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

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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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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.

2.5. Collection of samples

Animals were sacrificed by cervical dislocation, brains were removed and homogenized in phosphate buffer (pH = 7.4). The homogenates were than centrifuged at 3000 rpm for 15 min. The supernatant of homogenates were used for biochemical estimations as per the methods described below.

2.6. Estimation of brain acetyl cholinesterase (AChE) activity

The whole brain AChE activity was measured by the method of Ellman et al. (1961) with slight modifications (Voss and Sachsse, 1970). This was measured on the basis of the formation of yellow colour due to the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of brain cholinesterase was measured using a spectrophotometer. 0.5 ml of supernatant liquid of the brain homogenate was pipetted out into 25 ml volumetric flask and dilution was made with a freshly prepared DTNB {5,5'-dithiobis (2-nitro benzoic acid)} solution (10 mg DTNB in 100 ml of sorenson phosphate buffer, pH 8.0). From the volumetric flask, two 4 ml portions were pipetted out into two test tubes. Into one of the test tube, 2 drops of eserine solution were added. 1 ml of substrate solution (75 mg of acetylcholine iodide per 50 ml of distilled water) was pipetted out into both of the test tubes and incubated for 10 min at 30 °C. The solution containing eserine solution was used for zeroing the colorimeter and change in absorbance per min of the sample was read spectrophotometrically (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 420 nm. AChE activity was calculated using the following formula:

$$R = \frac{\delta \text{O.D.} \times \text{Volume of Assay (3 ml)}}{E \times \text{mg of protein}}$$

Where

R	rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed/minute/mg protein
δO.D.	change in absorbance/minute
E	extinction coefficient = 13,600/M/cm

2.7. Estimation of brain thiobarbituric acid reactive species (TBARS) level

The whole brain TBARS level was measured by the method of Ohokawa et al. (1979) with slight modifications. 0.2 ml brain homogenate was pipetted out in a test tube, followed by addition of 0.2 ml sodium dodecyl sulphate (SDS), 1.5 ml of 30% acetic acid (pH-3.5), 1.5 ml of 0.8% thiobarbituric acid (TBA) and made up the volume up to 4.0 ml with distilled water (DW). The test tubes were incubated at 95 °C for 60 min, and then cooled it. 1.0 ml of DW and 5.0 ml of *n*-butanol:pyridine (15:1 v/v) mixture was added to the test tubes and centrifuged at the 4000 ×g for 10 min. The absorbance of developed colour in organic layer was measured spectrophotometrically at 532 nm (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA). The absorbance from a standard curve generated using 1,1,3,3, tetra-methoxy propane as standard (range = 1 nmol–10 nmol) was extrapolated.

2.8. Estimation of brain GSH level

The whole brain GSH level was measured by the method of Beutler et al. (1963) with slight modifications. Tissue homogenate was taken and the proteins were precipitated with 10% w/v chilled trichloroacetic acid. Samples were kept in ice bath and were centrifuged after 30 min at 1000 ×g for 10 min at 4 °C. GSH levels were measured in the supernatant. 0.5 ml supernatant was mixed with 2.0 ml of 0.3 M disodium hydrogen phosphate solution and 0.25 ml of freshly prepared DTNB {5,5'-dithiobis (2-nitro benzoic acid)} solution (40 mg/100 ml in 1% w/v sodium citrate) was added just before measuring the absorbance spectrophotometrically at 412 nm (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA). Different concentration of GSH standard was also processed similarly to prepare a standard curve (5–50 µg) simultaneously. Results were expressed as nmole of GSH/mg of protein.

2.9. Estimation of brain total protein

For the estimation of total protein in brain, method of Lowry et al. (1951) with slight modifications was used. 150 µl of supernatant was taken in a test tube, volume was made up to 1 ml with distilled water than 5 ml of Lowry's reagent (freshly prepared mixture of 1% w/v copper sulphate, 2% w/v sodium potassium tartrate and 2% w/v sodium carbonate in 0.1 N NaOH in the ratio of 1:1:98 respectively), was added and mixed thoroughly. Mixture was allowed to stand for 15 min at room temperature and then 0.5 ml of 1:1 v/v diluted Folin–Ciacalteu reagent was added. Contents were vortexed and incubated at 37 °C for 30 min. Then absorbance was determined spectrophotometrically at 750 nm (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) against suitably prepared blank. A standard curved using 25–200 mg of BSA was plotted. The amount of total protein was expressed in mg.

3. Experimental protocol

Seventeen groups of mice were employed in the present study and each group comprised of ten mice.

3.1. Group I (control group)

Mice were administered distilled water (10 ml kg^{-1} i.p.) 30 min before acquisition trials conducted from day 1 to day 4 and 30 min before retrieval trial conducted on day 5.

3.2. Group II (CMC control group)

Mice, were administered 1% w/v sodium carboxymethyl-cellulose i.e. CMC, (10 ml kg^{-1} p.o.) daily for 10 days and then subjected to Morris water maze test. The administration of CMC (administered 45 min before) was continued during acquisition trials conducted from day 1 to day 4 and retrieval trial conducted on day 5.

3.3. Group III (CSF control group)

Mice, were injected artificial cerebro spinal fluid i.e. ACSF (25 mg ml^{-1}, 10 µl, intracerebroventricularly i.e. i.c.v.) in two dosage schedules (on 1st and on 3rd day) followed by exposure to Morris water maze test after 15 days.

3.4. Group IV (Celecoxib treatment group)

Mice, were administered Celecoxib (100 mg kg^{-1} p.o.) suspended in 1% w/v CMC, daily for 5 days and then subjected to Morris water maze test. The administration of celecoxib (administered 45 min before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5, these animals were administered vehicle (1% w/v CMC) only, and then subjected to retrieval test after 45 min.

3.5. Group V (Donepezil per se group)

Mice, were administered Donepezil (0.1 mg kg^{-1} i.p.) dissolved in distilled water, daily for 5 days and then subjected to Morris water maze test. The administration of Donepezil (administered 30 min before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5, these animals were administered vehicle (distilled water) only, and then subjected to retrieval test after 30 min.

3.6. Group VI (Donepezil + Celecoxib, group)

Mice, were administered Donepezil followed after 15 min by Celecoxib daily for 5 days and then subjected to Morris water maze test. The administration of Donepezil and Celecoxib (administered 60 min and 45 min, respectively before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5, these animals were administered vehicles (distilled water and CMC) only, and then subjected to retrieval test.

3.7. Groups VII and VIII (low/high dose Indinavir per se, groups)

Mice, were administered Indinavir (100/200 mg kg^{-1} p.o.) daily for 5 days and then subjected to Morris water maze test. The administration of Indinavir (administered 45 min before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5, these animals were administered vehicle (distilled water) only, and then subjected to retrieval test after 45 min.

3.8. Groups IX and X (low/high dose Indinavir + Celecoxib, groups)

Mice, were administered Indinavir (100 mg kg^{-1} p.o.)/ (200 mg kg^{-1} p.o.) followed after 15 min by Celecoxib (100 mg kg^{-1} p.o.) suspended in 1% w/v CMC, daily for 5 days The administration of Indinavir and Celecoxib (administered 60 min and 45 min, respectively before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5, these animals were administered vehicles (distilled water and CMC) only, and then subjected to retrieval test.

3.9. Group XI (STZ, treatment group)

Mice were injected Streptozotocin (3 mg kg^{-1}, 10 µl, i.c.v.) in two dosage schedules i.e. on 1st and on 3rd day followed by exposure to Morris water maze test after 15 days.

3.10. Group XII (Donepezil nineteen days per se, group)

Mice were administered Donepezil (0.1 mg kg^{-1} i.p.) dissolved in distilled water, daily for 15 days and then subjected to Morris water maze test. The administration of Donepezil (administered 30 min before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5, these animals were administered vehicle (distilled water) only, and then subjected to retrieval test after 30 min.

3.11. Group XIII (STZ + Donepezil, group)

STZ (i.c.v.) mice, were administered Donepezil (starting after second dose of STZ), daily for 15 days and then subjected to Morris water maze test. The administration of Donepezil (administered 30 min before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5, these animals were administered vehicle (distilled water) only, and then subjected to retrieval test after 30 min.

3.12. Groups XIV and XV (low/high dose, Indinavir, nineteen days per se, groups)

Mice, were administered Indinavir (100/200 mg kg^{-1} p.o.) daily for 15 days and then subjected to Morris water maze test. The administration of Indinavir (administered 45 min before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5, these animals were administered vehicle (distilled water) only, and then subjected to retrieval test after 45 min.

3.13. Groups XVI and XVII (STZ + low/high dose, Indinavir, groups)

STZ (i.c.v.) mice, were administered Indinavir (100/200 mg kg^{-1} p.o. starting after second dose of STZ), daily for 15 days and then subjected to Morris water maze test. The administration of Indinavir (administered 45 min before) was also continued during acquisition trials conducted from day 1 to day 4. On day 5, these animals were administered vehicle (distilled water) only, and then subjected to retrieval test after 45 min.

4. Statistical analysis

All results were expressed as mean ± S.E.M. Data was analyzed using one-way analysis of variance (ANOVA) followed by post hoc Tukey's multiple range test using Sigma Stat Statistical

Software, version 2.0. $p<0.05$ was considered to be statistically significant.

5. Results

Various pharmacological interventions employed in the present investigation did not show any mortality and daily injection of Donepezil produced no behavioral alteration. Further, no significant difference was observed between the results obtained from mice of either sex.

5.1. Effect of vehicles on escape latency time (ELT) and time spent in target quadrant

Administration of distilled water 45 min before acquisition trials conducted on day 1 to day 4, significantly decreased day 4 escape latency time (ELT) as compared to its value noted on day 1, reflecting acquisition or learning (Fig. 1). Further these mice, significantly spent more time in the target quadrant (Q4) in search of missing platform as compared to the time spent in other quadrants (Q1, Q2, Q3) during retrieval trial conducted on day 5, indicating memory or retrieval of acquisition (Fig. 2). Treatment of vehicles 1% w/v CMC or artificial cerebro spinal fluid did not show any significant effect on day 4 ELT (Fig. 3) and day 5 mean time spent in target quadrant of control animals (Figs. 2 and 4).

5.2. Effect of Celecoxib/Streptozotocin on acquisition and retrieval of memory

Celecoxib/Streptozotocin significantly prevented decrease in day 4 ELT of respective control group (Figs. 1 and 3) and markedly reduced time spent in target quadrant (Q4) in search of missing platform during retrieval trial (Figs. 2 and 4), reflecting impairment of both learning as well as memory.

5.3. Effect of Indinavir/Donepezil on acquisition and retrieval of memory

Indinavir (100 mg kg^{-1} p.o.; 200 mg kg^{-1} p.o.)/Donepezil (0.1 mg kg^{-1} i.p.) did not produce any significant effect on day 4 decrease in ELT (Figs. 1 and 3) and increase in time spent in target quadrant in search of missing platform during retrieval trial conducted on day 5 of control mice (Figs. 2 and 4).

5.4. Effect of Indinavir/Donepezil on Celecoxib/STZ induced dementia

Indinavir (100 mg kg^{-1} p.o.; 200 mg kg^{-1} p.o.)/Donepezil (0.1 mg kg^{-1} i.p.) significantly attenuated the day 4 rise in ELT (Figs. 1 and 3) and decrease in day 5, time spent in target quadrant of Celecoxib/STZ treated animals (Figs. 2 and 4).

5.5. Effect of Celecoxib, Streptozotocin, Indinavir, Donepezil on AChE activity of brain

Celecoxib/Streptozotocin significantly, increased the brain AChE activity when compared to control mice. Indinavir (100 mg kg^{-1} p.o.; 200 mg kg^{-1} p.o.)/Donepezil (0.1 mg kg^{-1} i.p.), *per se* did not produced any significant effect on the brain AChE level as compared to control mice (Table 1).

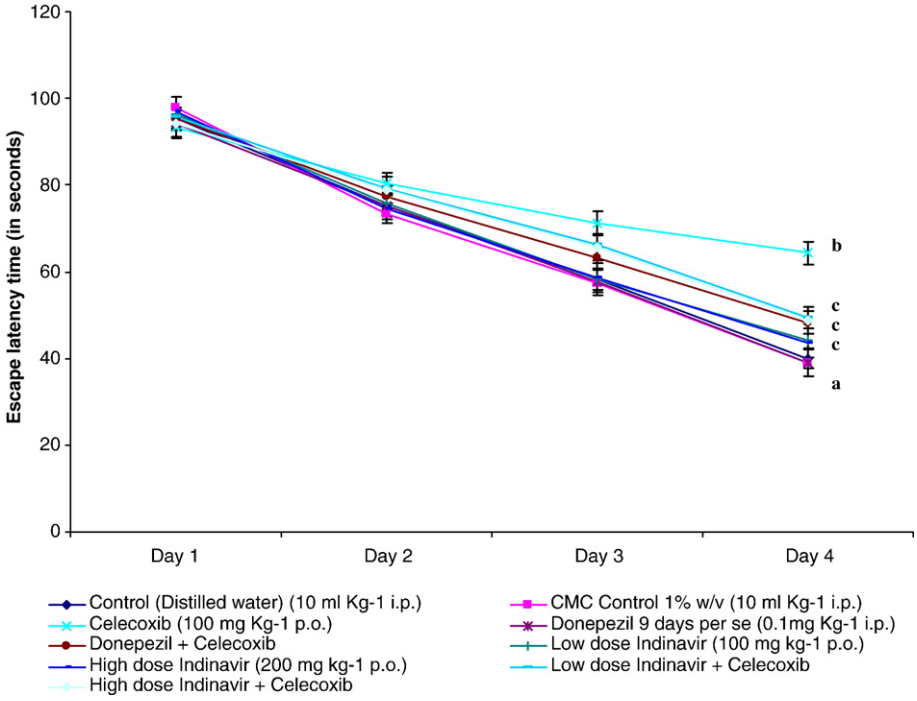

Fig. 1. Effect of Donepezil/Indinavir on Celecoxib induced increase in day 4 escape latency time (ELT). Each group ($n=10$) represents mean ± S.E.M. a = $p<0.05$ vs day 1 ELT in control. b = $p<0.05$ vs day 4 ELT in control. c = $p<0.05$ vs day 4 ELT in Celecoxib. ANOVA followed by Tukey's multiple range test.

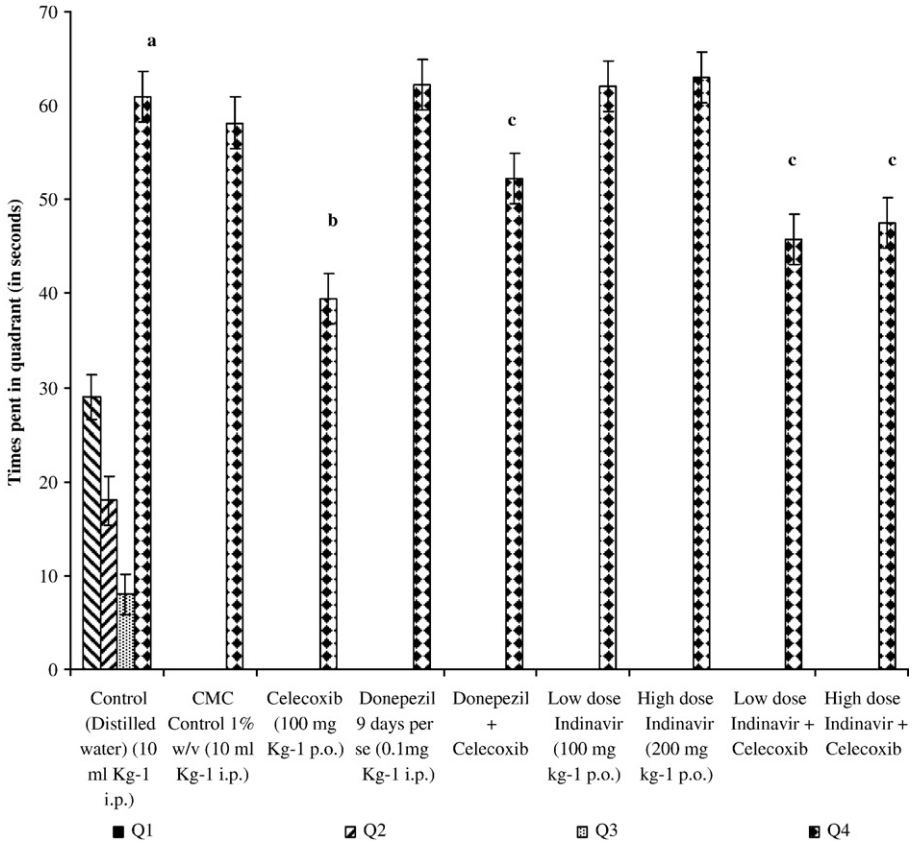

Fig. 2. Effect of Donepezil/Indinavir on Celecoxib induced decrease in time spent in target quadrant. Each group ($n=10$) represents mean±S.E.M., a = $p<0.05$ vs time spent in other quadrant, b = $p<0.05$ vs time spent in target quadrant of control group, c = $p<0.05$ vs time spent in target quadrant of Celecoxib treated group, ANOVA followed by Tukey's multiple range test.

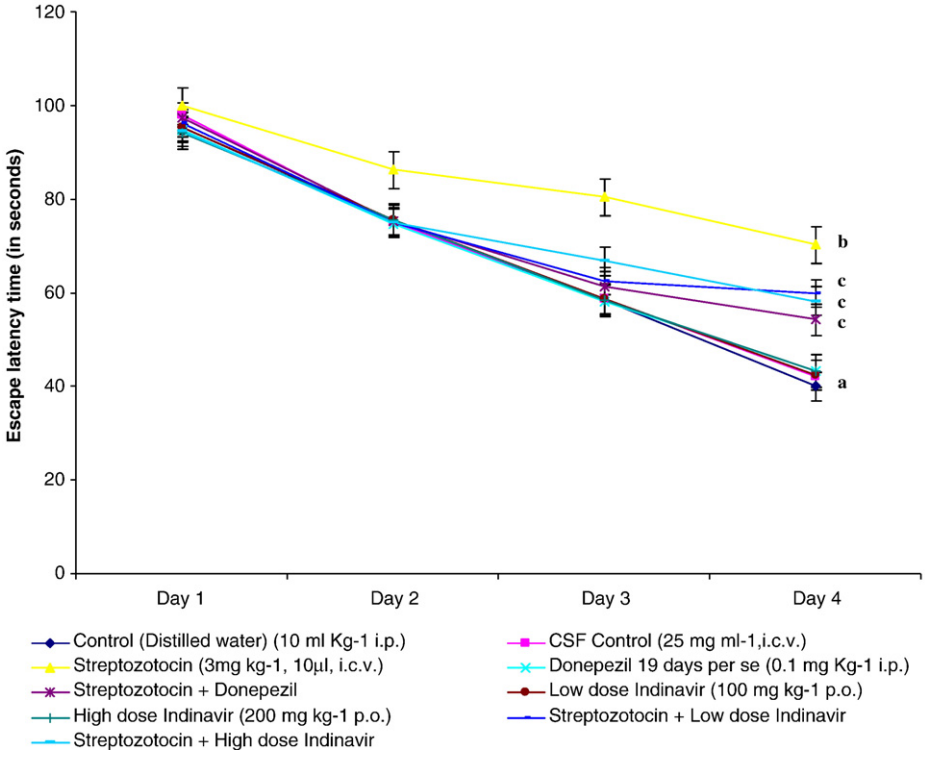

Fig. 3. Effect of Donepezil/Indinavir on Streptozotocin induced increase in day 4 escape latency time (ELT). Each group ($n=10$) represents mean±S.E.M., a = $p<0.05$ vs day 1 ELT in control, b = $p<0.05$ vs day 4 ELT in control, c = $p<0.05$ vs day 4 ELT in Streptozotocin, ANOVA followed by Tukey's multiple range test.

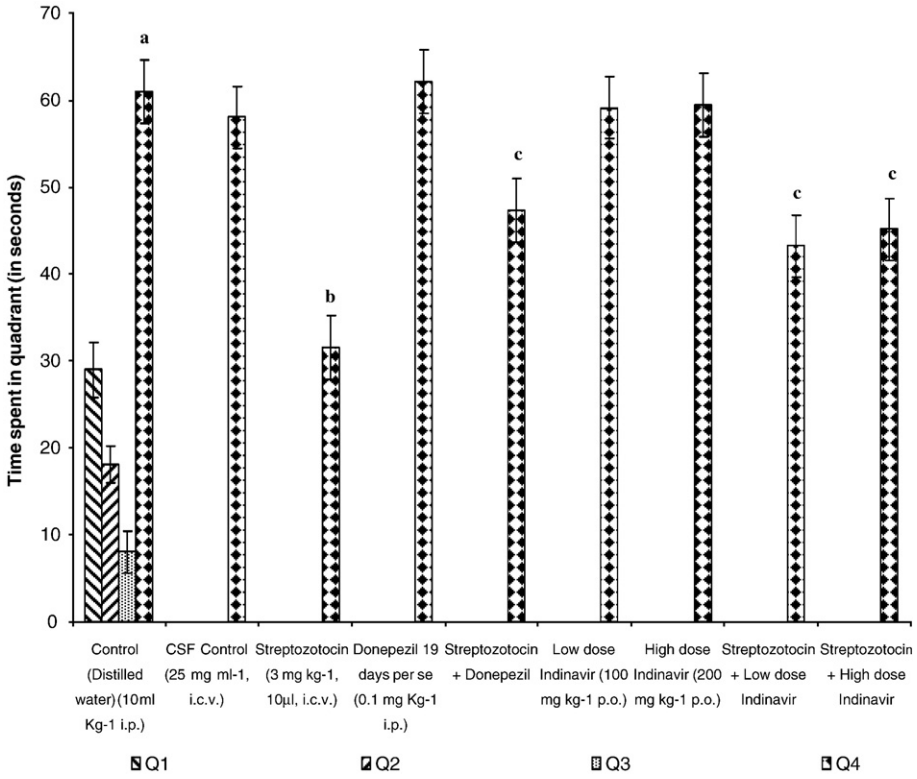

Fig. 4. Effect of Donepezil/Indinavir on Streptozocin induced decrease in time spent in target quadrant. Each group ($n=10$) represents mean±S.E.M., a = $p<0.05$ vs time spent in other quadrant. b = $p<0.05$ vs time spent in target quadrant of control group, c = $p<0.05$ vs time spent in target quadrant of Streptozotocin treated group, ANOVA followed by Tukey's multiple range test.

5.6. Effect of Indinavir/Donepezil on Celecoxib/STZ induced rise in brain AChE activity

Indinavir (100 mg kg^{-1} p.o.; 200 mg kg^{-1} p.o.)/Donepezil (0.1 mg kg^{-1} i.p.) significantly attenuated Celecoxib as well as STZ induced increase in brain AChE activity (Table 1).

5.7. Effect of Celecoxib, Streptozotocin, Indinavir, Donepezil on oxidative stress levels of brain

Celecoxib (100 mg kg^{-1} p.o.)/Streptozotocin (3 mg kg^{-1}) significantly, increased the brain TBARS level and reduced the brain GSH levels, compared to control group of animals,

Table 1
Effect of Donepezil/Indinavir on Celecoxib/Streptozotocin induced increase in brain acetyl cholinesterase (AChE) activity

Group	Treatment	Dose	*AChE activity
I	Control (distilled water)	10 ml kg^{-1} i.p.	125±3.8
II	CMC control 1% w/v	10 ml kg^{-1} i.p.	120±3.6
III	CSF control	25 mg ml^{-1}, i.c.v.	129±4.2
IV	Celecoxib	100 mg kg^{-1} p.o.	169±4.6[a]
V	Donepezil 9 days *per se*	0.1 mg kg^{-1} i.p.	121±3.7
VI	Donepezil+Celecoxib	0.1 mg kg^{-1} i.p.+100 mg kg^{-1} p.o.	134±3.7[b]
VII	Low dose Indinavir 9 days *per se*	100 mg kg^{-1} p.o.	122±4.4
VIII	High dose Indinavir 9 days *per se*	200 mg kg^{-1} p.o.	123±2.9
IX	Low dose Indinavir+Celecoxib	100 mg kg^{-1} p.o.+100 mg kg^{-1} p.o.	145±4.6[b]
X	High dose Indinavir+Celecoxib	200 mg kg^{-1} p.o.+100 mg kg^{-1} p.o.	149±4.6[b]
XI	Streptozotocin	3 mg kg^{-1}, 10 µl, i.c.v.	189±3.2[a]
XII	Donepezil 19 days *per se*	0.1 mg kg^{-1} i.p.	127.7±2.7
XIII	Streptozotocin+Donepezil	3 mg kg^{-1}, 10 µl, i.c.v.+0.1 mg kg^{-1} i.p.	156±3.8[c]
XIV	Low dose Indinavir 19 days *per se*	100 mg kg^{-1} p.o.	124±3.8
XV	High dose Indinavir 19 days *per se*	200 mg kg^{-1} p.o.	126±3.4
XVI	Streptozotocin+low dose Indinavir	3 mg kg^{-1}, 10 µl, i.c.v.+100 mg kg^{-1} p.o.	162±3.8[c]
XVII	Streptozotocin+high dose Indinavir	3 mg kg^{-1}, 10 µl, i.c.v.+200 mg kg^{-1} p.o.	160±4.8[c]

Each group ($n=10$) represents mean±S.E.M.
a = $p<0.05$ vs brain AChE activity of control.
b = $p<0.05$ vs brain AChE activity of Celecoxib.
c = $p<0.05$ vs brain AChE activity of Streptozotocin.
ANOVA followed by Tukey's multiple range test.
*AChE activity: acetyl cholinesterase activity of brain (in micromoles of ACh hydrolyzed/min/mg of protein).

Table 2
Effect of Donepezil/indinavir on Celecoxib/Streptozotocin induced increase in brain thiobarbituric acid reactive species (TBARS) level

Group	Treatment	Dose	*TBARS levels
			nM/mg of protein
I	Control (distilled water)	10 ml kg^{-1} i.p.	4.8±1.7
II	CMC control 1% w/v	10 ml kg^{-1} p.o.	5.02±1.3
III	CSF control	25 mg ml^{-1}, i.c.v.	5.6±1.6
IV	Celecoxib	100 mg kg^{-1} p.o.	13.4±1.9[a]
V	Donepezil 9 days *per se*	0.1 mg kg^{-1} i.p.	4.9±1.6
VI	Donepezil+Celecoxib	0.1 mg kg^{-1} i.p.+100 mg kg^{-1} p.o.	6.8±1.4[b]
VII	Low dose Indinavir 9 days *per se*	100 mg kg^{-1} p.o.	4.7±1.4
VIII	High dose Indinavir 9 days *per se*	200 mg kg^{-1} p.o.	5.03±1.2
IX	Low dose Indinavir+Celecoxib	100 mg kg^{-1} p.o.+100 mg kg^{-1} p.o.	8.1±1.6[b]
X	High dose Indinavir+Celecoxib	200 mg kg^{-1} p.o.+100 mg kg^{-1} p.o.	7.8±1.6[b]
XI	Streptozotocin	3 mg kg^{-1}, 10 µl, i.c.v.	18.9±2.2[a]
XII	Donepezil 19 days *per se*	0.1 mg kg^{-1} i.p.	5.1±1.7
XIII	Streptozotocin+Donepezil	3 mg kg^{-1}, 10 µl, i.c.v.+0.1 mg kg^{-1} i.p.	9.3±1.9[c]
XIV	Low dose Indinavir 19 days *per se*	100 mg kg^{-1} p.o.	4.9±1.4
XV	High dose Indinavir 19 days *per se*	200 mg kg^{-1} p.o.	4.8±1.4
XVI	Streptozotocin+Low dose Indinavir	3 mg kg^{-1}, 10 µl, i.c.v.+100 mg kg^{-1} p.o.	11.7±2.8[c]
XVII	Streptozotocin+High dose Indinavir	3 mg kg^{-1}, 10 µl, i.c.v.+200 mg kg^{-1} p.o.	10.3±2.3[c]

Each group (n=10) represents mean±S.E.M.
a = $p<0.05$ vs brain TBARS levels of control.
b = $p<0.05$ vs brain TBARS levels of Celecoxib.
c = $p<0.05$ vs brain TBARS levels of Streptozotocin.
ANOVA followed by Tukey's multiple range test.
*TBARS levels: thiobarbituric acid reactive species levels (in nanomoles/mg of protein).

reflecting enhanced oxidative stress. Indinavir (100 mg kg^{-1} p.o.; 200 mg kg^{-1} p.o.)/Donepezil (0.1 mg kg^{-1} i.p.), *per se* did not produce any significant effect on brain TBARS and GSH levels of control group animals (Tables 2 and 3).

5.8. Effect of Indinavir/Donepezil on Celecoxib/STZ induced enhancement of oxidative stress

Indinavir (100 mg kg^{-1} p.o.; 200 mg kg^{-1} p.o.)/Donepezil (0.1 mg kg^{-1} i.p.) significantly reversed the Celecoxib/STZ

Table 3
Effect of Donepezil/Indinavir on Celecoxib/Streptozotocin induced decrease in brain GSH (reduced glutathione) level

Group	Treatment	Dose	*GSH levels
			nanomole/mg of protein
I	Control (distilled water)	10 ml kg^{-1} i.p.	21.9±2.7
II	CMC control 1% w/v	10 ml kg^{-1} p.o.	20.1±2.9
III	CSF control	25 mg ml^{-1}, i.c.v.	21.5±3.2
IV	Celecoxib	100 mg kg^{-1} p.o.	13.4±2.1[a]
V	Donepezil 9 days *per se*	0.1 mg kg^{-1} i.p.	22.3±2.6
VI	Donepezil+Celecoxib	0.1 mg kg^{-1} i.p.+100 mg kg^{-1} p.o.	19.8±2.4[b]
VII	Low dose Indinavir 9 days *per se*	100 mg kg^{-1} p.o.	21.7±3.4
VIII	High dose Indinavir 9 days *per se*	200 mg kg^{-1} p.o.	20.8±3.2
IX	Low dose Indinavir+Celecoxib	100 mg kg^{-1} p.o.+100 mg kg^{-1} p.o.	19.1±1.6[b]
X	High dose Indinavir+Celecoxib	200 mg kg^{-1} p.o.+100 mg kg^{-1} p.o.	20.3±1.6[b]
XI	Streptozotocin	3 mg kg^{-1}, 10 µl, i.c.v.	11.2±2.2[a]
XII	Donepezil 19 days *per se*	0.1 mg kg^{-1} i.p.	22.1±2.7
XIII	Streptozotocin+Donepezil	3 mg kg^{-1}, 10 µl, i.c.v.+0.1 mg kg^{-1} i.p.	19.3±2.5[c]
XIV	Low dose Indinavir 19 days *per se*	100 mg kg^{-1} p.o.	22.1±2.4
XV	High dose Indinavir 19 days *per se*	200 mg kg^{-1} p.o.	21.7±3.4
XVI	Streptozotocin+Low dose Indinavir	3 mg kg^{-1}, 10 µl, i.c.v.+100 mg kg^{-1} p.o.	16.7±2.4[c]
XVII	Streptozotocin+High dose Indinavir	3 mg kg^{-1}, 10 µl, i.c.v.+200 mg kg^{-1} p.o.	18.3±3.3[c]

Each group (n=10) represents mean±S.E.M.
a = $p<0.05$ vs brain GSH levels of control.
b = $p<0.05$ vs brain GSH levels of Celecoxib.
c = $p<0.05$ vs brain GSH levels of Streptozotocin (ICV).
ANOVA followed by Tukey's multiple range test.
*GSH levels: reduced glutathione level nanomole/mg of protein.

induced rise in TBARS levels and fall in GSH levels of brain (Tables 2 and 3).

6. Discussion

Morris water maze (Morris, 1984) is employed in the present study as an exteroceptive model to evaluate spatial learning and memory. Extensive pretraining is not required in this model because animals learn rapidly to locate the hidden platform. Moreover escape from water itself acts as motivation and eliminates the use of other motivational stimuli such as food and water deprivation. Water provides uniform environment and eliminates interference due to olfactory clues (Morris, 1984). A marked decrease in escape latency time (ELT) control group animals during ongoing acquisition trials denotes normal acquisition of memory and an increase in time spent in target quadrant in search of missing platform during retrieval trial indicates retrieval of memory. These observations are in agreement with the results of our earlier studies (Parle and Singh, 2004, 2007) and reports from other laboratory (Packard et al., 1996). No effects of vehicles employed in the present study to prepare various solutions of drugs have been noted on acquisition and retrieval of memory. Therefore, the effect of pharmacological intervention on acquisition and retrieval of memory is due to them and not because of their vehicles.

Celecoxib in the present study produced impairment of acquisition and retrieval of memory as reflected by significant increase in day 4 escape latency time (ELT) and decrease in day 5 time spent in target quadrant respectively. Moreover, there was an enhancement of brain acetyl cholinesterase (AChE) activity and increase in oxidative stress as reflected by rise in brain TBARS and reduction in GSH levels. Recently, it has been reported that short-term Celecoxib treatment increases the amyloid beta-42 ($A\beta_{42}$) segment in brain cells (Kukar et al., 2005). Amyloid beta-42 ($A\beta_{42}$) is mainly responsible for formation of insoluble aggregates that result in the pathology of Alzheimer's disease (Jarrett et al., 1993; Kim et al., 2007). β-Amyloid has been shown to affect variety of neuronal functions including calcium signaling and impairment of mitochondrial redox activity (Etcheberrigaray et al., 1994; Chan et al., 1999). All these effects of β-amyloid may eventually lead to neuronal damage and dementia. Intracerebroventricular injection of beta-amyloid peptide has been shown to produce AD like dementia as well as to increase oxidative stress in mice (Song et al., 1998; Choi et al., 2001). Transgenic mice over-expressing beta-amyloid has been demonstrated to induce dementia of AD type (Sturchler et al., 1997; Kim et al., 2007). Therefore Celecoxib induced learning and memory deficits may be attributed to its stimulatory effect on brain beta-amyloid 42 ($AA\beta_{42}$) peptide segment level, brain AChE levels, and oxidative stress. The above noted effects on learning and memory are specific to Celecoxib. It has been reported that no other COX-2 inhibitor was able to modulate brain ($A\beta_{42}$) (Kukar et al., 2005). This contention is further supported by our earlier study, with Rofecoxib (another COX-2 inhibitor), which failed to show any impairment of learning and memory in mice, in contrast Celecoxib produced a marked loss of learning as well as memory (Sharma et al., accepted for publication and in press).

In our study, Streptozotocin (i.c.v.) significantly impaired learning and memory in mice. The STZ (i.c.v.) model has been described as an appropriate animal model for AD, typically characterized by progressive impairment of learning abilities and memory capacities (Lannert and Hoyer, 1998). Cerebral glucose and energy metabolism is associated with oxidative stress (Lannert and Hoyer, 1998 Sharma and Gupta, 2001). After i.c.v. administration, the highest concentration of STZ (3 mg kg^{-1}) reaches the fornix and periventricular white matter at the level of 3rd ventricle, which show the greatest damage (Shoham et al., 2003), and STZ (i.c.v.) induced amnesia is independent of its hyperglycemic effect (Mayer et al., 1990). Although the mechanism of action of STZ (i.c.v.) on memory impairment is not yet known, it probably involves the induction of oxidative stress (Feillet-Coudray et al., 1999; Reagan et al., 2000), to which myelin is particularly vulnerable (Smith et al., 1999). Damage to myelin by oxidative stress is seen in disorders such as AD with cognitive impairment (Braak et al., 2000). STZ (i.c.v.) in rats causes desensitization of insulin receptors and biochemical changes similar to that of AD or ageing brain (Hoyer et al., 1994; Hoyer, 2000a,b). In addition, reduced energy metabolism and synthesis of acetyl-CoA ultimately results in cholinergic deficiency and thereby memory deficit in STZ (i.c.v.) treated rats.

It is note worthy to mention here that in this study, the extent of memory impairment induced with Celecoxib as well as with STZ on animals of either sex was almost same and no significant difference was observed in male and female mice. Therefore, ruling out any interference due to gender as described by some studies.

In the present investigation, Donepezil reversed the memory deficits induced by Celecoxib/STZ. Central cholinergic system plays an important role in the process of learning and memory. Its hypofunction may induce aspect of dementia such as memory loss and disorientation in Alzheimer's disease (Dhingra et al., 2003). Loss of cortical cholinergic neurons is a prominent feature of dementia of AD (Parle and Singh, 2004). Cholinomimetic drugs have been reported to improve memory deficits, whereas anticholinergic drugs such as Scoplamine produce amnesia in animals (Itoh et al., 1990). Donepezil is recently approved AChE inhibitor and neuroprotective drug to be used clinically for memory impairment of AD. Therefore the noted reversal of Celecoxib/STZ dementia by Donepezil may be due to its AChE inhibitory and neuroprotective action.

Indinavir, a HIV protease inhibitor has significantly reversed Celecoxib/Streptozotocin induced memory deficits in our investigation. Further, significant decrease in brain AChE activity and oxidative stress levels were also noted. Studies have indicated that BACE has structural homology to the HIV protease (Hong et al., 2000) and thus, many inhibitors of the latter can modulate BACE activity (Nunan and Small, 2000), hence the amount of β-amyloid formation. Enhanced levels of beta-amyloid in the brain have been reported to induce memory dysfunction and dementia due to increase in oxidative stress and AChE activity (Song et al., 1998; Choi et al., 2001). Therefore, the observed ameliorative effect of Indinavir on memory deficits may be attributed to its potential anti-oxidative and anti-cholinesterase actions. It is further proposed that by virtue

the structural similarity of HIV protease with BACE, Indinavir might have exerted some inhibitory action on BACE activity and hence, decreased brain beta-amyloid load. However presently it is difficult to reconcile with the data in hand, the exact mechanism of action of Indinavir as a memory restorative agent should be explored in our next studies. Perhaps this is the first report highlighting the potential of Indinavir in memory impairment associated with dementia of AD type in mice.

7. Conclusion

Administration of Indinavir significantly attenuated memory deficits induced by Celecoxib/STZ in mice.

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