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Prolonged pretreatment with carvedilol prevents 3-nitropropionic acid-induced behavioral alterations and oxidative stress in rats

Puneet Kumar, Anil Kumar

Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, India

Correspondence: Anil Kumar, e-mail: kumaruips@yahoo.com

Abstract:
3-Nitropropionic acid (3-NP)-induced neurotoxicity causes a cellular energy deficit and oxidative stress *via* an irreversible inhibition of the mitochondrial enzyme succinate dehydrogenase (SDH). Systemic administration of 3-NP causes motor and cognitive deficits, particularly those associated with excessive free radical generation. Recently, carvedilol has been implicated as a neuroprotectant in the treatment of various neurological disorders. The present study was designed to investigate the neuroprotective effects of carvedilol against 3-NP-induced cognitive impairment and oxidative damage in rats. Intraperitoneal administration of 3-NP (20 mg/kg for 4 days) caused significant body weight reduction, impaired motor function (locomotor activity, movement pattern), induced vacuous chewing movements, led to poor retention of memory in the Morris water maze, and elevated plus maze task paradigms. Chronic treatment with carvedilol (1 and 2 mg/kg, *po*), once daily for a period of 8 days beginning 4 days before 3-NP administration, significantly reversed 3-NP-induced motor impairment and cognitive deficits. However, carvedilol (1 and 2 mg/kg, *po*) treatment significantly attenuated oxidative damage (reduced lipid peroxidation and nitrite levels, and restored depleted reduced glutathione and succinate dehydrogenase enzyme activity) in the rat brain. The results of the present study suggest that carvedilol has a neuroprotective effect against 3-NP-induced behavioral alterations and oxidative damage.

Key words:
carvedilol, motor activity, 3-nitropropionic acid, oxidative stress

Abbreviations: GSH – reduced glutathione, HD – Huntington's disease, 3-NP – 3-nitropropionic acid, MDA – malondialdehyde

Introduction

A plant and fungal toxin, 3-nitropropionic acid (3-NP), has been implicated in causing food poisoning in China following the ingestion of moldy sugarcane [21, 30]. Intoxication with 3-NP causes neurotoxicity in both animals and humans [19, 22, 23, 45], particularly affecting the striatum and cerebellum [17]. The underlying mechanism of 3-NP-induced neurotoxicity involves the irreversible inhibition of the mitochondrial enzyme succinate dehydrogenase (SDH), which is directly linked to the electron transport chain. Inhibition of SDH interferes with the electron cascade and interrupts oxidative phosphorylation. This leads to mitochondrial dysfunction and a cellular energy deficit that leads to decreased ATP production [24] and marked oxidative stress, as indicated by the decrease in antioxidant enzyme activities [4].

3-NP-induced neurotoxicity has been demonstrated to produce Huntington's disease (HD)-like symptoms in animals [6, 20, 22]. HD is a debilitating neurological disorder, characterized by progressive dementia, involuntary abnormal choreiform movements, psychological changes and cognitive impairment [29]. Recent evidence suggests that HD may be associated with impaired energy metabolism [6, 20]. Oxidative stress in HD patients, due to defects in energy metabolism, leads to excitotoxicity and increased free radical production [6, 20, 41].

Carvedilol [1-[carbazolyl-(4)-oxy]-3-[(2-methoxyphenoxyethyl)amino]-2-propanol], a multi-action drug indicated for hypertension and congestive heart failure, is an α- and β-adrenoceptor antagonist with strong antioxidant properties [26]. Carvedilol and some of its metabolites are potent antioxidants. It has also been suggested to behave as a neuroprotective agent, as it exerted antioxidant properties in an *in vitro* model of free radical-mediated neuronal injury. Its role in neuroprotection was also shown in an *in vivo* global ischemic model in which pretreatment with carvedilol limited injury in the CA1 hippocampal zone. In addition, carvedilol scavenged oxygen free radicals and inhibited lipid peroxidation in swine heart [25]. The antioxidant activity of carvedilol has been attributed to its carbazole moiety [36], and it is approximately 10-fold more potent as an antioxidant than vitamin E [26].

Thus, the present study was designed to investigate the neuroprotective effects of carvedilol against 3-NP-induced behavioral alterations and oxidative damage in rats.

Materials and Methods

Animals

Male Wistar rats weighing between 200–250 g were used. Animals were purchased from the Central Animal House, Panjab University, Chandigarh, India. Animals were acclimatized to laboratory conditions prior to experimentation. The animals were kept under standard conditions of light and dark cycles with food and water *ad libitum* in groups of 2 animals in plastic cages with soft bedding. All the experiments were carried out between 09:00 and 15:00 h. The protocol was approved by the Institutional Animal Ethics Committee and carried out in accordance with the Indian National Science Academy Guidelines for the use and care of animals.

Drugs and treatment schedule

3-NP (Loba Chem, India) was diluted with saline and adjusted to pH 7.4. Carvedilol (Sigma Chemicals Co., St. Louis, MO, USA) was suspended in a 0.05% sodium carboxymethylcellulose (CMC) solution and administered through canula by an oral route in a constant volume of 0.5 ml per 100 g of body weight. 3-NP was administered intraperitoneally (*ip*) at a dose of 20 mg/kg, once daily for four days. Carvedilol (1 mg/kg and 2 mg/kg) was administered orally (*po*) for 8 days beginning 4 days prior to the initiation of 3-NP treatment. Animals were randomly divided into six groups with 8 animals in each group. The first group, the vehicle-treated control group, received the vehicle for carvedilol (*po*) and normal saline (*ip*). The second group received 3-NP (20 mg/kg, *ip*) for four consecutive days. The third and fourth groups received carvedilol *per se* 1 mg/kg and 2 mg/kg once daily, respectively, for a period of 8 days. The fifth and six groups received carvedilol (1 mg/kg and 2 mg/kg) once daily for a period of 8 days and 3-NP (20 mg/kg, *ip*) administration was started four days after the initiation of carvedilol treatment, and it was given for four consecutive days. In the fifth and sixth groups, the 3-NP was given daily at 10:00 a.m. and carvedilol was given 1 h prior to 3-NP administration. Doses were selected on the basis of previous studies conducted in the laboratory and those reported in literature [38]. Each test was done on individual animals on a random basis. Three observers recorded the data on an individual basis. Observer was not aware about the conditions and treatment to the animals.

Measurement of body weight

Animal body weight was noted on the first and last days of the experiment. The change in the body weight during the experimental period was calculated as

(1st day body weight – 5th day body weight /1st day body weight) × 100.

Behavioral assessment

Assessment of motor activity

Assessment of gross behavioral activity (locomotor activity)

Gross behavioral activity was assessed on the 1st day (before 3-NP), 2nd day (24 h after the 1st dose of 3-NP) and 5th day (24 h after the last dose of 3-NP). Each animal was observed for a period of 5 min in a square (30 cm^2) closed arena equipped with infrared light-sensitive photocells using a digital photoactometer, and values were expressed as counts per 5 min. A 2 min adaptation period was given to each animal in the apparatus before starting the observation. The apparatus was placed in a ventilated, darkened, and light- and sound-attenuated testing room.

Orofacial movements

On the 5th day (24 h after the last dose of 3-NP), rats were placed individually in a small (30 × 20 × 30 cm) plexiglass cage for assessing vacuous chewing movements (VCMs) as described previously [22, 33]. The floor and the back wall of the cage consisted of mirrors to aid observation when the animal was facing away from the observer. VCMs were measured continuously for a period of 5 min.

Assessment of memory dysfunction

Elevated plus maze paradigm

The elevated plus maze consisted of two opposite open arms (50 × 10 cm) crossed with two closed arms of the same dimensions, and all with 40 cm high walls. The arms were connected with a central square (10 × 10 cm). The entire maze was elevated at a height of 50 cm. Acquisition of memory was assessed on the day 1 before initiating 3-NP treatment. Rats were placed individually at one end of an open arm facing away from the central square. The time taken by the animal to move from the open arm into the closed arms was recorded as the initial transfer latency. The animal was allowed to explore the maze for 30 s after recording the initial transfer latency and then returned to its home cage. If the animal did not enter an enclosed arm within 90 s, it was gently pushed in to the enclosed arm and the transfer latency was assigned as 90 s. Retention of memory was assessed by similarly placing a rat on an open arm, and noting the retention latency 24 h (day 2) and 4 days (day 5) after the initial transfer latency (ITL). These times are referred to as the first retention transfer latency and second retention transfer latency, respectively [46].

Morris water maze (spatial navigation task)

The acquisition and retention of the spatial navigation task was examined using the Morris water maze [12, 22]. Animals were trained to swim to a platform in a circular pool (180 cm diameter × 60 cm) located in a test room. The pool was filled with water (24 ± 2°C) to a depth of 40 cm. A movable circular platform, 9 cm in diameter and mounted on a column, was placed in the pool 1 cm above the water level (visible platform) for the maze acquisition test. Another movable platform, 9 cm in diameter and mounted on a column, was placed in the pool 1 cm below the water level (hidden platform) for the maze retention test.

Maze acquisition test (training): animals received a training session consisting of 4 trials in a day for four days before 3-NP administration. In all 4 days, the starting positions were different. The latency to find the escape platform was recorded up to a maximum of 2 min. The visible platform was fixed in the center of one of the 4 quadrants and remained there throughout the experiment. The time taken by a rat to reach the platform on the fourth day was recorded as the initial acquisition latency.

Maze retention test (testing for retention of the learned task): following 24 h (day 2) and 4 days (day 5) after the initial acquisition latency (IAL), a rat was randomly released at any one of the edges facing the wall of the pool and tested for the retention of the response. The time taken to reach the hidden platform on days 2 and 5 following initiation of 3-NP treatment was recorded and termed as the first retention latency and second retention latency, respectively.

Biochemical tests

Biochemical tests were carried out after the last behavioral test (on day 5 following 3-NP administration).

Tissue preparation

Animals were sacrificed by decapitation and the brains were removed and rinsed with ice-cold isotonic

saline. Brain tissue samples were then homogenized (10 times (w/v)) with ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 × g for 15 min, and aliquots of supernatant were separated and used for biochemical experiments.

Succinate dehydrogenase activity

Succinate dehydrogenase is a marker of impaired mitochondrial metabolism in brain. The quantitative measurement of brain succinate dehydrogenase levels was performed according to the method of Kumar et al. [20, 22]. A 0.3 ml volume of a sodium succinate solution was mixed with 50 µl of the homogenate. The mixture was incubated at 37°C for 10–20 min, and then 0.1 ml of a *p*-iodonitrotetrazolium violet solution was added and incubated for an additional 10 min. The reaction was stopped by adding 1 ml of a mixture of ethyl acetate:ethanol:tricholoroacetic acid 5:5:1 (v/v/w) and centrifuged at 15,000 rpm for 1 min, and the absorbance was determined at 490 nm using a Perkin Elmer Lambda 20 spectrophotometer. Results were calculated using the molar extinction coefficient of chromophore (1.36×10^4 $M^{-1}cm^{-1}$) and expressed as a percentage of the control.

Measurement of lipid peroxidation

The quantitative measurement of lipid peroxidation in the brain was performed according to the method of Wills [48]. The amount of malondialdehyde (MDA), a measure of lipid peroxidation, was measured by a 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 $M^{-1}cm^{-1}$) and expressed as a percentage of the control.

Estimation of reduced glutathione (GSH)

Reduced glutathione in the brain was estimated according to the method described by Ellman [8]. One milliliter of supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4°C for 1 h. The samples were centrifuged at 1,200 × g for 15 min at 4°C. One milliliter of this supernatant was added to 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of 5,5'dithio-bis(2-nitrobenzoic acid). A yellow color developed that was read immediately at 412 nm using a Perkin Elmer Lambda 20 spectrophotometer. Results were calculated using the molar extinction coefficient of chromophore (1.36×10^4 $M^{-1}cm^{-1}$) and expressed as a percentage of control.

Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide, was determined using a colorimetric assay with the Griess reagent (0.1% N-(1-naphthyl)ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green et al. [15]. Equal volumes of supernatant and the Griess reagent were mixed. Then, the mixture was incubated for 10 min at room temperature in the dark, and the absorbance was measured at 540 nm using a Perkin Elmer Lambda 20 spectrophotometer. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as a percentage of control.

Protein estimation

The protein content was measured by the biuret method using bovine serum albumin as standard [14].

Statistical analysis

Each specific group of rats was assigned to one specific drug treatment condition and each group comprised eight rats (n = 8). All the values are expressed as mean ± SEM. The data were analyzed using analysis of variance (ANOVA) followed by Tukey's test. In all tests, the criterion for statistical significance was $p < 0.05$.

Results

Effect of carvedilol on body weight in 3-NP-treated rats

There was no change in the initial and final body weight of vehicle-treated animals. However, 3-NP treatment caused a significant decrease in body weight (20.41 ± 4% changes in body weight) on day 5 as compared to the vehicle-treated group. Carvedilol *per se* (1 and 2 mg/kg, *po*) treatment had no effect on

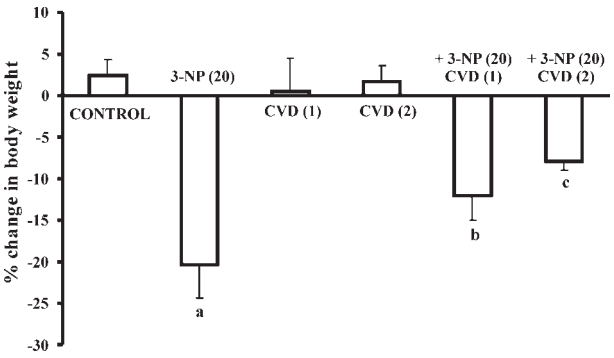

Fig. 1. Effect of carvedilol (CVD) (1 and 2 mg/kg, *po*) on % change in body weight in 3-nitropropionic acid (3-NP)-treated rats. Values are the mean ± SEM. [a] $p < 0.05$ as compared to the vehicle-treated control group; [b] $p < 0.05$ as compared to the 3-NP-injected group; [c] $p < 0.05$ as compared to the 3-NP- and 1 mg/kg carvedilol-treated groups. (One-way ANOVA followed by Tukey's test). n = 8 in each group

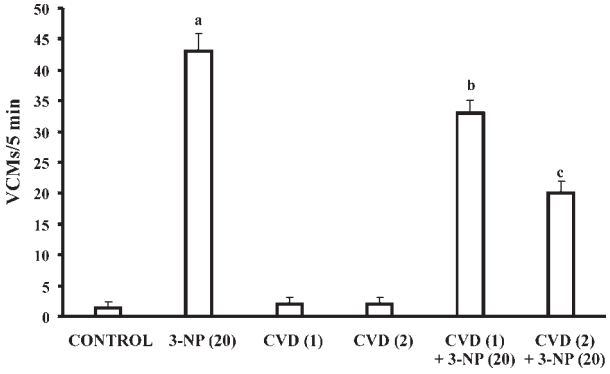

Fig. 3. Effect of carvedilol (CVD) (1 and 2 mg/kg, *po*) on 3-nitropropionic acid (3-NP)-induced vacuous chewing movement (VCMs) in rats. Values are the mean ± SEM. [a] $p < 0.05$ as compared to the vehicle-treated control group; [b] $p < 0.05$ as compared to the 3-NP-injected group; [c] $p < 0.05$ as compared to 3-NP- and 1 mg/kg carvedilol-treated groups. (One-way ANOVA followed by Tukey's test). n = 8 in each group

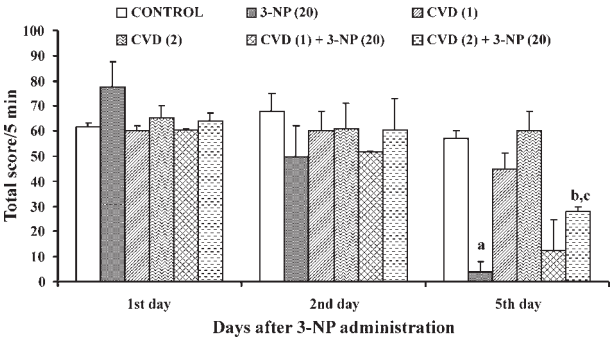

Fig. 2. Effect of carvedilol (CVD) (1 and 2 mg/kg, *po*) on locomotor activity in 3-nitropropionic acid (3-NP)-treated rats. Values are the mean ± SEM. [a] $p < 0.05$ as compared to the vehicle-treated control group; [b] $p < 0.05$ as compared to the 3-NP-injected group; [c] $p < 0.05$ as compared to 3-NP- and 1 mg/kg carvedilol-treated groups. (One-way ANOVA followed by Tukey's test). n = 8 in each group

body weight. By contrast, carvedilol (1 and 2 mg/kg, *po*) treatment significantly increased the body weight in 3-NP-treated rats (Fig. 1).

Effect of carvedilol on gross behavioral activity in 3-NP-treated rats

The mean scores of the gross behavioral activity on day 1 for each rat were relatively stable and showed no significant variation. The mean scores of the gross behavioral activity in the vehicle control rats did not change throughout the observation period. 3-NP treatment caused a significant decrease in motor activity as compared to the vehicle control group. Chronic administration of carvedilol (*per se*, 1 and 2 mg/kg, *po*) had no effect on the gross behavioral activity as compared to the vehicle control group on days 2 and 5. However, carvedilol (2 mg/kg, *po*) significantly reversed the motor abnormalities caused by 3-NP treatment in rats (Fig. 2, 3).

Effect of carvedilol on 3-NP-induced orofacial movements

Vehicle-treated control rats did not show any vacuous chewing movements throughout the observation period. However, 3-NP treatment significantly increased the VCMs in rats as compared to the vehicle control group. Similar to the vehicle control group, chronic administration of carvedilol (*per se*; 1 and 2 mg/kg, *po*) did not result in VCMs. Conversely, carvedilol treatment (1 and 2 mg/kg, *po*) significantly suppressed 3-NP-induced VCMs in rats (Fig. 3).

Effect of carvedilol on memory performance in elevated plus maze paradigm in 3-NP-treated rats

In this study, the mean ITL on day 1 before 3-NP treatment for each rat was relatively stable and showed no significant variation. All the rats entered the closed arm within 90 s. Following training, the vehicle-treated control and carvedilol-treated (*per se*; 1 and 2 mg/kg, *po*) rats entered the closed arm quickly, and the mean retention transfer latencies (1st

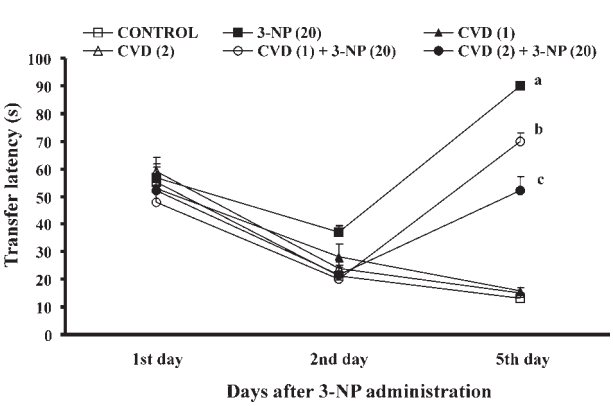

Fig. 4. Effect of carvedilol (CVD) (1 and 2 mg/kg, *po*) on memory performance in an elevated plus maze in 3-nitropropionic acid (3-NP)-treated rats. Values are the mean ± SEM. [a] $p < 0.05$ as compared to the vehicle-treated control group; [b] $p < 0.05$ as compared to the 3-NP-injected group; [c] $p < 0.05$ as compared to 3-NP- and 1 mg/kg carvedilol-treated groups. (One-way ANOVA followed by Tukey's test). n = 8 in each group

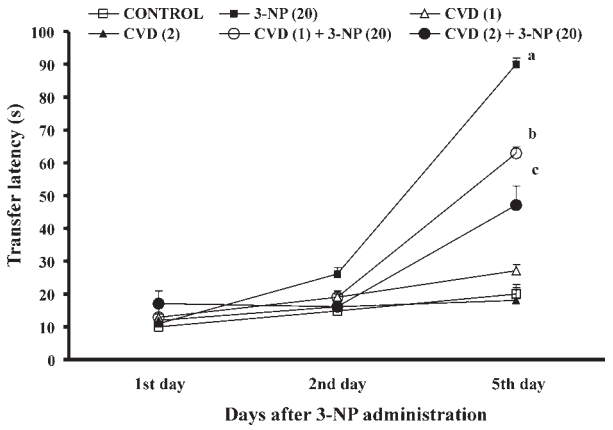

Fig. 5. Effect of carvedilol (CVD) (1 and 2 mg/kg, *po*) on memory performance in a Morris water maze in 3-nitropropionic acid (3-NP)-treated rats. Values are the mean ± SEM. [a] $p < 0.05$ as compared to the vehicle-treated control group; [b] $p < 0.05$ as compared to the 3-NP-injected group; [c] $p < 0.05$ as compared to 3-NP- and 1 mg/kg carvedilol-treated groups. (One-way ANOVA followed by Tukey's test). n = 8 in each group

Tab. 1. 3-Nitropropionic acid (3-NP) and carvedilol treatment-induced biochemical changes in rat brain

Treatment	MDA nmol/mg protein (% of control)	GSH nmol/mg protein (% of control)	Nitrite level µmol/mg protein (% of control)	SDH nmol/mg protein (% of control)
Vehicle	100 ± 3.0	100 ± 1.0	100 ± 7.5	100 ± 3.0
3-NP (20)	201 ± 6.1[a]	35 ± 4.0[a]	211 ± 6.7[a]	46 ± 3.0[a]
Carvedilol (1)	92 ± 4.0	110 ± 4.7	92 ± 8.0	101 ± 3.9
Carvedilol (2)	96 ± 5.0	100 ± 3.9	96 ± 1.0	107 ± 4.9
Carvedilol (1) + 3-NP (20)	162 ± 6[b]	64 ± 6.0[b]	176 ± 11.0[b]	57 ± 5.0[b]
Carvedilol (2) + 3-NP (20)	119 ± 8[b]	88 ± 7.3[b]	130 ± 15.0[b]	87 ± 10.0[b]

Values expressed as % of the vehicle-treated group. [a] $p < 0.05$ *vs.* vehicle-treated group, [b] $p < 0.05$ *vs.* 3-NP-treated group, n = 6–8 animals per group

RTL and 2nd RTL) to enter the closed arm on days 2 and 5 were shorter as compared to the ITL on day 1 of each group. In contrast, the 3-NP-treated rats performed poorly throughout the experiment, and an increase in the mean retention transfer latencies on days 2 and 5 was noted as compared to the pre-training latency on day 1, demonstrating that 3-NP induced cognitive dysfunction. In contrast, chronic administration of carvedilol (1 and 2 mg/kg, *po*) beginning prior to 3-NP treatment significantly decreased the mean retention latencies on day 5 following 3-NP administration ($p < 0.05$ *vs.* 3-NP-treated group), indicating an improvement in memory impairment induced by 3-NP (Fig. 4).

Effect of carvedilol on spatial navigation task in 3-NP-treated rats

All the animals quickly learned to swim directly to the platform in the Morris water maze on day 1 prior to the initiation of 3-NP treatment. In addition, they all showed an initial increase in escape latency, which declined with continued training during the acquisition of a spatial navigation task on day 1.

Following training, the mean retention latencies (1st and 2nd RL) to escape onto the hidden platform were not altered in the vehicle- and carvedilol *per se*-treated rats on days 2 and 5, as compared to the IAL on day 1. By contrast, the 3-NP-treated rats had

significantly higher mean retention latencies compared to the vehicle-treated group after the initial training in the water maze on days 2 and 5, ($p < 0.05$), suggesting that 3-NP caused significant cognitive impairment. However, chronic carvedilol treatment (1 and 2 mg/kg, *po*) starting before 3-NP injection resulted in a significant decline in the 1st and 2nd RL on day 5 ($p < 0.05$ *vs.* 3-NP-treated rats) and improved the retention performance in the spatial navigation task (Fig. 5).

Effect of carvedilol on brain succinate dehydrogenase levels in 3-NP-treated rats

Systemic 3-NP administration resulted in a significant decline in the brain SDH activity as compared to vehicle-treated rats. Carvedilol (1 and 2 mg/kg, *po*) *per se* treatment did not cause any changes in the brain SDH activity as compared to vehicle-treated rats. However, chronic oral administration of carvedilol (1 and 2 mg/kg, *po*) significantly attenuated the reduction in SDH activity compared to the 3-NP-treated group (Tab. 1).

Effect of carvedilol on brain lipid peroxidation and reduced glutathione levels in 3-NP-treated rats

Systemic administration of 3-NP caused a marked increase in free radical generation and lipid peroxidation in addition to a decline in antioxidant defense, as indicated by a significant rise in brain MDA levels and depletion of GSH as compared to the vehicle-treated rats. Further, there were no alterations in the brain MDA and GSH levels due to carvedilol (1 and 2 mg/kg, *po*) *per se* treatment as compared to the vehicle-treated rats. However, chronic carvedilol (1 and 2 mg/kg, *po*) administration significantly reduced the MDA elevation and GSH depletion. A higher dose of carvedilol showed an even more marked effect (Tab. 1).

Effect of carvedilol on brain nitrite levels in 3-NP-treated rats

There was no significant effect of carvedilol (1 and 2 mg/kg, *po*) *per se* treatment on brain nitrite levels as compared to vehicle-treated rats. Systemic 3-NP administration caused a significant increase in brain nitrite levels, which was significantly prevented by the chronic administration of carvedilol (1 and 2 mg/kg, *po*) (Tab. 1).

Discussion

In the present study, 3-NP administration caused both biochemical and behavioral alterations in rats. Pretreatment with carvedilol, a neuroprotective antioxidant, significantly attenuated the 3-NP-induced neurotoxicity in rats.

The effect of 3-NP administration on rodent behavior, physical dexterity, and neuropathology has previously been studied by several groups [5, 20]. 3-NP is unique among toxin models of HD as it can be used to mimic the two-stage (hyperactivity and hypoactivity) progression of HD [5]. 3-NP-induced striatal lesions and motor deficits as demonstrated by bradykinesia and gait abnormalities that closely mimic the signs and symptoms associated with HD [16, 21, 45]. Tsai et al. demonstrated a dose-dependent decrease in glutamine synthetase activity with 3-NP injections and an age-dependent increase in susceptibility toward 3-NP toxicity [43]. The decrease of glutamine synthetase activity coupled with the age-dependent susceptibility suggests an oxidative mechanism is underlying 3-NP toxicity [1, 18]. In addition, deficiencies in behavior and motor control are reminiscent of the loss of motor skills associated with increased brain protein oxidation [11].

Existing evidence indicates that excessive generation of free radicals might contribute to the onset of symptoms in HD and other movement disorders, such as dystonias and Parkinsonism [6, 28]. This effect can be related, at least in part, to a reduction in specific endogenous antioxidant mechanisms, such as a decrease in GSH levels and decreased activity of antioxidant defense enzymes, including SOD and catalase. Considerable evidence supports that the oxidative process significantly contributes to 3-NP toxicity [3, 10, 13, 22]. For example, 3-NP diminishes oxidative phosphorylation by interfering with the mitochondrial respiratory chain, reducing the level of available ATP, and thus causing metabolic inhibition [2, 47]. Furthermore, evidence indicates that the production of free radicals is a key contributing factor in the pathology of HD [6, 28].

In addition, the administration of 3-NP causes significant weight loss and movement abnormalities in rats. The cause of 3-NP-induced body weight changes may be partially due to factors outside the CNS. Intriguingly, treatment with carvedilol significantly increased the body weight in 3-NP-treated rats. 3-NP

treatment induced VCMs in rats, which were significantly attenuated by chronic carvedilol administration. This can be attributed to the antioxidant effect of carvedilol, as numerous reports in literature show that antioxidants are effective in ameliorating neurotoxin-induced VCMs in rodents [20, 32, 33]. 3-NP significantly impaired the memory on 5th day of treatment as observed in the Morris water maze and plus maze paradigms. It is reported that 3-NP produces lesions in hippocampal CA1 and CA3 pyramidal neurons, the area of brain that is associated with cognitive performance. Numerous studies show that antioxidants are effective against neurotoxin-induced memory impairment [21]. Some previous studies also report that β-adrenergic blockade also improves the memory in rats and other animals [39]. A pharmacological blockade of β-adrenoceptors prevents glucocorticoid-induced memory retrieval deficits in human subjects [7]. This supports the findings here that chronic carvedilol treatment was effective in reversing 3-NP-induced motor deficits and cognitive impairment. By contrast, the *per se* treatment of both doses of carvedilol did not effect the memory or locomotor activity in these animals.

Oxidative stress is considered to be one of the major determinants of 3-NP neurotoxicity [31, 41, 44], as demonstrated by increased MDA (a marker of lipid peroxidation) and nitrite levels, and the depletion of reduced glutathione (an endogenous antioxidant) levels [4]. The findings of the present study indicate that carvedilol has protective effects against 3-NP-induced oxidative injury. The antioxidant effects of carvedilol have been previously demonstrated and characterized in various *in vitro* and *in vivo* test models in animals and humans [27, 39]. Carvedilol has been shown to scavenge free radicals and inhibit lipid peroxidation [12] on swine ventricular membranes [26, 49] rat brain homogenate, and human low-density lipoproteins [49]. Carvedilol is a neuroprotective with neuromodulatory action at NMDA receptors and Na^+ channels [9, 25, 26]. Neuroprotection has also been shown in an *in vivo* global ischemic model in which pretreatment with carvedilol limited injury in the CA1 hippocampal zone. The antioxidant activity of carvedilol is due to the presence of the carbazole moiety that is unique to carvedilol [49]. Carvedilol preserves the endogenous antioxidant systems (i.e., vitamin E and glutathione), which are normally consumed when tissues or organs are exposed to oxidative injury induced by oxygen free radicals *in vitro* and from ischemia and reperfusion damage *in vivo* [26]. Carvedilol protects against peroxynitrite (ONOO-) toxicity and has been reported to increase glutathione levels [9, 42].

In the present study, systemic administration of 3-NP caused a marked decrease in SDH activity, which was significantly restored by carvedilol treatment. The blockade of free radical generation by carvedilol might be involved in the restoration of brain SDH activity [35]. Furthermore, various antioxidants, such as resveratrol, curcumin and melatonin, have also been reported to improve reduced SDH activity *in vivo* and *in vitro* [21, 22, 31, 44, 45]. Carvedilol is a highly liphophilic drug and easily crosses the blood brain barrier. By virtue of this property, carvedilol might be effective against 3-NP-induced neurotoxicity [37]. The present data clearly suggests that attenuation of oxidative stress by carvedilol might be responsible for its attenuation of SDH depletion in 3-NP-treated rats.

In conclusion, the present study demonstrates that carvedilol has neuroprotective effects against 3-NP-induced memory impairment and oxidative damage.

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