Activity exerted by a naphthalene-oxirane derivative on the ischemia/reperfusion injury.

Lauro Figueroa-Valverde¹*, Lenin Hau-Heredia¹, Elodia García-Cervera¹, Eduardo Pool-Gómez¹, Maria López-Ramos¹, Vera-Escobedo María Isabel¹, Francisco Díaz-Cedillo², Marcela Rosas-Nesticapa³

¹Laboratory of Pharmacochemistry, Faculty of Chemical and Biological Sciences, University Autonomous of Campeche. Av. Agustín Melgar, Col Buenavista C.P. 24039, Campeche Cam, México
³Facultad de Nutrición, Universidad Veracruzana. Médicos y Odontólogos s/n, 91010, Xalapa, Veracruz, México

Abstract

There are data, which indicate that myocardial ischemia/reperfusion injury is a serious problem involved on cardiovascular diseases. Some naphtalen-derivatives have been used as cardioprotective drugs on myocardial ischemia-reperfusion injury; however, their activity and the molecular mechanism involved on myocardial ischemia-reperfusion injury are very confusing. Therefore, the aim of this study was to evaluate the activity of a naphtalen-oxirane derivative on myocardial ischemia/reperfusion injury using an isolated heart model. Additionally, molecular mechanism involved in the activity exerted by the naphtalen-oxirane derivative on perfusion pressure and coronary resistance was evaluated by measuring left ventricular pressure in absence or presence of following compounds; prazosin, metoprolol, indomethacin and nifedipine. The results showed that the naphtalen-oxirane derivative reduce infarct size compared with the control conditions. Other results showed that the naphtalen-oxirane derivative significantly increases the perfusion pressure and coronary resistance in isolated heart in comparison the control. Finally, other data indicate that the naphtalen-oxirane derivative increase left ventricular pressure in a dose-dependent manner; however, this phenomenon was significantly inhibited by nifedipine. In conclusion, all these data suggest that the naphtalen-oxirane derivative exert a cardioprotective effect through calcium channels activation and consequently induce changes in the left ventricular pressure levels. This phenomenon results in decrease of myocardial necrosis after ischemia and reperfusion.

Keywords: Naphtalen-oxirane derivative, Ischemia/reperfusion injury left ventricular pressure, Nifedipine.

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Introduction

Several clinical and laboratory data indicate that myocardial infarction is a main cause of death worldwide [1,2]. In addition, there are studies that indicate that myocardial infarction can be induced by prolonged ischemia which consequently brings in cell viability, and ultimately cardiac function. Acute myocardial infarction can produce alterations in the topography of both the infarcted and non-infarcted regions of the ventricle [3]. There are some reports, which indicate the most effective method of limiting necrosis is restoration of blood flow; however, the effects of reperfusion itself may also be associated with tissue injury [4]. In the search of therapeutic alternatives to reduce the ischemia-reperfusion injury, some drugs have been evaluated; for example, there is evidence that methylene blue decreases ischemia-reperfusion injury by inhibiting xanthine oxidase, which could bring as a consequence changes on superoxide concentration using an in vitro model of ischemia and reoxygenation [5]. Other data indicate that the compound 5-(N,N-dimethyl) amiloride induced protective effects on ischemia-reperfusion injury in rat hearts via inhibition of the Na⁺-H⁺ exchanger [6]. In addition, a study showed the protective effect of magnolol on ischemia-reperfusion injury attributed to its antioxidant and anti-inflammatory activity using a hind limb ischemic-reperfusion animal model [7]. Other studies showed that glibenclamide reduces myocardial damage caused by ischemia/reperfusion in Guinea pig heart via activation of ATP-regulated K⁺ channels [8]. Also, other data indicates that Cyclosporin A can reduce the ischemia/reperfusion-injury in isolated rat hearts through changes in
cAMP levels [9]. In addition, some naphthalene derivatives have been developed to evaluate their biological activity in several animal models; for example, a study showed that the naphthalene derivative \((\pm)(R,S)-5,6\text{-dihydroxy-2-}
\text{methylamino-1,2,3,4-tetrahydro-naphthalenehydro-chloride})
can induces protective effects on ischemia-reperfusion injury in isolated heart rat via decrease of norepinephrine [10]. Other report, showed that the naphthalene derivative \((N\text{-}(6-
\text{aminothexyl})\text{-}5\text{-chloro-1-naphthalene sulfonamide}), exerts
cardioprotective effects on the isolated rat heart exposed to hypothermic and ischemic conditions by blocking the activity of calmodulin [11]. Other data indicates that compound \(1,5\text{-}
(\text{dimethylamino})\text{-}N\text{-}(3,4\text{-dimethyl-5-isoxazolyl})\text{-}1\text{-naphthalene}
sulfonamide, significantly improved the recovery of cardiac function during reperfusion-ischemia injury through of reduced
of creatine kinase (Figure 1) [12]. All these data show that
naphthalene derivatives induce inotropic effects in the cardiovascular system; nevertheless, the cellular site and molecular mechanism involved in its inotropic activity are very
confuse. Therefore, data information is needed to characterize
the activity induced by the naphthalen derivatives at cardiovascular level. To provide this information, the present
study was designed to investigate the effects of a naphthalen-
oxirane derivative on perfusion pressure and vascular
resistance in isolated rat hearts using the Langendorff
technique. In addition, to evaluate the molecular mechanism
involved in the inotropic activity induced by the naphthalen-
oxirane derivative on left ventricular pressure the following
compounds were used some pharmacological tools.

**Material and Methods**

All experimental procedures and protocols used in this
investigation were reviewed and approved by the Animal care
and use Committee of University Autonomous of Campeche
(No. PI-420/12) and were in accordance with the guide for the
care and use of laboratory animals [13]. Male Wistar rats;
weighing 200-250 g were obtained from University
Autonomous of Campeche.

**Reagents**

The compound \(3\text{-}(2\text{-Hydroxy-naphtalen-1-yl})\text{oxirane}-2-
carboxylic acid (2\text{-}{4\text{-}(3\text{-butyl-1-cyclohexyl-4-
(cyclohexylaminol)-azetidin-2-ylidenecarbamoyl})-
phenoxy[benzoylamino]-ethyl)-amide [14] was prepared with
methods previously reported. In addition, another reagents
used in this study were purchased from Sigma-Aldrich Co. Ltd.
All drugs were dissolved in methanol and different dilutions
were obtained using Krebs-Henseleit solution (≤ 0.01%, v/v).

**Experimental design**

Briefly, the male rat (200-250 g) was anesthetized by injecting
them with pentobarbital at a dose rate of 50 mg/Kg body
weight. Then the chest was opened, and a loose ligature passed
through the ascending aorta. The heart was then rapidly
removed and immersed in ice cold physiologic saline solution.
The heart was trimmed of non-cardiac tissue and retrograde
perfused via a non-circulating perfusion system at a constant
flow rate. The perfusion medium was the Krebs-Henseleit
solution \((pH = 7.4, 37°C) \text{ composed of (mmol);} \) 

\[117.8 \text{ NaCl; 6 KCl; 1.75 CaCl}_2; 1.2 \text{ NaH}_2\text{PO}_4; 1.2 \text{ MgSO}_4; 24.2 \text{ NaHCO}_3; 5 \text{ glucose and 5 sodium pyruvate. The solution was actively}
\text{ bubbled with a mixture of O}_2/\text{CO}_2 (95:5/5 \% ). The coronary
\text{ flow was adjusted with a variable speed peristaltic pump. An}
\text{ initial perfusion rate of 15 ml/min for 5 min was followed by a}
\text{ 15 min equilibration period at a perfusion rate of 10 ml/min. All}
\text{ experimental measurements were done after this}
\text{ equilibration period.}

**Perfusion pressure**

Evaluation of measurements of perfusion pressure changes
induced by drugs administration in this study were assessed
using a pressure transducer connected to the chamber where
the hearts were mounted and the results entered into a
computerized data capture system (Biopac).

**Evaluation of left ventricular pressure**

Contractile function was assessed by measuring left ventricular
developed pressure (LV/dP), using a saline-filled latex balloon
\((0.01 \text{ mm, diameter})\) inserted into the left ventricle via the left
atrium [15]. The latex balloon was bound to cannula, which
was linked to pressure transducer that was connected with the
MP100 data acquisition system.

**First stage**

**Ischemia/Reperfusion model:** After of 15-minute
equilibration time, the hearts were subjected to ischemia for 30
minutes by turning off the perfusion system [16]. After this
period, the system was restarted and the hearts were reperfused
by 30 minutes with Krebs-Henseleit solution. The hearts were
randomly divided into 2 major treatment groups with \(n=9:

Group I. Hearts were subjected to ischemia/reperfusion but
received vehicle only (Krebs- Henseleit solution).
Group II. Hearts were subjected to ischemia/reperfusion and treated with naphthalen-oxirane derivative before ischemia period (for 10 minutes) and during the entire period of reperfusion. At the end of each experiment, the perfusion pump was stopped, and 0.5 ml of fluorescein solution (0.10%) was injected slowly through a sidearm port connected to the aortic cannula. The dye was passed through the heart for 10 sec to ensure its uniform tissue distribution. The presence of fluorescein was used to demarcate the tissue that was not subjected to regional ischemia, as opposed to the risk region. The heart was removed from the perfusion apparatus and cut into two transverse sections at right angles to the vertical axis. The right ventricle, apex, and atrial tissue were discarded. The areas of the normal left ventricle non risk region, area at risk, and infarct region were determined using methods previously reported [16]. Total area at risk was expressed as the percentage of the left ventricle.

Second stage

Effect induced by the naphthalen-oxirane derivative on perfusion pressure: Changes in perfusion pressure as a consequence of increases in time (3 to 18 min) in absence (control) or presence of the naphthalen-oxirane derivative at a concentration of 0.001 nM were determined. The effects were obtained in isolated hearts perfused at a constant-flow rate of 10 ml/min.

Evaluation of effects exerted by the naphthalen-oxirane derivative on coronary resistance: The coronary resistance in absence (control) or presence of estrone and the naphthalen-oxirane derivative (0.001 to 100 nM) were administered and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of the naphtalen-oxirane derivative (0.001 nM) were evaluated. The effects were obtained in isolated hearts perfused at a constant flow rate of 10 ml/min. Since a constant flow was used changes in coronary pressure reflected the changes in coronary resistance.

Third stage

Effects induced by the naphthalen-oxirane derivative on left ventricular pressure through estrogen receptors: Intracoronary boluses (50 µl) of the naphthalen-oxirane derivative (0.001 to 100 nM) were administered and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of tamoxifen at a concentration of 1 nM (duration of preincubation with tamoxifen was by a 10 min equilibration period).

Effects induced by the naphthalen-oxirane derivative on left ventricular pressure through 1α-adrenergic receptor: Intracoronary boluses (50 µl) of the naphthalen-oxirane derivative (0.001 to 100 nM) were administered and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of prazosin at a concentration of 1 nM (duration of preincubation with prazosin was by a 10 min equilibration period).

The obtained values are expressed as average ± SE, using each heart (n=9) as its own control. The data obtained were put under Analysis of Variance (ANOVA) with the Bonferroni correction factor using the SPSS 12.0 program [18]. The
differences were considered significant when \( p \) was equal or smaller than 0.05.

**Results**

**First stage**

The results showed that the naphthalene derivative reduced infarct size (expressed as a percentage of the area at risk) compared with vehicle-treated hearts (Figures 2 and 3).

**Second stage**

In this study, the activity induced by a naphtalen-oxirane derivative on perfusion pressure and coronary resistance in isolated rat heart was evaluated. The results obtained from changes in perfusion pressure (Figure 3) as a consequence of increases in the time (3-18 min) in absence (control) or in presence of the naphtalen-oxirane derivative [0.001 nM], showed that this compound significantly higher the perfusion pressure \( (p=0.05) \) in comparison with the control conditions.

**Third stage**

Other result showed that coronary resistance, calculated as the ratio of perfusion pressure at coronary flow assayed (10 ml/min) was higher in the presence of the naphtalen-oxirane derivative \( (p=0.05) \) than in control conditions at a concentration of 0.001 nM (Figure 4).

On the other hand, other experiments (Figure 5) showed that the naphtalen-oxirane derivative increase the perfusion pressure in a dose dependent manner (0.001 nM) and this effect was not inhibited in presence of prazosin \( (p=0.05) \) and metoprolol \( (p=0.06) \) at a concentration of 1 nM. Other results showed that the naphtalen-oxirane derivative increase the perfusion pressure in a dose dependent manner (0.001 to 1 nM) and this effect was not inhibited in presence of indomethacin (1 nM).

Other results showed that the activity exerted by the naphtalen-oxirane derivative (0.001 nM) on perfusion pressure (Figure 6) in presence of flutamide was not blocked (1 nM); however, nifedipine or finasteride significantly inhibited \( (p=0.05) \) the activity of the naphtalen-oxirane derivative at a concentration of 1 nM.

Finally, other results showed in the Table 1 indicate that activity induced by the naphtalen-oxirane derivative [0.001 nM] increased the intracellular calcium levels as a consequence of increases in the time (3-18 min); nevertheless, this effect is significantly reduced \( (p=0.005) \) in presence of nifedipine [1 nM].
Figure 5. Effect exerted by the naphthalene-oxirane derivative on left Ventricular Pressure (LVP) via adrenergic system. Naphthalene-oxirane derivative [0.001 to 1 nM] was administered (intracoronary boluses, 50 μl) and the corresponding effect on the LVP was evaluated in absence and presence of prazosin [1 nM]. The results showed that activity induced by the naphthalene-oxirane derivative on LVP was not inhibited in presence of prazosin or metoprolol. Each bar represents the mean ± S.E. of 9 experiments.

Figure 6. Effects induced by the naphthalene-oxirane derivative on LVP through prostaglandins synthesis or calcium channel activation. Intracoronary boluses (50 μl) of the naphthalene-oxirane derivative [0.001 to 100 nM] were administered and the corresponding effect on the LVP was determined in the absence and presence of indomethacin or nifedipine. The results showed that the naphthalene-oxirane derivative higher the LVP in a dependent dose manner and this effect was not inhibited in the presence of indomethacin. However, the activity induced by the naphthalene-oxirane derivative on LVP was blocked by and nifedipine. Each bar represents the mean ± S.E. of 9 experiments. LVP: Left Ventricular Pressure.

Table 1. Activity induced by the Naphthalene-oxirane derivative [0.001 nM] on calcium intracellular [Ca^{2+}] in absence or presence of nifedipine [1 nM].

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Naphthalene-oxirane</th>
<th>Naphthalene-oxirane + nifedipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>$1.44 \times 10^{-3}$</td>
<td>$2.00 \times 10^{-4}$</td>
</tr>
<tr>
<td>6</td>
<td>$1.44 \times 10^{-3}$</td>
<td>$2.00 \times 10^{-4}$</td>
</tr>
<tr>
<td>9</td>
<td>$1.44 \times 10^{-3}$</td>
<td>$2.00 \times 10^{-4}$</td>
</tr>
<tr>
<td>12</td>
<td>$2.00 \times 10^{-3}$</td>
<td>$2.00 \times 10^{-4}$</td>
</tr>
<tr>
<td>15</td>
<td>$2.00 \times 10^{-3}$</td>
<td>$2.00 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Discussion

Several drugs have been used to treat the infarction/reperfusion injury resulting from myocardial ischemia [19-21]; nevertheless, there is scarce information about of activity exerted by the naphthalene derivatives on this phenomenon. Therefore, in this study the activity of a naphthalene-oxirane derivative on the infarction/reperfusion injury was evaluated using several strategies.

First stage

In this step, the activity of the naphthalene-oxirane derivative was evaluated on injury by ischaemia/reperfusion using an isolated heart model. The results showed in the Figure 2 indicate that naphthalene-oxirane derivative reduced infarct size compared with vehicle-treated hearts (control). This phenomenon could be because the naphthalene-oxirane derivative exerts some influence on blood pressure, which could result reduction in the infarct size, and decrease the myocardial injury after ischemia-reperfusion.

Analyzing these data, in this study the activity induced by the naphthalene-oxirane derivative on blood vessel capacity and coronary resistance; translated as changes in perfusion pressure was evaluated in an isolated rat heart model. The results showed that the naphthalene-oxirane derivative significantly increase the perfusion pressure over time compared with the control. This phenomenon could be via activation of some structure biological (p.e. ionic channels or specific receptors) involved in the endothelium of coronary artery [22] which might result as changes on coronary resistance. To evaluate this hypothesis, the effect induced by the naphthalene-oxirane derivative on coronary resistance was evaluated. The results showed that coronary resistance in presence of this compound was higher in comparison the control conditions.

Analyzing these results and other data which indicate that some substances can exert effects on coronary resistance and left ventricular pressure [23]; in this study, the activity of the naphthalene-oxirane derivative on left ventricular pressure was evaluated. The results showed that compound the naphthalene-oxirane derivative increase the left ventricular pressure in a manner dose dependent. These data indicated that this compound might activate some bioactive substance (ionic channels or specific receptors) which could result in changes in left ventricular pressure.

In the search of molecular mechanism involved in the effect exerted by the naphthalene-oxirane derivative on the left ventricular pressure on left ventricular pressure; in this study, also were analyzed some reports, which indicates that some...
compounds can induce changes on left ventricular pressure via activation adrenergic receptors [24,25]. Therefore, in this study the activity exerted by the naphthalen-oxirane derivative on left ventricular pressure in the absence or presence of prazosin, metoprolol was evaluated. The results showed that the naphthalen-oxirane derivative increases the left ventricular pressure in a manner dose dependent and this effect was not inhibited by prazosin or metoprolol. These results indicate that the molecular mechanism involved in the effects of this compound on left ventricular pressure was not via adrenergic activity.

Analyzing these data and another reports which indicate that some drugs may induce its effect on left ventricular pressure via prostaglandins synthesis [26]; in this study the activity exerted by the naphthalene derivative on left ventricular pressure in the absence or presence of indomethacin was analysed to evaluate the possibility that the activities exerted by the naphthalene derivative involve stimulation and secretion of prostaglandins. The results showed that the naphthalene derivative increased the left ventricular pressure and this effect was not blocked in presence of indomethacin. These data indicate that activity exerted by the naphthalene-oxirane derivative on left ventricular pressure was not via prostanooids synthesis and secretion.

Evaluating all this data and reports, which indicate that some drugs exert their activity on left ventricular pressure through activation of calcium channel [27], in this study the effect exerted by the naphthalene-oxirane derivative on left ventricular pressure was evaluated using nifedipine as pharmacological tool to characterize whether this mechanism was involved in their pharmacological activity. The results showed that the naphthalene-oxirane derivative increases the left ventricular pressure in a dose dependent manner; however this effect was inhibited in presence of nifedipine. These data indicate that the activity exerted by the naphthalene-oxirane derivative was through activation of L-type calcium channels. To evaluate this hypothesis and analyzing other reports which indicate that activity induced by some compounds on left ventricular pressure involve an increase of intracellular calcium concentration [28]; In this study, the effect exerted by the naphthalene-oxirane derivative on intracellular calcium levels were evaluated in absence or presence of nifedipine. The results showed that, the effect exerted by the naphthalene-oxirane derivative induces increase in the intracellular calcium levels as a consequence of increases in the time; nevertheless, this effect is significantly reduced by nifedipine. Analyzing these data, the effect exerted by naphtalen-oxirane derivative on the calcium concentration was evaluated. The results showed that the naphthalen-oxirane derivative increased the calcium levels in comparison with control conditions.

Conclusion
The naphthalene-oxirane derivative is a particularly interesting drug, because the activity induced for this compound on injury by ischemia/reperfusion involves a molecular mechanism different in comparison with other drugs. This phenomenon may constitute a novel therapy for ischemia/reperfusion injury.

References


*Correspondence to:
Lauro Figueroa-Valverde
Faculty of Chemical and Biological Sciences
University Autonomous of Campeche
México