

# Evaluation of analgesic activity of perindopril in albino mice

R. N. Suresha, Siddamma  
Amoghmath, P. S. Vaibhavi,  
S. L. Shruthi, M. K. Jayanthi,  
H. L. Kalabharathi

Department of Pharmacology, JSS  
Medical College, Mysore,  
Karnataka, India

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## ABSTRACT

The aim was to evaluate the analgesic activity of perindopril in chemical, thermal and mechanical pain on Swiss albino mice. A total of 54 albino mice (Swiss strain) weighing 25-30 g were allocated to each experimental model and in each model there were three groups. The control group received normal saline (25 ml/kg) per orally, standard group received pentazocine (10 mg/kg) intra-peritoneal and test groups received perindopril (1 mg/kg) per orally. Perindopril and normal saline was administered 2 h before, whereas the pentazocine was administered 15 min prior to Eddy's hot plate, writhing and tail clip methods. The decrease in number of writhes, the delay in reaction time in tail clip and Eddy's hot plate method denoted the analgesic activity. Perindopril decreased the number of writhes, delayed the reaction time in tail clip and Eddy's hot plate method considerably when compared with control (normal saline), but less when compared with standard (pentazocine). Perindopril exhibits analgesic activity in thermal, chemical, and mechanical pain models in albino mice.

**Key words:** Angiotensin II, angiotensin (1-7), endogenous opioid and prostaglandin E2

## INTRODUCTION

Angiotensin converting enzyme (ACE) catalyze the formation of angiotensin-II from angiotensin-I. This enzyme occurs not only in the plasma but also in the kidneys, brain, adrenal glands, ovaries, and possible other tissues. Angiotensin-II is a potent vasoconstrictor and blockade of its synthesis by ACE inhibitors are currently the medication of choice in management of hypertension and cardiac failure.<sup>[1]</sup>

Pain is very often associated with inflammation. Inflammation is a normal response to any noxious stimulus that threatens the host and may vary from

localized response to a generalized one. It is a complex process involving release of chemicals from tissues and migrating cells and various mediators such as prostaglandins, leukotriene's, and platelet activating factors.<sup>[2]</sup>

Angiotensin-II regulates vascular tone, stimulates the release of pro-inflammatory cytokines, activates nuclear factor-kappa B (NF-κB), increases oxidant stress and thus, it functions as an inflammatory molecule. Angiotensin-II increases the release of reactive oxygen species (ROS). ROS activate NF-κB (NF-κB, known to initiate inflammatory process) that increases the transcription of pro-inflammatory cytokines, adhesion molecules, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Angiotensin-II enhances ROS production by activating NADPH oxidase and stimulates the DNA-binding activity of NF-κB in human neutrophils. Angiotensin-II increases the synthesis and concentration of tumor necrosis factor-α, interleukin-6, and chemokine monocyte chemo attractant protein-1. It also elevates tissue levels of NF-κB, and results in inflammatory cell infiltration.<sup>[3]</sup>

Angiotensin-II increases the formation of prostaglandin E2 (PGE2) by inhibiting the enzyme PGE9-ketoreductase and increasing the cyclooxygenases 2 (COX 2) activity by increasing cyclic adenosine monophosphate. PGE2

### Address for correspondence:

Dr. Siddamma Amoghmath,  
Department of Pharmacology, JSS Medical College, SS Nagar,  
Mysore - 570 015, Karnataka, India.  
E-mail: siddama.a@gmail.com

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sensitizes pain receptors at efferent nerve endings to mediators of pain and amplify algia.<sup>[4]</sup>

Pain is also mediated by activating the afferent fibers in sympathetic nerves. Angiotensin-II increases the release of epinephrine and nor epinephrine from the sympathetic nerve terminals and adrenal medulla by stimulating autonomic ganglia.<sup>[5]</sup>

Most commonly employed pharmacotherapies for painful inflammatory conditions are nonsteroidal antiinflammatory drugs such as aspirin, ibuprofen, acetaminophen, naproxen, iteroxib and so on. These are known to cause side-effects such as erosive gastritis, peptic ulceration, increase in bleeding time, worsening of renal function in renal/cardiac/cirrhotic patients, hyperkalemia, higher risk of stroke, myocardial infarction, and osteoarthritis.<sup>[6]</sup>

Other drugs used to alleviate pain are opioids, which are known for side-effects like sedation, constipation, respiratory depression, tolerance, and dependence.<sup>[7]</sup> Thus, this study is aimed at evaluating drugs in the treatment of pain with less adverse effect and more or equi-efficacious compared to the existing drugs.

Perindopril (ACE inhibitor), which decreases the angiotensin-II formation after absorption is converted into perindoprilat, the active metabolite. It has the half-life of 1-17 h. And the peak plasma concentration is attained after 3-4 h. Perindopril is eliminated via urine (unchanged drug, as metabolite).<sup>[1]</sup>

### Hypothesis

Thus, it may be hypothesized that perindopril (ACE inhibitor) can exhibit analgesic activity by inhibiting the formation of angiotensin-II, decreasing the production of PGE2 and COX 2, decreasing the central sympathetic activity, increasing angiotensin (1-7) and endogenous opioid mechanism.

The aim of this study was

1. To evaluate the analgesic activity of perindopril
2. To compare the analgesic activity with that of standard drug (pentazocine).

The thermal pain was assessed through Eddy's hot plate Desh Biological Works, Ambala, chemical pain through Writhing method and mechanical pain through tail clip method.

## MATERIALS AND METHODS

The study was conducted after getting approval from Institution Animal Ethical Committee (IAEC). CPSEA approval number from IAEC of: JSSMC/PR/IAEC/17/26 (01)/2013-14.

Albino mice of either sex of average weight 30-50 g aged 3-4 months were used in experiments. The albino mice were bred in central animal house of JSS Medical College, Mysore. The study was done in Department of Pharmacology during September 2013. Animals were acclimatized to the laboratory conditions for at least 1 h before testing and were used during experiments. The doses of drugs were based on the human daily dose converted to that of mice according to Paget and Barnes (1962).

### Drugs and chemicals

Perindopril 1 mg (Serdia Pharmaceuticals, India) was dissolved in distilled water immediately before used orally, glacial acetic acid diluted in distilled water to provide 0.06% solution for intraperitoneal injection, pentazocine (Taj Pharmaceuticals, India) and normal saline.

The mice were divided into three groups containing six animals ( $n = 6$ ) in each group (control, standard, and test group). The test drug perindopril 1 mg/kg and normal saline 25 ml/kg was administered orally 2 h prior. Standard drug pentazocine 10 mg/kg was administered intra-peritoneal 15 min prior to the experiment. Significant analgesia of pentazocine occurs between 15 and 30 min.

- Group 1: Normal saline - 25 ml/kg (oral)
- Group 2: Pentazocine - 10 mg/kg (intra-peritoneal)
- Group 3: Perindopril - 1 mg/kg (oral).

### Analgesic activity

#### *Eddy's hot plate*

Mice weighing 20-30 g were used. Mice were placed on the hot plate, which consists of electrically heated surface. Temperature of the hot plate was maintained at 55°C. Responses such as jumping, withdrawal of the paws and licking of the paws were observed. The time period (latency period) when animals were placed and until responses occur was recorded by the stopwatch. Perindopril was administered orally and latency period was recorded after 0, 30, 60, 90 and 120 min. These values were compared with the standard drug pentazocine and control normal saline. This model evaluates the central pain.<sup>[8]</sup>

#### *Writhing method*

Mice weighing 20-30 g were used. Acetic acid 0.06% was injected intraperitoneally in each animal. The animal reacted with a characteristic stretching behavior that is, a series of constrictions occur that travel along the abdominal wall, sometimes accompanied by turning movements of the body and extension of the hind limbs. This response of writhing was recorded. Test group animals were administered perindopril prior to administration of acetic acid intraperitoneally. Later, mice were placed individually into glass chambers and number of writhes were recorded for 15 min. This model evaluates peripheral pain.<sup>[9]</sup>

$$\% \text{ of inhibition} = \frac{\text{Average number of writhes in control group} - \text{writhes in test group}}{\text{Writhes in the control group}}$$

The time period with the greatest percentage of inhibition was considered the peak time.

#### Tail clip method

Mice weighing 25-30 g were used. Haffner's clip was placed at the root of the tail of the mice to apply noxious stimulus. A quick response of the animal was seen as biting the clip or tail, where the clip was placed. The reaction time between application of the clip and the response was noted by a stopwatch. Test drug perindopril was administered orally. After 15, 30 and 60 min, same procedure was repeated and reaction time was measured. This model evaluates the central pain.<sup>[10]</sup>

#### Statistical analysis

The result was analyzed by calculating the mean values, standard deviation, and analysis of variance, *post-hoc* test (Bonferroni). IBM SPSS statistics © IBM Corporation and Other (s) 1989, 2012 software was used for statistical analysis purpose. To test the results of study for the corresponding degrees of freedom the values were compared at 0.05 level of significance.  $P < 0.05$  was considered as significant.

## RESULTS

#### Eddy's hot plate

Thus, the latency period of perindopril was significantly ( $P < 0.05$ ) good when compared to control at time period 30-120 min, whereas the latency period of the standard was more significant ( $P < 0.05$ ) when compared to perindopril at all-time intervals of experimentation [Tables 1a, 1b and 4].

#### Writhing

Perindopril which was given orally 2 h before intraperitoneal injection of acetic acid significantly reduced the number of writhes. Significant inhibition of the writhing response was observed after the administration of perindopril 1mg/kg when compared to normal saline control group.

The number of writhes of perindopril was less when compared to standard, whereas the number of writhes of standard drug (pentazocine) were less when compared to perindopril and normal saline. When compared to control, the percentage inhibition of perindopril was 56.39% and that of the standard was 84.35% [Table 2].

#### Tail clip

Thus, the mean reaction time of perindopril was significantly ( $P < 0.05$ ) good as compared to control at time period 30-120 min, whereas the latency period of the standard was more significant ( $P < 0.05$ ) when compared to perindopril at all-time intervals of experimentation [Tables 3a, 3b and 5].

## DISCUSSION

Pain is an unpleasant sensory and emotional experience associated with actual and potential tissue damage. Pain is produced by the excitation of nociceptors or their afferent free nerve endings. There are two types of pain, fast pain and slow pain, mediated through A $\delta$  fibers and C fibers. Nociception is the mechanism, whereby noxious peripheral stimuli are transmitted to the central nervous system. Nociceptive fibers terminate in the superficial layers of the dorsal horn, forming synaptic connections with transmission neurons running to the thalamus. Nociceptors release glutamate, substance P contributing to neurogenic inflammation.<sup>[11]</sup>

Transmission in the dorsal horn is subjected to various modulations constituting the gate control theory. Descending inhibitory pathways from the midbrain (periaqueductal grey area) and brain stem (nucleus raphe magnus) exert a strong inhibitory effect on dorsal horn transmission. Main transmitters in this pathway are enkephalin and 5-hydroxytryptamine. It causes both presynaptic and postsynaptic inhibition of incoming Type C and Type A $\delta$  pain fibers where they synapse in the dorsal horn.<sup>[12]</sup>

Nerve fibers derived from the periventricular nuclei and from the periaqueductal grey area secrete enkephalin at their endings. Fibers originating in this area send signals to the dorsal horns of the spinal cord neurons to secrete enkephalin. The enkephalin cause both pre synaptic and postsynaptic inhibition of incoming Type C and A $\delta$  pain fibers where they synapse in the dorsal horns. Thus, the analgesic system block pain signals at the initial entry point to the spinal cord.<sup>[13]</sup>

Angiotensin-II acts as an algescic and inflammatory molecule (as described earlier) and increases the sympathetic tone thus aiding to pain. Perindopril (ACE inhibitor) causes analgesic effect by decreasing the central sympathetic activity, inhibiting the synthesis of angiotensin-II and by eliminating the effect of angiotensin-II.

**Table 1a: The analgesic activity of perindopril in thermal pain model-Eddy's hot plate**

Groups	0 min	30 min	60 min	90 min	120 min
Control	0.89±0.07	1.08±0.06	2.1±0.07	2.31±0.04	2.19±0.06
Standard	2.95±0.11*	6.48±0.06*	7.04±0.07*	8.25±0.04*	10.21±0.05*
Perindopril	0.9±0.03	4.04±0.10*	6.01±0.08*	7.03±0.06*	5.44±0.08*

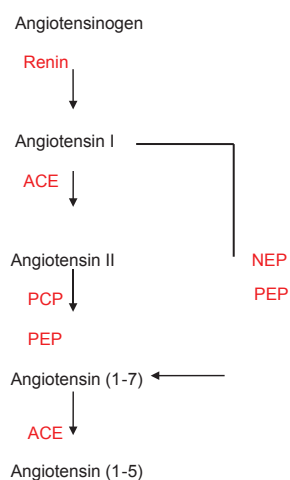
Data is expressed as mean±SD of n=6. \* $P < 0.05$  compared with control (normal saline). SD: Standard deviation

Important endogenous opioid substances are  $\beta$ -endorphan, met-enkephalin, leu-enkephalin and dynorphin. The two enkephalins are found in the brain stem and spinal cord are known to involve in analgesia. Perindopril (ACE-inhibitor) inhibit enkephalinase, this is the peptidase responsible for the hydrolysis of enkephalins, hence increasing the endogenous opioids.<sup>[13,14]</sup> Studies have shown that ACE inhibitors exert analgesic effect due to the action on central nervous system, which increases enkephalin and  $\beta$ -endorphan levels. The visceral antinoceptive effect of ACE inhibitor is due to opioid dependent mechanism.<sup>[15]</sup>

Perindopril (ACE inhibitor) increases the levels of angiotensin (1-7) by following two mechanisms,

1. Bypassing the requisite production of angiotensin-II and,
2. By inhibiting the hydrolysis of angiotensin (1-7).

Perindopril substantially augments circulating levels of angiotensin (1-7) and increases the peptide half-life. Angiotensin (1-7) increases n nitric oxide synthase-derived nitric oxide (NO) levels. Increased NO significantly decreases the discharge rate of spontaneous action potential in dorsolateral-periaqueductal gray (PAG) neurons. The midbrain PAG is a neural site for several physiological functions related to cardiovascular regulation, pain modulation and behavioral reactions.<sup>[16]</sup> Hence, angiotensin (1-7) is considered as an important biologically active component of the renin angiotensin system that plays an inhibitory role in the dorsolateral-PAG via a NO dependent signaling pathway. Therefore, angiotensin (1-7) is involved in pain modulation by acting on PAG through NO dependent signaling.



ACE: Angiotensin converting enzyme, NEP: Neutral endopeptidase, PEP: Prolyl endopeptidase, PCP: Prolyl carboxypeptidase

**Table 3a: The analgesic activity in mechanical pain mode-tail clip method**

Groups	0 min	30 min	60 min	90 min	120 min
Control	0.89±0.07	0.73±0.23	2.81±0.22	2.71±0.16	2.95±0.21
Standard	2.95±0.11*	8.84±0.12*	10.06±0.13*	10.20±0.07*	10.01±0.09*
Perindopril	0.9±0.03	2.03±0.11*	6.14±0.16*	6.50±0.18*	5.53±0.23*

Data are expressed as mean±SD of n=6. \*P<0.05 compared with control (normal saline). SD: Standard deviation

In this study, three analgesic models, acetic acid induced writhing reflex, Eddy’s hot plate and tail clip method were used to evaluate the analgesic activity of perindopril. These models involved the latency period, percent of inhibition and mean reaction time to a painful stimulus. The stimulus in these models are thermal (Eddy’s hot plate), chemical (acetic acid induced writhing) and mechanical (tail clip).

In Eddy’s hot plate model, when compared to control the latency period of perindopril was almost equal at 0 min and gradually increasing from 30 min, peaking at 60 min and 90 min. And the percent analgesic activity of perindopril was gradually increasing from 30 min. Although, the latency period and percent analgesic activity of standard, when compared to perindopril was more at all-time periods.

In writhing model, when compared to control perindopril significantly decreased the number of writhes and the percentage inhibition of 54.39% was observed.

In tail clip model, when compared to control the mean reaction time and percent analgesic activity of perindopril started increasing gradually at 30 min, peaking at 60 min and 90 min there by decreasing at 120 min. While, the mean reaction time and percent analgesic activity of standard, when compared to perindopril was more at all-time periods.

## CONCLUSION

The test drug perindopril shows significant analgesic activity when compared to that of control in all the three

**Table 1b: The percent analgesic activity of perindopril and control when compared to standard-Eddy’s hot plate**

Groups	0 min	30 min	60 min	90 min	120 min
Control	30.16	16.66	29.82	28	21.44
Perindopril	30.50	62.34	85.36	85.21	53.28

Data are expressed in percentage

**Table 2: The analgesic activity of perindopril in visceral pain model-writhing method**

Groups	Number of writhes	Percentage of inhibition
Control	35.16±2.85	0
Standard	5.5±2.42	84.35
Perindopril	15.33±2.16	56.39

Data are expressed as mean±SD of n=6 and in percentage. SD: Standard deviation



**Table 3b: The percent analgesic activity of perindopril and control when compared to standard-tail clip method**

Groups	0 min	30 min	60 min	90 min	120 min
Control	30.16	8.25	27.93	26.56	29.47
Perindopril	30.50	22.96	61.03	63.72	55.24

Data are expressed in percentage

**Table 4: The percent analgesic activity of perindopril when compared to control-Eddy's hot plate method**

Groups	0 min	30 min	60 min	90 min	120 min
Perindopril	0.34	45.68	55.54	57.21	31.84

Data are expressed in percentage

**Table 5: The percent analgesic activity of perindopril when compared to control-tail clip method**

Groups	0 min	30 min	60 min	90 min	120 min
Perindopril	0.34	14.71	33.1	37.16	25.77

Data are expressed in percentage

established experimental models of pain. The analgesic activity was maximum at 60 min and 90 min. The possible mechanism is due to decreasing the central sympathetic tone, increase in the release of  $\beta$ -endorphan and enkephalin levels in the spinal cord, increasing the angiotensin 1-7 levels and decreasing PGE2 and COX 2.

Thus, to conclude, perindopril possibly exhibits its analgesic activity both by central analgesic activity (Eddy's hot plate and tail clip) through release of  $\beta$ -endorphan and enkephalins and also peripheral analgesic action (writhing method) through inhibition of COX 2 and PGE2.

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