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RESEARCH ARTICLE

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GASTRIC ULCER PROTECTIVE PROPERTY OF CALCIUM CHANNEL BLOCKERS IN MALE ALBINO RATS

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ABSTRACT

Calcium ions have been considered as a serious contender for the development of various types of ulcers. Calcium ion influx seems to play an essential role in the stimulation-secretion coupling in mammalian oxyntic cells, an effect that can be inhibited by the calcium channel blockers. Calcium channel blockers (CCBs) are a class of drugs and natural substances that disrupt the calcium (Ca2+) conduction of calcium channels, the present study aims to evaluate comparative effect of Verapamil, Nifedipine, Diltiazem and Ranitidine against gastric ulcers induced in rats. The study was carried out in Sixty Wistar male albino rats (180–220 g) as two phase simultaneous study, one as acute and the other evaluated chronic effect. Gastric Ulcer was induced using aspirin (200 mg/kg) body weight (b.w) and treated orally using Verapamil (40mg/kg b.w) Nifedipine (40mg/kg b.w), Diltiazem (60mg/kg b.w) and Inj. Ranitidine 50mg/kg b.w intraperitoneally. The rats were anaesthetised under ether; Ulcer Pulse and Index were calculated.The results of comparison indicate that Nifedipine had shown but highly significant ulcer protective effect in both acute and chronic studies, than Verapamil, Diltiazem and Ranitidine.

KEYWORDS

Calcium channel blockers, Anti-ulcer property, Verapamil, Nifedipine, Diltiazem, Ranitidine

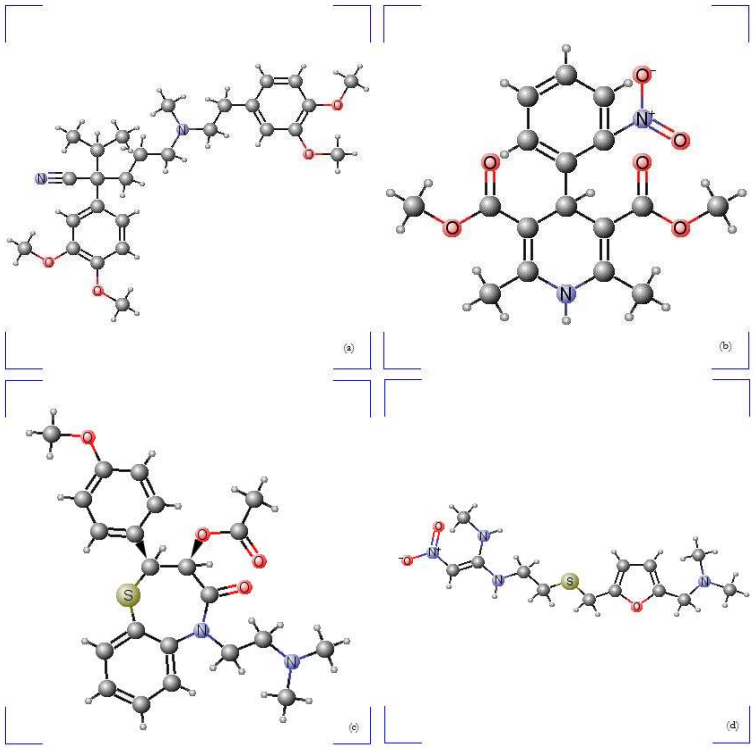
INTRODUCTION

Calcium ions play an important role in many exocrine and endocrine tissues. There is evidence that both gastric acid secretion and gastrin release are calcium dependent processes. The administration of calcium both orally or intravenously, stimulates acid secretion and increases circulating concentration of gastrin[1]. Stimulation of acid secretion by the parietal cells occurs by at least three major pathways: the cholinergic transmitter acetylcholine: histamine, which is locally released in the gastric epithelium and the hormone gastrin. The effect of histamine is mediated by increasing adenylate cyclase activity, whereas the effects of the acetylcholine and gastrin seem to involve an increase in cytosolic free calcium[2].

It has effects on many excitable cells of the body, such as cardiac muscle, i.e. heart, smooth muscles of blood vessels, or neurons. Drugs used to target neurons are used as antiepileptics. The main clinical usage of calcium channel blockers is to decrease blood pressure. It is for this action that they are used in individuals with hypertension. Our aim in this study was to find out the gastric ulcer protective property of calcium channel blockers in animal models (albino rats). The drugs used in this study Verapamil, Nifedipine, and Diltiazem. At present calcium channel blockers are mainly used for the management of cardiovascular disease like angina pectoris, arrhythmias, systemic hypertension and deceleration of atherosclerosis[3]. Some evidences show that there is a strong association of cardiovascular disease and peptic ulcer[3] and patients with atherosclerotic vascular disease have an increased risk of gastric and duodenal ulcers.

The frequency of gastric or duodenal ulcers has been found to be three fold higher in patients with abdominal aortic aneurysm than in a control population. The association of the peptic ulcer and cardiovascular disease may be related by common association with smoking personality trait or type A behaviour[4]. In addition to this, patients with peptic ulcer and angina pectoris have been noted to have increased urinary excretion of catecholamines, a stress related hormone. The association with of cardiovascular disease may also imply that peptic ulceration is related to the circulatory changes[5]. Some of the anti-hypertensive drugs produce undesirable gastric effects like gastric irritation or duodenal ulcer and even serious gastro intestinal bleeding. For example, the catecholamine depletors like reserpine and the vasodilators like hydrallazine are more prone to produce peptic ulcer. The loop diuretics like ethacrynic acid produce gastro intestinal bleeding[4]. The most commonly used anti-anginal drug, nitrates, produce gastric ulcer and there is a strong epidemiologic correlation between the incidence of esophageal and gasric carcinoma and the nitrate content. The most commonly used anti hypertensive anti-anginal drugs are capable of producing undesirable gastric effects, but the calcium channel blocking drugs are safe in this respect. This leads us to think whether they really possess any gastric antiulcer activity in addition to cardiovascular activities. In addition to that, as there is a strong link between ischaemic heart disease and peptic ulcer, an anti ulcer therapy will be needed along with the used anti-anginal drug like nitrates[4]. If the calcium channel blockers are having the antiulcer effect as the other standard anti ulcer drugs like H2 blockers, they can be safely used for patients having hypertension and angina along with peptic ulcer disease for their dual action.

Figure 1
2D Structure of (a) Verapamil (b) Nifedipine (c) Diltiazem (d) Ranitidine



Retrospective studies have indicated that as many as 30% of patients with hyperparathyroidism may suffer from peptic ulcer. As a potential mechanism for a positive association, it has been suggested that hypercalcemia not only directly stimulates the gastric parietal cells, but also enhances the release of gastrin and its secretagogue effect on the stomach[5].

Calcium channel blockers have been tried for diseases like bronchial asthma, subarachnoid haemorrhage, intermittent claudication, migraine, peptic ulcer and Raynaud's phenomenon[6], though peptic ulcer is very common in our country, the information about the use of calcium channel blockers in patients with hyper secretary states is lacking[7]. There are controversies regarding their gastro protective effect in some animal studies also. So we wanted to conduct an animal study to find out the antiulcer activity of calcium channel blockers.

MATERIALS AND METHODS

2.1 Experimental animals

The study was carried out in Sixty Wistar male albino rats (180–220 g) that were obtained from the Central Animal Facility of Annamalai University, Chidambaram, Tamil Nadu, India (11° 20' 55" North, 79° 41' 36" East). The experimental studies were performed according to the guidelines of Institutional Ethics Committee (IEC) and followed CPCSEA requirements under Reg.418/01/A/dt.04.06.2001. In brief, they were fed with standard rat feed for a week. They were divided in to thirty rats for study I (acute study) and the thirty rats for study II

(chronic study). In each study, thirty were further sub-divided in to five groups of six each, Group A, B, C, D and E. Each group of rats dotted with five different colours, for identification. The animals were left in cages with a false bottom of wide mesh wire gauze to prevent conception of their own faeces (coprophagy). The rats were housed in cages at ambient temperature and provided with food and water ad libitum. The animals were acclimatized to the laboratory conditions and were fasted for 72 h before experimentation.

2.2 Drugs and chemicalsn

All chemicals used in the study were of analytical grade and were procured from Sigma Aldrich, USA. Verapamil, Nifedipine, Diltiazem, and Ranitidine were obtained from Glaxo, USA in brand and Aspirin was obtained from USV Pharmaceuticals, Mumbai, India.

2.3 Study I (acute study)

In this study the animals were fasted for three days but had free access to water. The gastric ulceration was induced by aspirin followed by pyloric ligation[8], on the fourth day. Drug administration for the different groups was given as follows:

Group A, [AC] Control (Aspirin Alone): All the six received aspirin in the dose of 200mg/kg, once daily at 11a.m.for three days. This solution was administered by intra gastric feeding cannula.

Group B, [AV] (Aspirin + Verapamil): Along with Aspirin, Verapamil was given in the dose of 40mg/kg orally to six rats daily in the morning, for three days.

Group C, [AN] (Aspirin + Nifedipine): Rats received Nifedipine in the dose of 40mg/kg along with aspirin in the same dose in the same time orally, for three days.

Group D, [AD] (Aspirin +Diltiazem): These rats received diltiazem in the dose of 60mg/kg along with aspirin orally, for three days. The group B, C and C animals received the test drugs, 30mts before aspirin administration.

Group E, [AR] (Aspirin+Ranitidine): The group E animals received Ranitidine, 30 minutes before aspirin administration. Inj. Ranitidine 50mg/kg was given intraperitoneally to all the six rats in this group along with oral aspirin. On the fourth day the pyloric ligation was done as per the method of Shay et al[9].

2.4 Pyloric ligation

The rats were anaesthetised with light ether. Abdomen was opened by a vertical, midline incision. T he stomach was identified and the pylorus was ligated. Then the wound was closed in layers and the animals were left in the cages. The animals were fasted for four hours after pyloric ligation and then sacrificed. The abdomen was opened again and the cardiac end of stomach was ligated. Stomach was taken out and cut open along the greater curvature and the mucosa was washed under slow running tap water. Then the stomach was mounted on a moistened paper. The ulcers were examined with the help of a magnifying glass macroscopically. The numbers of ulcers were noted and the severity was scored in pulses (1 to 4 pulses) and the ulcer index was calculated as described by Sing et al method[10].

2.5 Pulses

+ states Haemorrhagic spots, ++, +++, ++++ states Superficial ulcers, Deep ulcers, and Perforation respectively.

2.6 Ulcer Index

The sum total number of ulcers with severity in each pulses (XI+X2+X3+X4) was calculated. Thus a rat having the ulcers in each degree of severity is described as in table1.

Table 1
The ulcer index can be counted as 18 (described by Goel et al) (8).

+	++	+++	++++
6	3	2	0
X1	X2	X3	X4

2.7 Study II (chronic study)

This study was done for 21 days. The type of animals and the study population were the same as in acute study, except for the duration of the drug administration. Here also we had five groups of animals namely aspirin group, Aspirin + Verapamil group, Aspirin + Nifedipine group, Aspirin +Diltiazem group and Aspirin + Ranitidine group.

2.8 Drug Administration

All the rats belonging to the different groups were fasted for three days and received the respective drugs as in study I. From the fourth day onwards all rats were given regular feed along with drugs for 21 days. On the twenty second day the animals were fasted 4 hours, following which pyloric ligation was done. Four hours after pyloric ligation the animals were sacrificed. The number of ulcers and the ulcer index were listed.

2.9 Data Analysis

Student's t test was used to compare light and dark periods. One-way analysis of variance with Dunnett's test was used for multiple comparisons. A difference of $p < 0.05$ was regarded as significant.

3. RESULTS AND DISCUSSION

Voltage-sensitive calcium channels (VSCC) mediate the entry of calcium ions into excitable cells and are also involved in a variety of calcium-dependent processes, including muscle contraction, hormone or neurotransmitter release, gene expression, cell motility, cell division and cell death[11]. Isoform alpha-1I gives rise to T-type calcium currents. T-type calcium channels belong to the low-voltage activated (LVA) group and are strongly blocked by nickel and Verapamil, Diltiazem and Ranitidine[12]. A particularity of this type of channels is an opening at quite negative potentials, and a voltage-dependent inactivation. T-type channels serve pace making functions in both central neurons and cardiac nodal cells and support calcium signalling in secretory cells and vascular smooth muscle. They may also be involved in the modulation of firing patterns of neurons which is important for information processing as well as in cell growth processes. Gates in voltage ranges similar to, but higher than alpha 1G or alpha 1H. In addition they have specific function in Potassium voltage-gated channel subfamily H member 2 Voltage-gated signal transduction. In brief, Pore-forming (alpha) subunit of voltage-gated inwardly rectifies potassium channels, Channel properties are modulated by cAMP and subunit assembly. CCB mediates the rapidly activating component of the delayed rectifying potassium current in heart (IKr). Isoform 3 has no channel activity by itself, but modulates channel characteristics when associated with isoform 1. Apart from these they have many specific functions in signal transduction that classifies calcium channel blocker as a class IV anti-arrhythmia[13, 14] agents but their gastric ulcer protective property have not been studied so far. We have attempted to reveal its gastric ulcer protective property in two different studies; the former was Comparative analysis of mean ulcer index of CCB in Acute Renal Kidney failure, whereas the latter was Comparative analysis of mean ulcer index of CCB in chronic Renal Kidney failure condition using rat animal models.

The results of study I and study II were depicted in figure 2. The ulcer index in animal was calculated by totalling all the individual scores in that animal and expressed as Mean S.E.M. of 6 animals, of groups in both the studies (Acute and Chronic). The significance of the differences between mean values of the ulcer index for various drug groups were tested using the students 't' test as described by Gosh[15] They were indicated in figure 3. Calcium channel blockers are found to possess important pharmacological actions and therapeutic uses. As mentioned earlier, though the literatures revealed that they could be of use in the treatment of peptic ulcer, much research was not done in this field. So we conducted a preclinical study with various calcium channel blockers in drug induced ulcers, in rats. We have evaluated in both acute and chronic conditions with three different calcium channel blockers – verapamil, nifedipin and diltiazem. At the end of the trial, we sacrificed the animals and analysed the results, statistically. The aspirin group (Group A) was kept as the standard for our study. The verapamil group did not show any significant effect in acute study. Whereas, in chronic study verapamil produced a significant improvement in ulcer index compared to others.

Figure 2

Comparative analysis mean ulcer index of Various Calcium channel blockers in Acute Renal Kidney failure (Values are expressed as mean ± SE)

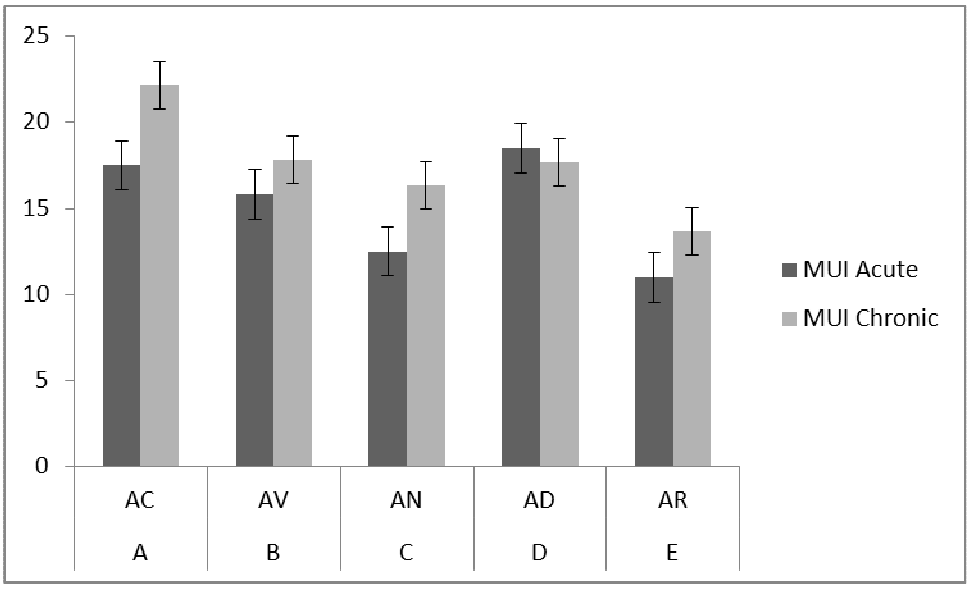
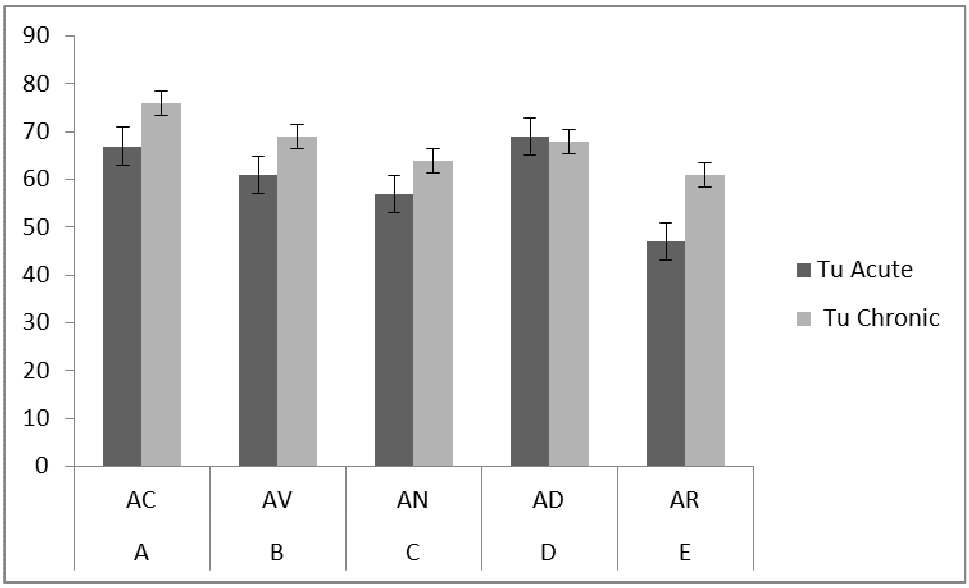


Figure 3
Comparative analysis of total ulcer of various calcium channel blockers in chronic renal kidney failure (Values are expressed as mean ± SE)



This is in contrast to the results published by the Rueil Malmaison et al[16]. But our results go well with reports produced by different authors like, Wait et al[17], Ghanayem et al[18] Glavin et al[19] and Wong et al[20].

Further studies with verapamil might solve the controversies over the results. The animals which received nifedipine showed positive findings both in acute and chronic studies. In acute study the results were significant and in chronic study the results were highly significant. The highly significant results of our chronic study were in accordance with the studies Tsimmerman et al[21] Glavin et al[22] and Suzuki et al[23].

In acute study, the animals belonging to diltiazem group did not show any significant ulcer protecting effect. Albeit, in chronic study, diltiazem showed significant improvement in the ulcer index compared to the other CCB. This is similar to that of the studies conducted by Ghanayem et al[20], Glavin et al[21]. The results obtained in our acute study with verapamil and diltiazem may be due to the fact that calcium channel blockers are associated with mild gastro intestinal irritation in the initial phase. But these effects subside on prolonged administration[24]. Our results of the chronic study go parallel with this observation.

4. CONCLUSION

On comparing the antiulcer activity of calcium channel blockers in animals, Nifedipine had shown highly significant ulcer protective effect in both acute and chronic studies, than Verapamil Diltiazem and Ranitidine.

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CONFLICTS OF INTEREST

The authors declare no potential conflict of interest in terms of affiliation, finance and/or any other means.

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