JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 1665–1672

Received May 22, 2009
Accepted September 16, 2009
DOI 10.1211/jpp/61.12.0012
ISSN 0022-3573

Research Paper

Effect of chronic treatment of carvedilol on oxidative stress in an intracerebroventricular streptozotocin induced model of dementia in rats

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Abstract

Objectives Oxidative stress is emerging as an important issue in the pathogenesis of dementia. This study was conducted to investigate the possible neuroprotective effects of carvedilol against streptozotocin induced behavioural alterations and oxidative damage in rats.

Methods An intracerbroventricular cannula was implanted in the lateral ventricles of male Wistar rats. Various behavioural (locomotor activity, Morris water maze task) and biochemical parameters (lipid peroxidation, nitrate concentration, catalase, acetylcholinesterase, reduced glutathione and protein) were assessed.

Key findings Intracerebroventricular administration of streptozotocin caused a significant memory deficit as evaluated in the Morris water maze task paradigms, and caused marked oxidative damage as indicated by significant increases in malondialdehyde and nitrite levels, and depletion of superoxide dismutase, catalase and reduced glutathione levels. It also caused a significant increase in acetylcholinesterase activity. Chronic administration of carvedilol (1 and 2 mg/kg, i.p.) for a period of 25 days starting 4 days before streptozotocin administration resulted in an improvement in memory retention, and attenuation of oxidative damage and acetylcholinesterase activity.

Conclusions This study demonstrates the effectiveness of carvedilol in preventing cognitive deficits as well as the oxidative stress caused by intracerbroventicular administration of streptozotocin in rats. Carvedilol may have potential in the treatment of neurodegenerative diseases.

Keywords carvedilol; dementia; neuroprotection; oxidative stress; streptozotocin

Introduction

Alzheimer's disease is a devastating neurodegerative disorder and the most common cause of dementia. Clinically, it presents with intellectual deterioration that involves not only memory, orientation and language functions but also components of higher function, such as personality, judgement, perception, problem solving, calculation, visual/spatial and constructional abilities.[1] Neuropathological hallmarks of the disease include neurofibrillary tangles, neurite (senile) plaques, amyloid angiopathy, granulovacuolar degeneration and Hirano bodies.[2] Even though the neuropathological characteristics of Alzheimer's disease are well defined, its aetiology remains a mystery, which makes the treatment of Alzheimer's disease a challenge. The results of extensive experimental work indicate that free radicals appear to be a major factor in the cause of cellular damage. Several studies provide evidence that links neuronal damage with excessive generation of free radicals, which may be due to factors such as oxidative stress,[3] DNA damage and neuronal disruption,[4] neuroinflammation,[5] abnormal proteins[6] or unknown factors. It has been observed that the use of antioxidants and neuroprotective agents may decrease the risk of memory deficits associated with Alzheimer's disease.[7,8]

Drugs that can modulate reactive oxygen species may be potentially useful in the management of Alzheimer's disease. Carvedilol is an antihypertensive drug with non-selective β-adrenergic and selective α-adrenergic blocking activities, and associated pleiotropic effects (antioxidant activity, α_1-adrenoceptor blocking effect, vasodilatation, inhibition of apoptosis,[9] anti-inflammatory,[10] mitochondrial protective,[11] non-competitive

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inhibitor of *N*-methyl D-asperate receptor and calcium channel blocker).[12] Carvedilol has been shown to exert neuroprotective effects in several models of transient focal stroke[9] and tardive dyskinesia,[13] cardioprotective effects in several models of cardiovascular ischaemia and reperfusion,[14] and nephroprotective effects.[15] These effects have been attributed in part to the free radical scavenging and metal chelating properties of carvedilol.[16] Suzuki *et al.* have shown that carvedilol suppresses lipid autoxidation and protein carbonyl formation in brain homogenate in a dose-dependent manner.[17] The antioxidant activity of carvedilol emanates from the carbazole moiety.[18] Studies have also shown that carvedilol prevents the conversion of β amyloid into the biologically active form, thereby acting as a novel antifibrillar agent.[19] The dementia model is based on the generation of free radicals in the brain, which increases lipid peroxidation and decreases the antioxidant glutathione, together with causing memory impairment.[20] Intracerbroventricular streptozotocin at subdiabetogenic doses causes prolonged impairment of brain glucose and energy metabolism in rats. This is accompanied by impairment of learning and memory in addition to a decrease in choline acetyltransferase levels in the hippocampus.[21,22] The present study was designed to investigate the effects of carvedilol on streptozotocin induced cognitive dysfunction and oxidative damage in rats.

Materials and Methods

Animals

Male Wistar rats (Central Animal House, Panjab University, Chandigarh, India), 180–200 g, were used. Animals were acclimatized to laboratory conditions at room temperature before experimentation. After surgery, animals were kept in groups of two in plastic cages with soft bedding, under standard conditions with a 12-h light/dark cycle and free access to food and water. All the experiments were carried out between 0900 and 1700 hours. The protocol was approved by the Institutional Animal Ethics Committee and carried out in accordance with the Indian National Science Academy guidelines for the care and use of animals.

Surgery and intracerebroventricular administration of streptozotocin

Surgery was performed according to the previously described protocol.[20] All animals were anaesthetized with thiopental sodium (45 mg/kg, i.p.) and positioned in a stereotaxic apparatus. The head was positioned in a frame and a midline saggital incision was made in the scalp. Two holes were drilled in the skull for the placement of the injection cannula on both sides over the lateral cerebral ventricle using the following coordinates as described by Paxinos and Watson:[23] 0.8 mm posterior to bregma, 1.5 mm lateral to saggital suture, 3.6 mm beneath the cortical surface of brain. The scalp was then closed with a suture and dental cement. After surgery, all animals received gentamicin (5 mg/kg, i.p.) to prevent sepsis. Animals were given bilateral intracerebroventricular (i.c.v.) injections of streptozotocin (3 mg/kg) in two divided doses (Day 1 and Day 3) using a Hamilton microsyringe through the cannula. Streptozotocin was prepared in artificial cerebrospinal fluid and a solution of 25 mg/ml was made. This solution was freshly made just before injection. Each rat was given 10-μl injections at each site. The injection of streptozotocin (3 mg/kg) was repeated on Day 3. In the artificial cerebrospinal fluid (aCSF) group, aCSF (in mmol/l: 147 NaCl, 2.9 KCl, 1.6 $MgCl_2$, 1.7 $CaCl_2$ and 2.2 dextrose) was injected (10 μl at each site) on the same days as the streptozotocin group. To promote diffusion, the microsyringe was left in place for a period of 1 min following injection. Special care of the animals was taken during the postoperative period.

Drugs and treatment schedule

Streptozotocin (Sigma Chemicals Co., St Louis, MO, USA) and carvedilol (Zydus Medica, Ahmedabad, India) solutions were freshly made at the beginning of each experiment. Carvedilol was dissolved in distilled water and administered intraperitoneally in a constant volume of 0.5 ml/100 g bodyweight. Streptozotocin (3 mg/kg) was prepared in aCSF and delivered in two doses (10 μl injection volume) by intracerbroventricular administration on Day 1 and Day 3. Animals were randomly divided based on bodyweight into seven groups of eight animals each. Group 1 served as the vehicle control group: animals received a cut in the mid-brain region and were administered vehicle for carvedilol (distilled water) intraperitoneally. Animals in group 2 were given aCSF (10 μl, i.c.v.) and administered vehicle for carvedilol. Animals in group 3 were given streptozotocin (3 mg/kg, 10 μl, i.c.v.) and administered vehicle for carvedilol. Group 4 and 5 animals received 1 and 2 mg/kg carvedilol only, respectively. Animal in groups 6 and 7 were given streptozotocin (3 mg/kg, 10 μl, i.c.v.) together with 1 and 2 mg/kg carvedilol, respectively, daily for 21 days. Figure 1 outlines the protocol design.

The doses of carvedilol were selected based on previous studies in our laboratory and reports in the literature.[13]

Behavioural assessment

Assessment of cognitive performance: spatial navigation task

The acquisition and retention of a spatial navigation task was evaluated by using the Morris water maze.[24] Animals were trained to swim to a visible platform in a circular pool (180 cm in diameter and 60 cm in height) situated in a brightly light room with four fluorescent overhead lights providing consistent illumination. In principle, rats can stop swimming by climbing onto the escape platform and over time the rats apparently learn the spatial location of the platform from any starting position on the circumference of the pool. Thus, the platform offers no local cues to guide the escape behaviour of the rats. The only spatial cues are those outside of the tank, primarily the visual cues. The pool was filled with pure drinking water to a height of 40 cm and made opaque with non-toxic water colour (28 ± 2°C). A movable circular platform (9 cm diameter), mounted on a column, was placed in the pool 2 cm above the water level during the acquisition phase. A similar platform was placed in the pool 2 cm below the water level for the maze retention phase. During both the phases, the platform was placed in the centre

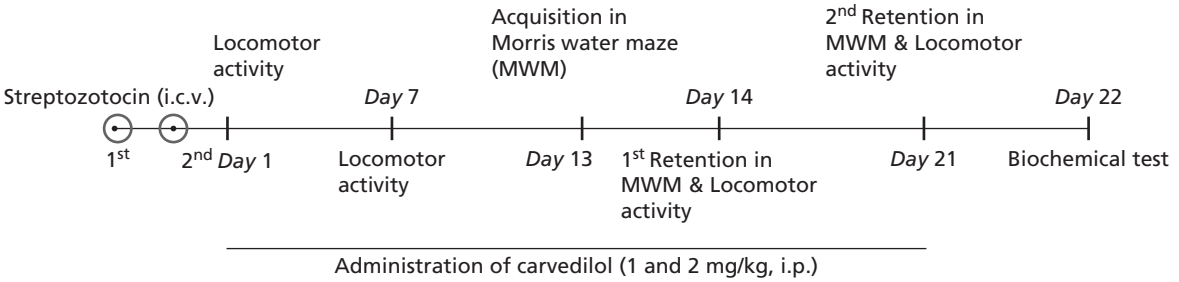

Figure 1 Protocol design

of one of the quadrants. Four equally spaced locations around the edge of the pool were used as starting points and this divided the pool into four equal quadrants.

In the maze acquisition phase (training), rats received a training session comprising four trials on Day 13. In all four trials, the starting position was different. A trial began by releasing a rat into the maze facing towards the wall of the pool. The latency to find the escape platform was recorded to a maximum of 90 s. If the rat did not escape onto the platform within this time it was guided to the platform and was allowed to remain there for 20 s. The time taken by each rat to reach the platform was taken as the initial acquisition latency. At the end of the trial, the rat was returned to the home cage and a 5-min gap was allowed between subsequent trials.

In the maze retention phase (testing for retrieval of the learned task), at 24 h (Day 14) and 8 days (Day 21) after the initial acquisition latency, each rat was released randomly at one of the edges facing the wall of the pool and tested for retention of response. The time taken to find the hidden platform on Day 14 and Day 21 following administration of streptozotocin was recorded and termed the first retention latency and second retention latency, respectively.

Assessment of gross behavioural activity

Gross behavioural activity was observed on Days 1, 7, 14 and 21 following intracerebroventricular streptozotocin injection. Each animal was placed in a square (30 cm) closed arena equipped with infrared light sensitive photocells using a digital photoactometer. The animals were observed for a period of 5 min and the values were expressed as counts/5 min. The horizontal locomotor activities were carried out between 1000 and 1200 hours. The apparatus was placed in a darkened, light and sound attenuated, and ventilated test room.[25]

Biochemical assessments

Biochemical tests were conducted 24 h after the last behavioural test. The animals were killed by decapitation under deep ether anaesthesia. Brains were removed and rinsed with ice-cold isotonic saline. Brains were then homogenized with ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenate (10%, w/v) was then centrifuged at 10 000*g* for 15 min and fractions of supernatant were separated and used for the biochemical estimations.

Measurement of lipid peroxidation

The extent of lipid peroxidation in the brain was determined quantitatively according to the method described by Wills.[26] The amount of malondialdehyde (MDA) was measured by reaction with thiobarbituric acid at 532 nm using a Perkin Elmer Lambda 20 spectrophotometer. The values were calculated using the molar extinction coefficient of chromophore (1.56×10^5 $M^{-1}cm^{-1}$).

Estimation of reduced glutathione

Reduced glutathione was estimated as described by Ellman.[27] A 1-ml sample of supernatant was precipitated with 1 ml 4% sulfosalicylic acid and cold digested for 1 h at 4°C. The samples were then centrifuged at 1200*g* for 15 min at 4°C. To 1 ml of the supernatant obtained, 2.7 ml phosphate buffer (0.1 M, pH 8) and 0.2 ml 5,5′dithio-bis (2-nitrobenzoic acid) (DTNB) were added. The yellow colour that developed was measured at 412 nm using a Perkin Elmer Lambda 20 spectrophotometer. Results were calculated using the molar extinction coefficient of the chromophore (1.36×10^4 $M^{-1}cm^{-1}$).

Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide, was determined by a colorimetric assay with Greiss reagent (0.1% *N*-(1-naphthyl) ethylene diamine dihydrochloride, 1% sulfanilamide and 5% phosphoric acid) according to Green *et al*.[28] Equal volumes of the supernatant and the Greiss reagent were mixed and the mixture was incubated for 10 min at room temperature in the dark. The absorbance was measured at 540 nm using a Perkin Elmer Lambda 20 spectrophotometer. The concentration of nitrite in the supernatant was determined from the sodium nitrite standard curve.

Superoxide dismutase activity

Superoxide dismutase activity was assayed by the method of Kono.[29] The assay system consisted of 0.1 mM EDTA, 50 mM sodium carbonate and 96 mM nitro blue tetrazolium. The above mixture (2 ml), hydroxylamine (0.05 ml) and 0.05 ml of the supernatant were added to a cuvette and the autoxidation of hydroxylamine was measured for 2 min at 30-s intervals by measuring the absorbance at 560 nm using a Perkin Elmer Lambda 20 spectrophotometer.

Catalase activity

Catalase activity was assessed by the method of Luck,[30] in which the breakdown of H_2O_2 is measured. Briefly, the assay mixture consisted of 3 ml H_2O_2 phosphate buffer and 0.05 ml of the supernatant of the tissue homogenate. The change in absorbance was recorded for 2 min at 30-s

intervals at 240 nm using a Perkin Elmer Lambda 20 spectrophotometer. The results were expressed as micromoles of hydrogen peroxide decomposed/min per mg protein.

Acetylcholinesterase activity

Acetylcholinesterase is a marker of extensive loss of cholinergic neurons in the forebrain. The acetylcholinesterase activity was assessed by the Ellman method.[31] The assay mixture contained 0.05 ml of supernatant, 3 ml of sodium phosphate buffer (pH 8), 0.1 ml of acetylthiocholine iodide and 0.1 ml of DTNB (Ellman reagent). The change in absorbance was measured for 2 min at 30-s intervals at 412 nm using a Perkin Elmer Lambda 20 spectrophotometer. Results were calculated using the molar extinction coefficient of the chromophore (1.36×10^4 $M^{-1}cm^{-1}$) and expressed as percentage of control.

Protein estimation

The protein content was estimated by the Biuret method[32] using bovine serum albumin as a standard.

Statistical analysis

Values are expressed as mean ± SEM. The behavioural assessment data were analysed by a repeated measures two-way analysis of variance with drug-treated groups as the between-subject and sessions as the within-subject factors. The interaction drug treatment session was considered to test for drug effect on retention. The biochemical estimations were separately analysed by one-way analysis of variance. Post hoc comparisons between groups were made using Tukey's test and $P < 0.05$ was considered significant.

Results

Effect of carvedilol on the spatial navigation task

Animals in the vehicle control, aCSF and carvedilol only groups quickly learned to swim directly to the platform in the Morris water maze on Day 13. Animals in the streptozotocin plus vehicle group showed an initial delay in escape latency, which improved with continued training during the acquisition phase of the spatial navigation task on Day 13. Carvedilol only groups performed similarly during the acquisition phase of the spatial navigation task on Day 13 as compared with the vehicle control group. There was a significant difference in the mean initial acquisition latency of the streptozotocin plus vehicle group compared with the aCSF treated group on Day 13, indicating that intracerebroventricular streptozotocin impaired acquisition of the spatial navigation task ($P < 0.05$). In contrast, carvedilol treatment (1 and 2 mg/kg, i.p.) significantly decreased the initial acquisition latency to reach the platform in the pre-trained rats as compared with the streptozotocin plus vehicle group on Day 13 after streptozotocin administration (Table 1).

After training, the mean retention latencies (first and second) to escape onto the hidden platform was significantly decreased in the vehicle control and aCSF treated rats on Days 14 and 21, respectively, as compared with the initial acquisition latency on Day 13 after streptozotocin administration. On the other hand, the performance in the streptozotocin plus vehicle group was changed after initial training in the water maze on Days 14 and 21, with a significant increase in mean retention latencies compared with the initial acquisition latency on Day 13. The results suggest that streptozotocin caused significant cognitive impairment. However, chronic carvedilol treatment (1 and 2 mg/kg, i.p.) showed a significant decline in the first and second retention latencies as compared with the streptozotocin plus vehicle group on Days 14 and 21, respectively, after streptozotocin administration (Table 1) and improved the retention performance of the spatial navigation task.

Effect of carvedilol on locomotor activity

In this series of experiments, the mean scores of locomotor activity for each rat were relatively stable and showed no significant variation (F value 42.92). The mean scores in the vehicle control, aCSF and streptozotocin plus vehicle groups remained unchanged. Chronic administration of carvedilol only (1 and 2 mg/kg, i.p.) had no effect on the locomotor activity as compared with aCSF treated rats on Days 14 and 21. Carvedilol treatment (1 and 2 mg/kg, i.p.) in intracerebroventricular streptozotocin treated rats did not cause any alteration in the locomotor activity as compared with streptozotocin plus vehicle rats on Days 14 and 21 (Table 2).

Table 1 Initial acquisition latency and retention latencies after intracerebroventricular streptozotocin injection in the Morris water maze

Treatment group	Initial acquisition latency	Retention latency	
	Day 13	Day 14 (first)	Day 21 (second)
Vehicle control (carvedilol vehicle)	48 ± 3.464	23 ± 3.215	14 ± 4.163
Artificial cerebrospinal fluid + vehicle	63 ± 2.646	27.667 ± 2.603	15.333 ± 2.963
Streptozotocin (3 mg/kg) + vehicle	75 ± 4.041[a]	79 ± 3.999[a]	80 ± 5.099[a]
Carvedilol (1 mg/kg)	54.5 ± 2.9	38.0 ± 1.8	22.8 ± 2.5
Carvedilol (2 mg/kg)	50.5 ± 2.4	32.1 ± 1.4	20.5 ± 2.2
Streptozotocin (3 mg/kg) + carvedilol (1 mg/kg)	72.333 ± 4.333[b]	45.333 ± 4.807[b]	37.333 ± 5.457[b]
Streptozotocin (3 mg/kg) + carvedilol (2 mg/kg)	69.667 ± 4.256[b]	42.333 ± 7.219[b]	26 ± 5.292[b]

Carvedilol was dissolved in distilled water (vehicle) and administered intraperitoneally. Values are expressed as mean ± SEM. [a]$P < 0.05$ significantly different compared with the artificial cerebrospinal fluid treated group; [b]$P < 0.05$ significantly different compared with the streptozotocin plus vehicle group (repeated measures two-way analysis of variance followed by Tukey's test for multiple comparisons).

Table 2 Effect of carvedilol on locomotor activity in intracerebroventricular streptozotocin treated rats

Treatment group	Day 1	Day 7	Day 14	Day 21
Vehicle control	304 ± 7.81	263.66 ± 4.05	233.66 ± 6.38	208.33 ± 6.38
Artificial cerebrospinal fluid + vehicle	294 ± 6.65	273.33 ± 5.23	247.33 ± 4.66	199.66 ± 5.60
Streptozotocin (3 mg/kg) + vehicle	314.33 ± 3.93	277.66 ± 4.41	238.33 ± 3.75	214.33 ± 4.91
Streptozotocin (3 mg/kg) + carvedilol (1 mg/kg)	254.66 ± 4.05	223.66 ± 4.25	195.33 ± 6.36	188.66 ± 7.31
Streptozotocin (3 mg/kg) + carvedilol (2 mg/kg)	265 ± 4.01	227 ± 4.04	207.33 ± 3.71	199.33 ± 7.21

Carvedilol was dissolved in distilled water (vehicle) and administered intraperitoneally. Values are expressed as mean ± SEM. Data was analysed by two-way analysis of variance.

Effect of carvedilol on brain lipid peroxidation, nitrite and reduced glutathione levels, superoxide dismutase and catalase activity

Intracerebroventricular administration of aCSF had no effect on brain MDA, nitrite levels, reduced glutathione, superoxide dismutase and catalase levels as compared with vehicle control rats. Intracerebroventricular streptozotocin plus vehicle administration caused a marked increase in free radical generation and a significant rise in brain MDA, nitrite levels, depletion of reduced glutathione, superoxide dismutase and catalase levels as compared with aCSF treated rats. Further, there were no alterations in brain MDA levels, nitrite levels, reduced glutathione, superoxide and catalase levels as a result of carvedilol only (1 and 2 mg/kg, i.p.) treatment as compared with aCSF treated rats. However, chronic carvedilol administration (1 and 2 mg/kg, i.p.) significantly prevented the increase in MDA, nitrite levels and depletion of reduced glutathione (Table 3). It also caused a significant increase in the levels of superoxide dismutase and catalase (Table 3).

Effect of carvedilol on brain acetylcholinesterase levels

Intracerebroventricular administration of aCSF had no effect on brain acetylcholinesterase levels as compared with vehicle control rats. In contrast, the intracerebroventricular streptozotocin plus vehicle group showed a significant increase in brain acetylcholinesterase activity as compared with aCSF treated rats. Chronic administration of carvedilol (1 and 2 mg/kg, i.p.) significantly ameliorated the increase in acetylcholinesterase activity compared with the streptozotocin plus vehicle group (Figure 2).

Discussion

This study demonstrated that chronic treatment with carvedilol caused a significant improvement in the memory performance tasks and marked attenuation of oxidative stress as indicated by a decrease in the lipid peroxidation and nitrite concentration, and restoration of the reduced glutathione levels, catalase and superoxide dismutase. Intracerebroventricular streptozotocin administration increased acetylcholinesterase activity, which was ameliorated by carvedilol chronic treatment. These findings suggest that the central administration of streptozotocin causes deterioration of cognition, cerebral glucose and energy metabolism as well as the presence of oxidative stress.[20,21] In the present study, the Morris water maze was used as an exterioceptive model for evaluation of spatial learning and memory. Extensive pre-training is not required as the platform is placed 1 cm above the water. After acquisition, animals searched for the hidden platform 1 cm below the surface of the water in the retrieval period with the help of external clues. Water provides a uniform environment and eliminates the interference due to olfactory clues. In our study of intracerebroventricular streptozotocin treated rats, there was a significant impairment of learning and memory as indicated by no marked change in latency time and no improvement in retention latency time. These results are in agreement with other studies on the impairment of learning and memory by intracerebroventricular administration of streptozotocin to rats.[33,34]

Chronic administration of carvedilol was found to improve not only the memory retention but also reduced oxidative damage induced by intracerebroventricular streptozotocin administration. Carvedilol has been reported to scavenge free radicals and inhibit lipid peroxidation in swine ventricular membranes[35] and rat brain homogenates.[36] It has also been shown to inhibit superoxide ion release from activated neutrophils.[37] Carvedilol has also been shown to preserve the endogenous antioxidant system, that is vitamin E and reduced glutathione, which are normally consumed when tissues or cells are exposed to oxidative stress.[38,39] This may explain the fact that carvedilol treatment was able to restore the levels of reduced glutathione in the intracerebroventricular streptozotocin treated rats.

Our results show that nitric oxide concentrations are increased in intracerebroventricular streptozotocin treated rats. It is well established that nitric oxide acts as a precursor for the peroxynitrite free radical.[40,41] Overproduction of nitric oxide causes neurotoxicity to cholinergic neurons.[42,43] This explains that centrally administered streptozotocin caused a significant increase in nitrite concentrations in the brain and carvedilol treatment was able to decrease the nitrite levels. It has been reported in the literature that carvedilol acts as a nitric oxide quenching agent in vascular endothelial cells and cell-free systems.[44] Carvedilol is approximately 10-times more potent than vitamin E as an antioxidant and, being lipophilic in nature, it easily crosses the blood–brain barrier. As well as the parent compound, even its metabolites are powerful antioxidants and may be responsible for the neuroprotective effect of carvedilol.[9]

Activated microglia and astrocytes can release cytokines, reactive oxygen species and nitric oxide, which may

Table 3 Effect of carvedilol on intracerebroventricular streptozotocin induced oxidative stress parameters in rat brain

Treatment group	MDA levels (nmol MDA/mg protein) (% of control)	Nitrite levels (μmol/mg protein) (% of control)	Reduced glutathione (nmol/mg protein) (% of control)	Catalase (μmol of hydrogen peroxide decomposed/min per mg of protein) (% of control)	Superoxide dismutase (units/mg protein) (% of control)
Vehicle control	79.667 ± 0.882 (100)	200 ± 5.774 (100)	0.68 ± 0.0208 (100)	6.706 ± 0.01 (100)	0.673 ± 0.517 (100)
Artificial cerebrospinal fluid + vehicle	84.93 ± 7.24 (106.6)	228.333 ± 9.3 (114.16)	0.6 ± 0.0232 (88.23)	6.244 ± 0.01 (93.11)	0.827 ± 0.335 (122.88)
Streptozotocin (3 mg/kg) + vehicle	423.93 ± 6.54 (532.12)[a]	815 ± 5.951 (407.5)[a]	0.198 ± 0.0852 (29.11)[a]	2.436 ± 0.05 (36.32)[a]	0.119 ± 0.011 (17.68)[a]
Carvedilol (1 mg/kg)	95.22 ± 2.75 (119.52)	225.5 ± 3.681 (112.75)	0.58 ± 0.0257 (85.29)	5.785 ± 0.02 (86.26)	0.598 ± 0.425 (88.85)
Carvedilol (2 mg/kg)	82.64 ± 1.82 (103.73)	198.32 ± 4.6 (99.16)	0.62 ± 0.0225 (91.17)	6.321 ± 0.01 (94.25)	0.621 ± 0.487 (92.27)
Streptozotocin (3 mg/kg) + carvedilol (1 mg/kg)	198.02 ± 7.357 (248.55)[b]	416.667 ± 9.42 (208.33)[b]	0.354 ± 0.084 (52.05)[b]	5.082 ± 0.03 (75.78)[b]	0.339 ± 0.0629 (50.37)[b]
Streptozotocin (3 mg/kg) + carvedilol (2 mg/kg)	125.301 ± 5.32 (157.28)[b,c]	340 ± 8.65 (170)[b,c]	0.441 ± 0.141 (64.85)[b,c]	5.172 ± 0.05 (77.124)[b,c]	0.513 ± 0.059 (76.22)[b,c]

Carvedilol was dissolved in distilled water (vehicle) and administered intraperitoneally. Values are expressed in mean ± SEM. MDA, malondialdehyde. [a]$P < 0.05$ significantly different compared with the artificial cerebrospinal fluid treated group; [b]$P < 0.05$ significantly different compared with the streptozotocin plus vehicle treated group; [c]$P < 0.05$ significantly different compared with the streptozotocin + carvedilol (1 mg/kg) group (repeated measures one-way analysis of variance followed by Tukey's test for multiple comparisons).

Figure 2 Effect of carvedilol on acetylcholinesterase activity in intracerebroventricular streptozotocin treated rats. Carvedilol was dissolved in distilled water (vehicle) and administered intraperitoneally. Values are mean ± SEM, $n = 8$ in each group. aCSF, artificial cerebrospinal fluid. [a]$P < 0.05$ significantly different compared with the aCSF treated group; [b]$P < 0.05$ significantly different compared with the streptozotocin plus vehicle treated group (repeated measures one-way analysis of variance followed by Turkey's test for multiple comparisons).

contribute to memory deficits.[45] It has been found that microglia activation occurs in a discrete area of the brain in intracerebroventricular streptozotocin treated rats. Streptozotocin also increased the number of activated astrocytes in the CA_1 and other regions of the hippocampus. Carvedilol has been shown to act as an anti-inflammatory agent by suppressing the mRNA expression of inflammatory cytokines and interleukin 1β. It has also been reported that it may inhibit the activator signal transduction pathway of nuclear factor κB.[46] All these effects may contribute to the neuroprotective effect of carvedilol by suppressing activated microglia and astrocytes. The brain inflammatory response to injury is mediated in part by the up-regulation of proinflammatory cytokines, tumour necrosis factor α and interleukin 1β.[47] Another related mechanism to explain the neuroprotective effects of carvedilol is the down-regulation of inflammatory cytokine gene expression as an anti-adrenergic agent. Immune cells express various adrenergic receptors sensitive to transmitters of the synaptic nervous system. β-Adrenergic stimulation prevents the release of interleukin 1β and tumour necrosis factor α from activated macrophages and microglia. Based on these observations, it seems that carvedilol attenuated neuroinflammatory damage and related inflammatory cascade. However, prolonged exposure to β-blockers supersensitizes the β-adrenoceptor, enabling lower concentrations of norepinephrine and epinephrine to suppress cytokine release. The cholinergic system is responsible for storage and retrieval of memory and is by far the most affected in Alzheimer's disease. Central administration of streptozotocin resulted in a decrease in the activity of glycolytic enzymes, leading to reduced formation of acetylCoA and acetylcholine,[48,49] thereby causing a cholinergic deficit. In the present study, streptozotocin caused a significant increase in the acetylcholinesterase activity, which is responsible for the degradation of acetylcholine. Chronic administration of carvedilol (1 and 2 mg/kg, i.p.) caused a marked depletion of acetylcholinesterase activity. It

is also plausible that increased free radical formation causes macromolecular changes in cholinergic neurons and leads to the increase in acetylcholinesterase activity that contributes to learning and memory deficits. The antioxidant action of carvedilol has been attributed to its radical scavenging and iron chelating property, however the mechanistic dynamics that underlie these effects are not yet known. Our results confirm that carvedilol has a protective effect against intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats and it represents a valid rationale for the use of carvedilol in the prevention of sporadic dementia such as occurs in Alzheimer's disease.

Conclusions

The present study suggests that enhancement of acetylcholinesterase and oxidative damage are the causes of memory impairment in intracerebroventricular streptozotocin induced rats. Carvedilol exerts beneficial effects on memory processing that may be attributed to its effect of lowering acetylcholinesterase levels and its antioxidative action. The study demonstrates the beneficial effect of carvedilol in intracerebroventricular streptozotocin induced cognitive deficits, however further investigation is required to elucidate the neurochemical and molecular mechanisms involved in the neuroprotective effect of carvedilol.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

Financial support from the Indian Council of Medical Research, New Delhi, India, is gratefully acknowledged.

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