The Effect of Carvedilol on the Nitric Oxide Inducing Properties of Bacterial Lipopolysaccharide in Mice

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Abstract

The aim of this study was to investigate the effect of carvedilol on the ability of lipopolysaccharide (LPS) to induce nitric oxide (NO) production in mice.
Groups of BALB/c mice were injected with carvedilol and/or LPS. Animals were then bled 1, 3, 6 and 9 hours post-injection and serum NO levels were measured using the Griess reagent.

Carvedilol did not induce NO production, however, it enhanced its induction by LPS.

If this finding could be translated to humans, then patients on carvedilol who come down with a Gram negative septicemia should be considered.

**Keywords:** Carvedilol, LPS, Mice, Nitric oxide

**List of abbreviations**

<table>
<thead>
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<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>cAMP</td>
<td>Adenosine 3’, 5’ Cyclic monophosphate</td>
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<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
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<td>DIC</td>
<td>Disseminated intravascular coagulation</td>
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<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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**Introduction**

Carvedilol (Dilatrend®) \[1-[carbazolyl-(4)-oxy]-3-[(2-methoxyphenoxyethyl) amino]-2-propanol\] \[4\] is a non selective β-adrenoceptor blocker commonly used to treat patients with heart problems and hypertension [4, 9]. It inhibits the action of cathecolamines, which indirectly leads to the down-regulation of cAMP and therefore causes smooth muscle relaxation [5]. The drug is also a α1-adrenoceptor blocker, an antioxidant and a vasodilator [4].

The reports on the effect of carvedilol on NO production are scarce. One *in vivo* study using rats indicated that the drug decreased arterial pressure whilst it increased NO production [3]. However, *in vitro* studies are controversial. On one hand, “Pecivova et al. [12]” reported that carvedilol does not affect the expression of inducible nitric oxide synthase (iNOS) by macrophages and “Yoshioka et al. [14]”
reported that carvedilol is a scavenger of NO. On the other hand, “Kurosaki et al. [10]” reported that carvedilol stimulated the expression of iNOS in cardiac myocytes.

Lipopolysaccharide (LPS) is a major constituent of the cell wall of Gram negative bacteria. It consists of a polysaccharide moiety (O-antigen and core polysaccharide) and a lipid moiety (lipid A). LPS is responsible for the fever, hypotension, disseminated intravascular coagulation (DIC) and other symptoms seen in certain Gram negative septicemias [6].

LPS induces the production of NO, an important mediator in septic shock and a contributing factor to the loss of vascular tone. As early as 1994, “Horton et al. [7]” reported an increase in hepatic nitric oxide synthetase in rats injected intraperitoneally with LPS. One year later, it was shown that LPS induces the formation of inducible nitric oxide synthase (iNOS), rather than the endothelial nitric oxide synthase (eNOS) [13].

Since reports indicate that carvedilol and LPS modulate NO production it was of interest to determine what affect, if any, carvedilol has on the induction of NO production by LPS in mice.

Materials and Methods

Animal model

Four to six weeks old female BALB/c mice (average weight: 30g) were obtained from and housed at the Animal House, Diana Tamari Sabbagh Building at the American University of Beirut and were fed with a normal diet. Mice were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals following the approval of the Animal House Ethical Committee of our institution.

Materials

Lipopolysaccharide (LPS) from Salmonella enterica serovars Minnesota was purchased as lyophilized powder prepared by phenol-water extraction. (Sigma Chemicals co., MO, USA). Three milligrams of LPS were dissolved in 10 ml of sterile phosphate buffered saline (PBS) and 0.2 ml/30g (ml/mouse) were injected intraperitoneally.
A 1 mg/ml solution of Carvedilol was prepared by dissolving the appropriate number of Dilatrend® tablets (Roche, Basel, Switzerland) in saline. The solution was filtered to remove starch and other inactive material. Appropriate dilutions were prepared for injection using normal saline according to Table 1.

Ketamine, 50mg/ml (Panpharma, Fougères, France).

Xylazine, 20mg/ml (Inter chemie, Castenray, Holland).

All agents used in this study were confirmed to be endotoxin-free using the Limulus Amebocyte Lysate assay.

Experiment protocol

Five groups of 12 mice each were injected intraperitoneally with one of the different agents listed in Table 1. Each of the 5 groups was divided into subgroups, comprising 3 mice each. Mice in the first, second, third and fourth subgroup were bled and sacrificed 1 hour, 3 hours, 6 hours and 9 hours respectively after the injection of the appropriate solution. On the day of bleeding and sacrifice, mice were anesthetized with a mixture of ketamine and xylazine (250mg/kg of ketamine + 25mg/kg of xylazine). Blood was collected by cardiac puncture after opening the thoracic cavity.

Nitric oxide quantification

Blood collected from each subgroup was pooled, centrifuged and the separated serum was used for the quantification of nitric oxide using the Griess reagent kit (World Precision Instruments Inc., USA) according to the manufacturer’s instructions. Samples were run in triplicates along with 6 standards of different concentrations.

Results were obtained by spectrophotometry and a standard curve was drawn from which NO concentrations were deduced. Results are presented as the average of the triplicate readings for each sample followed by the standard deviation (Figure1).
Results and Discussion

Carvedilol and LPS have been reported to induce NO synthesis. Hence, the aim of this study was to assess the combined effect of these two entities on NO production in mice.

NO was not detected in sera obtained at all times post-carvedilol and post-PBS (control group) treatment. However, it was detected in sera collected at all times post-LPS treatment. A peak was recorded 6 hours post-LPS treatment (Figure 1). LPS causes hypotension followed by shock [1, 2]. One of the mechanisms involved is its ability to induce nitric oxide production [13], as indicated by the results obtained. It has been reported that carvedilol also causes the elevation of NO levels [8]. However, in this study carvedilol, in the doses tested, did not do so (Figure 1).

When LPS and carvedilol were given in combination, the level of NO was significantly higher than when LPS was injected alone with a peak in specimens obtained 9 hours post-treatment. This increase was more apparent when the lower dose of carvedilol (2µg/g) was used (Figure 1). In a murine study, it was reported that LPS caused a decrease in cyclic AMP levels and this decrease was reduced when epinephrine was injected with LPS [11]. Since carvedilol is a beta adrenergic receptor antagonist, it most probably causes a decrease in cyclic AMP levels as well. What bearing does cyclic AMP have on the production of NO is yet to be determined but it can be hypothesized that decrease in cyclic AMP levels is associated with the production of NO. It is worth noting that a 2µg/g dose of carvedilol was more effective than the 4µg/g in enhancing the NO inducing effect of LPS. One would wonder if doses less than 2µg/g of carvedilol would induce NO production.

In conclusion, carvedilol appeared to enhance the NO-inducing property of LPS. If this finding could be translated to humans, then patients on carvedilol who come down with a Gram negative septicemia should be considered.

References


**Table 1. Protocol for the assessment of the effect of carvedilol on the NO-producing property of LPS.**

<table>
<thead>
<tr>
<th>Group number</th>
<th>Injected with</th>
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<tbody>
<tr>
<td>1</td>
<td>autoclaved PBS solution</td>
</tr>
<tr>
<td>2</td>
<td>LPS (2μg/g)</td>
</tr>
<tr>
<td>3</td>
<td>Carvedilol (4μg/g)</td>
</tr>
<tr>
<td>4</td>
<td>LPS (2μg/g) and Carvedilol (2μg/g)</td>
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<tr>
<td>5</td>
<td>LPS (2μg/g) and Carvedilol (4μg/g)</td>
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</table>
Figure 1. The effect of carvedilol and/or LPS on serum nitric oxide (NO) levels in mice.
Effect of carvedilol

Figure legend:

Figure 1. The effect of carvedilol and/or LPS on serum nitric oxide (NO) levels in mice

NO concentrations (in μM) in mice sera after treatment with carvedilol and/or LPS. The mice were injected with the appropriate solution at time (t) = 0, then bled at t = 1 hour, t = 3 hours, t = 6 hours and t = 9 hours. The collected blood was centrifuged and NO quantification was performed on the sera using the Griess reagent kit (the sera of 3 mice from each subgroup were pooled together and run in triplicates for the assessment of the NO concentration). A standard curve was drawn from which NO concentrations were deduced. NO was not detected in sera obtained at all times post-carvedilol and post-PBS treatment.

- Standard deviations are indicated above each NO concentration in the figure.
- Control group: NO level = 13 ± 12.8 μM.

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