Possible Hepatoprotective Effects of Lacidipine in Irradiated DOCA-Salt Hypertensive Albino Rats

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Abstract: Calcium channel blockers are increasingly used for the treatment of hypertension. Hypertension is an important risk factor for liver damage and several other circulatory abnormalities. The aim of this study was to determine the effects of lacidipine in a irradiation-induced hepatocellular damage model in Deoxy Corticosterone Acetate (DOCA)-salt-induced hypertensive model in rats. In this study, animals were divided into five groups as follows: control (Group 1), hypertensive (Group 2), irradiated (Group 3), irradiated and hypertensive (Group 4) and irradiated, hypertensive and lacidipine-treated (Group 5). At the end of the experiment, the livers were removed and their homogenates were biochemically examined. Significant differences were found between values of all groups (p<0.05). Group 3 and particularly Group 4 showed significant increase in lipid peroxidation and Nitric Oxide (NO) and serum tumor necrosis factor-α (TNF-α) with a significant reduction in serum level of alanine aminotransferase (ALT) enzyme and in superoxide dismutase in red blood cells lysates. Lacidipine-treated group (5) showed a significant reduction in elevated systolic blood pressure together with a great protection of ALT and SOD enzymes from the destructive effects of irradiation and hypertension. Additionally, this CCB reduces hepatic NO and serum TNF-α levels that were increased in groups (2,3,4). The present study suggests that lacidipine has some important protective effects on liver of hypertensive irradiated albino rats.

Key words: Hypertension, irradiation, liver, peroxidation, SOD and albino rats

INTRODUCTION

The survival rates of cancer patients are extremely improved by radiotherapy (Bentzen, 2006). Meanwhile, these patients suffer from various hepatic disorders up to hepatocellular damage and an increase in risk of liver failure as well as many other important side effects (Bentzen, 2006). An increase in inflammatory cytokines as tumor necrosis factor-alfa (TNF-α). Interleukin-1, interleukin-6 is reported with irradiation-induced adverse effects as hepatocellular damage or bone loss (Park et al., 2004). Additionally, irradiation is always accompanied by an increase in serum cortisol level together with a reduction in antioxidant markers due to oxidative stress-induced by irradiation (Ozgoemen et al., 2007).

Calcium channel blockers (CCB) are commonly used in the treatment of many clinical disorders e.g. angina pectoris, myocardial infarction, arrhythmia, left ventricular diastolic dysfunction, Raynaud's syndrome, migraine, osteoporosis, esophageal spasm and subarachnoid bleeding (McIntyre et al., 1999; Yagmurdur et al., 2002; Galisteo et al., 2004; Halici et al., 2008).

Lacidipine is a L-type Calcium Channel Blocker (CCB). It is a very effective antihypertensive drug. In previous studies, it was proven to induce many beneficial effects in its different therapeutic uses that may be related to its antioxidant and Nitric Oxide (NO) modulation (Garzotti, 2003). Hypertension is recognized as a "silent killer" disease whose pathogenesis includes the production of reactive oxygen species (ROS) by vascular cells and alterations in antioxidant enzymes (McIntyre et al., 1999). The antioxidant effects of CCBs against these harmful actions are not yet well recognized.

A study conducted in 1993 proved that dihydropyridine CCBs can modulate many inflammatory markers mediated via inhibition of induction of Nitric Oxide Synthase (NOS) enzyme. This inhibition prevents the production of detrimental NO by cultured macrophages under the effect of endotoxins (Szabo et al., 1993). This inhibitory effect protects against damage of the vascular endothelium especially in cases of atherosclerosis and hypertension with reduction in the risk of hypertension-related complications (Kunz, 2000; Chobanian et al., 2003).

Hypertension induces many vascular disorders in the liver which is a rich organ in blood supply. Irradiation has a hepatotoxic effect, however hepatic cells can regenerate themselves but this regeneration was found to be greatly reduced if the condition is accompanied by a vascular...
disease such as hypertension (Bentzen, 2006). Chronic
radiation hepatitis is a serious adverse effect of exposure
to irradiation (Mornex et al., 1997).

The literature about the effects of irradiation on the
liver of hypertensive patients is still inadequate. The
present study aimed to determine some possible
hepatoprotective effects of lactidipine, as a
dihydropyridine CCB, in irradiated albino rats.

MATERIALS AND METHODS

Materials: Lacidipine ester was provided as powder by
GlaxoSmithKline, Philadelphia, PA, USA.
Deoxycorticosterone acetate (DOCA) (Sigma chemicals
co. available as powder suspended in corn oil).
Superoxide dismutase enzyme [SOD] kit (RANSOD, by
Randox Laboratories). ALT enzyme Kit was obtained from
Biodiagnostic (Cairo, Egypt) and serum TNF-α ELISA was
purchased from Ani Biotech, Finland. Any other chemical
was purchased from Sigma Chemicals Co. Lisinopril was
dissolved in 0.5% methylcellulose and it was freshly
prepared.

Animal protocols: Sixty albino rats weighing 180-200 g,
each were randomized into 5 groups [N = 12, in each
group based on sample size done on microlab system
using α<0.05 and β>80% values].

DOCA-Salt Hypertension (McIntyre et al., 1999).
Thirty-six albino rats were treated twice weekly with
DOCA and administered subcutaneously (15 mg kg⁻¹) and
1% NaCl was added to their tap water for drinking. Two
weeks after the start of DOCA-salt treatment, these rats
were randomly divided into 3 groups (each group, n = 12).
Animal grouping:

(Duration of the study = 8 weeks)

• **Group 1 (Control non-treated):** It received standard
  volume of 0.5% methylcellulose equal to that injected
in lactidipine-treated rats as a solvent of lactidipine

• **Group 2:** It was DOCA-induced hypertensive rats
  without any treatment. [Rats are already rendered
  hypertensive as mentioned above]

• **Group 3 (Irradiated group):** It was exposed to 15 GY
  whole body γ-irradiation fractionated over 8 weeks
  (3 Gy/week, 1 Gy day after day) using the facilities of
  Misr Center for Radiation-Egypt using Cesium-137
  irradiation unit (Gamma cell-40) produced by the
  Atomic Energy of Cananda Limited at a dose rate
  of 0.46 Gy min⁻¹

• **Group 4:** It was DOCA salt-induced hypertension+γ-irradiation as mentioned above in
  groups 2 and 3

• **Group 5:** It was DOCA salt-induced hypertension+γ-irradiation as mentioned above in
  groups 2 and 3 + Lacidipine in a dose of 1 mg kg⁻¹
day⁻¹ ip for 8 weeks

Assessment of Systolic Blood Pressure (SBP) changes
(Bunag, 1973): SBP was measured by a tail-cuff
sphygmomanometer (UR-5000, Ueda Co, Ltd, Japan). SBP
measurements were conducted before starting treatment
with lisinopril then at the end of the 8th week of treatment.
Measurements were made at 2:00 to 5:00 p.m. (5 to 6 h
after treatment administration) to minimize circadian
influences. For each animal an average of at least three
consecutive measurements was taken to reduce variability.

At the end of the 8th week, each rat was anesthetized
with urethane [1 mg kg⁻¹ i.p.], slaughtered and liver was
removed and divided into 2 parts, part of it was
homogenized for TBARS measurement and the other part
was used to measure liver tissue content of NO as total
nitrite (NOx). Additionally, blood samples were collected
from rats of all groups for measurement of serum ALT
enzyme and TNF-α levels, SOD enzyme concentrations in
erythrocyte lysates using commercially available
colorimetric assay kit.

Measurement of serum levels of alanine amine
transferase (ALT enzyme): Serum levels of ALT was
measured using biochemistry automatic analyzer
(Hitachi7600).

Measurement of hepatic thiobarbituric acid-reactive
substances (TBARS) as a marker of lipid peroxidation
(Gutteridge and Quinlan, 1983): Liver homogenates were
rinsed with cold 0.14 M sodium chloride and
homogenized in 25% ice-cold 50 mM Tris-HCl buffer
(pH 7.4). A 150 μL of the tissue supernatant of samples
were diluted to 500 μL with deionized water. Two-hundred
and fifty microliter of 1.34% thiobarbituric acid was
added to each tube, followed by the addition of an equal
volume of 40% trichloroacetic acid. The mixtures were
then shaken and incubated for 30 min in a boiling water
bath. Tubes were allowed to cool to room temperature and
the absorbance was then read at 532 nm, using zero
concentration.

Measurement of liver tissue of NO (Green et al., 1982):
Liver tissue content of NO was measured as total nitrite
(NOx), the stable degradation products of NO, after
reduction of nitrate to nitrite by copper-cadmium alloy and measuring total nitrite (nitrite+nitrate) using Griess reagent.

Determination of SOD enzyme level in erythrocyte lysates: At the end of the study, blood samples were collected from rats from all groups for measurement of SOD levels in erythrocyte lysates, using commercially-available colorimetric assay kits, based on an indirect xanthine-xanthine oxidase (Halliwell and Chirico, 1993) and results were expressed in IU mL⁻¹.

Measurement of serum TNF-α as a pro-inflammatory mediator: Serum TNF-α was assayed using ELISA reagent kit.

Protein determination: The protein content of liver homogenates was determined by spectrophotometer according to the method of Bradford (1976). The aim is to relate the oxidative marker concentrations to the total tissue protein.

Data analysis: The results are presented as mean± Standard Deviation (SD) and evaluated using one-way analysis of variance (ANOVA), followed by Tukey’s post hoc determination, using GraphPad Prism (version 3.00; GraphPad Software, La Jolla, CA, USA).

RESULTS

- Effect of lacidipine on Systolic Blood Pressure (SBP) in irradiated DOCA-salt treated albino rats SBP was significantly (p<0.05) lowered by lacidipine (group 5) compared to non-treated irradiated DOCA-salt administered albino rats (group 3 and 4). Results were comparable to that reported with control rats (group 1) (Fig. 1). The mean±SD of SBP for each group remained constant all over the 8-hours period of measurement of SBP
- Effect of lacidipine on serum ALT in U L⁻¹ and liver tissue contents of TBARS in nmol mg⁻¹ tissue protein and liver tissue NOx in μM g⁻¹ and SOD in IU mL⁻¹ RCS lysates in irradiating DOCA-salt treated hypertensive albino rats (Table 1)
- Effect of lacidipine on serum serum TNF-α in Pg mL⁻¹ of irradiated DOCA-salt treated hypertensive albino rats (Table 2)

![Graph](https://via.placeholder.com/150)

Fig. 1: Effect of lacidipine on Systolic Blood Pressure (SBP) on irradiated DOCA-salt treated albino rats, *Significant (p<0.05) increase in SBP in DOCA-salt treated without lacidipine (group 2-4) compared to group 1, **Significant (p<0.05) decrease in SBP in lacidipine-treated group (5) compared to group 2-4

Table 1: Showed a significant (p<0.05) reduction in TBARS and in serum ALT concentration with a decrease in liver NOx, SOD enzyme level in RBC lysates in lacidipine-treated group (5) in comparison to the untreated hypertensive and/or irradiated group (2,3,4).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Control non-treated group 1</th>
<th>DOCA-salt treated group 2</th>
<th>Irradiated group 3</th>
<th>DOCA-salt treated+irradiated group 4</th>
<th>DOCA-salt treated+irradiated +lacidipine group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT in U L⁻¹</td>
<td>42.46±1.8</td>
<td>129.5±13.73*</td>
<td>138.4±15.06*</td>
<td>266±37.61*</td>
<td>42.94±3.44**</td>
</tr>
<tr>
<td>TBARS nmol mg⁻¹</td>
<td>0.58±0.11</td>
<td>11.76±1.5*</td>
<td>13.09±1.8*</td>
<td>21.65±1.24*</td>
<td>0.37±0.03**</td>
</tr>
<tr>
<td>Tissue protein</td>
<td>44.49±3.8</td>
<td>91.48±8.5*</td>
<td>93.64±4.3*</td>
<td>96.64±6.7*</td>
<td>40.32±3.5**</td>
</tr>
<tr>
<td>Liver tissue NOx (μM g⁻¹)</td>
<td>35.67±4.9</td>
<td>12.13±0.8*</td>
<td>12.23±0.8*</td>
<td>8.16±1.3*</td>
<td>65.73±4.3**</td>
</tr>
</tbody>
</table>

*p<0.05 significant increase in TBARS and serum ALT concentration and liver NOx with reduction in SOD enzyme level in groups (2,3,4) compared to the control non-treated group (1) **p<0.05 significant reduction in TBARS with decrease in ALT enzyme concentration and liver NOx and SOD enzyme level in RCS lysates of lacidipine-treated group (5) in comparison to groups (2,3,4)

Table 2: Showed a significant (p<0.05) reduction in serum TNF-α concentration in lacidipine-treated group (5) in comparison to the untreated hypertensive and/or irradiated group (2,3,4).

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</tr>
</thead>
<tbody>
<tr>
<td>Serum TNF-α (Pg mL⁻¹)</td>
<td>7.6±1.0</td>
<td>80.48±8.0*</td>
<td>83.97±4.6*</td>
<td>4.08±4.6*</td>
<td>18.38±1.4**</td>
</tr>
</tbody>
</table>

*p<0.05 significant increase in serum TNF-α concentration in groups (2,3,4) compared to the control non-treated group (1) , **p<0.05 significant reduction in TNF-α concentration of lacidipine-treated group (5) in comparison to groups (2,3,4)
DISCUSSION

Exposure of DOCA-salt treated hypertensive albino rats to γ-irradiation, in the present study, revealed a significant increase in hepatic TBARS, a marker of lipid peroxidation, an elevation in deleterious NOx content and in serum level of TNF-α with a significant reduction in serum levels of ALT enzyme and SOD enzyme in RBCs lysates. Eight-weeks treatment with lacidipine, as once daily dose, reverses all the above mentioned results with improvement of the detrimental effects induced by γ-irradiation on albino rats.

The radiation-induced liver disease, often called radiation hepatitis, occurs approximately 2 weeks to 4 months after hepatic irradiation. The late lesions may be associated with signs of chronic radiation hepatitis (Mornex et al., 1997). Radiation-induced hepatic injury is a form of veno-occlusive disease due to fibrous obliteration of the terminal hepatic venules leading to postsinusoidal obstruction (Lawrence et al., 1995).

In an attempt to explain the results of the present study, similar experimental studies demonstrated a state of chronic oxidative stress in lungs of rats exposed to γ-irradiation (Vujaskovic et al., 2001). Hypoxia was induced in the rat lung by the administration of a single dose of 28 Gy for 6 weeks. There were significant increase in macrophage activity, fibrosis and production of pro-inflammatory mediators in the post-irradiation period. Hypoxia has been shown to lead to increased Reactive Oxygen Species (ROS) production with a significant reduction in antioxidant and/or antioxidant enzyme production e.g., Super Oxide Dismutase enzyme (SOD) (Li and Jackson, 2002).

An increase in malondialdehyde levels, as a marker of lipid peroxidation, was reported in the lungs of mice after exposure to γ-irradiation. These mice were described to be exposed to a model of radiation-induced chronic oxidative stress (Kang et al., 2003).

Lacidipine, as a one of dihydropyridine Calcium Channel Blockers (CCBs), is an effective antihypertensive drug together with its great efficacy in reducing vascular Intima-Media Thickness (IMT) than other classes of antihypertensive drugs due to their vascular effects. An experimental study in 2012 revealed that 30 μM lacidipine inhibited about two-thirds of the oxidized-low density lipoprotein (ox-LDL). This ox-LDL has a major role in induction of ROS production. This study has demonstrated that lipophilic CCB, lacidipine, may inhibit ox-LDL induced proliferation and oxidative stress of vascular smooth muscle cells (VSMCs) (Zou et al., 2012). This anti-oxidant effect could be implicated on the results of the present study as the increase in SOD enzyme content in association with a significant reduction in hepatic lipid peroxidation.

Another study evaluated the extent to which lacidipine possessed antioxidant properties. The authors investigated the expression of intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin on human umbilical vein endothelial cells, induced by different pro-oxidant signals such as oxidized Low Density Lipoprotein (LDL) and tumor necrosis factor-alpha (TNF-alpha) in the presence of this lipophilic CCB.

Results of this study showed that the incubation of 5 μ mol L⁻¹ Cu⁺-oxidized LDL not only caused a dose-dependent increase in ICAM-1, VCAM-1 and E-selectin, but also synergically increased their TNF-alpha-induced expression. The addition of lacidipine to human umbilical vein endothelial cells significantly reduced the expression of ICAM-1, VCAM-1 and E-selectin induced by TNF-alpha alone or with oxidized LDL. These results pointed to the inhibitory effect of lacidipine to the pro-inflammatory mediators such as TNF-α and nitric oxide. So, lacidipine may have protective and therapeutic effects in atherosclerosis as a disease associated with pro-inflammatory mediators and oxidative stress.

Similarly, the present study could conclude that lacidipine could provide a hepatoprotective effect against pro-inflammatory mediators and lipid peroxidation induced by exposure of albino rats to 8-weeks γ-irradiation as it possessed an inhibitory effect to ROS as well as to the pro-inflammatory mediators in addition to its powerful vascular protective effect as an effective antihypertensive drug.

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REFERENCES


