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Research Article

EVALUATION OF ANALGESIC ACTIVITY OF BENZAPRIL IN ALBINO MICE

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ABSTRACT

Objective: To evaluate the analgesic activity of benzapril in chemical, thermal and mechanical pain onswiss albino mice

Methods: Thirty 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 group received benzapril (5mg/kg) per orally. Benzapril and normal saline was administered 1 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.

Results: Benzapril 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).

Conclusion: Benzapril exhibits analgesic activity in thermal, chemical and mechanical pain models in albino mice.

Keywords: Benzapril, Angiotensin II, Analgesicactivity, Opioid and Angiotensin – (1-7).

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 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 increasese 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, peptic ulceration, increase in bleeding time, worsening of renal function in renal/cardiac/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.

Benzapril (ACE inhibitor) which decreases the angiotensin II formation. It has the half-life of 10-11hours and the peak plasma concentration is attained after 1-2 hrs. Benzapril is eliminated by renal excretion. Some amount is also excreted non renal (biliary). [8]

Hypothesis: Thus it may be hypothesized that benzapril (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 present study was

1) To evaluate the analgesic activity of benzapril

2) 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). IAEC: 26(A)

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 1hr 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: Benzapril 5mg(Novartis, Mumbai) 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 containing6 animals (n=6) in each group[control, standard and test group].The test drug benzapril 5mg/kg and normal saline 25ml/kg was administered orally

1hr. 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:-Benzapril-5mg/kg (oral)

Analgesic activity

1) 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 55^{o}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. Benzapril are administered orally and latency period is 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]

2) Writhing method: Mice weighing 20-30gms wereused. 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 benazepril 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]

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

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

3) 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 benzapril was administered orally. After 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 were analyzed by calculating the Mean values, Standard Deviation, and the analysis of variance (ANOVA), post hoc test (Bonferroni). The value was compared at 0.05 level of significance to test the results of the study for the corresponding degrees of freedom. P<0.05 will be considered as significant.

RESULTS

1) Eddy's hot plate

Table 1: Results of benzapril by hot plate reaction time in mice

	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
Benzapril	0.90±0.03	5.2±0.05	6.12±0.05*	8.09±0.03*	5.71±0.04

Values are givenas mean± SD for groups of six animals; * p<0.05

Table 2: Results of benzapril by writhing method in mice

Groups	No. of writhes	% of inhibition
Control	35.16±2.85	0
Standard	5.5±2.42	84.35
Benzapril	13.6±1.63	61.14

Values are givenas mean± SD for groups of six animals

3) Tail clip

Table 3: Results of benzapril by tail clip method in mice

Groups	30min	60min	90min	120 min
Control	0.73±0.23	2.81±0.22	2.71±0.16	2.95±0.21
Standard	8.84±0.12	10.06±0.13	10.20±0.07	10.01±0.09
Benzapril	3.10±0.03	5.53±0.23*	6.28±0.27*	5.01±0.06

Values are givenas mean± SD for groups of six animals; *p<0.05

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

The latency period of the standard drug(pentazocine)was more when compared to benzapril atall-time intervals.

The latency period of benzapril was insignificant at 0 min, but started increasing thereafter up to 120 min, showing peak activity at 60 min and 90 min.

Thus, the latency period of benzapril was significant 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.

2) Writhing: Benzapril which is given orally 1 hour before intra peritoneal injection of acetic acid significantly reduced the number of writhes. Significant inhibition of the writhing response was observed after the administration of benzapril 5mg/kg as compared to normal saline control group.

The mean reaction time of the standard drug (pentazocine) was more when compared to benzapril at all the time intervals. The mean reaction time of benzapril was insignificant at 0 min, but started increasing thereafter up to 120 min showing peak activity at 60 min and 90 min. Thus, the latency period of benzapril 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.

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. Fast pain is by Aδfibers and slow pain is by 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 .Benzapril (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.

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. **B**enzapril (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]

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

1) Bypassing the production of Angiotensin II and

2) By inhibiting the hydrolyses of Angiotensin(1-7).

Benzapril(ACE inhibitor) 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.

CONCLUSION

The test drug benzapril shows significant analgesic activity when compared to that of control in all the 3 established experimental models of pain. The maximum activity was at 60 min and 90 min.The possible mechanism is by decreasing the central sympathetic tone, increase in release of β endorphin and enkephalin levels in the spinal cord increasing the Angiotensin 1-7 levels and decreasing PGE2 and COX 2 action. Thus, to conclude, perindopril exhibits its analgesic activity both by central analgesic activity (Eddy's hot plate and tail clip) through release of β endorphin and enkephalins and also peripheral analgesic action (writhing method) through inhibition of COX 2 and PGE2.

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