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EFFECTS OF CAPTOPRIL AND LOSARTAN ON THERMAL AND CHEMICAL INDUCED PAIN IN MICE

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Abstract : Angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers antagonists (ARAs) are widely used compounds in various cardiovascular disorders. ACEIs, but not ARAs, inhibit the enzyme dipeptidyl carboxypeptidase which is involved in the conversion of angiotensin I to II and degradation of kinins like bradykinin and substance P. Bradykinin and substance P are potent mediators of inflammation and pain. Hence the study was undertaken to evaluate the effects of captopril (an ACEI) and losartan (an ARA-AT_1 receptor antagonist) on thermal and chemical induced nocioception by employing hot plate and acetic acid induced writhing tests respectively in mice. Inbred albino mice weighing between 25-30 g were used and they were divided into two sets, each set containing 7 groups. Control groups received normal saline and the remaining six groups received three doses (0.5, 1 and 2 mg/kg) of captopril and three doses (0.5, 1 and 2 mg/kg) of losartan. Drugs were administered intraperitoneally fifteen minutes before placing the animal over the hot plate or 30 minutes before injecting 0.6% acetic acid. Both drugs dose dependently reduced the reaction time in hot plate method. In chemical induced writhing test, both the drugs reduced the latency of onset of writhing and in captopril pretreated groups, acetic acid induced sustained abdominal contraction without any intermittent relaxation. However, in losartan pretreated animals acetic acid just increased the number of writhings without sustained abdominal contraction. Thus, our study suggests that both drugs have hyperalgesic effects.

Key words : captopril losartan writhing nocioception hyperalgesia hot plate

INTRODUCTION

Angiotensin converting enzyme inhibitors (ACEIs) and Angiotensin receptor antagonists (ARAs) are widely used compounds in various cardiovascular disorders (1). Angiotensin converting enzyme (carboxy - dipeptidylpeptidase), converts angiotensin - I to angiotensin - II and also involved in degrading the kinins like bradykinin and substance P (1). Thus ACEIs, but not ARAs, decrease the plasma

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concentration of angiotensin-II and increase the tissue concentration of kinins. The role of Angiotensin II in pain perception is not clear. It is reported to produce analgesia on intra-cerebro-ventricular administration that could be blocked by naloxone (2). Evidence also indicates that angiotensin II has pro-nocioceptive activity and has anti-opioid activity (3). Involvement of bradykinin in inflammation and pain is well established. Bradykinin is a potent mediator of inflammation and pain (4, 5). Both preclinical and clinical studies have indicated the hyperalgesic effect of ACEIs (6, 7, 8) but such reports for ARAs like losartan is limited. A clinical study (9) has reported that both the angiotensin converting enzyme inhibitor enalapril and the AT1 receptor blocking agent losartan acted similarly on pain threshold, pain sensitivity being increased during these two anti-hypertensive treatments. The blood pressure reduction during drug treatment could not be accounted for the pain sensitivity changes observed. On the other hand a preclinical study (10) has suggested the antinocioceptive effects of ACEIs like spirapril, trandolapril (but not enalapril) and an ARA, losartan. These conflicting results tempted us to evaluate and compare the effects of captopril and losartan on chemical and thermal induced pain by employing hot plate and acetic acid induced writhing tests in mice.

MATERIAL AND METHODS

Animals : Inbred albino mice (Swiss strain) weighing between 25–30 g of either sex were used for the study. They were housed in clean polypropylene cages in groups of four and maintained at room temperature between 27–31°C with standard laboratory feed and water ad libitum. Animals were divided into two sets. Each set consisting of 7 groups with 6 animals in each group. Group I animals were administered normal saline (vehicle), Groups II to IV received captopril 0.5, 1 and 2 mg/kg and Groups V to VII received losartan 0.5, 1 and 2 mg/kg body weight respectively. The ethical clearance for the use of animals was obtained from the committee constituted for the purpose.

Drags : Both test drugs, captopril (Wokhardt Ltd., Mumbai) and losartan (Wokhardt Ltd., Mumbai) were dissolved in normal saline and injected intraperitoneally. The vehicle and the drugs were administered in the volume of 10 ml/kg body weight.

Methods :

a. Hot plate method (11)

In this method heat is used as a source of pain. All animals were placed individually on the Eddy's hot plate (Techno instruments, India) maintained at constant temperature, 55°C and the time taken by the animal for the reaction either by licking the paw or jumping or raising the limbs which ever was observed first taken as the end point. Animals having basal reaction time not exceeding 15 seconds were included in the study. Reaction time was noted before and 15, 30, 60, 90 and 120 minutes after the drug or vehicle administration in each animal.

b. Writhing test (12)

Freshly prepared 0.6% acetic acid

solution in the volume of 10 ml/kg was administered intraperitoneally to each animal which received either the vehicle (Group I) or the test drugs captopril (Groups II–IV) or losartan (Groups V–VII) 30 minutes before the challenge. The time of onset writhing and the number of abdominal contractions or writhing in the following 15 minutes were recorded.

Statistical analysis

All data were expressed as mean ± SEM. and was analyzed by using one-way ANOVA followed by Turkey-Kramer multiple comparison test. P values < 0.05 were considered significant as compared to control vehicle treated group.

RESULTS

Hot plate method : (Table I) Captopril produced dose dependent reduction in basal reaction time at all doses tested (0.5, 1 and 2 mg/kg) throughout the period of observation (till 120 minutes) when compared to vehicle treated control group. Losartan also at the entire doses tested (0.5, 1 and 2 mg/kg) dose dependently decreased the reaction time throughout the period of observation (120 minutes), except, with 0.5 mg/kg dose, where at the end of 120 minutes the reduction in reaction time was not significant.

Writhing test : (Table II) In comparison to control, a dose dependent reduction in the time for onset of writhing induced by intraperitoneal administration of 0.6% acetic acid was observed at all the doses tested in the Captopril pretreated groups ($P<0.05$). The abdominal contraction induced by acetic acid was sustained and all animals remained immobile throughout the period of observation (15 minutes) in these groups of animals.

In losartan pretreated groups (Groups V, VI and VII), time for onset of writhings with 0.6% acetic acid was significantly reduced ($P<0.05$). The number of abdominal writhings when compared to vehicle treated control group showed a significant increase ($P<0.05$).

TABLE I: Effects of captropril and losartan on basal reaction time of mice in hot plate method.

Drug (N)	*Dose/kg*	*Reaction time before drug admn*	*Reaction time after drug admn*				
			15 min	*30 min*	*60 min*	*90 min*	*120 min*
Control normal saline (6)	10 ml	3.09±0.31	2.63±0.34	3.21±0.26	4.40±1.18	3.15±0.52	3.79±0.79
Captopril (6)	0.5 mg/kg	3.35±0.24	1.52±0.13**	1.68±0.29***	1.51±0.40*	1.63±0.47*	1.04±0.14***
Captopril (6)	1.0 mg/kg	2.66±0.42	0.84±0.10***	1.27±0.20***	1.48±0.36*	1.15±0.11*	0.90±0.09***
Captopril (6)	2.0 mg/kg	3.24±0.14	0.78±0.12***	0.86±0.09***	0.93±0.10**	0.90±0.03**	0.90±0.08***
Losartan (6)	0.5 mg/kg	3.32±0.22	1.38±0.17**	1.25±0.13***	1.41±0.19*	1.48±0.02**	2.26±0.24
Losartan (6)	1.0 mg/kg	3.17±0.31	1.39±0.15**	1.28±0.13***	1.39±0.25*	1.20±0.11***	1.75±0.11*
Losartan (6)	2.0 mg/kg	2.61±0.37	1.35±0.10**	1.26±0.08***	1.30±0.19*	1.09±0.05***	1.25±0.10**

All value are Mean±SEM, *=P<0.05; **=P<0.01; ***=P<0.001.

TABLE II : Effects of captropril and losartan on 0.6% acetic acid induced abdominal writhings in mice.

Drugs	*Dose/kg*	*Time for onset of writhing (In minutes)*	*Total number of writhings in 15 minute*
Control normal saline (6)	10 ml	3.78±0.54	28.833±6.590
Captopril (6)	0.5 mg/kg	0.82±0.11*	Sustained abdominal contraction
Captopril (6)	1.0 mg/kg	0.79±0.14*	Sustained abdominal contraction
Captopril (6)	2.0 mg/kg	0.39±0.03*	Sustained abdominal contraction
Losartan (6)	0.5 mg/kg	1.25±0.09*	56.66±2.45*
Losartan (6)	1.0 mg/kg	0.93±0.30*	73.16±2.78*
Losartan (6)	2.0 mg/kg	0.41±0.017*	93.66±2.33*

Values are Mean±SEM, *=P<0.001.

DISCUSSION

The present study suggests that both captopril and losartan at the doses studied have hyperalgesic effect in both the experimental models studied. On dose-to-dose basis captopril exhibited higher and long lasting hyperalgesic effect than losartan as indicated by the persistent reduction in reaction time in hot plate and the quick onset of writhing and sustained abdominal contraction in acetic induced writhing tests.

The role of angiotensin II in pain perception is not clear. Modulations of pain with ACE inhibitors suggest that there is interplay between renin angiotensin system and pain perception (2, 6). Intra-cerebro ventricular administration of angiotensin has been shown to produce analgesic effect, which could be blocked by naloxone (2). Evidence also indicates that angiotensin II by acting centrally at area postrema regulates the blood pressure and pain perception (13, 14). In hypertensive patients who were showing the hypalgesia, enalapril treatment normalized the pain perception (8). When spontaneously hypertensive rats are treated with enalapril and losartan they reduced the hot plate latency (15). All these evidence suggest the role of angiotensin in pain and the hyperalgesic effect of drugs that reduce the angiotensin activity (15). Our findings do not go with the findings of Takai et al (10) who has stated that on single administration of ACEIs like spirapril, trandolapril (but not enalapril) and an ARA, losartan have no effect on pain perception, while chronic administration they had antinocioceptive effect.

Kinins and prostaglandins are important chemical substances involved in inflammation and pain perception. Evidence indicates the involvement of bradykinin and prostaglandins in chemical induced pain (4, 5, 7, 16). Captopril has been shown to antagonize kaolin induced writhing which is associated with increased prostaglandin level (16). Thus the hyperalgesia produced by ACE inhibitors like Captopril might be due to – 1) decreased angiotensin II concentration. 2) increased bradykinin and 3) enhanced prostaglandin level.

There is not much data about the enhanced activity of bradykinin and prostaglandins with the administration of losartan. However, an experimental study found an increased renal bradykinin concentration following AT1 receptors blockade, due to the unopposed action of angiotensin II on AT2 receptors (17) hence,

the possibility of involvement of bradykinin and prostaglandins in hyperalgesic effect of ARAs is yet to be determined.

Both these drugs are the commonly used anti- hypertensives. Patients who are on these drugs may show higher sensitivity to various noxious stimuli. Our findings suggest that both drugs enhance the pain perception. Care should be taken while prescribing these drugs to patients in the presence of pain.

Enhanced sensitivity to pain by ACE inhibitors and angiotensin receptor blockers may be helpful. Hypertensive patients show a reduced sensitivity to pain (8) and a generalized impairment of pain sensitivity has been associated with silent ischemia (18, 19, 20). ACE inhibitors and the angiotensin receptor blockers by facilitating the perception of painful stimuli, at least in hypertensive patients with coronary artery disease, may possibly interfere with silent episodes (8).

To conclude, both captopril and losartan showed hyperalgesic effect in both experimental models, thermal and chemical induced pain in mice. It can also be inferred that captopril produced more hyperalgesia than losartan on dose to dose basis.

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