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Archives of Applied Science Research, 2010, 2 (4): 85-94

(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Modulatory role of Gabapentin alone and on co-administration with Verapamile or Nimesulide in acute inflammatory condition

Otari Kishor Vasant[1*], Taranalli Ashok Dundappa[2], Shete Rajkumar Virbhadrappa[1], Singh Vijay Pal[3] and Harpallani Anand Nirmal[2]

[1]RD's College of Pharmacy, Bhor, Dist. Pune, Maharashtara (India)
[2]KLES's College of Pharmacy, Belgaum, Karnataka (India)
[3]Gautam College of Pharmacy, Banglore, Karnataka (India)

ABSTRACT

Gabapentin, a novel anticonvulsant drug, was found to be an effective anti-nociceptive in neuropathic pain. The effect of gabapentin has been studied in several experimental paradigms of pain particularly, neuropathic pain. Also gabapentin was shown to reduce hyperalgesia and inhibit C-fiber responses to noxious stimuli in animal models of inflammatory pain (injection of formalin or carrageenan). The present research work was therefore, focused to investigate the effect of gabapentin in acute inflammatory condition in carrageenan-induced rat paw edema. Further, the effect of gabapentin on myeloperoxidase activity, lipid peroxidation and histopathlogical changes in the rat footpad tissues were evaluated. In addition, in combination study the effect of sub-effective dose combinations of gabapentin with verapamile or nimesulide were studied in carrageenan-induced rat paw edema. Gabapentin (10, 30 & 100 mg/kg, po) was demonstrated inhibition of carrageenan-induced rat paw inflammation. Also significant inhibition of the myeloperoxidase activity, generation of lipid peroxides and morphological injury in footpad tissues, induced by carrageenan, was evident at higher dose tested. On co-administration, the sub-effective dose of nimesulide (0.5 mg/kg, po) or verapamil (2 mg/kg, po) significantly enhanced the anti-inflammatory effect of sub-effective dose of gabapentin (5 mg/kg, po) against carrageenan induced-paw edema in rats when compared to the effect per se. It was suggested that, by possibly binding to the $\alpha_2\delta$ subunit, gabapentin might affect Ca^{2+} currents, which might modulate neurotransmitter release, neuronal excitability or release or synthesis of inflammatory mediators thereby alleviating the inflammatory conditions. In addition the results of combination study suggested that the co-administration of gabapentin with drugs acting through other transaction pathways (COX, Ca^{2+} etc) in inflammation might pose a beneficial effect in the clinics for the treatment inflammatory conditions.

Key words: Gabapentin; Inflammation; Myeloperoxidase; Lipid peroxidation; Calcium channel blocker; Nimesulide.

INTRODUCTION

Gabapentin, 1-(amino methyl) cyclohexane acetic acid, is a novel anticonvulsant drug that is active in a variety of animal seizure models and prevents and is used as anticonvulsant, both in add-on and monotherapy. Recently, it has been shown that gabapentin prevents nociceptive responses from hyperalgesia in animal models and also has analgesic actions in clinical reports. [1]

Gabapentin has antihyperalgesic and antiallodynic properties. [2] In particular, gabapentin prevents pain-related responses in several models of neuropathic pain in rats or mice (e.g. spinal nerve ligation models, streptozocin-induced diabetes model, spinal cord injury model, acute herpes zoster infection model). [3]

After gabapentin was found to have an analgesic effect in patients with intractable neuropathic pain, its antinociceptive effects had been reported in several animal pain models, including the phase 2, but not phase 1, of formalin inflammatory, neuropathic, postoperative, lumbar adhesive arachnoiditis, and cancer-induced bone pain models. It seems that gabapentin is effective in inflammatory and tissue-injury induced pain models. [4]

In animal models of inflammatory pain (injection of formalin or carrageenan), gabapentin was shown to reduce hyperalgesia and inhibit C-fiber responses to noxious stimuli. [5] Recent studies have shown that gabapentin possesses antihyperalgesic actions in animal models of inflammatory and neuropathic pains. It has been reported that gabapentin selectively blocks the second phase of the formalin response and carrageenan-induced thermal and mechanical hyperalgesia. [6]

The effects of gabapentin and all types of Ca^{2+} channel blockers on nociceptive behaviors in various animal pain models have been summarized. It demonstrates that intrathecal L-type Ca^{2+} channel blockers are ineffective in most of the pain models except the acute and visceral pain models. The P-type Ca^{2+} channel blocker is effective in certain inflammatory and acute pain models. The T-type Ca^{2+} channel blockers, when administered intrathecally, are exclusively effective in the formalin test, while they are effective when given systemically in both acute and inflammatory pain models, but not in neuropathic, postoperative, and visceral pain models. However, N-type Ca^{2+} channel blockers are effective in almost all models. It is interesting to note that Bay K 8644, the L-type Ca^{2+} channel opener, reversed the antiallodynic effect of gabapentin in the postoperative pain model. [4]

Comparing the effectiveness of gabapentin in various animal pain models, it was found that intrathecal administration of N-type, but not L-, T-, and P /Q-type, Ca^{2+} channel blockers produced antinociceptive effects in the gabapentin-sensitive models, including the nerve ligation neuropathic, postoperative, formalin, and other inflammatory pain models. It is, therefore, suggested that spinal N-type Ca^{2+} channels may be involved in the antinociceptive effect of intrathecal gabapentin. This does not mean that gabapentin selectively interacts with N-type Ca^{2+} channels but that the N-type Ca^{2+} channels are important analgesic targets in the spinal cord. The possibility remains that other types of Ca^{2+} channels are involved in other therapeutic actions of gabapentin. [4]

However there are no reports found regarding the effects of gabapentin on acute inflammatory conditions. This indicates that anticonvulsant drugs can be a good option as anti-inflammatory drug. The present research work was therefore, be focused to further investigate the effect of

gabapentin in acute inflammatory conditions in rodents. Further, the present study was aimed to assess any possible modulation of anti-inflammatory effect of sub-effective dose of gabapentin when co-administered with of sub-effective dose of verapamil (a selective L-type calcium channel blocker) or nimesulide (a preferential cyclooxygenase -2 inhibitor) against carrageenan-induced rat paw edema.

MATERIALS AND METHODS

***Animals*:**
Healthy albino Wistar rats (150-250 g) of either sex (n = 6 per group) were used. Animals were procured from Venkateshwara Enterprises, Bangalore and housed in standard environmental condition in the institutional animal house. The animals were fed with standard pellet rodent diet (Lipton India Ltd., Mumbai) and water was provided *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethical Committee of K.L.E.S's College of Pharmacy, Belgaum (Karnataka), India.

***Drugs and regimen*:**
Gabapentin (generously gifted by Dr. Vijay Pal Singh), nimesulide (Nise® tablet); Verapamil HCL 5mg/2ml (VPL® Injection); carrageenan (Type IV) (Sigma, USA) were used. Gabapentin was dissolved in distilled water. Nimesulide was suspended in 0.5 % carboxy methyl cellulose and verapamil was diluted in distilled water. All drugs were administered per orally (*po*).

***Effect of gabapentin on carrageenan induced inflammatory changes*:**
Carrageenan-induced rat paw edema (inflammation): [7-9]
Rats from respective groups were received vehicle (saline), nimesulide (4 mg/kg) or gabapentin (10, 30 or 100 mg/kg) and thirty minutes later, challenged with subcutaneous injection of freshly prepared carrageenan (type IV) (0.1 ml, 1% w/v solution) into the plantar side of the left hind paw. The right (contra-lateral) paw was served as control (non-inflamed paw) for comparison (0.1 ml/paw of normal saline). The paw volumes were measured using plethysmometer (UGO Basile, Italy) at various time intervals after carrageenan or saline injection up to 5 h. The changes in paw volume were expressed as percent inhibition calculated using the formula:

$$[(V_T - V_0)_{control} - (V_T - V_0)_{treated}] \text{ X } 100/ (V_T - V_0)$$

Where V_0 is the paw volume measured immediately, and V_T is the paw volume at one particular time interval after carrageenan or saline injection.

Myeloperoxidase (MPO) activity in the rat footpad:
Myeloperoxidase activity was determined following technique reported earlier. [10, 11] Four hours (time corresponding to maximum inflammation) after carrageenan injection, rats were sacrificed and hind paws from different treatment groups were collected. The isolated segments from different treatment groups were individually homogenized in 5 ml of phosphate buffer (0.01M). Homogenized tissue was centrifuged at 10,000 rpm. Supernatant collected mixed with *o*-phenylenediamine (660 µg/ml in phosphate buffer) and 300 mM of H_20_2 were used to initiate the reaction. Absorbance was observed at 492 nm at an interval of 30 sec. for 5 min. Change in the optical density per minute was calculated and the results were expressed as percentage increase of myeloperoxidase activity considering 100 % myeloperoxidase activity in control (normal paw).

Lipid peroxidation assay in the rat footpad:
The extent of lipid peroxidation was measured as malondialdehyde formed after reaction with thiobarbituric acid by the technique reported earlier [12]. In brief, four hours (time corresponding to maximum inflammation) after carrageenan injection, rats were sacrificed and hind paws from different treatment groups were collected. The isolated segments from different treatment groups were individually homogenized in 10 %, 0.1 M potassium phosphate buffer (pH 7.4) (1 g of wet tissue to 9 ml buffer). The peroxidation in homogenate was stopped by addition of 400 µl of 35 % perchloric acid. The homogenate was then centrifuged (5000 X g for 10 min) to obtain supernatant. To the supernatant, 400 µl of 1.2 % thiobarbituric acid was added, and the mixture was placed in water bath (95-100°C) for 30 min. The solution was then cooled; the absorbance was measured spectrophotometrically at a wavelength of 532 nm. The results were expressed as % lipid peroxidation assuming control (normal paw) values as 100 %.

Histopathlogical changes in the rat footpad: [13]
In brief, four hours (time corresponding to maximum inflammation) after carrageenan injection, rats were sacrificed and tissue sections (1 cm) from the plantar surface of hind paws was collected and washed with normal saline. The sections were fixed in 10 % neutral-buffered formaldehyde, embedded in paraffin, sectioned and stained with haemtoxylin and eosin and observed for inflammatory events under microscope

***Combination study*:**
Rats from respective groups were received vehicle, nimesulide 0.5 mg/kg, verapamil 2 mg/kg, gabapentin 5 mg/kg, gabapentin 5 mg/kg + nimesulide 0.5 mg/kg or gabapentin 5 mg/kg + verapamil 2 mg/kg. The anti-inflammatory effect of combinations was evaluated using the test described as above under carrageenan-induced rat paw edema.

Statistical analysis:
Data presented as mean±SEM. and analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Dunnet's test. $p<0.05$ was considered to be significant.

RESULTS

Effect of gabapentin on carrageenan induced inflammatory changes:

Table 1: Effect of gabapentin on carrageenan-induced paw edema in rats

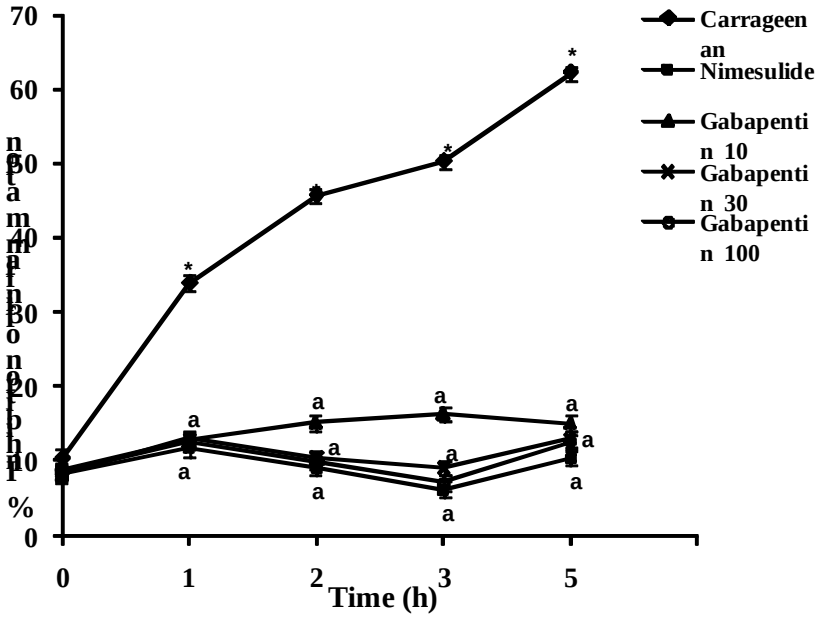
Group/ Treatments	Dose (mg/kg)	Change in paw volume (ml)				
		0 h	1 h	2 h	3 h	5 h
Carrageenan control	---	0.100±0.013	0.183±0.017*	0.275±0.017*	0.35±0.018*	0.25±0.018*
Nimesulide	4	0.092±0.015	0.133±0.011a	0.100±0.013a	0.067±0.011a	0.117±0.011a
Gabapentin	10	0.100±0.013	0.150±0.018a	0.183±0.017a	0.200±0.013a	0.183±0.024a
	30	0.092±0.015	0.150±0.013a	0.117±0.011a	0.100±0.013a	0.150±0.013a
	100	0.092±0.015	0.142±0.015a	0.108±0.008a	0.075±0.011a	0.142±0.008a

*Data expressed as mean±SEM (n = 6). *p<0.05 as compared to the volume of contra-lateral paw, ap<0.05 as compared with carrageenan control.*

Carrageenan-induced rat paw edema (inflammation):
The challenge with carrageenan (1% w/v, 0.1 ml) in the hind paw of rat produced the significant increase in the paw volume at 3-5 h of carrageenan injection as compared to the volume of contra-lateral paw. Gabapentin (10, 30 & 100 mg/kg, *po*) produced significant but not dose-

dependent inhibition of paw edema as compared to carrageenan control. The peak effect for gabapentin was observed at 3 h. Nimesulide (4 mg/kg, *po*) produced significant anti-inflammatory effect in rats as compared to carrageenan control. (Table 1 & Figure 1)

Figure 1: Effect of gabapentin on carrageenan-induced paw edema in rats



*Data expressed as mean±SEM (n = 6). *p<0.05 as compared to the volume of contra-lateral paw, [a]p<0.05 as compared with carrageenan control*

Myeloperoxidase (MPO) activity in the rat footpad:

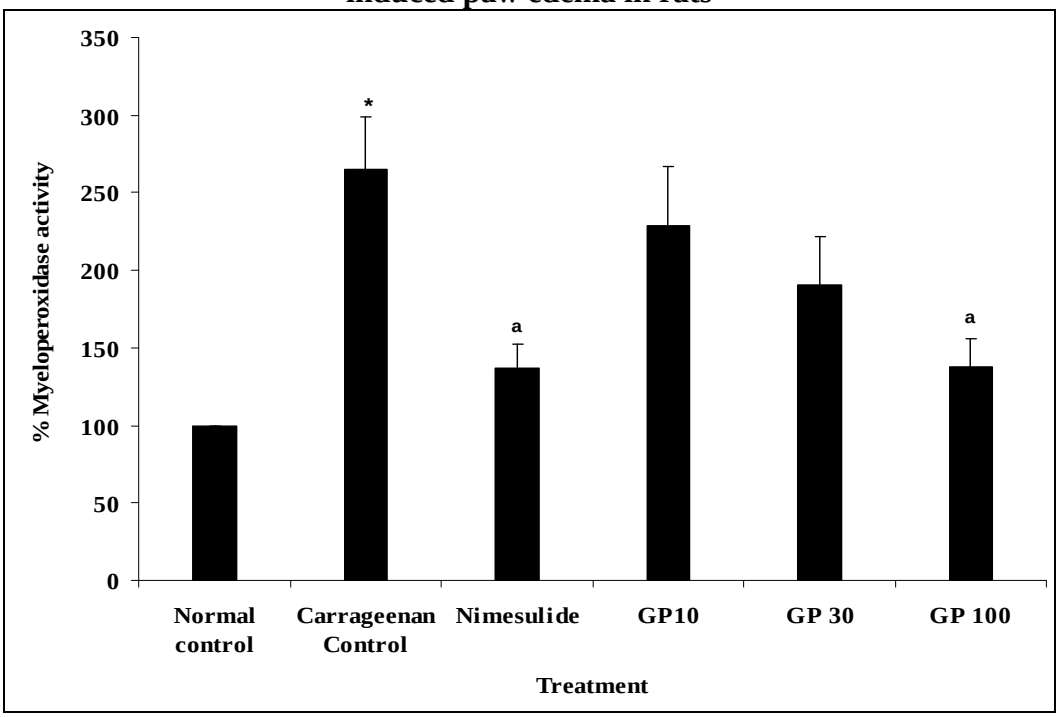
The MPO activity was tested at 4 h (time of peak inflammation) of carrageenan challenge. Intraplantar injection of carrageenan (0.1 % w/v, 0.1 ml) in rat hind paw induced marked neutrophil infiltration in the paw, as measured by MPO activity, which was significantly prevented by gabapentin 100 mg/kg but not by 10 and 30 mg/kg. (Table 2 & Figure 2)

Table 2: Effect of gabepentin on myeloperoxidase (MPO) activity in carrageenan-induced paw edema in rats

Groups/ Treatment	Dose (mg/kg)	Absorbance	% MPO activity
Normal control	--	0.156±0.0104	100
Carrageenan control	--	0.408±0.0487*	264.9±33.62*
Nimesulide	4	0.210±0.0183[a]	136.6±16.05[a]
Gapapentin	10	0.346±0.0351	229.1±37.65
Gapapentin	30	0.295±0.0419	191.0±30.93
Gapapentin	100	0.213±0.0244[a]	137.8±18.00[a]

*Data expressed as mean±SEM. *p<0.05 compared with the normal control, [a]p<0.05 compared with carrageenan control. Percent MPO activity was calculated with respect to the untreated paw (Normal control), which was considered 100 %*

Figure 2: Effect of gabepentin on myeloperoxidase (% MPO) activity in carrageenan-induced paw edema in rats



*Data expressed as mean±SEM. *p<0.05 compared with the normal control, [a]p<0.05 compared with carrageenan control. Percent MPO activity was calculated with respect to the normal control, which was considered 100 %*

Lipid peroxidation (LP) assay in the rat footpad

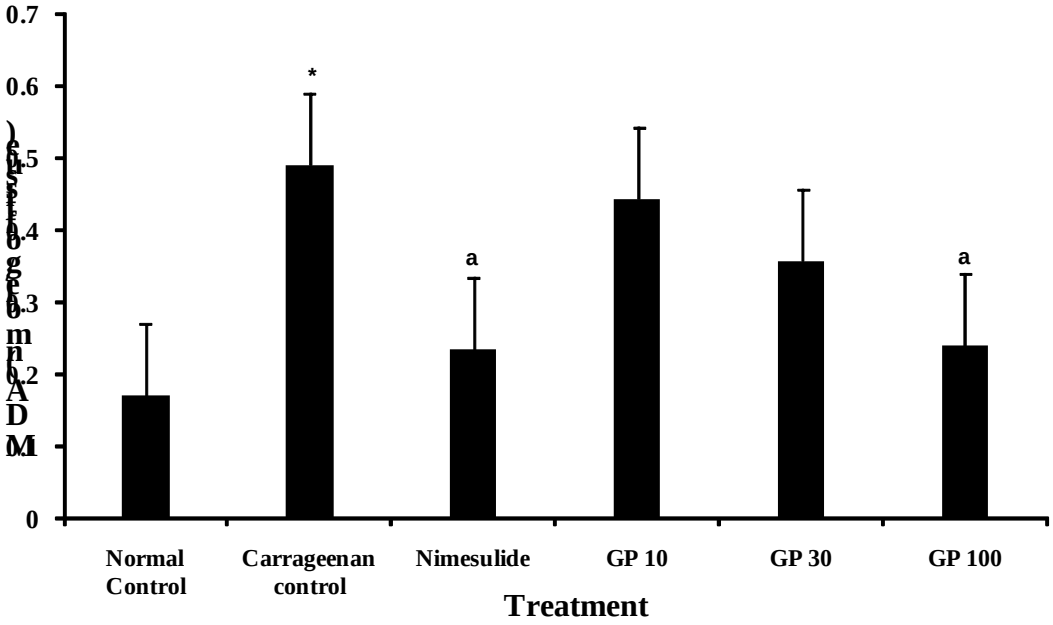
The LP was tested at only 4 h (time of peak inflammation) of carrageenan challenge. Intraplantar injection of carrageenan (0.1 % w/v, 0.1 ml) in rat hind paw induced marked LP in the paw, measured as MDA levels, which was significantly prevented by gabapentin 100 mg/kg but not by 10 and 30 mg/kg (Table 3 & Figure 3).

Table 3: Effect of gabepentin on lipid peroxidation (LP) in carrageenan-induced paw edema in rats

Groups/ Treatment	Dose (mg/kg)	Absorbance (532 nm)	MDA (nmols/mg of tissue)	% Lipid peroxidation
Normal control	--	0.105±0.013	0.171±0.021	100
Carrageenan control	--	0.301±0.042*	0.491±0.069*	287.588
Nimesulide	4	0.143±0.010[a]	0.234±0.016[a]	136.885
Gapapentin	10	0.271±0.034	0.445±0.055	259.135
Gapapentin	30	0.218±0.027	0.356±0.044	208.372
Gapapentin	100	0.147±0.010[a]	0.240±0.017[a]	140.691

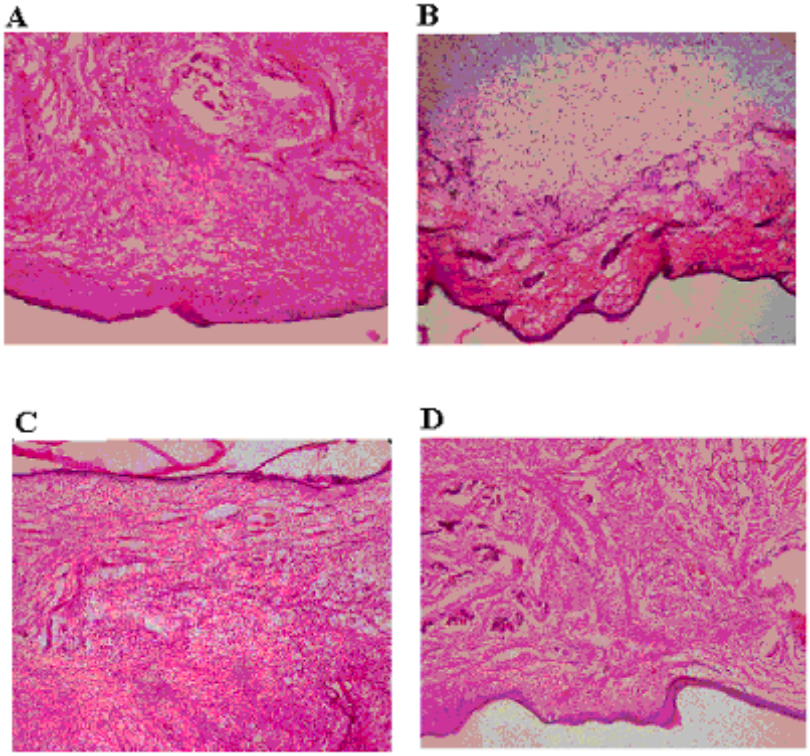
*Data expressed as mean±SEM. *p<0.05 as compared with the normal control, [a]p<0.05 compared with carrageenan control. Percent LP was calculated with respect normal control, which was considered 100 %.*

Figure 3: Effect of gabepentin on lipid peroxidation (LP) in carrageenan-induced paw edema in rats



*Data expressed as mean±SEM. *p<0.05 as compared with the normal control, [a]p<0.05 compared with carrageenan control*

Figure 4: Effect of gabepentin on histopathlogical changes in the carrageenan challenged rat footpad



Representative microphotographs of hind footpad sections from rats from (A) Normal control, (B) Carrageenan control (C) Gabapentin (100 mg/kg, po) and (D) Nimesulide (4 mg/kg, po). 40 X magnification.

Histopathlogical changes in the rat footpad

The tissue sections from hind footpads of carrageenan-treated rats showed pathological changes that can be correlated with inflammation. In particular, edematasis and extravasation of polymorphonuclear (PMN) cells (indicator of cellular infiltration) was evident (Figure 4: A & B).

The treatment with gabapentin 100 mg/kg reduced the histological injury to paw tissue sections. Most of the histological changes were minimized and found negligible (Figure 4 C). Also, the treatment with nimesulide (4 mg/kg, *po*) completely prevented occurrence of carageenan-induced inflammatory changes in the rat hind paw (Figure 4 D).

Combination study

Co-administration of sub-effective dose of gabapentin (5 mg/kg, *po*) with sub-effective dose of nimesulide (0.5 mg/kg, *po*) or verapamil (2 mg/kg, *po*) showed an enhanced anti-inflammatory effect as compared to *per se* effect. (Table 4)

Table 4: Effect of combination of gabapentin with nimesulide or verapamil in carrageenan-induced paw edema in rats

Groups/ Treatments	Dose (mg/kg)	% Inhibition of paw edema (Inflammation)				
		0 Hr	1 Hr	2 Hr	3 Hr	5 Hr
Carrageenan control	---	---	---	---	---	---
Nimesulide	0.5	15.28±6.944	40.43±1.801	57.37±2.704	48.69±2.058	41.02±1.884
Verapamil	2	15.28±6.944	32.49±2.606	47.06±1.097	42.26±1.951	38.97±1.303
Gabapentin	5	5.56±5.556	32.45±2.720	37.68±1.407	42.26±1.462	37.54±1.780
Gabapentin + Nimesulide	5 + 0.5	9.72±6.242	63.80±2.300*	84.66±2.232*	85.36±1.790*	81.53±0.946*
Gabapentin + Verapamil	5 + 2	9.72±6.242	63.62±2.842*	85.85±1.749*	88.61±1.037*	86.43±1.456*

*Data expressed as mean±SEM. *p<0.05 as compared with carrageenan control. Percent inhibition of paw edema (Inflammation) was calculated with respect to the carrageenan control*

DISCUSSION

The present study was aimed to evaluate the anti-inflammatory effect of gabapentin against carrageenan-induced paw edema in rat hind paw. Intraplantar injection of carrageenan in the rat provokes a local, acute inflammatory reaction that is a suitable method for evaluating the anti-inflammatory agents.

In the present study, treatment with gabapentin (10, 30 & 100 mg/kg, *po*) demonstrated inhibition of paw inflammation and also prevented the neutrophil migration at the site of inflammation, evident from the histopathological observations. The effect was comparable to that produced by nimesulide (a preferential COX-2 inhibitor).

The efficacy of gabapentin on neutrophil migration was further confirmed by its effect on myeloperoxidase activity in rat paw, and histopathology of rat footpad tissues. Gabapentin treatment significantly inhibited the myeloperoxidase activity and also reduced morphological injury and neutrophil infiltration in footpad tissues at higher dose tested.

Carrageenan injection into the rat paw provokes a local, acute inflammatory reaction that is a suitable method for evaluating the anti-inflammatory agents.[14] The carrageenan-induced on site inflammation consists of two phases (biphasic event). A rapid early phase (up to 2 h) triggered by the concerted release of histamine, 5-hydroxytryptamin and then peaked at 180 min to release bradykinins, cyclooxygenase products and a more sustained late phase (2 to 5 h)

regulated by neutrophil infiltration and sustained production of arachidonic metabolites (prostanoids) (primarily by cyclooxygenase) or nitric oxide from inducible nitric oxide synthase).[14, 15, 16] The production of arachidonic metabolites is the main factor responsible for the both the first and second phases of the carrageenan-induced inflammation. The second phase of the carrageenan-induced inflammation is also related to PMN accumulation. [14]

The drugs are often combined clinically in variety of situation. Combination therapy using multiple drugs or modalities that target multiple mechanisms is common practice in the treatment of chronic pain. The benefit of combination therapy is purported to lie in its ability to provide improved efficacy than either of the drug administered separately and allow a reduction in the required dose with reduced toxicity. [17] The present study was therefore planned to assess the anti-inflammatory effect of co-administration of sub-therapeutic doses of gabapentin with nimesulide (a preferential COX-2 inhibitor) or verapamil (a calcium channel antagonist).

In the present study the co-administration of sub-effective dose of gabapentin (5 mg/kg, *po*) with sub-effective dose of nimesulide (0.5 mg/kg, *po*) or verapamil (2 mg/kg, *po*) significantly enhanced anti-inflammatory effect against carrageenan induced-paw edema in rats when compared to the effect *per se*.

Earlier, studies involving co-administration of gabapentin with other auxiliary drugs such as clonidine, naproxen, morphine [18], tramadol [19], metamizol [20] or diclofenac [21] reported a therapeutic advantage over the individual drug for clinical treatment of pain and inflammation.

Nimesulide, a preferential COX-2 inhibitor, possesses anti-inflammatory effect by inhibiting COX-2 enzyme and thus the synthesis of prostaglandins, leukotrienes, and thromboxanes. Verapamil, on then other hand, is a selective L-type calcium channel blocker and is also reported to exert anti-inflammatory effect [22, 23]. Gabapentin has too been shown, in present study, to possess inflammation attenuating effect possibly by modulating calcium channels.

CONCLUSION

It might be presumed that the anti-inflammatory activity of gabapentin is related to the inhibition of the release or synthesis of cyclooxygenase products through direct or indirect inhibition of neutrophil infiltration. Additionally, gabapentin treatment also showed an inhibitory effect on generation of lipid peroxides. This might be an important aspect in the anti-inflammatory effect of gabapentin in preventing the damage to cell membranes and subsequent availability of precursors (free arachidonic acid) for generation of pro-inflammatory mediators. The results of the present study hence showed the anti-inflammatory effect of gabapentin against carrageenan-induced paw edema in rats.

The results of combination study in present research work therefore suggested the potential of combining the secondary drugs that are acting through other transaction pathways (COX, Ca^{2+} etc) in inflammation, together for a better control of inflammation with reduced dose of each drug. This might pose a beneficial effect in the clinics for the treatment inflammatory conditions. However further studies for the clinical use of such combinations might be needed to be confirmed for their other potential systemic effects.

Acknowledgement

Sincere thanks to Dr. F.V. Manvi, Principal, KLES's College of Pharmacy, Belgaum, Karnataka, India for providing necessary facilities required for research work.

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