



GABAPENTIN IS EFFECTIVE IN VISCERAL & BIPHASIC ANIMAL PAIN MODELS: AN EXPERIMENTAL STUDY

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ABSTRACT

Pain syndrome involves a variety of etiopathogenesis and temporal domains, management of pain as such requires consideration of many factors that may dictate appropriate therapeutic management. Carbamazepine is an established drug for trigeminal neuralgia while Gabapentin has been tried in postoperative pain but its effectiveness perse and when compared to conventional analgesics needs to be evaluated. The present study was planned to study the analgesic effects of Gabapentin in various pain models like writhing and formalin test and to compare it with conventional analgesics like Diclofenac sodium and Tramadol in various acute pain models. This study has been carried out in department of Pharmacology, HIMS, Dehradun, for evaluation of Gabapentin for its antinociceptive effect in rats and mice. In the writhing test, a reduction in number of writhes, though insignificant, was found in the Gabapentin pre-treatment group. However, in the first phase of Formalin test which is characterized by licking and biting, Gabapentin produced no significant effect in comparison to control values. In the second phase of leg raising (LR), all three drugs, i.e. Gabapentin and the two positive controls i.e. diclofenac and tramadol produced significant decrease ($p < 0.05$) in episodes when compared to the control group.

Key words: pain, neuropathic pain, animal models

INTRODUCTION

Pain is one of the most common presentation in a clinical set up and needs immediate and appropriate attention of the treating physician. As pain syndromes involve a variety of etiopathogenesis and temporal domains, management of pain as such requires consideration of many factors that may dictate appropriate therapeutic management.

NSAIDs and opioids are the most potent and commonly used group of established analgesic but their use is associated with a greater degree of adverse drug reactions. Since treatment of pain, especially the neuropathic pain, continues to be a challenge, a variety of drugs like Anticonvulsants, Gabapentin, Tricyclic Antidepressants have been evaluated from time to time as newer unconventional analgesic drugs. Some of these drugs are being empirically used, the rationality of their use for neuropathic pain is still ill defined. They show variable effect on animal pain models (both acute and chronic pain) and abuse liability (1,2).

Evaluation of drugs in chronic pain models, is quite cumbersome, time consuming and costly, and needs to be uniformly standardized, however, acute pain models are well established and they have been in use for many years. So the present study was planned to verify the effects of Gabapentin with conventional analgesics in acute pain models of Formalin induced rat paw edema and writhing test since these are reversible experiments that can be done with ease.

MATERIALS AND METHODS

This study was carried out in department of Pharmacology, HIMS, Dehradun for evaluation of analgesic effects of various drugs in animal models of pain after clearance from institutional animal

ethics committee. Ethical committee no is - pc secrecy /3445 /09/2006.

Animals used

Adult albino rats of either sex, weighing 150-200 gm.
Adult mice of either sex, weighing 18-30 g

Drugs

The following drugs were used to evaluate their antinociceptive effects in our study. The drugs were given per orally (p.o), 1 hr. before the experimentation. The control group of 6 animals was run simultaneously and given saline/double deionized water per orally (p.o). All the experiments were done at the same time in the morning hours on all days of experimentation.

Drug Doses

Gabapentin	50mg/kg (20)
Diclofenac	5mg/kg (11)
Tramadol	10mg/kg (11, 12)

Commercial preparations of these drugs have been used. Tramadol (Lupin Ltd. Santacruz Mumbai) was dissolved in saline as it is water soluble. Gabapentin (Sunpharma, Dadra, New Delhi) and Diclofenac (Novartis India Ltd. Pune, India) were suspended in 5 % acacia & double deionized water. All drugs were administered per oral by gavage.

Procedures: For antinociceptive evaluation (4,5)

Writhing test

For the writhing test 0.55% acetic acid solution was prepared and injected i.p to the mice (3). Mice were placed individually into glass beakers and 5 minutes were allowed to elapse. The test drug was injected intraperitoneally to the mice. The mice were observed for a period of 10 min and the number of writhes were recorded for each animal for a period of 30 minutes. The animals reacted with a characteristic stretching behavior, which is called writhing (14). Treatment groups were compared with appropriate control groups using 'student t-test'

Formalin test (Table2)

The formalin test has been used as a model of tonic inflammatory pain. Rat was administered 0.05 ml of 10% formalin into the dorsal portion of the front paw. Formalin injection leads to occurrence of two characteristics phases of increased pain sensitivity in rats. The first phase lasts for a period of 0-15 minutes and phase II for 30 minutes. Pain has been quantified by counting the incidence of spontaneous flinches, shakes and jerks, leg raising, licking and biting of the formalin in the injected paw. Analgesic response was indicated if both paws are resting on floor with no obvious favoring of injected paw (18,19)

RESULTS

Writhing test

The Writhing test in mice which denotes inflammatory and visceral pain, revealed that Diclofenac and tramadol (both used as positive controls) produced significant decrease in writhes in comparison with control ($p < 0.01$). A reduction in number of writhes though insignificant was found in the Gabapentin pre-treatment group. (Table I)

Formalin test

In the first phase characterized by leg raising (LR), licking and biting in formalin test, both positive controls, (tramadol and diclofenac) produced significant decrease in leg raising ($p < 0.05$). Gabapentin however, produced no significant effect on leg raising in comparison to control values. In the second phase of raising foot (LR), all three drugs, i.e. Gabapentin and the two positive controls i.e. diclofenac and tramadol produced significant decrease ($p < 0.05$) when compared to the control values. Maximum effect was seen by tramadol ($p < 0.01$) followed by other drugs ($p < 0.05$) in suppressing leg raising (LR). In the licking and biting episodes of second phase, all drugs exerted a significant effect ($p < 0.02$) in comparison to control. The decrease observed in licking and biting (LB) with diclofenac and tramadol was more ($p < 0.001$) as compared to control values than with the experimental drug ($p < 0.02$) versus control values. (Table 2)

Writhing test: Pain was induced by injection of 0.55% acetic acid (0.55% given as .01ml/gm i.p)in the peritoneal cavity of mice. All test drugs was administered 1 hr before giving acetic acid (i.p) in mice, abdominal contractions (writhes) were recorded after 5 min of injected acetic acid till 30 min. Animals were divided into four groups of six animals each

Table 1

Group	Dose and Route of Administration of drugs	No. of Albino Mice	No. of Writhes
			Mean \pm SE
Control (NS)	0.09% p.o	6	40.00 \pm 1.81
Diclofenac	5 mg/kg p.o	6	28.00** \pm 1.80
Tramadol	10 mg/kg p.o	6	32.33** \pm 1.59
Gabapentin	50 mg/kg p.o	6	37.33 \pm 1.48

** $p < 0.01$ vs. control values

Table 2

Group	No of Albino Rats	Dose and Route of Administration of drugs	Raising Foot (Mean \pm SE)		Licking & Biting (Mean \pm SE)	
			First Phase	Second Phase	First Phase	Second Phase
Control	6	0.09% p.o.	13.8 \pm 2.9	6.2 \pm 1.2	23.0 \pm 3.0	15.3 \pm 1.8
Diclofenac	6	10 mg/kg p.o	5.8 \pm 1.3*	2.7 \pm 0.5*	9.2 \pm 1.0**	6.3 \pm 0.7***
Tramadol	6	5 mg/kg p.o.	5.2 \pm 1.1*	2.3 \pm 0.3**	8.2 \pm 1.2**	5.8 \pm 0.8***
Gabapentin	6	50 mg/kg p.o.	13.7 \pm 3.3	2.5 \pm 0.2*	22.0 \pm 3.3	6.8 \pm 1.4**

* $p < 0.05$ vs control values ** $p < 0.02$ vs control values *** $p = 0.001$ vs control values

Formalin Test

Albino rats were administered dilute formalin (0.05 ml of 10% in NS, i.d.) on the dorsal surface of the right forepaw. Experimental drugs and positive controls (tramadol and diclofenac) were administered p.o 1 h before and the number of raising foot and licking and biting responses were measured in 2 phases, 1st at 0-15 min and 2nd at 45-75 min.

DISCUSSION

In the writhing test, tramadol and diclofenac showed significant analgesia, while gabapentin produced some analgesic effect, though not statistically significant. In formalin test, gabapentin and the two

positive controls produced significant analgesic effect only in phase II of the experiment.

Writhing test in mice denotes inflammatory and visceral pain. Diclofenac and tramadol (both used as positive controls) produced significant decrease in writhes in comparison with control ($p < 0.01$). Tramadol produced significant antinociceptive effect in writhing test as observed in a previous study whereby tramadol, 5 mg/kg & 10 mg/kg, i.p. produced a marked decrease in the number of writhes induced by acetic acid (1%v/v), suggesting a strong antinociceptive effect (13). Further, in another study, tramadol 2.09 - 4.31 mg/kg, i.p. induced a dose dependent inhibition of the writhing response when administered to mice (6).

Tramadol is an atypical opioid agent that also modulates the monoaminergic pathway (7) and acts on central pathways of pain to modulate pain perception and reaction to pain. Its weak opioid agonist activity is mainly due to its active metabolite i.e. o-desmethyl tramadol. Earlier studies with Tramadol 10 mg/kg, i.v. have produced significant analgesic activity in hot plate and paw pressure test and p-phenyl benzoquinone induced writhing test (9).

Further, Tramadol, 1.7 mg/kg, p.o. & 19.5 mg/kg, s.c. produced dose related antinociception in rat in the 'air induced abdominal constriction' & 'hot plate' tests respectively (12), which further establishes the strong antinociceptive influence of tramadol in animal models of visceral and phasic pain a good candidate for positive control in present study.

In writhing test, in present study, Gabapentin pre-treatment produced a decrease, though not significant ($p > 0.05$) in number of writhes. It has been earlier documented in human trials that only 10-30% patients respond to ion channel blockers like Gabapentin and this could be the reason for poor antinociceptive response to the drug in our study. In an earlier study however, Gabapentin in a dose of 100 mg/kg, i.p. & 70 mg/kg, i.p. reduced acetic acid (0.6% & 0.75% v/v respectively) induced nociception (13,20).

This difference can be explained on the basis of higher oral doses (70 mg/kg, p.o.) used in previous study unlike 50 mg/kg, p.o. in the present study(20). Use of higher dose could have led to an antinociceptive response and this was one of the limitations of our study.

We anticipate that if the study would have been conducted using a 'dose range' rather than a single dose, we would have been able to reach to a more conclusive antinociceptive dose in animal model of mice.

In formalin test, diclofenac produced significant analgesic effect in both phase 1 and phase 2 which has also been observed in an earlier in which diclofenac at a dose of 5,10 and 20 mg/kg, i.p. study produced significant antinociceptive effect in both phases of Formalin test (8).

A previous study revealed that diclofenac, 5mg/kg, i.v. had produced analgesic effect alone or in combination with opioid and pretreatment with local diclofenac, 25-200 mg/paw in formalin test in the past (10,13).

Our study reveals that Gabapentin inhibits late phase of Formalin test. The second or the late phase of formalin test represents the central sensitization to pain which plays a key role in perpetuation of pain, especially in neuropathic pain syndromes even after the painful stimulus has been withdrawn(16,17).

The very fact that Gabapentin showed significant pain relief in second phase of Formalin test indicates that Gabapentin is a useful drug for neuropathic pain where central sensitization assumes a key role. Gabapentin which is a structural analogue of GABA and acts by binding to a subunit of voltage gated Calcium channel in the brain reverses not only the central component of pain but also its peripheral component by suppressing ectopic discharges in the peripheral nerves, as has been documented earlier, where GABAA and GABAB receptor agonists inhibit Pain due to substance P while GABAA agonists decrease NMDA induced nociception (14,15).

The beneficial effect of Gabapentin in Writhing model, which though not significant ($p > 0.05$) throws light on peripheral mechanisms involved in antinociceptive effect of Gabapentin (19).

Hence it is plausible to conclude that Gabapentin acts as an antinociceptive agent by more mechanisms than one and makes it an interesting drug for further evaluation and research for painful conditions unresponsive to conventional drugs and also for breaking the vicious cycle of Chronic pain that self perpetuates in neuropathic pain syndromes.

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