

# Anti-inflammatory activity of calcium channel blocker lercanidipine hydrochloride

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## ABSTRACT

**Objective:** To evaluate the anti-inflammatory effect of the third-generation CCB, lercanidipine. **Materials and Methods:** The ability of lercanidipine to reduce inflammation in carrageenan-induced paw edema in rats was evaluated, using different doses and its anti-inflammatory effect was compared with that of diclofenac sodium. Mast cell degranulation effect of lercanidipine was also carried out. **Results:** Lercanidipine produced a significant reduction in the inflammatory paw edema at all doses and at all intervals ( $P < 0.001$ ). The percentage of reduction in edema was found to be proportionate to the doses of lercanidipine. Lercanidipine also significantly reduced the percentage of propranolol-induced mast cell degranulation. **Conclusions:** It was found that lercanidipine exerts a positive anti-inflammatory effect in a dose-dependent manner and was superior to diclofenac sodium.

**Key words:** Carrageenan, diclofenac sodium, lercanidipine, mast cells

## INTRODUCTION

The second half of the 20<sup>th</sup> century has seen a constant decrease in cardiovascular mortality in the western industrialized nations.<sup>[1]</sup> This decrease has occurred concomitantly with an improved control in the incidence of hypertension.

Elevated blood pressure has been related to the risks of major coronary events, such as myocardial infarction and coronary death.<sup>[2]</sup> Blood pressure levels have also been positively and continuously related to the risk of stroke,<sup>[2,3]</sup> cerebral hemorrhage, cerebral infarction, and dementia.<sup>[3]</sup> It is also noteworthy that people are now confronted currently

with the rapid development of a 'second wave' epidemic of cardiovascular disease that is hitting the developing countries.

Basically, there are six major drug classes used in the treatment of blood pressure. There include diuretics, beta-blockers, calcium-antagonists (CA), angiotensin-converting-enzyme inhibitor (ACE-inhibitors), angiotensin II-antagonists, and alpha-adrenergic blockers. All subgroups of CA are effective and well tolerated in lowering blood pressure. While several studies have cast an unfavorable light on the use of calcium channel blockers (CCBs) as first-line antihypertensive agents,<sup>[4-7]</sup> morbidity and mortality results of other trials have evidenced that CA are definitely useful in the treatment of hypertension.

The search for new designs of CA with extended properties has superseded the earlier search for new clinical indications for these drugs.<sup>[8,9]</sup> Such improved qualities of new dihydropyridines (DHP) would be prolonged action, less side effects, single dosing (improved compliance), and less negative inotropic and chronotropic actions. A second generation of CA has addressed this problem with variable success.

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While drugs like nitrendipine,<sup>[10]</sup> or amlodipine,<sup>[11]</sup> have done reasonably well, others such as mibefradil,<sup>[12]</sup> or isradipine in the Multicenter Isradipine Diuretic Atherosclerosis Study (MIDAS) trial,<sup>[13]</sup> have not shown such a favorable outcome.

The third generation of DHP has added an additional property to this class of drugs: High lipophilicity. Currently, one of these drugs commercially available is lercanidipine. As a result of the lipophilic character, this compound is relatively quickly cleared from plasma building up within the phospholipid bilayer of cell membranes. The DHP thus accumulated can interact with its target, the site of the target, and the L-type calcium channel that lies within the double layer of the cell membrane as well. This phenomenon explains on one hand the slow onset, while on the other hand, the long duration of action.

Several agents with CA activity, DHP-derivatives, emerged on the market as early as 1998. The first of these drugs was amlodipine, a DHP with 30-50 hours clinical half-life and the most recent ones are lacidipine and lercanidipine, the latter being well known more for its anti-hypertensive property.<sup>[14,15]</sup>

The beneficial effects of lercanidipine include myocardial protection during ischemia, antagonism to the vasopressor activity of endothelin-1,<sup>[16]</sup> improving all indices of mitochondrial function,<sup>[17]</sup> decreasing fasting blood-glucose, glycosylate-hemoglobin, and improved oral glucose tolerance.

While third-generation DHP-CA Manidipine has been investigated for its pro-inflammatory cytokines secretion reduction property<sup>[18]</sup> and cardiometabolic properties,<sup>[19]</sup> drugs such as amlodipine, lacidipine, and nicardipine were evaluated for anti-inflammatory activity on the paw edema produced by carrageenan and compared with the activity of indomethacin,<sup>[9]</sup> that of the third-generation drug lercanidipine on carrageenan-induced rat hind paw edema has not so far been demonstrated, although a few other aspects of this drug have been investigated.<sup>[20-22]</sup>

Hence, the current study aims to evaluate the anti-inflammatory potential of the drug lercanidipine.

## MATERIALS AND METHODS

### Animals

Adult male Wistar rats (150-200 g) were used. The rats were housed in a temperature-controlled room in a 12 h/12 h dark/light cycle, and a standard dry diet (pellets) and water were made available *ad libitum*. All experiments were performed according to the guidelines of the Institutional Ethics Committee as well as those of CPCSEA. All efforts were made to minimize pain and suffering and to reduce the number of animals used.

### Methods

Two methods were employed to verify the anti-inflammatory effects of lercanidipine.

#### Carrageenan-induced paw edema

Lercanidipine was evaluated for anti-inflammatory activity by carrageenan-induced rat paw edema method.<sup>[23]</sup> Rats were divided into five groups of six animals each. Group 1 served as the negative Control, which received dimethyl sulfoxide (DMSO) (solvent for lercanidipine) 0.1 ml intraperitoneally as well as carrageenan. Group 2 was the positive control and received diclofenac sodium, i.p., at the dose of 2.5 mg/kg BW as well as carrageenan. Groups 3, 4, and 5 were experimental and received carrageenan and lercanidipine hydrochloride dissolved in DMSO, in different doses at the rate of 300, 600, and 900 µg/kg BW. Lercanidipine i.p. was administered 1 hour before the carrageenan injection, and the latter was procured from Sigma-Aldrich. Lercanidipine was bought from Tocris Bioscience, UK.

#### Induction and measurement of the inflammation

Inflammatory edema was induced as recommended by Winter *et al.*,<sup>[23]</sup> by injection of 0.1 ml of freshly prepared suspension of carrageenan (0.1 ml 1% w/v) solution in the sub-plantar region of right hind paw of rats.

The hind paw volumes were measured by a plethysmometer 1, 2, and 3 h after carrageenan injection and the difference between the treated and untreated hind paw volumes in the same rat was taken as the edema volume.

The percentage inhibition of edema (anti-inflammatory activity) of the drugs was calculated by the following equation: % inhibition of edema =  $(1 - D/C) \times 100$ . *D* represents the paw volume in drug-treated animals and *C* represents the paw volume of control group. The results are expressed as means ± S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA) followed by a *post hoc* Tukey-Kramer Multiple Comparisons Test.

#### Mast cell degranulation

*In vitro* test for mast cell degranulation was carried out by the method of Kelley and Weiner,<sup>[24]</sup> using the following formula: % of degranulation =  $(1 - T/C) \times 100$ , where *T* = mast cell count in the experimental tissues and *C* = mast cell count in the control.

Male albino rats were sacrificed by cervical dislocation, the mesentery carefully removed and cut into small bits of about 1 sq cm. These bits of tissues, belonging to the experimental group, were incubated for about 5 min in Tyrode solution containing various doses of lercanidipine hydrochloride (5, 10, 20 mg/ml) and propranolol, a degranulating agent, (50 µg/ml) while the control tissue was incubated with propranolol only.

The tissues were removed after 10 minutes and carefully spread over glass slides and stained with 1% toluidine blue. Mast cells are counted in five random fields under high power (X400). The percentage of degranulated mast cells is calculated in each treatment group. Sodium cromoglycate (10 µg/ml), a mast cell stabilizer, was used as standard.

**Statistical analysis**

Data were expressed as mean ± SEM. The results were analyzed by ANOVA followed by Tukey-Kramer multiple comparisons test. *P* < 0.05 was considered as a significant difference.

**RESULTS**

**Carrageenan-induced paw edema**

Injection of carrageenan produced an obvious paw edema as seen from the paw volume data at 1, 2, and 3 h after carrageenan injection [Table 1]. Administration of lercanidipine produced a clear and significant reduction in the edema at all doses and intervals (*P* < 0.001). The higher the dose, the higher was the percentage of reduction of edema. Diclofenac sodium, administered i.p., also reduced the edema but when compared with the efficacy of lercanidipine, the difference in the level of percentage of inhibition of the latter was much greater than that of diclofenac at doses of 600 and 900 mg at all time intervals. At the dose of 300 mg, the difference was not significant.

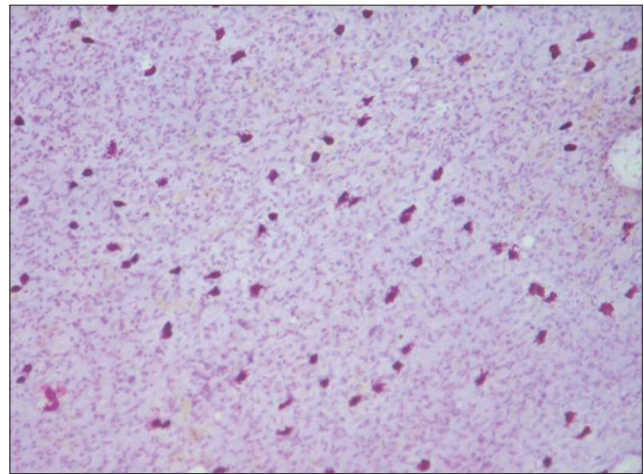
**Mast cell degranulation**

Comparison was made in the number of mast cells degranulating after incubation with propranolol only and along with lercanidipine. The results are shown in Table 2. Figure 1 shows the appearance of mast cells in a normal piece of rat mesentery simply incubated in Tyrode solution without addition of any agents. Figures 2a and b demonstrate the appearance of degranulation. Microscopic examination of bits of rat mesentery incubated at 37°C with propranolol alone showed a mean of 65.9% of mast cells degranulating, while the percentage of degranulation in the mesentery of rats incubated with lercanidipine at a concentration of 5, 10, and 20 mg/ml showed values of 38.6%, 21.5%, and 14.2%, respectively [Table 2].

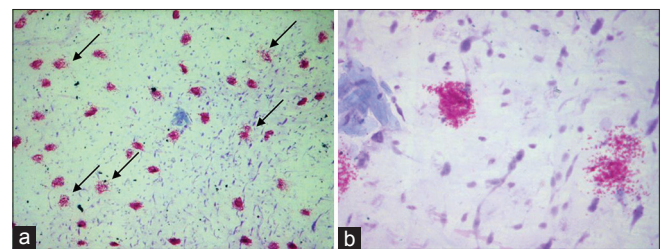
The dose of propranolol (P) remained the same (50 µg/ml). The dose (L) refers to the concentration of lercanidipine in the Tyrode solution. All the values are highly significant. Counting was done at a magnification of ×400.

**DISCUSSION**

Inflammation and increased capillary permeability are important factors in the pathogenesis of many diseases including atherosclerosis, while oxidative stress (OS), inflammation, and insulin resistance are among the mechanisms that have been recently implicated in pathogenesis of



**Figure 1:** Normal mast cells in the mesentery of control rats. Note the intact mast cells. Toluidine Blue stain, ×100



**Figure 2:** (a and b) Degranulating mast cells in the rat mesentery after incubation with propranolol. Note the damage to the cell membrane and spilling out of granules from the mast cells. a = ×400, b = ×1000

**Table 1: Changes in carrageenan-induced mean paw volume after administration of diclofenac and lercanidipine in different doses**

| Groups                 | Dose (kg) | Paw volume±S.E.M (ml) |         |            |        |             |         |
|------------------------|-----------|-----------------------|---------|------------|--------|-------------|---------|
|                        |           | 1 h                   |         | 2 h        |        | 3 h         |         |
|                        |           | OV                    | OI (%)  | OV         | OI (%) | OV          | OI (%)  |
| -Control (DMSO+Carr)   | -         | 0.21±0.004            | -       | 0.25±0.006 | -      | 0.39±0.0135 | -       |
| + Control (Diclofenac) | 2.5 mg    | 0.18±0.013            | 14.29   | 0.21±0.007 | 16     | 0.29±0.003  | 25.65   |
| Lercanidipine          | 300 µg    | 0.14±0.011            | 33.34*  | 0.18±0.008 | 28*    | 0.24±0.010  | 38.47*  |
| Lercanidipine          | 600 µg    | 0.11±0.009            | 47.62*∅ | 0.13±0.005 | 48*∅   | 0.20±0.006  | 48.72*∅ |
| Lercanidipine          | 900 µg    | 0.09±0.013            | 57.15*∅ | 0.11±0.005 | 56*∅   | 0.16±0.002  | 58.8*∅π |

F=21.853 (1 h), 82.412 (2 h), and 124.69 (3 h), OV=Edema volume, OI=Edema inhibition, in terms of percentage as compared with the values in negative control, \**P*<0.001 as compared to negative control group in all 3 hourly measurements, ∅ *P*<0.00 as compared with diclofenac in all 3 hourly measurements, π *P*<0.001 between L 300 vs L 900 in all 3 hourly measurements, by ANOVA and post hoc Tukey-Kramer Multiple Comparisons Test



**Table 2: Mesentery cell count data, showing number of cells per field in control (normal), and experimental rat mesenteries exhibiting degranulating mast cells**

| Control cells/field (P) | %    | Experimental   |                 |             |
|-------------------------|------|----------------|-----------------|-------------|
|                         |      | Dose (L) mg/ml | Cells/field (L) | % P         |
| 31±4.6                  | 65.9 | 5              | 17±7.2          | 38.6 <0.001 |
|                         |      | 10             | 11±6.5          | 21.5 <0.001 |
|                         |      | 20             | 7±6.8           | 14.2 <0.001 |

essential hypertension (EH). Peripheral polymorphonuclear leukocytes (PMNLs) are primed in EH patients, releasing uncontrolled superoxide anion contributing to OS and chronic low-grade inflammation in these patients. PMNL priming correlates with insulin resistance and PMNL intracellular calcium ((Ca<sup>2+</sup>) i). Japanese Society of Hypertension Committee published Guidelines for the Management of Hypertension in 2009 (JSH2009),<sup>[25]</sup> which recommend CCBs as one of the first choice of antihypertensive drugs among angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, diuretics, and beta-adrenergic blockers. Because DHP-CCBs have the greatest hypotensive efficacy without affecting organ blood flow, they are indicated in the patients with complications and the elderly.

Recent studies have attributed additional anti-oxidative characteristics to the anti-hypertensive drug lercanidipine (Vasodip), a third-generation CCB. It has been shown that the low-grade systemic inflammation and insulin resistance detected in EH patients may be attenuated by the use of lercanidipine, adding new unknown anti-inflammatory properties to this CCB.

Carrageenan-induced rat paw edema is the most suitable and commonly used investigation to look for anti-inflammatory potential of a drug, evaluated by its anti-edematous effect. Occurrence of edema secondary to carrageenan serves as an index of acute inflammatory changes and can be determined from differences in the paw volume measured immediately after carrageenan injection and then every hour for 3-6 hours. Carrageenan-induced edema is said to be biphasic, with the first phase occurring during the first hour after the injection, which is brought about by the release of histamine and serotonin and the second one due to the release of prostaglandins and cyclo-oxygenase products, with kinins providing continuity between the two phases.

In our study, carrageenan was used to induce paw edema and various doses of lercanidipine were used to primarily verify whether lercanidipine exerts any anti-inflammatory action at all like its related compounds, such as nifedipine and verapamil, since such a study has not so far been carried out. Various doses of lercanidipine were also employed in order to find out whether the anti-inflammatory effect, if present, is dose-dependent.

Diclofenac sodium was also used in one group of rats as a contrast measure to compare the efficacy of lercanidipine.

Carrageenan has caused inflammation as shown by the values of paw volume data. Statistical analysis of the experimental data suggests a highly significant anti-inflammatory activity by lercanidipine ( $P < 0.001$ ). The effect is also dose-dependent as seen from the values that after a dose of 900 µg/kg BW, the values of paw volume are comparatively the lowest, much lower than the values after diclofenac sodium injection. Unlike verapamil, the anti-inflammatory potency of which is effective at low dosage, lercanidipine's efficacy is dose-dependent similar to that of nifedipine.<sup>[26]</sup>

There is significant evidence that mast cells, basophils, and their released mediators are primary effectors of allergic inflammation.<sup>[27-29]</sup> It is believed that many of the deleterious effects that mast cells mediate are due to a variety of proinflammatory molecules exocytosed with exposure to antigen. By inhibiting the degranulation process of mast cells, lercanidipine has proved its anti-inflammatory potential.

## CONCLUSIONS

Lercanidipine exerts a dose dependent anti-inflammatory activity and the effect is superior to diclofenac sodium.

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