ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Analgesic Activity of Fosinopril in Albino Mice.

Siddamma A, and Lokraj Subedee*

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

ABSTRACT

To evaluate the analgesic activity of fosinopril in chemical, thermal and mechanical pain on swiss albino mice. Fifty four albino mice (Swiss strain) weighing 25-30 grams were allocated to each experimental model and in each model there were three groups. The control group received normal saline (25ml/kg) per orally, standard group received pentazocine (10mg//kg) intra -peritoneal and test groups received fosinopril (5mg/kg) per orally. Fosinopril and normal saline was administered 2 hr before, while 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. Fosinopril decreased the number of writhes, delayed the reaction time in tail clip and eddy's hot plate method considerably when compared to control (normal saline) but less when compared to standard (pentazocine). Fosinopril exhibits analgesic activity in thermal, chemical and mechanical pain models in albino mice.

Keywords: Fosinopril, angiotensin II, analgesic activity, opioid and Angiotensin – (1-7).

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Angiotensin converting enzymes (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 medications of choice in management of hypertension and cardiac failure [1].

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, leukotrienes and platelet activating factors [2].

Angiotensin-II regulates vascular tone, stimulates the release of pro-inflammatory cytokines, activates NF- κB, increases oxidant stress and thus, it functions as an inflammatory molecule. Ang-II increases the release of reactive oxygen species (ROS).ROS activate NF-κB (nuclear factor-kappa B, known to initiate inflammatory process) that increases the transcription of pro-inflammatory cytokines, adhesion molecules, and NADPH oxidase. Ang-II enhanced ROS production by activating NADPH oxidase and stimulated the DNA-binding activity of NF- κB in human neutrophils. Ang II increases the synthesis and concentrations of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and chemokine monocyte chemo attractant protein-1 (MCP-1), elevated tissue levels of NF-κB, and inflammatory cell infiltration. These events ultimately cause inflammation [3].

Angiotensin II increases the formation of PGE2 by inhibiting the enzyme PGE 9-ketoreductase and increasing the cyclo-oxygenase 2 (COX 2) activity by increasing cyclic AMP (adenosine monophosphate).PGE2 sensitizes pain receptors at efferent nerve endings to mediators of pain and amplify algesia [4].

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

Most commonly employed pharmacotherapies for painful inflammatory conditions are NSAIDS like aspirin, ibuprofen, acetaminophen, naproxen, iterocoxib etc. are known to cause side effects like erosive gastritis and peptic ulceration, increase in bleeding time, worsening of renal function in renal/cardiac and cirrhotic patients, hyperkalemia, higher risk of stroke, myocardial infarction and osteoarthritis [6].

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

Fosinopril (ACE inhibitor) which decreases the Angiotensin II formation after absorption is converted into fosinoprilat, the active metabolite. It has the half-life of 12hrs. and the peak plasma concentration is attained after 3 hrs. Fosinoopril is eliminated via urine and feces [8].

HYPOTHESIS

Thus it may be hypothesized that fosinopril (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 the study of the present study was

- To evaluate the analgesic activity of fosinopril
- To compare the analgesic activity with that of standard drug (pentazocine).

The thermal pain was assessed through Eddy's hot plate, chemical pain through writhing method and mechanical pain through tail clip method.

MATERIALS AND METHODS

The study was conducted after the approval of IAEC (Institution Animal Ethical Committee).

Albino mice of either sex of average weight 30-50gms 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 hr 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

Fosinopril 5mg (Candila pharmaceuticals, India) was dissolved in distilled water immediately before used orally, glacial acetic acid diluted in distilled water to provide 0.6% solution for i.p injection, Pentazocine (Taj pharmaceuticals, India) and Normal saline.

The mice were divided into 3 groups containing 6 animals (n=6) in each group [control, standard and test group].The test drug fosinopril 5mg/kg and normal saline 25ml/kg was administered orally 2 hr. prior. Standard drug pentazocine 10mg/kg was administered intra peritoneal 15 min prior to the experiment. Significant analgesia of pentazocine occurs between 15 to 30 min.

Group 1:-Normal saline- 25ml/kg (oral)
Group 2:-Pentazocine-10mg/kg (intra peritoneal)
Group 3:- Fosinopril-5mg/kg (oral)

Analgesic activity

Eddy's hot plate

Mice weighing 20-30gms were used. Mice were placed on the hot plate, which consists of electrically heated surface. Temperature of the hot plate was maintained at 55oC.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. Fosinopril was administered orally and latency period was recorded after 0min, 30min, 60min, 90min and 120min.These values were compared with the standard drug pentazocine and control normal saline.This model evaluates the central pain.[9]

Writhing method

Mice weighing 20-30gms were used. Acetic acid 0.06% was injected intraperitonally in each animal. The animal reacted with a characteristic stretching behavior,i.e., 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 fosinopril prior to administration of acetic acid intraperitonally. Then mice were placed individually into glass chambers and numbers of writhes were recorded for 15 min.This model evaluates peripheral pain.[10]

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

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

Tail clip method

Mice weighing 25-30gms 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 clip has been placed. The reaction time between application of the clip and response was noted by a stopwatch. Test drug fosinopril was administered orally. After 0, 15, 30 and 60 min, same procedure was repeated and reaction time was measured.This model evaluates the central pain[11]

Statistical analysis

The results was analyzed by calculating the Mean values, Standard Deviation, and the analysis of variance (ANOVA), post hoc test (Bonferroni). The values was compared at 0.05 level

ISSN: 0975-8585

of significance to test the results of the study for the corresponding degrees of freedom. P<0.05 will be considered as significant.

RESULTS

Eddy's hot plate

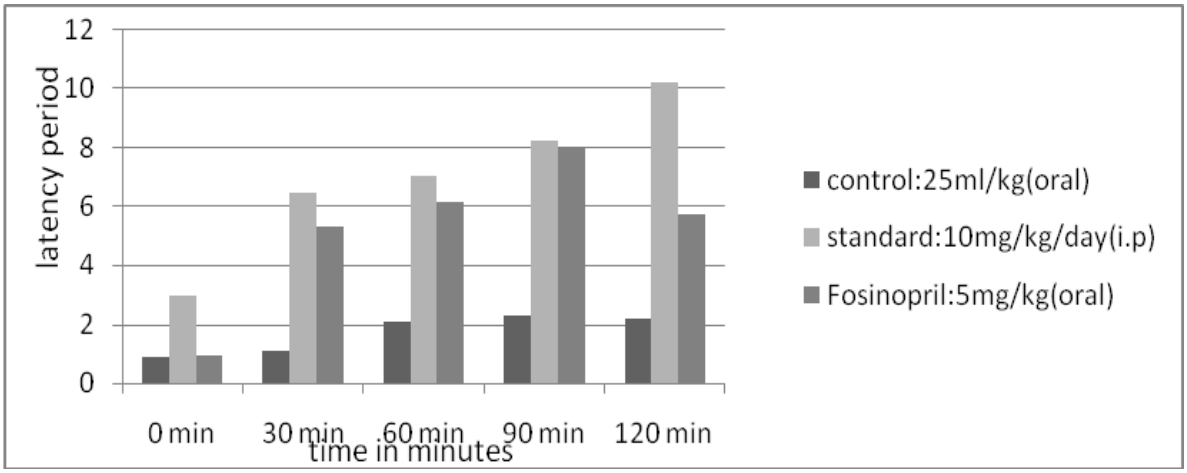
Table 1:-The analgesic activity of fosinopril in thermal pain model - Eddy's hot plate

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
Fosinopril	0.92±0.04	5.32±0.05	6.18±0.04*	8.02±0.03*	5.72±0.05

Data is expressed as mean ± SD of n=6, *p<0.05

The latency period of the standard drug (pentazocine) was more when compared to fosinopril at all the time intervals.The latency period of fosinopril was insignificant at 0 min, but started increasing there after upto 120 min,showing peak activity at 60min and 90 min.Thus, the latency period of fosinopril was significantly good compared to control at all time periods i.e.,30 to 60 min and less significant compared to standard at all time intervals of experimentation.

Graph 1: **The analgesic activity of fosinopril in thermal pain model - Eddy's hot plate**



Writhing

Fosinopril which is given orally 2hr before i.p injection of acetic acid significantlyreduced the number of writhes. Significant inhibition of the writhing response by was observed after the administration of fosinopril 5mg/kg as compared to normal saline control group.

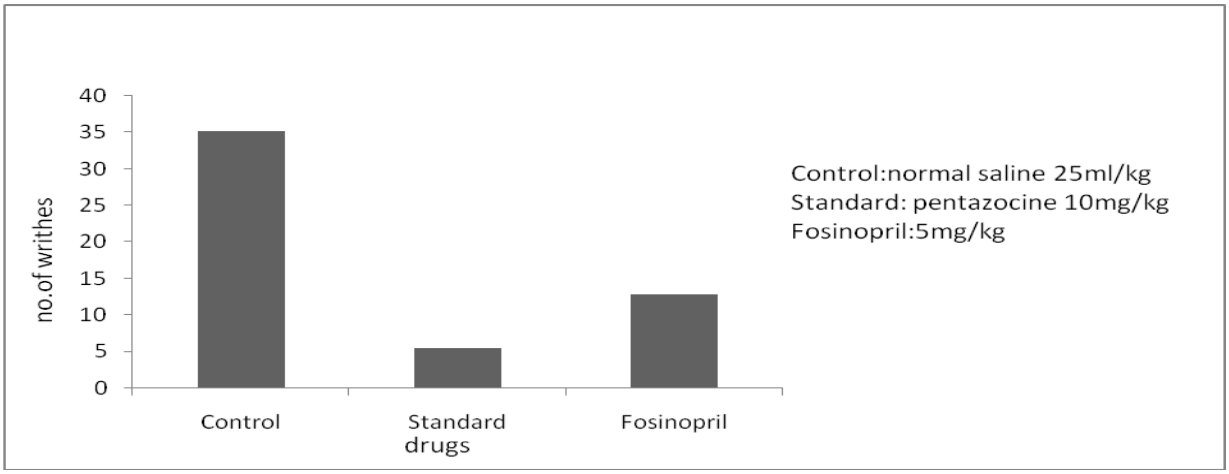
ISSN: 0975-8585

Table 2.The analgesic activity of fosinopril in visceral pain model - writhing method

Groups	No. of writhes	% of inhibition
Control	35.16±2.85	0
Standard	5.5±2.42	84.35
Fosinopril	12.8±1.47	63.59

Data is expressed as mean ± SD of n=6,

Graph 2: The analgesic activity of fosinopril in visceral pain model - writhing method



The no. of writhes of standard drug (pentazocine) is less when compared to test drug fosinopril and normal saline. However, no. of writhes of fosinopril was also less when compared to that of control and more when compared to standard.

TAIL CLIP

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

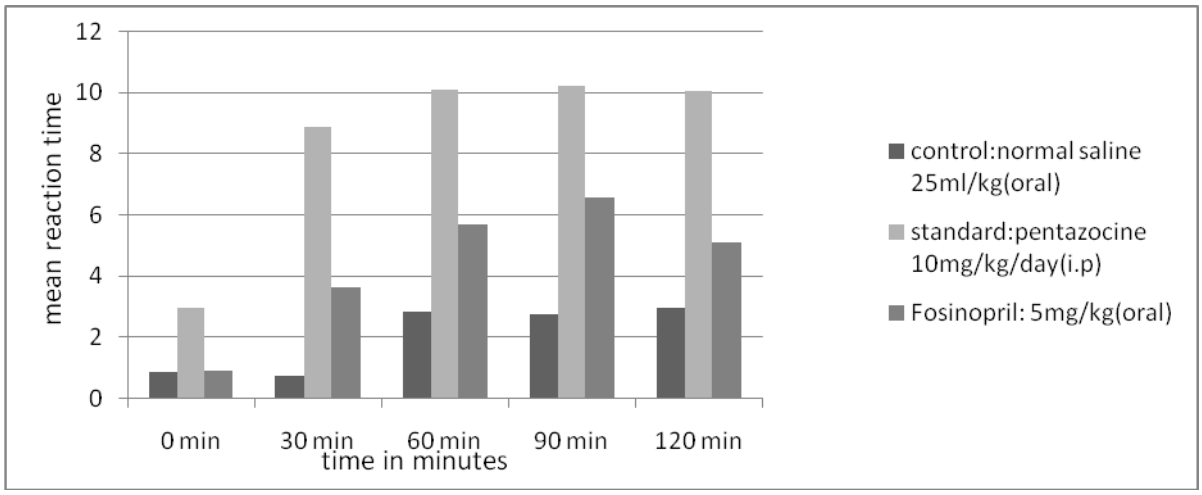
Groups	0 min	30min	60min	90min	120 min
Control	0.89±0.07	0.73±0.23	2.81±0.22	2.71±0.16	2.95±0.21
Standard	2.95±0.11	8.84±0.12	10.06±0.13	10.20±0.07	10.01±0.09
Perindopril	0.88±0.05	3.61±0.08	5.66±0.09*	6.55±0.08*	5.07±0.07

Data is expressed as mean ± SD of n=6, *p<0.05

The mean reaction time of the standard drug (pentazocine) was more when compared to fosinopril at all the time intervals.The mean reaction time of fosinopril was insignificant at 0 min, but started increasing there after upto 120 min,showing peak activity at 60 min and 90 min.Thus, the latency period of fosinopril was significantly good compared to control at all time periods i.e.,30 to 60 min and less significant compared to standard at all time intervals of experimentation.

ISSN: 0975-8585

Graph 3: The analgesic activity in mechanical pain mode - tail clip method.



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 Aδ 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 [12].

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-HT.It causes both presynaptic and post synaptic inhibition of incoming Type C and type Aδ pain fibers where they synapse in the dorsal horn [13].

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 post synaptic inhibition of incoming Type C and Aδ 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 [13].

Angiotensin II acts as an algesic and inflammatory molecule (as described earlier) and increases the sympathetic tone thus aiding to pain. Fosinopril (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.

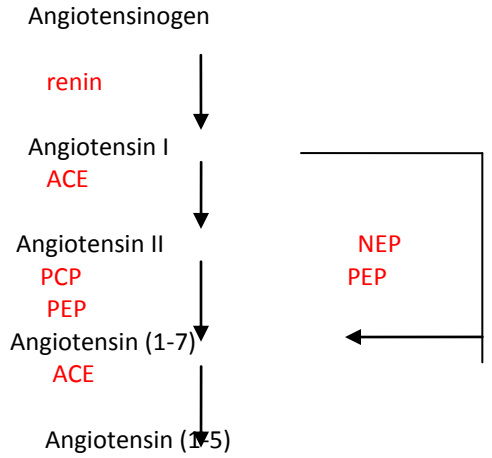
ISSN: 0975-8585

Important endogenous opioid substances are β-endorphan, met-enkaphalin, leu-enkephalin and dynorphin.The two enkephalins are found in the brain stem and spinal cord are known to involve in analgesia.Fosinopril (ACE-inhibitor) inhibit enkephalinase, this is the peptidase responsible for the hydrolysis of enkephalins, hence increasing the endogenous opioids [14, 15] .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 ACE inhibitor is due to opioid dependent mechanism [16].

Fosinopril (ACE inhibitor) increases the levels of Angiotensin (1-7) by following 2 mechanisms,

- Bypassing the requisite production of Angiotensin II and,
- By inhibiting the hydrolyses of Angiotensin (1-7).

Fosinopril substantially augment circulating levels of Angiotensin (1-7) and increase the peptide half-life. Angiotensin (1-7) increase nNOS-derived NO levels. Increased NO significantly decreases the discharge rate of spontaneous Action Potential in dorsolateral-PAG neurons. The midbrain periaqueductal gray (PAG) is a neural site for several physiological functions related to cardiovascular regulation, pain modulation and behavioral reactions [17].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

CONCLUSION

The test drug fosinopril shows significant analgesic activity when compared to that of control in all the 3 established experimental models of pain.. The activity was maximum at 60

min and 90 min.The possible mechanism is due to decreasing the central sympathetic tone, increase in release of β endorphan and enkephalin levels in the spinal cord increasing the Angiotensin 1-7 levels and decreasing PGE2 and COX 2 action.

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

REFERENCES

[1] Randa hilal-dandan, Lawrencebruton. Renin and angiotensin .The pharmacological basis of therapeutics, 12 th edition. China: Mcgraw hill;2011.721-723.

[2] VinayKumar, Abul K Abbas, Nelson Fausto. Robbins, Cotran. Acute and Chronic Inflammation..Robbins and Cotron Pathological Basis of Diseases,7thedition.Noida : Elseiver ;2008.p.48-55

[3] UN Das. JAPI 2005;53:472-4

[4] Helmy M. Siragy and Robert M. Carey. J Clin Invest 1996;97(8):1980-82

[5] Richard Harvey, Pamela Champe. Drugs affecting the cardiovascular system. Lippincott's illustrated reviews, 4th edition. New Delhi: Lippincott Williams and Wilkins;2009.p.184-189

[6] Daniel E Furst, Robert D Ulrich Cissy Varkey- Alta miranong. Non-steroidal anti-inflammatory drugs,. Basic and clinical pharmacology,11thedition.Mumbai:McGraw Hill;2009.p.181-185

[7] Mark.A.Schumacher Allan I Basbaum Walter L Way . Opoid Analgesic and Antagonist. Basic and clinical pharmacology,11th edition. Mumbai: McGraw Hill;2009.622-629

[8] Randa hilal-dandan, Lawrence bruton. Renin and angiotensin. The pharmacological basis of therapeutics,12thedition.China: Mcgraw hill;2011.p.733-735

[9] Shravan Kumar G, RajeshK, Sengottuvelu S. Int Res J Pharm 2011;2(12):230-4

[10] Koster R, Anderson M, De-Beer EJ. Fed Proc1959;18:412–8.

[11] Bianchi C, Francbschini J. Br J Pharmacol Chemother 1954;9:280–284.

[12] SK Gupta. Analgesic agents. Drug screening methods ,2nd edition. New Delhi: Jaypee, 2009.p.462-468

[13] John E Hall. Somatic sensations II pain,headache and thermal sensations. Text book of medical physiology,12th edition . New Delhi: Saunders ;2012.p.583-588

[14] Rabinowitz I, Reis S. Isr Med Assoc J 2001;3(12):963-4.

[15] Keller S, Frishman WH. Cardiol Rev 2003;11(2):73- 93.

[16] Omar M E Abdul Salam Siham El-ShenaveySalwa M Nofal. J Pharmacol Toxicol 2007;2(6):533-541

[17] Jihong Xing, JianKong, Jianhua Li. Neurosci Lett 2012; 522(2): 156–161.