner.

2.4. Morris water maze (MWM)

Morris water maze test was employed to assess learning and memory of the animals (Morris, 1984). MWM is a swimming based model where the animal learns to escape on to a hidden platform. It 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 white colored non-toxic dye. The tank was divided into four equal quadrants with help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (10 cm^2), painted in white was placed inside the target quadrants of this pool, 1 cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive training trials on each day with inter-trial gap of 5 min. The mouse was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 s to locate submerged platform. Then, it was allowed to stay on the platform for 20 s. If it failed to find the platform within 120 s, it was guided gently onto platform and allowed to remain there for 20 s. day 4 escape latency time (ELT) to locate the hidden platform in water maze was noted as index of acquisition or learning. Animal was subjected to training trials for four consecutive days, the starting poison was changed with each exposure as mentioned below and target quadrant (Q 4) remained constant throughout the training period.

Day 1	Q1	Q2	Q3	Q4
Day 2	Q2	Q3	Q4	Q1
Day 3	Q3	Q4	Q1	Q2
Day 4	Q4	Q1	Q2	Q3

On fifth day, platform was removed and each mouse was allowed to explore the pool for 120 s. Mean time spent in all four quadrants was noted. The mean time spent by the animal in target quadrant searching for the hidden platform was noted as index of retrieval or memory.

The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory serving, as prominent visual clues were not disturbed during the total duration of study. All the trials were completed between 09.00 and 17.00 h. All the time indexes were noted manually with the help of stop watches.