Role of Gabapentin as Anti-Inflammatory Agent Alone and Its Modulatory Effect on Co-Administration with Diclofenac Sodium in Rat Paw Edema

Authors

Rajiv Kumar¹, Akhilesh Kumar², Manju Gari³, Rima⁴, Sumit Kumar Mahato⁵

¹Professor, Department of Pharmacology, RIMS, Ranchi
²Junior Resident, Department of Pharmacology, RIMS, Ranchi
³Professor and Head of Department, Department of Pharmacology, RIMS, Ranchi
⁴Dental College, RIMS
⁵Junior Resident, Department of Pharmacology, RIMS, Ranchi

Corresponding Author

Akhilesh Kumar
Junior Resident, Dept of Pharmacology, 4th Floor, Rims, Ranchi, Bariatu, Jharkhand, Pin Code 834009 India
Mob No 9110156584, Email: docranchi@gmail.com

Abstract

Objective: To evaluate the anti-inflammatory effects of Gabapentin alone and as adjuvant with diclofenac by using formalin induced paw edema model.

Methods: After acclimatization of one week, adult winstar albino rats were divided into two groups: control and treatment (n=6). Treatment group received diclofenac (10 mg/kg), gabapentin (20 mg/kg), and gabapentin (20 mg/kg) + diclofenac (10 mg/kg) respectively. Paw edema was produced by injecting 0.2ml of 2% formalin subcutaneously on the dorsal surface of right hind paw. Animals received drug treatment 30 minutes before injection of formalin and paw volume was measured at 0, 30, 60, 120 and 240 minutes after formalin challenge with help of water plethysmometer.

Results: Both gabapentin alone and in combination with diclofenac caused a significant reduction (p<0.01) in rat paw edema when compared to group given saline only. Reduction in paw edema with gabapentin and diclofenac was significantly superior when compared with either drug alone.

Conclusions: Combination of gabapentin and diclofenac showed synergistic anti-inflammatory effect as compared to either drug alone or combination with diclofenac groups.

Keywords: Diclofenac, Gabapentin, Water Plethysmometer, Inflammation.

Introduction

Inflammation is the response of living tissue to injury.¹ Inflammation leads to increased vascular permeability; cellular response like chemotaxis of neutrophils; free radical production (H2O2,OH).² These inflammatory response are mediated through release of Prostaglandin, cytokines release (IL-1, TNF-alpha, IL-6,IL-11,IL-8), serotonin, bradykinin, substance P.³ The source of these mediators are the injured tissue itself as well normal tissue, present at the site of injury . Formalin produces inflammatory pain when injected in hind paw of rat. Formalin induces rapid Ca²⁺ influx via native TRPA-1 channel. Influx of
Ca^{2+} causes release of neurotransmitter at synapse which further augments inflammation. So formalin represent an ideal inflammatory model to investigate anti-inflammatory effect of test compounds. Gabapentin a structural analogue of GABA and a novel anticonvulsant has been reported to selectively blocks the inflammatory response of the formalin response and carrageenan induced thermal and mechanical hyperalgesia. Both are the animal models of inflammatory pain. The action of gabapentin is mediated through inhibition of the α2δ subunits of voltage-gated Ca^{2+} channels that resemble the Trap-1 channel induced by formalin at nerve terminal. Despite extensive investigations on analgesic mechanisms of gabapentin, not much has been studied about their anti-inflammatory role as there are only few reports in their supports. The present research work is therefore focused to further investigate and evaluate the effect of gabapentin in inflammatory conditions in rodents by creating inflammatory models using formalin. Further, the present study was aimed to assess any possible modulation of anti-inflammatory effect of gabapentin when co-administered with diclofenac in formalin induced rat paw edema.

Aims and Objectives
1. To study anti-inflammatory activity of drug Gabapentin and compare this effect with diclofenac
2. To Evaluate the Modulatory role of gabapentin on anti-inflammatory effect of Diclofenac when both used in combination and compare this with control group.

Methods
The study was conducted after the approval of IAEC (Institutional Ethical Committee). Adult healthy albino rats of Wistar strain of either sex, weighing between 150-200 gm aged 3-4 months were selected from the Central animal facility. They were kept at a constant temperature of 26±20C and relative humidity of 30-70% under a 12 h dark/light cycle. The animals were fed with standard diet and water ad libitum. The rats were acclimatized to the laboratory conditions for seven days prior to test before assigning animals to treatment group. The doses of drugs were based on human daily dose converted to that of rats according to Paget and Barnes (1962).

Drugs and Chemicals: The following drugs were used Gabapentin (Gabapin,300 mg capsule, INTAS, India), Diclofenac (Voveran D, 50 mg tablets, Novartis, India)

Grouping of animals: Twenty four rats were divided into four groups of six animal each. Group 1 were served as control and received comparable amount of normal saline. group 2 served as standard and received diclofenac (10.0 mg/kg body wt.). Group 3 and 4 received test compound, gabapentin (20.0 mg/kg body wt.) alone and gabapentin with diclofenac in combination respectively.

Anti-inflammatory activity: Thirty minutes after oral feeding of winstar rat with drug, edema was produced in all groups by injecting 0.2 ml of 2% formalin subcutaneously on the dorsal aspect of hind paw of the rats. The paw of each rat was marked with the ink at the level of lateral malleolus and immersed in water of plethysmometer up to this mark. For the assessment of the anti-inflammatory activity, the paw volume was measured plethysmographically at 0 min , 30 min , 60 min, 90 min, 120 min and 240 min. The 0th min reading was considered as the initial paw size of the rats. The change in the paw volume in the test groups was compared with the untreated control groups. two parameters were recorded (1) reduction in paw volume (mL) with the help of water plethysmometer and (2) percentage inhibition of paw edema which was calculated by using the following formulas:

% inhibition of paw edema = [(Cf-Ci)-(Tf-Ti)]*100/[Cf-Ci]

where, at a particular time, Cf = final paw volume of control group; Ci = initial paw volume of control group; (Cf-Ci) = change in paw volume of control group; Tf = final paw volume of test
group; Ti = initial paw volume of test group; (Tf - Ti) = change in paw volume of test group.

**Statistical Formulas:** Paw volume (mL) was calculated as (Mean ±SEM). To compare with different groups with the saline groups, one way analysis of variance (ANOVA) was done followed by, p <0.05 was considered significant.

**Results and Discussion**

The present study was aimed to evaluate the anti-inflammatory effect of Gabapentin in formalin induced edema in rat hind paw using digital plethysmometer. In our study vehicle control group (Formalin induced) showed increase in mean paw volume at regular interval. The displaced volume of water which was 0.71ml at 0 min rose to 1.04 ml at 180 min as shown in Fig.1 showing there is increase in rat paw edema. Intraplantar injection of formalin provokes a local acute inflammatory reaction. It also produced writhing movement in rats which indicates occurrence of pain in rats. Our finding was similar to finding by Goyal et al (2015) that also showed increase in paw volume over time. The possible explanation could be that formalin causes tissue injury that induces a cascade of cellular reactions in the lesion area, accompanied with the release of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL 6, IL- 8 and other substances like bradykinins, serotonin, nitric oxide, which is then followed by subsequent inflammatory reactions. Prostaglandins such as PGE1 and PGE2, which are produced at elevated levels in inflamed tissues, increase local blood flow and potentiate the effects of mediators such as bradykinin that induce vascular permeability. Formalin excites afferent sensory neurons by directly activating TRPA-1 channel that induces rapid Ca2+ influx via which formalin activate pain pathway. In formalin induced inflammation there is increased leukocytes migration as well nociceptive fibers activation leading to pain as suggested by Sir Charles Scott Sherrington. Our study with Gabapentin revealed that with its use there was initial increase in paw edema value from 0 to 60 min, value ranging from 0.77 ml to 0.95ml. The mean volume of rat paw start decreasing at 90 min till 180 min ranging from 0.87ml to 0.81ml respectively as shown in table 3. Corresponding percentage inhibition also shows the increase in value from 7% at 30 min to 35% at 90 min till 44% at 180 min. The decrease in paw edema beginning at 90 min and there after continuously decreasing is supported by various test done on gabapentin and various inflammatory model. The findings were similar to study done by Jordana et al (2014). This increase in values was explained on the basis of pharmacokinetics properties of gabapentin which reveals that peak plasma concentration of gabapentin is reached in 2-3 hours i.e. the time when % inhibition was showing an increasing trend. In our study when compared with vehicle control group the gabapentin showed there is constant increase in the % inhibition and decrease mean paw volume as shown in Fig.4. The possible explanation to this effect can be that gabapentin by binding to α2δ subunit of voltage dependent L type calcium channel (VDCC), inhibits release of excitatory neurotransmitters. These neuropeptide is known to produces vasodilatation, plasma extravasations, edema, and leukocyte influx, a process termed “neurogenic inflammation. Gabapentin reduces inflammation have been illustrated in various study. Dias et al have found that GBP decreases Myeloperoxidase (MPO), TNF-α, IL-1β, oxidative stress marker like Malonaldehyde (MDA) and Glutathione (GSH). Normally there level are increased in inflammation. Camara et al have found GBP increases IL-10 level in tissues, an anti-inflammatory cytokines thus inhibiting flaring of inflammation. It has been found that during neuropathic inflammation activated macrophages releases IL-2 and more TNF-α, there level was found to decrease in gabapentin pre-treated mice. Gabapentin diminish the release of “pain neuromodulators, fractalkine, Excitatory amino acid like glutamate, substance P, ATP and activation of microglia in the spinal cord by
modulating VDCC \( \alpha_{2\delta}-1 \) subunits, leading to a reduction in thermal hyperalgesia and inflammation.\(^{15}\)

In our study the percent inhibition of rat paw edema after oral administration of diclofenac at 30min, 60min, 90min, 120 min, 180 min were 21\%, 22\%, 45\%, 64\%, 74\% respectively as shown in Fig.1, 2, 3 and table 1. when compared with % edema at 0 min p-value was found to be <0.01 which was highly significant at all time interval supporting the hypothesis that diclofenac was able to decrease inflammation. These finding were supported by earlier studies by Singh et al (2010), Mulla et al (2010) which have stated 68.2\%, 61\% inhibition at 180 min.\(^{16,17}\) The mean paw volume that changed during this time interval were 0.75 ml to 0.76ml at 0 min and 180 min respectively as shown in table 3 and Fig.4. The possible reason to increased % inhibition could be that the second phase of formalin test is attributed to the release of nociceptive mediators, such as histamine, serotonin, prostaglandin, and bradykinin.\(^{18,19}\)

These causes increased production of PG occurs via COX enzyme pathway in the vicinity of injured tissue. Prostaglandins are the major mediators of inflammation. Their inhibition by diclofenac will produce maximum relief from inflammation. When compared with Gabapentin, Diclofenac showed higher % of edema inhibition and more decreased in mean paw volume as shown in fig1-4 and table 1-2 The possible explanation could be that diclofenac cause direct inhibition of PGs synthesis rather than indirect method as done by gabapentin where inhibiting channels to influence neurotransmitter release and up-regulating IL-10 to inhibit pro-inflammatory cytokines release. Our study also revealed that % inhibition by Diclofenac was maximum at 90, 120, 180 min. This occurred in spite of peak plasma plasma concentration is achieved at 35 min but owing to high binding to plasma protein (99.7\%) the maximum concentration at the site of inflammation are achieved after 2-3 hours of peak plasma level.

Diclofenac when combined with Gabapentin the % inhibition of paw edema were found to be superior to either drug when used alone. % inhibition at 30, 60, 90, 120, and at 180 min were 35\%, 36.36\%, 58\%, 69\%, 82\% respectively. Corresponding mean paw volume also changed from 0.74ml to 0.73 at 0 min and 180 min respectively as shown in table 2. Gabapentin and diclofenac combination groups showed a significant (\( p< 0.01 \)) reduction in paw volume at various intervals of time in comparison to saline treated control group as shown in table 2. This finding were similar to study done by Goyal et al(2015) who found decrease in mean paw volume and % inhibition of 63\% and 76\% at 120, 240 min interval.\(^3\) The possible explanation could be combined COX inhibition by Diclofenac and neurotransmitter mediated inflammatory reaction inhibition by Gabapentin at site of tissue injury and inhibiting neuropathic inflammation development thus having greater inhibitory effect on paw edema development in rats.

**Observation**

**Table1:** Percentage inhibition of rat paw edema in different treated groups

|       | 0 min | 30 min | 60 min | 90 min | 120 min | 180 min |
|-------|-------|--------|--------|--------|---------|---------|
| Group A | -     | -      | -      | -      | -       | -       |
| Group B | 0     | 7%     | 18%    | 35%    | 41%     | 44%     |
| Group C | 0     | 21%    | 22%    | 45%    | 64%     | 74%     |
| Group B and C | 0 | 35% | 36.36% | 58% | 69% | 82% |
Table 2. Mean and Standard Deviation with p value of water volume displacement digital plethysmometer

| Drugs                  | 0 min | 30 min | 60 min | 90 min | 120 min | 180 min |
|------------------------|-------|--------|--------|--------|---------|---------|
| Normal Saline          | Mean±S.D | 0.71±0.075 | 0.85±0.049 | 0.95±0.062 | 0.99±0.072 | 1.02±0.065 | 1.04±0.64 |
|                        | P value | 0.001 | 0.003 | 0.001 | 0.000 | 0.001 |         |
| Gabapentin             | Mean±S.D | 0.77±0.095 | 0.90±0.079 | 0.95±0.101 | 0.87±0.068 | 0.83±0.67 | 0.81±0.59 |
|                        | P value | 0.003 | 0.042 | 0.005 | 0.014 | 0.015 |         |
| Diclofenac             | Mean±S.D | 0.75±0.076 | 0.86±0.80 | 0.92±0.058 | 0.84±0.056 | 0.79±0.58 | 0.76±0.073 |
|                        | P value | 0.001 | 0.005 | 0.001 | 0.016 | 0.013 |         |
| Gabapentin+Diclofenac  | Mean±S.D | 0.74±0.077 | 0.83±0.066 | 0.9±0.036 | 0.81±0.047 | 0.76±0.50 | 0.73±0.056 |
|                        | P value | 0.002 | 0.003 | 0.006 | 0.010 | 0.049 |         |

Figure 1: % inhibition in paw volume in different treated groups

Figure 2: % inhibition in paw volume in different treated groups and their combination
Figure 3: Bar diagram showing % inhibition in paw volume in different groups and their combination.

Figure 4: Line diagram showing changes in mean volume of formalin induced rat paw using Normal saline, Gabapentine, Diclofenac and their combination.

**Limitation**

1. Histo-pathological study of inflamed paw tissue could not be done which would have shown the changing nature of inflammatory process.
2. Only limited drug dose study was done as it was not possible in our settings.

**Conclusion**

From the finding of our study, we can conclude that Gabapentin has a significant anti-inflammatory property in formalin induced paw edema in albino rats. The result is significantly better if it is co-administered with diclofenac as compared, when the drug are used individually.

We can predict that further clinical study of these drugs may give influencing results in patients requiring regular medication for chronic conditions.
inflammatory and arthritic pain who are at risk of NSAIDs toxicity. There is need of big sample size research with more sensitive & specific methods to established gabapentin as a novel anti-inflammatory drug in chronic pain and further human studies are required to establish the same.

Reference
1. Rankin JA. Biological mediators of acute inflammation. AACN Clin Issues. 2004 Jan-Mar;15(1):3-17.
2. Manish Mittal, Mohammad Rizwan Siddiqui, Khiem Tran, Sekhar P. Reddy, and Asrar B. Malik. Reactive Oxygen Species in Inflammation and Tissue Injury. Antioxidant & Redox signalling. 2014;20(7):7.
3. Jun-Ming Zhang and Jianxiong An. Cytokines, Inflammation and Pain. Int Anesthesiol Clin. 2007 Spring. 2007; 45(2): 27–37.
4. Kumar, Abbas, Aster. Inflammation and Repair. Robbins and Cotran Pathologic Basis of Disease. 9th edition. Chapter 3, page no 73-96.
5. Goyal, Sarita. (2015). Modulatory Role of Morphine and Gabapentin as Anti-inflammatory Agents Alone and on Coadministration with Diclofenac in Rat Paw Edema. American Journal of Pharmacology and Pharmaco-therapeutics 2015;2(1):056-061
6. Xianxin Hua, Xuedong Liu, Dominic O. Ansari, and Harvey F. Lodish,1998. Synergistic cooperation of TFE3 and Smad proteins in TGF-β-induced transcription of the plasminogen activator inhibitor-1 gene. Genes & Dev. 1998;12: 3084-3095.
7. Yoshida T., Yamagishi S., Nakamura K., Matsui T., Imaizum T. Telmisartan inhibits AGE-induced C-reactive protein production through down regulation of the receptor for AGE via PPAR-γ activation. Diabetologia. 2006;49:3094–3099
8. Colleen R. McNamara, Josh Mandel-Brehm, Diana M. Bautista, Jan Siemens, Kari L. Deranian, Michael Zhao, Neil J. Hayward, Jayhong A. Chong, David Julius, Magdalene M. Morin, Christopher M. Fanger Proc Natl Acad Sci U S A. 2007 Aug 14; 104(33): 13525–13530.
9. Sherrington CS. The Integrative Action of the Nervous System. New York: Charles Scribner’s Sons; 1906
10. Jordana Maia Dias, Tarcisio Vieira de Brito, Diva de Aguair Magalhães, Pammela Weryka da Silva Santos, Jaide Arruda Batista, Evelia Gabriela do Nascimento Dias, Heliana de Barros Fernandes, Samara Rodrigues Bonfim Damasco, Renan O. Silva, Karoline S. Aragão, Marcellus H. L. P. Souza, Jand-Venes R. Medeiros, and André Luiz R. Barbosa. Gabapentin, a Synthetic Analogue of Gamma Aminobutyric Acid, Reverses Systemic Acute Inflammation and Oxidative Stress in Mice. Inflammation. 2014; 37 (5):1826-36.
11. Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN (1996). The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. J Biol Chem.1996; 271: 5768–5776.
12. Schmelz M, Petersen LJ. Neurogenic inflammation in human and rodent skin. News Physiol Sci. 2001;16:33–37
13. C. R. Cámara-Lemarroy, F. J. Guzmán-de La Garza, and N. E. Fernández-Garza, “Molecular inflammatory mediators in peripheral nerve degeneration and regeneration.” NeuroImmuno Modulation.2010;17(5):314–324.
14. Fregnan F, Muratori L, Simões AR, Giacobini-Robecchi MG, Raimondo S. Role of inflammatory cytokines in peripheral nerve injury. Neural Regeneration Research. 2012;7(29):2259-2266. doi:10.3969/j.issn.1673-5374.2012.29.003.
15. Yang JL, Xu B, Li SS, Zhang WS, Xu H, Deng XM, Zhang YQ. Mol Brain. 2012 May 30;5:18. doi: 10.1186/1756-6606-5-18.

16. Singh M, Kumar V, Singh I, Gauttam V, Kalia AN. Anti-inflammatory activity of aqueous extract of Mirabilis jalapa Linn. leaves. Pharmacognosy Research. 2010;2(6):364-367.

17. Mulla W, Kuchekar S, Thorat V, Chopade A, Kuchekar B. Antioxidant, Antinociceptive and Anti-inflammatory Activities of Ethanolic Extract of Leaves of Alocasia indica (Schott.). Journal of Young Pharmacists: JYP. 2010;2(2):137-143. doi:10.4103/0975-1483.63152

18. Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain. 1992; 51: 5-17

19. de Queiroz, D. P. de Lira, T. D. L. M. F. Dias et al., “The antinociceptive and anti-inflammatory activities of Piptadenia stipulacea Benth. (Fabaceae),” Journal of Ethnopharmacology. 2010;128(2): 377–383, 2010.