

CALCIUM ENHANCES ANTI-INFLAMMATORY ACTIVITY OF ASPIRIN

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ABSTRACT

The objective of present study is to evaluate the effects of calcium carbonate and calcium gluconate on acute and subacute inflammation and to study their possible interactions with Aspirin. Calcium carbonate (10 mg/kg) and calcium gluconate (5 mg/kg) were administered individually and also co-administered along with sub therapeutic dose Aspirin (50mg/kg) to study their interaction. The inflammation was induced by carrageenan or a foreign body. Both calcium carbonate and calcium gluconate could not show significant anti-inflammatory activity on their own in acute as well as subacute inflammation models. Aspirin at sub-anti-inflammatory dose (50mg/Kg) when co-administered along with calcium salts produced the significant anti-inflammatory response which was comparable to anti-inflammatory response of aspirin at therapeutic dose (200mg/Kg). Also co-administration minimized the gastro-toxicity of aspirin.

KEYWORDS: Acetylsalicylic acid, anti-inflammatory agent, calcium salt, aspirin.

INTRODUCTION

Inflammation continues to be an area of great interest for research, probably due to the non availability of safer and more effective anti-inflammatory agents. The conventional nonselective NSAIDs like aspirin, diclofenac, indomethacin or ibuprofen possess side effects like gastropathy or impairment of renal functions. Gastrointestinal conditions such as dyspepsia, peptic ulcer disease and GI haemorrhage are exacerbated by aspirin¹. Topical use of diclofenac was reported to cause severe corneal toxicity². It also causes acute renal toxicity. The newer COX-2 selective agents such as meloxicam, nimesulide, celecoxib etc retain the anti-inflammatory effects characteristic of NSAIDs with a marked increase in gastrointestinal (GI) tolerability as compared to classical non selective ones. But even these agents showed cardiotoxic and hepatotoxic side effects³. In recent years increased understanding of the inflammatory mechanism and the mediators involved has led to the development of newer anti-inflammatory agents like monoclonal antibodies and antagonists of inflammogens. Interestingly, several other drugs like minocycline, ascorbic acid and calcium salts (calcium dobesilate, calcium hydroxide, calcium pentosan polysulfate) have also been reported to possess anti-inflammatory property. Earlier, calcium chloride was advocated for the treatment of urticaria, acute oedema, pruritus and erythema, calcium carbonate and calcium gluconate for the treatment of insect stings and calcium

hydroxide to suppress peri apical inflammation in dental practice. Interaction of calcium supplementation and NSAIDs had shown a protective effect on colorectal neoplasia⁴. These reports indicate that calcium salts possess anti-inflammatory property⁵.

The present study was planned to investigate the effect of calcium carbonate and calcium gluconate on acute and subacute inflammation in Wistar rats. The other objective was to investigate the interaction of calcium salts with aspirin.

MATERIALS AND METHODS

Drugs and chemicals

Aspirin, Calcium gluconate, calcium carbonate, carrageenan and all other chemicals used were of analytical grade obtained from Sigma Aldrich Chemical Company, St Louis, USA.

Animals

Twelve week-old healthy Wistar rats (150–200 g) of either sex procured from inbred facility of Srinivas College of Pharmacy, Mangalore were used for this study. They are maintained under standard conditions (temperature $22 \pm 2^\circ\text{C}$, relative humidity $60 \pm 5\%$ and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water ad libitum. Experiments were conducted between 9:00 to 14:00 h. Each rat was used only once. The Institutional Animal Ethics Committee approved the experimental protocol. All the animals received humane

care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

Acute inflammation

The animals were starved overnight with water ad libitum prior to the day of experiment. All the treatment was done orally. The different groups of animals (n=6) are assigned as below.

Group I: Control group (0.5 ml of 1% gum acacia suspension).

The Group II: aspirin (200mg/Kg).

Group III: calcium carbonate (10mg/Kg)

Group IV: calcium carbonate (10mg/Kg) + aspirin (50mg/Kg)

Group V: calcium gluconate (10mg/Kg)

Group VIII: calcium gluconate (25mg/Kg)

Group IX: calcium gluconate (50mg/Kg)

Group X: calcium gluconate (10mg/Kg) + aspirin (50mg/Kg)

Thirty minutes after drug administration, acute inflammation was induced by injecting 0.05 ml of 1% carrageenan in normal saline into the subplantar region of the left hind paw. A mark was applied on the leg at the malleolus to facilitate subsequent readings. The paw oedema volume was measured by mercury displacement with the help of a plethysmograph at 0, 0.5, 1, 3 and 5 h after injecting carrageenan. The difference between 0 h and subsequent readings was considered as oedema volume. The percentage inhibition of oedema in various groups was calculated using the formula:

$$\% \text{ oedema} = 1 - V_t/V_c \times 100$$

V_t and V_c were oedema volume in the drug-treated and control groups respectively.⁶

Subacute inflammation

Under light ether anaesthesia, hair in the axilla and the groin were clipped, and two sterile cotton pellets weighing 10 mg each were implanted subcutaneously, through a small incision, either in the axilla or the groin, at random. The wounds were then sutured and the animals were caged individually after recovery from anaesthesia. Aseptic precautions were taken throughout the experiment. The different groups of animals (n=6) are assigned as below.

Group I: Control group (0.5 ml of 1% gum acacia suspension).

The Group II: aspirin (200mg/Kg).

Group III: calcium carbonate (10mg/Kg)

Group IV: calcium carbonate (10mg/Kg) + aspirin (50mg/Kg)

Group V: calcium gluconate (05mg/Kg)

Group VI: calcium gluconate (05mg/Kg) + aspirin (50mg/Kg)

The treatment was started on the day after the implantation and was repeated every 24 h, regularly for 10 days.

On the 11th day the rats were sacrificed with an overdose of ether anaesthesia, and the cotton pellets and the stomachs were removed. The pellets, freed from extraneous tissue, were dried overnight at 60°C and their dry weight measured. Net granuloma formation was calculated by subtracting the initial weights of cotton pellet from the final weights. Mean granuloma dry weight for the various groups was calculated and expressed as mg/100 g body weight.⁶

Ulcer index

The stomachs were cut open along the greater curvature and gently washed with normal saline. Gastric mucosa was examined for the presence of erosions, haemorrhagic spots, ulcer and perforation, if any, with the help of a magnifying lens. The severity of the ulcers was determined by an arbitrary scoring system. The ulcer index was calculated as a mean score of ulcer severity in all the treated groups and was compared with that of control.⁷

Statistical analysis

Data were expressed as mean±SEM and were analyzed by the one-way ANOVA followed by the Dunnett's test.

RESULTS

Acute studies

Treatment with aspirin significantly inhibited the Carrageenan induced paw edema at all time intervals. The corresponding inhibitions in calcium carbonate and calcium gluconate treated groups were not significantly differing with control group animals. On the contrary, sub-anti-inflammatory dose of aspirin (50 mg/Kg) when co administered along with calcium carbonate or calcium gluconate showed significant inhibition of paw edema, which was comparable with aspirin alone at anti-inflammatory dose (200mg/Kg).

Subacute studies

Aspirin treatment significantly ($P < 0.001$) reduced the mean granuloma dry weights. Also co administration of aspirin at sub-anti-inflammatory dose (50 mg/Kg) along with calcium carbonate and calcium gluconate resulted in significant reduction ($P < 0.001$) in the mean granuloma dry weights. Administration of both calcium carbonate and calcium gluconate alone also reduced mean granuloma dry weights but level of significance was less ($P < 0.05$).

In the gastric-mucosal study the mean ulcer index of the aspirin (200 mg/Kg) group was significantly ($P < 0.01$)

higher than the control group. The ulcer index in other groups was comparable to that of the control.

DISCUSSION

Results of the present study indicate the anti-inflammatory property of both calcium carbonate and calcium gluconate of their own in sub acute models of inflammation. A survey of literature reveals that in different models of inflammation, calcium salts like calcium dobesilate⁸, calcium pentosan polysulfate⁹ and calcium hydroxide¹⁰ possess anti-inflammatory property. Findings of the present study corroborate the anti-inflammatory property of calcium salts.

The sub-anti-inflammatory doses of calcium carbonate (10 mg/kg) and calcium gluconate (5 mg/kg) co-administered with sub-anti-inflammatory doses of aspirin (50 mg/kg) exhibited significant anti-inflammatory activity in acute as well as in sub-acute models of inflammation. Such an interaction between calcium salts and aspirin appears to be poorly documented in literature.

The mechanism of the anti-inflammatory action of calcium cannot be proposed on the basis of the present findings. However, several mechanisms have been proposed in earlier reports. Piller has speculated that calcium dobesilate reduced the number of circulating monocytes and also blocked the action of macrophage in order to suppress inflammation⁸. It has also been shown that calcium dobesilate can suppress platelet aggregating-factor production in endothelial cells in a dose-dependent manner¹¹. The above mechanisms may play a role in the anti-inflammatory activity of calcium carbonate and calcium gluconate too.

The other proposed anti-inflammatory mechanisms of calcium salts include the precipitant action of calcium on a cement substance¹² and enhanced superoxide anions scavenging through increased activity of superoxide dismutase, peroxidase, glutathione peroxidase and glutathione reductase, which are reported to be increased by calcium gluconate¹³. It is well known that such enzymes suppress inflammation. It is not known whether calcium salts like calcium carbonate and calcium gluconate have a property similar to that of calcium gluconate, which could explain their anti-inflammatory action. Detailed studies are needed to explain the exact mechanism of action.

Calcium being an essential element for the vascular smooth muscle contraction can prevent effusion and thereby exert its anti-inflammatory effect. However such a possibility appears to be quite remote since calcium salts failed to prevent effusion in earlier studies¹⁴. In some smooth muscles, calcium, through the calcium-sensitive potassium channels, can lead to

hyperpolarization¹⁵. If this is true for the vascular smooth muscle, then calcium can produce vasodilatation. In fact, calcium has been reported to produce vasodilatation by stabilizing the cell membranes^{16, 17}. Due to vasodilatation the interendothelial cell gaps may be reduced, leading to decreased effusion, which is one of the events of inflammation.

With aspirin, calcium interaction appears to be of a pharmacodynamic nature. The extracellular actions of calcium as mentioned above, might add to the insignificant anti-inflammatory activity of a low dose of aspirin.

Studies on the gastric mucosa showed some significant ulcerogenic activity of aspirin (200 mg/kg), whereas both, the calcium salts and the combination of sub-anti-inflammatory doses of aspirin and calcium carbonate were free from ulcerogenic action despite exhibiting significant anti-inflammatory activity. It is well known that calcium salts possess astringent as well as antacid properties, which may explain the lack of their ulcerogenic potential. In fact, the availability of aspirin and calcium combinations in the market, their extensive use in clinical practice and the controversial reports regarding the anti-inflammatory property of calcium were the main initiatives for the present study.

The results of the present study favour the combined use of aspirin and calcium, if the present findings could be extrapolated to clinical situations. The advantages of such combined preparations are obviously a reduction in the dose of aspirin that could still produce significant anti-inflammatory action, and a possible protection against aspirin-induced gastric toxicity. It is worthwhile to evaluate such preparations through clinical trials.

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Table 1: Effect of various treatments in rats on carrageenan induced paw oedema.

Drug (mg/Kg)	Paw volume in ml			
	0h	0.5h	1h	3h
Control (1%gum acacia)	1.30±0.06	2.51±0.07	3.21±0.05	3.25±0.08
Aspirin (200)	1.30±0.05	1.60±0.02 **	1.71±0.03 **	1.75±0.02 **
Calcium carbonate (10)	1.29±0.01	2.35±0.04	2.33±0.01	2.32±0.03
Calcium carbonate (10) + Aspirin (50)	1.29±0.05	1.62±0.04**	1.74±0.03**	1.79±0.06**
Calcium gluconate (05)	1.30±0.05	2.33±0.07	2.31±0.06	2.20±0.04
Calcium gluconate (05) + Aspirin (50)	1.30±0.08**	1.59±0.04**	1.71±0.04**	1.74±0.06**

Values represents mean±SEM. **P<0.001 compared with control (ANOVA followed by Dunnett's test).

Table 2: Effect of various treatments in rats on granuloma dry weight and ulcer index.

Drug (mg/Kg)	Granuloma dry weight (mg% of body wt)	Ulcer Index
Control (1% gum acacia)	42.89±5.32	-
Aspirin (200)	26.85±3.12**	29.32±2.35**
Calcium carbonate (10)	33.21±2.13*	-
Calcium carbonate (10) + aspirin (50)	28.32±3.21**	11.12±2.13*
Calcium gluconate (05)	31.85±3.84*	-
Calcium gluconate (05) + aspirin (50)	27.52±2.56**	11.84±3.10*

Values represents mean±SEM. *P<0.05, **P<0.001 compared with control (ANOVA followed by Dunnett's test).

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