Protection Effects of Carvedilol Against Stress-induced Oxidative Damage in Rats Brain

Abdulaziz A. Al-Yahya

The objective of this investigation was to test the hypothesis that carvedilol having neuroprotective activities with as a potent antioxidant properties by measuring glutathione (GSH) and malondialdehyde (MDA) concentrations in brain of restraint stressed rats. Repeated immobilization of adult male Wistar albino rats for 4 h daily for 5 consecutive days per week was used as a model to induce oxidative stress. A time course study was conducted for 1, 2 and 3 consecutive weeks to detect the peak time of stress and on the drug response. Carvedilol (2.5 mg kg⁻¹, i.p.) was treated 30 min before each immobilization session. Animals were sacrificed immediate after the last stress session of each week. Brain and liver tissues were analyzed for GSH, MDA, RNA and total protein concentrations. Repeated immobilization stress induced a significant decrease in the level of GSH, RNA and total protein, while the levels of MDA were found elevated in brain and liver tissues as compared to controls. Parallel treatment with carvedilol to restraint rats found affective in enhancing the levels of GSH, RNA and total proteins and decreasing MDA concentrations in brain and liver tissues. In conclusion, carvedilol can be use as a potential drug to treat stress-induced oxidative damage.

Key words: Immobilization stress, carvedilol, malondialdehyde, glutathione, RNA, total protein

Dr. Abdulaziz A. Al-Yahya
Department of Pharmacology,
College of Pharmacy,
King Saud University,
P.O. Box 2457, Riyadh-11451,
Saudi Arabia

Tel: 00-966-1-4677174

Department of Pharmacology, College of Pharmacy, King Saud University,
P.O. Box 2457, Riyadh-11451, Saudi Arabia
INTRODUCTION

Reactive Oxygen Species (ROS) are generated by a variety of physiological and pathological conditions and despite their vital importance to normal function including proliferation, growth, signaling and apoptosis (Sen and Packer, 1996; Reid, 1997) they cause continuous damage to lipids, proteins and DNA (Ames et al., 1993; Beckman and Ames, 1997; Radak et al., 2001; Goto and Nakamura, 1997). There are several studies that are related to the effects of stress on the antioxidant system and induction of lipid peroxidation in brain after immobilization-induced stress model (Madrigal et al., 2001; Kashif et al., 2004; Sahin and Gunuslu, 2004). It is reported that the balance disturbance in favor of activation of free radical processes was observed with maximal changes in the brain after immobilization stress in the study Voronych and Fennel’ ianenko (1994). Brain tissue contains large amounts of polyunsaturated fatty acids, which are particularly vulnerable to free radical attacks (Reuter, 1995; Olanow, 1993; Muller, 1997; Cui et al., 2004; Gutteridge, 1995). One common method to determine the degree of lipid peroxidation is by measuring the level of malondialdehyde, a by-product of the lipid peroxidation process (Millan-Plano et al., 2003; Topal et al., 2004). Additionally, measuring changes in the concentration of reduced glutathione, an endogenous antioxidant, has also been used as an indicator of lipid peroxidation severity (Abd El-Gawad and El-Sawalhi, 2004; Zaidi and Banu, 2004).

Carvedilol 1-(4-carbazolyl oxy)-3-[2-(methoxy) ethylamino]-2-propanol is novel adrenergic antagonist that competitively blocks \( \beta_1 \) and \( \beta_2 \) vascular receptors, primarily through a selective \( \alpha_1 \)-blockade (Ruffolo et al., 1991). The drug has been introduced for the treatment of congestive heart failure, mild to moderate hypertension and myocardial infarction (Matsui et al., 1999; Feuerstein et al., 1997). It has been shown to be a multiple-action neurohormonal antagonist, has been shown to have greater cardioprotective efficacy than other \( \beta \)-blockers in animal models of cardiac ischemia (Feuerstein et al., 1992). It has been shown to scavenge oxygen free radicals (Yue et al., 1994) and to inhibit lipid peroxidation in biological systems (Yue et al., 1992; Kramer and Weglicki, 1996). Several reports suggest the cardioprotective activity of carvedilol may be through its antioxidant activity, which is not shared by all \( \beta \)-adrenergic receptor antagonists (Ruffolo et al., 1990; Nichols et al., 1991; deMey et al., 1994; Yue et al., 1992). Moreover, the antioxidant protection of carvedilol occurs through a chain-breaking mechanism in postischemic rat hearts (Kramer and Weglicki, 1996).

The results of these studies are likely to contribute to understanding the potential of antioxidant effects of carvedilol in preventing/alleviating stress induced diseases involving oxidative damage to cellular constituents.

MATERIALS AND METHODS

The present study was performed in Department of Pharmacology, College for Pharmacy, King Saud University, Riyadh, Saudi Arabia during 2005.

Animals: Seventy-two male Wistar albino rats were received from Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, of roughly the same age, weighing 200-250 g were used for the present studies. All the animals were maintained under controlled condition of temperature (24±1°C), humidity (50-55%) and light (12 h light/dark cycle) and were provided with Purina chow and water ad lib. They were divided in 4 groups (18 rats in each group) as followed, 1) control group, 2) carvedilol (2.5 mg/kg/day i.p.) group, 3) immobilization stress group and 4) carvedilol + immobilization stress group.

Carvedilol tablets (25 mg) was powdered and dissolved in 0.25% carboxymethyl cellulose (CMC) solution and 0.25 mL kg\(^{-1}\) body weight as final volume by calculating the dose of 2.5 mg kg\(^{-1}\) was injected intraperitoneally (i.p.), to rats for 5 consecutive days per week. Control and stress groups of rats received 0.25% CMC solution as vehicle treatment. Treatment with carvedilol was done 30 min before the 4 h immobilization session per day for 5 consecutive days per week (treatments stress sessions were made from Saturday to Wednesday and the animals were kept two days without treatment and stress).

Immobilization of rats: The method used in this study was a modified method for immobilization stress as earlier used by different research groups (Nadeem et al., 2005; Zaidi et al., 2003; Zaidi and Banu, 2004). In a review Pare and Glavin (1986) concluded that, placing the animals in exact size tube for immobilization stress is good type of methods involves minimum pain with minimum movement including that of the tail. Immobilization stress accomplished by placing individual animal in plastic, well ventilated, of their size tubes. The rats were deprived of food and water during stress exposure (Liu et al., 1996). The animals subjected to a 4 h stress period beginning at the same time each day for five consecutive days per week. Rats in stress group were sacrificed immediate after the last immobilization stress session. Brain and liver
tissues from all the animals were taken out quickly, washed with chilled normal saline, immediately dipped in liquid nitrogen for a minute and kept at -70°C till estimation of GSH, MDA, RNA and total protein concentrations.

**Estimation of GSH:** Glutathione concentration was assayed using the method of Sedlak and Lindsay (1968). A cross sectional piece of brain and liver tissues (200 mg) were dissected and homogenized in ice-cold 0.02 M ethylenediaminetetraacetic acid (EDTA). Aliquots of 0.5 mL of the tissue homogenates were mixed with 0.2 M Tris buffer, pH 8.2 and 0.1 mL of 0.01 M Ellman’s reagent, [5,5’-dithiobis-(2-nitro-benzoic acid)] (DTNB). Tubes were centrifuged at 3000 g at room temperature for 15 min. The absorbance of the clear supernatants was read in a spectrophotometer at 412 nm in 1 cm quartzs cells. The concentrations were estimated by using the standard curve.

**Estimation of MDA:** The method described by Okawara et al. (1979) was used to estimate MDA concentrations in brain and liver. A cross sectional piece of brain and liver tissues (200 mg) were homogenized in trichloroacetic acid (TCA) and the homogenates were suspended in thiobarbituric acid (TBA) reagent in test tubes. The test tubes were closed by glass stoppers and placed them in a boiling water bath for 15 min. Tubes were allowed to cool down at room temperature and then centrifuged at 3000 rpm for 15 min. Optical density of the clear pink supernatants was read at 532 nm. Malondialdehyde bis (dimethyl acetal) was used as an external standard.

**Estimation of total RNA and protein:** The method of Bregman (1983) was used to determine levels of RNA in rat brain and liver tissues. Tissues were homogenized in ice-cold distilled water. The homogenates were extracted in different concentrations of cold and hot TCA and 95% ethanol. The final extraction with 5% TCA was incubated in water bath at 90°C for 15 min. After cool down the tubes were centrifuged at 3000 rpm for 10 min and 0.4 mL supernatant was mixed with 2.6 mL of 5% TCA and 3 mL of orcinol reagent. After shaking tubes were placed in boiling water bath for 20 min then transferred to ice-cold water bath. After 10 min the samples were readout on spectrophotometer at the wavelength of 660 nm against reagent blank. Concentrations of the unknown samples were estimated by using the standard curve. Total proteins were estimated by the modified Lowry method of Schaeferle and Pollack (1973).

**Statistical analysis:** The various parameters studied were subjected to statistical analysis with Student’s t-test.

**RESULTS**

**Effect of restraint stress and/or carvedilol on GSH concentrations:** Carvedilol treatments failed to cause any changes in brain or liver GSH levels (p>0.05) as compared to the values in the control group. Immobilization stress induced significant decrease in brain GSH concentrations after 1 week (p<0.01), 2 weeks (p<0.001) and 3 weeks (p<0.001) as compared to controls and this inhibition were significantly increased by carvedilol treatment for 1 week (p<0.05), 2 weeks (p<0.05) and 3 weeks (p<0.01) respectively. Restrained stress for 2 weeks (p<0.05) and 3 weeks (p<0.001) caused decreased liver GSH levels as compared to controls. The depleted liver GSH concentrations caused by stress was significantly (p<0.01) increased with carvedilol treatment for 3 weeks (Table 1).

**Effect of restraint stress and/or carvedilol on MDA concentrations:** Neither brain nor liver MDA levels altered significantly after treating with carvedilol up to three consecutive weeks. Immobilization stress started inducing oxidative damage form 1st week as it showed significant increase in MDA concentrations of brain and liver. Carvedilol treatment to stressed rats showed significant decrease against the MDA increase by restraint stress in brain after 1st week (p<0.01), 2 weeks (p<0.001) and 3 weeks (p<0.01), respectively. Such protection has seen in liver, after two (p<0.05) and three (p<0.01) weeks treatment with carvedilol to stressed rats (Table 2).

**Table 1: Effect of carvedilol on glutathione (μg g⁻¹ wet tissue) concentrations in brain and liver tissues of restraint stressed rats for 1, 2 and 3 weeks (Mean±SE)**

<table>
<thead>
<tr>
<th>Treatment dose (mg kg⁻¹ body weight)</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brain</td>
<td>Liver</td>
<td>Brain</td>
</tr>
<tr>
<td>Control (0.25% CMC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>127.5±4.73</td>
<td>214.4±6.87</td>
<td></td>
<td>109.3±4.11</td>
</tr>
<tr>
<td>Carvedilol (2.5 mg kg⁻¹, i.p.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>126.7±6.88</td>
<td>211.3±10.60</td>
<td></td>
<td>111.68±6.58</td>
</tr>
<tr>
<td>Stress (0.22% CMSO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95.33±3.15</td>
<td>195.23±4.95</td>
<td></td>
<td>66.99±4.05**</td>
</tr>
<tr>
<td>Carvedilol-Stress (2.5 mg kg⁻¹, i.p.)</td>
<td>124.3±5.11*</td>
<td></td>
<td>197.31±10.58</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 and ***p<0.001 Student’s t-test, Groups 2 and 3 were statistically compared with group 1, Group 4 was statistically compared with Group 3.
Table 2: Effect of carvedilol on malondialdehyde (nmol g⁻¹ wet tissue) concentrations in brain and liver tissues of restraint stressed rats for 1, 2 and 3 weeks (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment dose (mg kg⁻¹ body weight)</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.25% CMC)</td>
<td>354.00±44.90</td>
<td>270.61±42.78</td>
<td>345.24±63.10</td>
</tr>
<tr>
<td>Carvedilol (2.5 mg kg⁻¹, i.p.)</td>
<td>392.50±6.54</td>
<td>277.21±10.72</td>
<td>343.57±13.71</td>
</tr>
<tr>
<td>Stress (0.25% CMS)</td>
<td>467.67±6.54**</td>
<td>324.29±13.26*</td>
<td>445.96±11.71***</td>
</tr>
<tr>
<td>Carvedilol-Stress (2.5 mg kg⁻¹, i.p.)</td>
<td>410.17±3.12**</td>
<td>322.46±10.66</td>
<td>393.15±9.83***</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 and ***p<0.001 Student's t-test. Groups 2 and 3 were statistically compared with group 1. Group 4 was statistically compared with Group 3.

Table 3: Effect of carvedilol on RNA (µg/100mg wet tissue) concentrations in brain and liver tissues of restraint stressed rats for 1, 2 and 3 weeks (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment dose (mg kg⁻¹ body weight)</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.25% CMC)</td>
<td>214.33±4.88</td>
<td>655.96±28.84</td>
<td>179.70±7.27</td>
</tr>
<tr>
<td>Carvedilol (2.5 mg kg⁻¹, i.p.)</td>
<td>195.00±4.92</td>
<td>674.05±15.72</td>
<td>176.13±8.12</td>
</tr>
<tr>
<td>Stress (0.25% CMS)</td>
<td>603.57±26.65</td>
<td>618.89±22.80*</td>
<td>141.53±6.94**</td>
</tr>
<tr>
<td>Carvedilol-Stress (2.5 mg kg⁻¹, i.p.)</td>
<td>205.17±8.72*</td>
<td>652.68±11.09</td>
<td>175.71±4.26*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 and ***p<0.001 Student's t-test. Groups 2 and 3 were statistically compared with group 1. Group 4 was statistically compared with Group 3.

Table 4: Effect of carvedilol on total protein (mg/g wet tissue) concentrations in brain and liver tissues of restraint stressed rats for 1, 2 and 3 weeks (Mean±SE)

<table>
<thead>
<tr>
<th>Treatment dose (mg kg⁻¹ body weight)</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.25% CMC)</td>
<td>4.65±0.17</td>
<td>14.08±0.44</td>
<td>4.86±0.28</td>
</tr>
<tr>
<td>Carvedilol (2.5 mg kg⁻¹, i.p.)</td>
<td>4.49±0.19</td>
<td>14.36±0.24</td>
<td>4.60±0.16</td>
</tr>
<tr>
<td>Stress (0.25% CMS)</td>
<td>3.79±0.13</td>
<td>13.79±0.29</td>
<td>3.64±0.11**</td>
</tr>
<tr>
<td>Carvedilol-Stress (2.5 mg kg⁻¹, i.p.)</td>
<td>4.00±0.16</td>
<td>13.77±0.19</td>
<td>4.31±0.11*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 and ***p<0.001 Student's t-test. Groups 2 and 3 were statistically compared with group 1. Group 4 was statistically compared with Group 3.

Effect of restraint stress and/or carvedilol on RNA and total protein levels: Carvedilol treatment for three consecutive weeks to normal rats failed to induce any significant change in brain and liver RNA concentrations compared to control group of rats. Immobilization stress from the first week showed significant (p<0.01) reduction in brain RNA levels. However, this reduction has seen after 2 (p<0.01) and 3 (p<0.001) weeks of stress in liver tissue. Carvedilol, significantly (p<0.05) increased the RNA levels from first week against the decreased caused by the restraint stress. However, in liver such effect (p<0.05) has seen after three consecutive weeks treatment (Table 3).

Brain and liver total protein concentrations were significantly decreased after 2 consecutive weeks of restraint stress to rats. Two consecutive weeks treatment with carvedilol to stressed rats showed protection (p<0.05) against the decreased brain total protein concentrations caused by immobilization stress (Table 4).

**DISCUSSION**

All cells in the body are exposed chronically to oxidants from both endogenous and exogenous sources, but are also equipped with an antioxidant system. Reactive oxygen and nitrogen species, if unchecked, can contribute to chronic disease development by oxidatively modifying lipids, nucleic acids and proteins (Liu et al., 1996). Of all the organs, the brain is thought to be the most vulnerable to oxidative damage due to its high oxygen consumption, presence of high levels of polyunsaturated fatty acid (PUFA) and nondegenerative nature of neurons, which may lead to various neurodegenerative diseases (Floyd and Carney, 1992; Halliwell, 2001; Cui et al., 2004). Recently Zaidi and colleagues (2005) reported that, stress plays a potential role in aggravating liver diseasses like hepatic inflammation through ROS generation.

Restraint/immobilization stress is a well-known method for the production of chronic physical and emotional stress (Singh et al., 1993). In the present study, 4 h immobilization stress per day for 5 consecutive days per week resulted in the generation of oxidative stress/reactive ROS in the brain and liver of rats by decreasing the levels of GSH and enhancing the level of lipid peroxidation reactive substances MDA, which is an indicative of lipid peroxidation. However, the restraint stress effect found higher in brain than liver, as it has been reported earlier in literature (Reiter, 1995; Olawu, 1993; Muller, 1997; Cui et al., 2004; Gutteridge, 1995). It may conclude that, ROS is propagating the initial attack on lipid rich membranes of the brain to cause lipid
peroxidation (Das and Kanna, 1997). The enhanced lipid peroxidation may also be due to marked depletion of GSH content of brain, which acts as one of the guarding factors against oxidative stress (Lewine, 1982). Glutathione plays an important role in the detoxification of ROS in brain. It reported that stress reduces GSH levels and leads to increased levels of ROS (Liu et al., 1994). Evidence has been presented that the neuronal defense against H$_2$O$_2$, which is the most toxic molecule to the brain, is mediated primarily by the GSH system (Dringen, 2000). Madrigal et al. (2001) also found a decreased level of GSH in brain of rats exposed to immobilization stress. The ROS may propagate the initial attack of lipid rich membranes of the brain to cause lipid peroxidation (Das and Kanna, 1997). Furthermore, several reports confirm our findings that, brain is more vulnerable to oxidative damage due to its high oxygen consumption, presence of high levels of PUFA and neurodegenerative nature of neurons, which may lead to various neurodegenerative diseases (Floyd and Carney, 1992; Cui et al., 2004).

Carvedilol, a β-receptor blocker with additional vasodilating activity, is used for the treatment of hypertension, ischemic heart disease and congestive heart failure (Behn et al., 2001). It has demonstrated that patients with cardiovascular disease have higher level of oxidative stress, as determined by MDA test (Castro et al., 2002). Several reports suggest the cardioprotective of carvedilol may be through its antioxidant activity, which is not shared by all β-adrenergic receptor antagonists (Ruffolo et al., 1990; Nichols et al., 1991; deMey et al., 1994; Yue et al., 1992). In the present study, carvedilol treatment showed protection against the oxidative stress induced by immobilization as significant increase GSH and decrease MDA concentrations in both brain and liver tissues compared to untreated stressed rats. Present results are in parallel with earlier reports that, carvedilol shown to scavenge oxygen free radicals (Yue et al., 1994) and to inhibit lipid peroxidation in biological systems (Yue et al., 1992; Kramer and Weglicki, 1996). Recently, Castro et al. (2005) reported that, carvedilol treatment in patients with chronic heart failure reduced oxidative stress.

To determine whether carvedilol provided neuroprotection in ischemic brain, Lysko and colleagues (1992a) studied a model of gerbil forebrain ischemia where animals were exposed to 6 min of bilateral carotic artery occlusion. Seven days after ischemia, 52% protection of CA1 hippocampal neurons was achieved by both pretreatment and posttreatment with subcutaneous injections of carvedilol. In continuation to explore the potential molecular mechanisms of carvedilol-mediated neuroprotection, the same group of researchers had more studies and concluded that, in addition to free radical scavenging, carvedilol may have utility as a low-affinity antagonist of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor channel (Lysko et al., 1992b; 1994), as a Na"+ channel blocker to prevent glutamate neurotransmitter release and its metabolites may have a membrane-fluidizing effect Lysko et al. (1998).

In conclusion, repeated immobilization of rats generated higher oxidative stress in rat brain by decreasing GSH and increasing the lipid peroxidation. Treatment with carvedilol found more effective in enhancing the levels of GSH, RNA and total proteins and decreasing lipid peroxidation. Therefore, in addition to its ability to offer protection from myocardial ischemia by virtue of β-blockade, vasodilatation and free radical scavenging, carvedilol may have additional therapeutic value to reduce the risk of cerebral ischemia and stroke.

ACKNOWLEDGEMENT

The author is thankful to King Saud University, School of Pharmacy Research Center, Riyadh, Kingdom of Saudi Arabia, for sanction of grant to conduct research on stress-induced oxidative damages.

REFERENCES


