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ANTI-INFLAMMATORY ACTIVITY OF CALCIUM CHANNEL BLOCKERS

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ABSTRACT

Objective: To explore the anti-inflammatory activity of calcium channel blockers.
Material and Methods: The anti-inflammatory activity was determined by producing inflammation in rats of either sex. Acute inflammation was produced by injecting carrageenan, formalin and histamine in hindpaw of rats. For sub-acute inflammation the cotton pellets granuloma method was employed. Formaldehyde induced arthritis was used as a model of chronic inflammation.
Result: The acute inflammation produced by carrageenan, histamine and formaldehyde was significantly reduced by pretreatment with Ca^{++} channel blockers of di-hydropyridine group $P<0.01$ exception being Nimodipine which showed varied results. Similarly in sub-acute inflammation model of cotton pellet implantation the drugs showed significant inhibition of inflammation exception being Nimodipine which showed significant protection in high dose 40mg/kg oral $P<0.01$.In chronic arthritis induced by formaldehyde there was a dose dependent reduction in paw oedema varying from $P<0.05$ at 10mg/kg oral dose to $P<0.01$ at 40mg/kg dose.
Conclusion: These findings suggest that CCBs exert significant dose dependent anti-inflammatory activity against acute, sub acute and chronic models of inflammations.

Keywords: CCBs (Calcium channel blockers) of 1,4 di-hydropyridine group, anti-inflammatory activity,phlogestic agents, Acute, Sub acute and Chronic models of inflammation.

INTRODUCTION

Rheumatoid arthritis (RA) is globally prevalent chronic inflammatory disease that occurs due to interplay of genetic risk, environmental insults and random somatic changes in musculoskeletal and immune system. The term inflammation has come from the Latin word "inflammare" which means "to burn" (Kanaan SA, 1996). During inflammation there is dilatation of capillaries and increased vascular permeability with resulting oedema; cellular infiltration phagocytosis; and proliferation of fibroblasts to synthesize new connective tissue for repair of the injury.

Since RA is a common chronic disease, the effort to find newer anti-inflammatory medicines and study their mechanisms continues (Bahl AK et al., 1994 ; Salvatore A & Carson D, 1965). Many synthetic and indigenous products have been explored with the hope to get an efficacious, safe, anti-inflammatory and anti-arthritic medicine which can have efficacy similar to non-steroidal anti inflammatory medicines (NSAIMs) and steroids, without their associated adverse effects (ADRs).
Influx of Ca^{++} is an essential step in the synthesis and release of prostaglandins (PGs) which are the main

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mediators in inflammation. Hence, CCBs are expected to have anti-inflammatory role because CCBs also alter the local concentration of the inflammation mediators. Hence this study was planned to study the anti-inflammatory effect of CCBs in comparison to NSAIMs (Turner RA, 1965; Parmar NS & Ghosh MN, 1978 ; Kelled DN, 1966).

MATERIAL AND METHODS

The experiment was designed in adherence to the Institutional Research Board. Five groups of six male Wistar albino rats aged six to eight months, weighing between 100-150 g were used. The CCBs used were Nimodipine, Amlodipine and Felodipine, which were administered intragastrically in doses of 10, 20 and 40 mg/kg respectively. Phenylbutazone 50mg/kg intragastric was used as a standard anti-inflammatory agent for comparison. The control group was administered 5 ml propylene glycol:glycerine:distilled water in the ratio of 10:6:1.

1. MODELLING ACUTE INFLAMMATION
Five groups of six rats were administered different doses of CCBs and the standard anti-inflammatory agent. Acute inflammation was induced after one hour of drug treatment by injecting different phlogistic agents into sub-plantar tissue of right hind paw. Pre and post drug paw volumes were measured by plethysmograph (Inco Rat Paw Plethysmograph-Mercury Model) (Chattopadhaya RN et al., 1986 ; Wills A L, 1969 ; Winter CA et al., 1962 ; Ghosh MN, 1984).

1.1   Carrageenin induced hind paw oedema: It was produced by injecting 0.1ml of 1% solution of carrageenin in distilled water and the paw volume was measured before and 3 hrs after the injection.

1.2   Formalin induced hind paw oedema: 0.05ml of 3.5% formalin in 0.9% sodium chloride solution was injected and the paw volume was measured before and after 1.5, 4 and 24 hrs.

1.3 Histamine induced hind paw oedema: 0.1ml of 0.1% solution of histamine acid phosphate [5] was

injected and the paw volume was recorded before and 1 hr after the injection.

2. MODELLING SUBACUTE INFLAMMATION:
Sub-acute inflammation was produced by granuloma pouch method by implanting cotton pellets (Kelled D N, 1966). Four cotton pellets of 10 mg each, sterilized at 160°C for 30 min, were implanted in axilla and groin of each rat under ether anaesthesia. The drugs were administered once daily intragastrically for five days from the day of implantation. On the sixth day the rats were anaesthetised with ether and pellets were dissected out, dried in the oven at 60°C for 24 hrs and the dry weight of the four pellets were recorded.

3. MODELLING CHRONIC INFLAMMATION:
Formaldehyde induced arthritis: 0.1ml of 2% formaldehyde was injected in the hind paw of rats just beneath the plantar aponeurosis on day 1 and day 3 (Brownlee G, 1966). The rats were treated daily with different doses of CCBs for five days. Daily change in paw volume was recorded by a plethysmograph. Results were analysed using One Way ANOVA followed by Tukey's HSD post hoc.

RESULTS

In the acute anti-inflammatory studies with carrageenin induced inflammation all three CCBs viz. Nimodipine, Amlodipine and Felodipine were effective in doses of 10, 20 and 40 mgs /kg respectively in rats [Table I].
Histamine induced inflammation was prevented by Felodipine in 20 and 40 mg/kg dose and in 10, 20 and 40 mg/kg dose by Amlodipine while Nimodipine was ineffective in all the given doses [Table1]. In the sub-acute studies only Felodipine in the dose of 10 mg/kg produced a significant reduction in granulation tissue formation (62±3.84%). Felodipine and Amlodipine were effective in reducing granulation tissue formation in dose of 20 mg/kg. The 40 mg/kg dose of all the drugs was effective as it prevented significantly the percent rise in weight of cotton pellets [Table 2].
In the chronic studies the 10mg/kg dose of Felodipine and Amlodipine reduced the

inflammation significantly (p<0.05 and p<0.01) on fifth day. Nimodipine reduced the percent increase in paw volume (p<0.05) on fifth day [Table 3]. The 20mg/kg dose of Amlodipine prevented rise in formaldehyde induced hind paw oedema (p<0.01). On administration of high dose of 40mg/kg, of Nimodipine, there was mortality in rats [Table 3].

TABLE –1. *Effect of Dihydropyridines and Phenylbutazone on rat hind paw inflammation (acute inflammatory model) induced by carrageenin, histamine and formalin (n = 6)*

DRUG	DOSE mg/kg/oral	Percentage increase in hind paw volume ± SE		
		Carrageenin	Histamine	Formalin
		3 hr	1 hr	24 hr
Control	5 ml	167 ± 32.34	55.40 ± 6.0	113.54 ± 15.36
Phenylbutazone	50	19.22 ± 3.81**	11.40 ± 5.95***	23.86 ± 4.41***
Nimodipine	10	28 ± 5.15**	117 ± 13.50	69.24 ± 5.02*
	20	20 ± 3.14**	88 ± 14.03	46 ± 8.22**
	40	24 ± 5.64**	76 ± 17.32	36.23 ± 3.86***
Felodipine	10	37 ± 5.61*	75 ± 18.03	81 ± 9.34
	20	38 ± 4.03*	79 ± 7.27*	64.20 ± 4.20*
	40	30 ± 4.13**	62 ± 4.66*	52.22 ± 10.26**
Amlodipine	10	36 ± 5.79*	73 ± 6.65*	63.22 ± 4.48*
	20	35 ± 3.38*	69 ± 6.99*	32.44 ± 6.64***
	40	21 ± 1.82**	34 ± 3.91**	30.20 ± 4.44***

*P<0.05, **P<0.01, ***P<0.001 when compared to control group.
Control (vehicle) was propylene glycol, glycerine and distilled water in the ratio of 10:6:1

TABLE -2. *Effect of Dihydropyridines and Phenylbutazone on cotton pellet implantation (sub acute inflammatory model) in albino rats (n=6)*

DRUG	DOSE mg/kg/oral	% increase in weight of pellets	
		mg ± SE	% ± SE
Control	5 ml	81.66 ± 13.21	104 ± 13.27
Phenylbutazone	50	50.83 ± 6.03**	26 ± 6.25**
Nimodipine	10	73.33 ± 6.22	83 ± 6.25
	20	80.83 14.66	93 ± 14.72
	40	50.66 ± 3.18**	26 3.89**
Felodipine	10	65 ± 3.82*	62 ± 3.84*
	20	55.33 ± 3.57**	38 ± 3.59**
	40	45.66 ± 3.27***	14 ± 3.28***
Amlodipine	10	73.66 ± 3.82	87 ± 3.84*
	20	53.83 ± 2.44**	34 ± 2.45**
	40	47 ± 3.82***	17 ± 3.84***

Initial weight of pellets was 40mg
*P<0.05, **P<0.01, ***P<0.001 when compared to control group.
Control (vehicle) was propylene glycol, glycerine and distilled water in the ratio of 10:6:1

TABLE -3. *Effect of Dihydropyridines and Phenylbutazone on formaldehyde induced arthritis (chronic inflammatory model) in hind paw of albino rats (n=6)*

DRUG	Dose mg/kg/ oral	5TH DAY PERCENT INCREASE IN RAT HIND PAW VOLUME (MEAN ± SE)
Control	5ml	137.54 ± 16.39
Phenylbutazone	50	34.25 ± 5.48***
Nimodipine	10	89.58 ± 8.45*
	20	69.16 ± 8.54**
	40	
Felodipine	10	38.33 ± 4.61**
	20	39.58 ± 3.96**
	40	34.99 ± 1.05**
Amlodipine	10	50.00 ± 6.48**
	20	39.58 ± 3.96**
	40	41.66 ± 6.47**

*P<0.05, **P<0.01, ***P<0.001 in relation to control group.
Control (vehicle) was propylene glycol, glycerine and distilled water in the ratio of 10:6:1

DISCUSSION

Calcium channel blockers have proven efficacy in ischemic heart disease, cardiac failure, and cardiac arrhythmias [12, 13]. These have been also successfully used in inflammatory injuries, epilepsy and withdrawal symptoms from ethanol, and morphine (Khaksari M et al., 2004). Inhibition of transmembrane influx of Ca^{++} decreases the total quantum of the cation reaching intracellulary. Ca^{++} mobilization pathways being multiple, CCBs bring out this action by blocking the receptor operated and voltage sensitive Ca^{++} channels – the slow L-type of Ca^{++} channels [14]. Influx of Ca^{++} being essential for release of histamine and 5HT from mast cells and synthesis and release of PGs, which are the main mediators of inflammation, the CCBs decrease all.

The inflammatory response is a plurifactorial, polyphasic tissue reaction, ranging from rapid short lived increase in vascular permeability to a prolonged cellular infiltration and proliferation. Hence it is important that an anti-inflammatory agent be accessed in acute, subacute and chronic inflammation to cover all the processes of inflammation. We found dose dependant anti-inflammatory activity of 1,4 dihydropyridines viz. Nimodipine, Felodipine and Amlodipine. The results obtained by us were variable but significant. In acute inflammation induced by carrageenin, histamine and formalin, there was significant inhibition of paw oedema by Amlodipine in all the doses. Nimodipine showed varied significance in all the doses while Felodipine was more effective in preventing paw oedema induced by carrageenin, histamine and formalin in the higher dose only. These findings on Nimodipine, Felodipine and Amlodipine corroborate with the findings of others who reported that Nifedipine was more potent anti-inflammatory agent in carrageenin induced rat hind paw oedema and carrageenin induced granuloma pouch in rats [12]. However now it is realised that suppression of rat hind paw oedema does not give a valid assessment of clinical anti-inflammatory property of drugs.

In subacute model of cotton pellet implant, all the CCBs were found to be efficacious by interfering with the proliferative phase. Measuring granulation tissue formation with cotton wool pellets has been of value in evaluating drug action. This test represents a similar phase of the inflammatory response mediated by PGs in connective tissue.

The CCBs effect the proliferative phase of inflammation by inhibiting the synthesis of PGs, alike NSAIMs. That CCBs suppress the proliferative phase was also evident from their efficacy against the chronic inflammation produced by the injection of formaldehyde. Formaldehyde induced chronic inflammation is representative of secondary inflammation and differs from subacute inflammation in migration and accumulation of fibroblasts at the inflammation site. The CCBs therefore seem to affect the cellular infiltration as well.

No known anti inflammatory agent is equally effective in suppressing all the components of inflammatory response and many act either on one aspect or a single phase of one component. Salicylates and cortisone are known to diminish acute as well as chronic phases of inflammation. In our study CCBs of the 1,4 dihydropyridine group exhibited significant and promising anti inflammatory activity in all models, except Nimodipine which showed significant protection against rat hind paw oedema in various models of inflammation in the 20 and 40 mgs/kg doses. This could be because of the tendency of Nimodipine to act upon the brain tissues where it concentrates in high doses and is more effective than at the periphery where the accumulation is less.

Anti-inflammatory activity in CCBs reflects their similarity in action to NSAIMs, since the results were comparable to the standard NSAIM - Phenylbutazone. It seems reasonable to speculate that CCBs might be acting through the same mechanisms as NSAIMs. Inhibition of PGs synthesis appears to be more logical as their mechanism of action. It is however not clear whether such inhibition of PG synthesis is due to inhibition of cyclo oxygenase or the inhibition occurs at the higher levels of eicosanoid chain i.e. phospholipase A_2.

REFERENCES

Many animal studies and clinical trials have shown that CCBs especially the L-type, selectively potentiate opioid induced analgesia as a function of the pain assay used (pressure induced pain *versus* cold induced pain). According to the study on the anti-inflammatory effect of L-type CCBs the possible mechanisms involved may be through:

1) Reduction of Ca^{++} concentration in blood, causing a decrease in the vessel resistance, consequent reduction of hydrostatic pressure in the capillaries.
2) Inhibition of pro-inflammatory mediators.
3) Reduction of Ca^{++} leading to inhibition of activity of PLA2 and / or PLC the enzymes responsible for the synthesis of eicosanoids and leukotrienes.
4) Stabilization of the cell membrane integrity by inhibiting Ca^{++} influx, thus preventing tissue injury and inflammation.

The present status of anti-inflammatory effect of L-type CCBs is supported by the growing evidence that pretreatment with CCBs suppresses the expression of plasminogen receptors (Plg-Rs) on macrophages – Plasminogen being an important mediator of macrophage recruitment (Khaksari M et al., 2004 ; Das R et al., 2009)

CONCLUSION

This study suggests that possession of anti-inflammatory activities in CCBs reflects their similarity in action to NSAIMs. Inhibition of prostaglandin synthesis appears to be a logical approach to their mechanism of action. It is how ever not clear that this inhibition of prostaglandin synthesis is due to inhibition of cyclooxygenase or the inhibition occurs at higher level of eicosanoid chain i.e. phospholipase A2 Further studies are needed to explore the role of calcium channel blockers in the biosynthesis and release of chemical mediatorsofinflammation.

1. Kanaan SA. Endotoxin induced local inflammation and hyperalgesia in rats and mice: A new model for inflammatory pain. Pain.1996; 66: 373-9.

2. Bahl AK, Dale MM, Foreman JC. The effects of non-steroidal anti-inflammatory drugs on the accumulation & release of interleukin-1 like

activity by peritoneal macrophage from the mouse. Br J Pharmac.1994; 113 :809-14.

3. Salvatore A, Carson D editors. Etiology and pathogenesis of rheumatoid arthritis. Arthritis and Allied conditions. Text book of Rheumatology.13th ed 1: 979-90.

4. Turner RA.Nonnarcotic Analgesics. Screening methods in Pharmacology.New York: Academic Press.1965;1,114-5

5. Parmar NS, Ghosh MN. Anti-inflammatory activity of gossypin a bioflavonoid isolated from Hibiscus vitifolius Linn. Ind J Pharmac. 1978; 10: 277-93.

6. Kellet DN. The mechanism of the Anti-inflammatory activity of Hexadimethrine bromide . Br J Pharmac.1966; 26:351-7.

7. Chattopadhyay RN, Chattopadhyay R , Roy S ,Moitra SK. A simple method for plethysmometric measurement of paw volume of small laboratory animals in the evaluation of anti-inflammatory effects. Bull Calcutta Sch. Trop. Med. 1986; 34: 5-8.

8. Wills AL. Release of histamine, kinin & prostaglandin during carrageenin induced inflammation in the rats. In Mantegazza P & Horton E Q, editors. Prostaglandins, peptides and amines Academic press, London. 1969.p. 31–8.

9. Winter CA, Risley EA, Nuss GM: Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc.Soc.Exp.Biol.Med.1962; III: 544-7.

10. Ghosh MN. Fundamentals of experimental pharmacology. 2nd ed Calcutta : Science Book Agency;1984. 146.

11. Brownlee G. Effect of deoxycortisone & ascorbic acid on formaldehyde induced arthritis in normal and adrenalectomized rats. Lancet. 1966; 1:157-9.

12. Aditya GN, Chattopadhyay RN, Mandal S, Roy RK, Lahiri HL, Maitra SK. Preliminary study on anti-inflammatory effect of calcium channel blockers in albino rats. Ind J Pharmac. 1997; 29:132-4.

13. Pitt B, Byington RP, Furberg CD, Hunninghake DB, Mancini GBJ, Miller ME,et al . Effect of Amlodipine on the progression of Atherosclerosis and the occurrence of clinical events. Circulation. 2000; 102:1503.

14. Khaksari M, Mahani SE, Mahmoodi M. Calcium channel blockers reduce inflammatory edema in the rat. Ind J Pharmac. 2004; 36:335-51.

15. Das R, Burke T, David R, Wagoner V, Plow EF. L-Type calcium channel blockers exert an Anti-inflammatory effect by suppressing expression of Plasminogen receptors on macrophages. Circulation. 2009; 105:167-75.