CHAPTER 10:
INTERACTIONS BETWEEN CYCLOOXYGENASE INHIBITORS AND GABAPENTIN IN ACUTE NON-INFLAMMATORY AND INFLAMMATORY PAIN
10.1. INTERACTIONS BETWEEN CYCLOOXYGENASE INHIBITORS AND GABAPENTIN IN ACUTE VISCERAL AND INFLAMMATORY PAIN

10.1.1. INTRODUCTION

Noxious insult to the peripheral and central nervous system arising as a result of tissue damage or inflammation can induce sensitization of peripheral nociceptor terminals and increase neuronal hyperexcitability within the spinal cord (Woolf and Salter 2000). The major therapy for acute non-inflammatory and inflammatory pain is NSAIDs, which reduce prostanoids levels (Simmons et al., 2004). It is well known that NSAIDs produces analgesic and anti-inflammatory activates by inhibiting the COX enzyme and thereby prevents the synthesis of PGs which sensitize nerve endings and cause inflammation. Despite their ability to reduce pain and inflammation, NSAIDs cause wide variety of reported adverse events, paramount among them are upper gastrointestinal side effects, such as dyspepsia, peptic ulceration, hemorrhage and perforation, leading to death in some patients (Kulkarni et al., 2000; Peskar, 2001; Peng and Duggan, 2005).

Gabapentin is an anticonvulsant that was originally developed as a spasmolytic agents and adjuvant in the management of generalized or partial epileptic seizures resistant to conventional therapies (Crawford et al., 1987). Its pharmacological actions are different from other substances that interact at GABAergic synapses because it does not bind to GABA receptors or any of the known neurotransmitters receptors (Rock et al., 1993; Taylor et al., 1998). A number of preclinical studies has reported that gabapentin, unlike opiates, had no effect on transient physiological nociceptive responses but possessed antihyperalgesic and antiallodynic properties in animal models of inflammatory, surgical, and neuropathic pain (Field et al., 1997; Hunter et al., 1998; Gustafsson et al., 2003). These studies have demonstrated that gabapentin has a good side effect profile compared with other existing agents used to treat neuropathic pain. In addition, analgesic effects of gabapentin in relieving acute inflammatory and non-inflammatory pain have also been demonstrated in few studies (Shimoyama et al., 1997; Feng et al., 2003; Gustafsson et al., 2003).

The development of new pain strategies involves combinations of analgesics belonging to different pharmacological classes. The potential advantage of using
combinations is to achieve one or more therapeutic goals such as facilitating patient compliance, improving analgesic efficacy without increasing adverse effects, or decreasing adverse effects without loss of efficacy (Kehlet and Dahl, 1993; Raffa, 2001).

Thus, the present study was designed to investigate pharmacodynamic interactions using acetic acid-induced writhing and formalin-induced tonic pain in mice since these models are analogous to human non-inflammatory visceral pain and inflammatory postoperative pain, respectively. The accumulating evidence for the presence of the COX enzymes both in the periphery and in the spinal cord and analgesic efficacy of COX inhibitors and gabapentin in relieving acute non-inflammatory and inflammatory pain prompted to carry out pharmacodynamic analgesic interaction between NSAIDs and gabapentin to assess any beneficial interactions, if any existed, in these acute nociceptive models.

10.1.2. MATERIALS AND METHODS

10.1.2.1. Experimental animals
As per 1.2.1.
Albino Laka mice (20 – 25 g; bred in Central Animal House of Panjab University, Chandigarh, India) of either sex were used. Each mouse was used for a single treatment and each group consisted of 6 or 8 animals.

10.1.2.2. Behavioral assessment of pain
10.1.2.2.1. Acetic acid-induced writhing
As per 1.2.2.1.

10.1.2.3. Formalin-induced tonic pain
As per 1.2.2.2.

10.1.2.3. Drugs and treatment schedule
The drugs used in the study were naproxen, valdecoxib (Panacea Biotec Ltd., Lalru, India) and gabapentin (SUN Pharma, India) and the reagents were acetic acid and formalin (Loba chemicals, India).

The solutions of all the drugs alone or in combination for intraperitoneal administration were freshly prepared by solubilizing them in distilled water with one or two drops of Tween 80 except gabapentin, which was dissolved in distilled water only and administered 1 ml/100 g body weight 30 min before algesiometric test. Control animals received equivalent volume of saline intraperitoneally.

10.1.2.4. Experimental design
10.1.2.4.1. Dose-response studies for NSAIDs and gabapentin

Six to eight animals were used at each of at least four doses to determine a dose-response curve. Dose-response curves were constructed to assess antinociceptive activity of NSAIDs and gabapentin administered intraperitoneally in both the nociception models. Several doses of naproxen (5, 10, 20, 50 or 100 mg/kg, i.p.), valdecoxib (1, 5, 10, 20 or 50 mg/kg, i.p.), and gabapentin (10, 20, 50, 10 or 200 mg/kg, i.p.) were administered to plot dose-response curve. The same doses were used in evaluating antinociceptive activity in both the models. ED\textsubscript{50} (effective dose estimated to produce 50%MPE) with its 95% confidence intervals (CI) using standard linear regression analysis of log dose-response curve was calculated for each drug.

10.1.2.4.2. Drug-interaction studies

Isobolographic analysis was used as per 9.1.2.4.2. Alternatively, a modified method described by Porreca et al. (1990) was used to characterize the interaction between drugs when one drug lacks efficacy. In brief, mixtures of fixed ratio (1:1 or 1:3 of ED\textsubscript{50} value) of effective drug and ineffective drug were used in combination studies and dose-response curve was constructed as mentioned above. The doses for ineffective drug(s) were taken as multiples of ED\textsubscript{50} fractions of the effective drug for intraperitoneal administration. In this interaction study, Z\textsubscript{add} becomes equal to the ratio between ED\textsubscript{50} of the effective drug and its proportion in combination i.e. Z\textsubscript{add} = Z\textsubscript{2}/p\textsubscript{2} considering drug 1 is ineffective. The remaining procedure is same as above mentioned to analyze statistical significance. In case of both the drugs lack efficacy or minimal efficacy, then mixtures of ineffective drugs in a fixed dose ratio (1:1 of weights) were used in combination studies and dose-response curve was constructed as mentioned above and then %MPE of drug(s) alone and in combination were compared at the same dose level.

The theoretical and experimental ED\textsubscript{50} values of the studied combination were also contrasted by calculating the interaction index (γ) as described in 9.2.2.4.2.

γ = Experimental ED\textsubscript{50} of combination / Theoretical ED\textsubscript{50} of combination

10.1.2.5. Statistical analysis

As per 2.1.2.5 and 9.1.2.5.

10.1.3. RESULTS

10.1.3.1. Dose-response studies for NSAIDs and gabapentin

10.1.3.1.1. Acetic acid-induced writhing
Intraperitoneal administration of a solution of 1% acetic acid resulted in characteristic writhing response in control animals that received saline intraperitoneally with mean writhes count of 39.50 ± 1.41 during 20 min period of observation. Intraperitoneal administration of naproxen produced dose-dependent antinociceptive effect, as there was significant reduction in the number of writhes as compared to control animals in the algesiometric assay (Fig. 10.1.1). However, valdecoxib and gabapentin did not show antinociceptive activity in this algesiometric assay after intraperitoneal administration (Fig. 10.1.2).

10.1.3.1.2. Formalin-induced tonic pain

The administration of formalin subcutaneously into the hind paw of mice induced a typical biphasic licking/biting response with an early and a late phase. Valdecoxib (up to 50 mg/kg, i.p.) failed to have any effect on either phase of the formalin assay (Fig. 10.1.5 and 10.1.6B). Similarly, both naproxen and gabapentin had no effect on the early phase of the formalin response, however, naproxen (5 – 100 mg/kg, i.p.) and gabapentin (10 – 200 mg/kg, i.p.) dose dependently and significantly blocked the development of late phase (Fig. 10.1.5 and 10.1.6A).

10.1.3.2. Drug-interaction studies

10.1.3.2.1. Acetic acid-induced writhing

Naproxen and gabapentin were combined in the ratio of 1:3 of the ED$_{50}$ of naproxen to evaluate possible interaction between these agents on acetic acid-induced writhing (Fig. 10.1.1). A modified method as described by Porreca et al. (1990) was used to characterize the interaction between gabapentin and naproxen, as the former drug alone did not show analgesic efficacy in acetic acid-induced writhing. The theoretical ED$_{50}$ for this combination was calculated differently. As gabapentin alone lacked analgesic effect, it was assumed that the total analgesic effect observed in combination study could be attributed to naproxen alone. Thus, theoretical ED$_{50}$ for this combination becomes ED$_{50}$ of naproxen. Interestingly, the experimental ED$_{50}$ value was significantly higher than theoretical ED$_{50}$ value for this combination when administered intraperitoneally. On careful examination of results revealed that the analgesic effect (%MPE) of naproxen in the mixture was same as that was observed with the naproxen alone in dose-response studies. This was further confirmed by isobolographic analysis clarifying that gabapentin merely increased the bulk of the combined dose rather than any significant effect on writhing response (Fig. 10.1.3). Hence, the amount of naproxen in experimental ED$_{50}$ value was compared with the
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theoretical ED\textsubscript{50} value, which was not significantly different (Table 10.1.1). The
%MPE of the combination of gabapentin and valdecoxib in 1:1 fixed dose ratio of
their weight (i.e. 10/10, 20/20, and 50/50 mg/kg of naproxen/gabapentin) was not
statistically different from that of each drug alone on the writhing response revealed
no potential interaction when administered intraperitoneally (Fig. 10.1.2 and 10.1.4).

10.1.3.2.2. Formalin-induced tonic pain

The combination of gabapentin and naproxen showed dose dependent analgesic
activity when administered intraperitoneally in the late phase of the formalin test (Fig.
10.1.6A). Isobolographic analysis using fixed ratio (1:1) of the ED\textsubscript{50} fractions
revealed a significant additive interaction between naproxen and gabapentin after
intraperitoneal (Fig. 10.1.7A) administration in the late phase the formalin test. The
point for experimental ED\textsubscript{50} for this combination was obtained almost adjacent to the
theoretical additive line with in the confidence intervals indicating possible additive
interaction. Further, the experimental ED\textsubscript{50} dose was not significantly different from
(p < 0.05) the calculated additive ED\textsubscript{50} dose thereby demonstrating an additive
interaction (Table 10.1.1). This was further evidenced by interaction index where \( \gamma \) for
this combination was nearer to 1 indicating additive interaction only (Table 10.1.1).

Valdecoxib and gabapentin were combined in the ratio of 1:1 of the ED\textsubscript{50} of
gabapentin to evaluate possible interaction between these agents on formalin-induced
nociceptive behavior. The interaction between these agents was characterized using
modified method as described by Porreca et al. (1990) as valdecoxib alone had no
analgesic effect in the formalin test. The analgesic effect (%MPE) of gabapentin in
the mixture (1:1) was same as that was observed with the gabapentin alone in dose-
response studies (Fig. 10.1.6B). Isobolographic analysis for this combination revealed
that valdecoxib did not contribute to antinociceptive effect in formalin-induced
nociceptive responses instead it increased the bulk of the mixture (Fig. 10.1.7B).
Further, there was no significant difference between the amounts of gabapentin in
experimental ED\textsubscript{50} value was compared with that of the theoretical ED\textsubscript{50} value (Table
10.1.1). In these interaction studies, each agent alone or in combination with
gabapentin did not alter phase 1 nociceptive responses of the formalin test (Fig.
10.1.8). The experimental ED\textsubscript{50} with 95% CI obtained for each agent and their
combinations and the corresponding calculated theoretical ED\textsubscript{50} with 95% CI for such
combinations in the writhing and formalin tests are given in Tables 10.1.1.
Fig. 10.1.1. Dose-response curves for intraperitoneal administration of naproxen (Nap), gabapentin (Gab) and mixture of naproxen and gabapentin (1:3 of ED$_{50}$ of naproxen) on acetic acid-induced writhing response. Each dose point on graph represents mean %MPE ± SEM.

Fig. 10.1.2. Dose-response curves for intraperitoneal administration of valdecoxib (Val), gabapentin (Gab) and mixture of valdecoxib and gabapentin (1:1 ratio; 10/10, 20/20, and 50/50 mg/kg of Val+Gab). Each dose point on graph represents mean %MPE ± SEM.
Fig. 10.1.3. Isobologram for interaction between intraperitoneally administered naproxen and gabapentin (1:3 of ED\textsubscript{50} of naproxen) in the writhing test. The straight solid line is the theoretical additive line and the open point corresponds to theoretical ED\textsubscript{50} ± SEM and the filled point corresponds to experimental ED\textsubscript{50} ± SEM of the mixture. The experimental points were not significantly different from the calculated additive points indicating an additive interaction (Student’s t-test).

Fig. 10.1.4. Effect of valdecoxib (Val; 10, 20, 50 mg/kg, i.p.), gabapentin (Gab; 10, 20, 50 mg/kg, i.p.) and co-administration of 1:1 mixture of valdecoxib and gabapentin (Val+Gab; 10+10, 20+20, 50+50 mg/kg, i.p.) on acetic acid-induced writhing response. The data represent the mean ± S.E.M. of sum of writhes.
Fig. 10.1.5. Effect of intraperitoneally administered naproxen 100 mg/kg (Nap 100), valdecoxib 50 mg/kg (Val 50) and gabapentin 200 mg/kg (Gab 200) on nociceptive behavior during the early phase (0 - 10 min) and the late phase (10 – 60 min) of the formalin test in mice. The data represent the mean ± S.E.M. of sum of formalin-induced licking and biting responses in seconds during the early and late phase. * P < 0.05 as compared to control animals (one way ANOVA followed by Dunnett’s test).

(A)
Fig. 10.1.6. Dose-response curves for intraperitoneal administration of (A) naproxen (Nap), gabapentin (Gab) and their combination (Nap + Gab), (B) valdecoxib (Val), gabapentin (Gab) and their combination (Val + Gab) during the late phase (10 - 60 min) of the formalin test in mice. Drugs alone or in combination were intraperitoneally administered 30 min before nociceptive assay. Each dose point on graph represents mean %MPE ± SEM.
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Gabapentin (mg/kg, i.p.)

Fig. 10.1.7. Isobologram for interaction between intraperitoneally administered (A) naproxen and gabapentin (1:1 of their ED$_{50}$ fractions) and (B) valdecoxib and gabapentin (1:1 of ED$_{50}$ of gabapentin) during the late phase (10 – 60 min) of the formalin test. The straight solid line is the theoretical additive line and the open point corresponds to theoretical ED$_{50}$ ± SEM and the filled point corresponds to experimental ED$_{50}$ ± SEM of the mixture. The experimental points were not significantly different from the calculated additive points, indicating an additive interaction (Student’s t-test).

![Graph showing isobologram for Gabapentin and Valdecoxib](image)

**Fig. 10.1.8.** Effect of intraperitoneal administration of naproxen (Nap; ED$_{50}$ = 50.84 mg/kg), gabapentin (Gab; ED$_{50}$ = 52.88 mg/kg), their combination (Nap + Gab; 103.72 mg/kg), valdecoxib (Val; 52.88 mg/kg) and co-administration of 1:1 mixture of valdecoxib and gabapentin (Val+Gab; 105.76 mg/kg) on formalin-induced nociceptive responses. The data represent the mean ± S.E.M. of sum of nociceptive responses during early phase of the formalin test.
Table 10.1.1. Theoretical and experimental ED$_{50}$ values with 95% confidence intervals (CI) and interaction index for the antinociceptive effect of naproxen, valdecoxib, gabapentin alone and their combination with gabapentin administered intraperitoneally.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Acetic acid-induced writhing</th>
<th>Formalin-induced nociception</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED$_{50}$ (mg/kg; 95% CI)</td>
<td>Interaction index (γ)</td>
</tr>
<tr>
<td></td>
<td>Theoretical</td>
<td>Experimental</td>
</tr>
<tr>
<td>Naproxen</td>
<td>26.74</td>
<td>(20.46 – 23.67)</td>
</tr>
<tr>
<td>Valdecoxib</td>
<td>-----</td>
<td>NA</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>-----</td>
<td>NA</td>
</tr>
<tr>
<td>Naproxen + Gabapentin</td>
<td>26.74</td>
<td>98.88</td>
</tr>
<tr>
<td></td>
<td>(22.04 – 29.19)</td>
<td>(88.15 – 116.75)</td>
</tr>
<tr>
<td>Gabapentin #</td>
<td>24.72</td>
<td>(22.04 – 29.19)</td>
</tr>
<tr>
<td>Valdecoxib + Gabapentin</td>
<td>-----</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gabapentin #</td>
<td>-----</td>
<td>NA</td>
</tr>
</tbody>
</table>

# Proportion of effective analgesic agent in experimental ED$_{50}$ value of the mixture; NA: not achieved; * γ values are nearer to 1 indicating additive interaction
10.1.4. DISCUSSION

The present study demonstrated the possible antinociceptive interactions between COX inhibitors and gabapentin investigated in mouse inflammatory and non-inflammatory pain models. Intraperitoneal administration of a solution of acetic acid produces characteristic writhing response in animals and widely used for evaluating non-inflammatory pain. Acetic acid directly activates visceral and somatic nociceptors innervating the peritoneum and induces inflammation not only in subdiaphragmatic visceral organs, but also in subcutaneous muscle walls (Cervero, 1995). In contrast, the diluted formalin when injected into hind paw of mice and rats shows characteristic biphasic licking and biting behaviors to continuous (tonic) noxious stimuli and widely used for evaluating inflammatory pain. The early phase (0 – 5 min) consisted of intense licking and biting response followed by a quiescent period of little activity and the late phase (10 to 60 min) after formalin injection and involved periods of licking and biting of the injected paw (Murray et al., 1988). The first phase is thought to be a model of acute chemical pain resulting from immediate increase in the activity of slowly conducting C-fibres, whereas the second phase reflects a facilitated state of central sensitisation driven by the persistent primary afferent inputs (Abbadie et al., 1997; Malmberg and Yaksh, 1992). These nociceptive assays have been used for the evaluation of the analgesic activity of various pharmacological agents such as NSAIDs and opioids.

In the present study, intraperitoneal administration of naproxen, but not valdecoxb, alone resulted in the dose-dependent antinociception in the writhing test. In addition, naproxen also decreased late phase, but not early phase, nociceptive behavior in the formalin test. In contrast, valdecoxib, a selective COX-2 inhibitor, lacked analgesic efficacy in both the tests. Accumulating data demonstrated that selective COX-1 and non-selective COX-1/2 inhibitors showed antinociceptive effects whereas selective COX-2 inhibitors were ineffective in altering acute visceral and formalin-induced nociception (Dirig et al., 1997; Jain et al., 2001; Matsumoto et al., 1998). In deed, selective COX-1 inhibition, but not COX-2 resulted in marked attenuation of writhing and formalin-induced nociceptive behaviors (Dirig et al., 1997; Ochi et al., 2000). Further, COX-2 mRNA and COX-2 protein in the spinal cord and at the inflammation site increased 3 – 6 h following peripheral inflammation (Beiche et al., 1996; Hay et al., 1997; Itchitani et al., 1997). Therefore, it is possible that acetic acid-induced writhing for 20 min and formalin-induced licking and biting
for 60 min would not be sufficient to activate COX-2 mRNA and the generation of COX-2 and PGs. It is likely that COX-2 may not be associated with spinal prostanoid synthesis acutely or with facilitated nociception, which occurs within limited time frame of acute analgesic tests. These data along with the present observations implicate that COX-1 inhibition is required for alleviating non-inflammatory pain and formalin-induced late phase nociceptive behavior.

Several studies demonstrated the efficacy of gabapentin in a variety of pain syndromes (Field et al., 1997; Hunter et al., 1998; Gustafsson et al., 2003). Although its binding to α5δ subunit of high voltage-gated Ca\(^{2+}\) channels could explain the inhibitory actions of gabapentin on voltage-gated Ca\(^{2+}\) channels and on Ca\(^{2+}\) influx into synaptosomes, its significance for Ca\(^{2+}\) channel function is far from established (Gee et al., 1996; Stefani et al., 1998; Fink et al., 2000). Other studies have found that gabapentin is able to increase GABA synthesis and release in brain regions, indirectly activates GABA\(_B\) receptors (Loscher et al., 1991; Bertrand et al., 2001). It has also been suggested that gabapentin action could result from its interaction with synthesis, degradation, or the reuptake of neurotransmitters (Kocsis and Honmou 1991; Silverman et al., 1991). However, the mechanisms in particular of its analgesic action are still unknown. In the present study, systemic administration of gabapentin alone resulted in the dose-dependent antinociception in the formalin test, but not in the writhing test indicating that gabapentin could not act by inhibiting direct chemical stimulation of peripheral nociceptors. The results are in consistence with previous reports where gabapentin lacked analgesic efficacy in acute nociceptive assays (Shimoyama et al., 1997; Gustafsson et al., 2003). However, its ability to decrease central sensitization and peripheral inflammatory response as reported in other nociceptive assays could explain the attenuation of late phase of the formalin test (Field et al., 1997; Hunter et al., 1998).

The combinations of analgesic agents from different classes are often used in clinical practice to control pain. The potential advantage of using combination therapy is that the analgesic efficacy can be maximized while the incidence of adverse side effects is minimized (Kehlet and Dahl, 1993; Raffa, 2001). An interesting finding that has emerged from the reported interaction studies is that drug synergism may occur with combinations in which one of the constituents lacks efficacy as a sole agent. Therefore, it was expected that if gabapentin when combined with COX inhibitors
might result in enhancement of total analgesic efficacy that would clarify any substantial involvement of COX-2 and gabapentin in these acute nociceptive assays. The combination of gabapentin with COX inhibitors did not result in any interaction in visceral pain test. In contrast, an additive interaction was resulted when naproxen and gabapentin were combined, instead no such interaction was observed between valdecoxib and gabapentin in the formalin test. The isobolographic analysis and interaction index for the combination of naproxen and gabapentin showed only additive effect. Moreover, the sum of licking and biting behaviors observed with combination of valdecoxib and gabapentin was not significantly different from that observed with ED$_{50}$ dose of gabapentin alone. This confirms that the observed antinociceptive effect of this combination in the interaction studies was contributed by gabapentin alone. These observations clearly demonstrated only an additive interaction between naproxen and gabapentin in this inflammatory pain model.

An additive effect refers to the interaction between two drugs such that, when co-administered, the resultant effect approaches the maximum effect or the sum of the effects of the drugs administered individually, however, the incidence of adverse effects can be reduced. Previous drug-interaction analysis also demonstrated an additive effect when COX inhibitors combined with $\kappa$-opioid or adenosine agonist in the formalin test and with glucosamine in the writhing test (Malmberg et al., 1993; Tallarida et al., 2003). The only potential advantage of naproxen and gabapentin combination is 50% reduction of the respective dose to achieve pain relief and could result in decrease in adverse effects of each agent when used alone.

It is well known that nociceptive assays of pain in experimental animals are analogous to different pain conditions in humans. It is well reported that the high affinity of gabapentin to $\alpha_2\delta$ subunit of high voltage-gated Ca$^{2+}$ channels binding site play an important role in the spinal nociceptive processing. Its efficacy has been well established in chronic pain situations like neuropathic pain in animals and humans (Gee et al., 1996; Luo et al., 2001). Recent findings demonstrated the efficacy of COX inhibitors, especially selective COX-2 inhibitors, on the development and maintenance of chronic pain, such as arthritic, inflammatory and neuropathic pain (Dirig et al., 1998; Yaksh et al., 2001; Hay et al., 1997; Ma and Eisenach 2003a). In addition, increased expression of $\alpha_2\delta$ subunit of high voltage-gated Ca$^{2+}$ channels or COX-2 expression and PGE synthase expression in animal models of neuropathic
pain have been reported (Luo et al., 2001 and 2002; Ma and Eisenach 2003a,b). Because the combination of naproxen and gabapentin resulted in only additive effect in relieving acute inflammatory pain, it would be prudent to determine the effects of combination COX inhibitors with gabapentin in other preclinical models of acute and chronic pain.

In conclusion, isobolographic analysis indicated an additive interaction between non-selective COX inhibitor and gabapentin, when administered systemically, in relieving inflammatory pain. Further, additional experiments are warranted to study the nature interaction between COX inhibitors and gabapentin in other clinically relevant acute and chronic pain models.
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10.2. INTERACTIONS BETWEEN CYCLOOXYGENASE INHIBITORS AND GABAPENTIN IN ACUTE LONG TERM INFLAMMATORY PAIN

10.2.1. INTRODUCTION

It is well known that acute peripheral injection of formalin or carrageenan causes tissue injury that displays spontaneous pain behavior and an exaggerated response to moderate stimuli, a state otherwise referred to as hyperalgesia. Consistent with the behavioral effects, injury or inflammation produces enhanced discharge rate and stimulus intensity and spontaneous activity in otherwise silent small primary afferent axons (Pitcher and Henry, 2002; Pogatzki et al., 2002). Both these models are the commonly employed for acute nociceptive studies as they differ in stimulus intensity, time course, and the type of behavioral measures made. Although, both the agents cause inflammation, the diluted formalin when injected subcutaneously into paw displays an acute, short-term, and biphasic spontaneous nociceptive behavior lasting up to 60 min whereas paw carrageenan injection displays an acute, long-term, hyperalgesic behavior that lasts up to few days (Dirig et al., 1997, 1998; Yaksh et al., 2001). Further, there are the differential mechanisms involved in hyperalgesia and spontaneous behaviors.

It has been reported that injury to and inflammation of tissue results in profound changes to the chemical environment of the peripheral terminal of nociceptors (Mizumura, 1997; Willis, 2001). Thus, sensory nerve endings close to the area of injury acquire a state of hyperresponsiveness known as peripheral sensitization (Willis, 2001; Rahman et al., 2003). It is well known that the central nociceptor transmission neurons in the dorsal horn of the spinal cord can also be sensitized in the same way that the peripheral terminal of the nociceptor can be sensitized. Central and peripheral sensitizations are the major causes of hypersensitivity to pain after injury (Urban and Gebhart, 1999; Julius and Basbaum, 2001).

The NSAIDs, the commonly used analgesics, act by inhibiting the COX enzyme thereby blocking the generation of PGs. Importantly, PGs released both centrally and peripherally play an important role in pain and inflammatory processes and produce both localized and central hypersensitivity (Willingale et al., 1997;
However, their usage is severely hampered by a variety of adverse events, particularly upper gastrointestinal side effects, such as peptic ulceration, hemorrhage and perforation, leading to death in some patients (Kulkarni et al., 2000). Gabapentin, an anticonvulsant with relatively low toxicity and different spectrum of side effects than the other commonly used analgesic, has been shown to reduce hypersensitivity or produce antinociception after inflammation or nerve injury in animals and shows effectiveness in the treatment of clinical chronic pain (Shimoyama et al., 1997; Feng et al., 2003; Gustafsson et al., 2003). It has been reported that systemic administration of gabapentin reduced hyperalgesia after paw carrageenan injection (Singh et al., 1996; Stanfa et al., 1997).

It has been demonstrated that the nature of the pharmacological interaction between two agents depended on the type of test measure and the extent and intensity of that measure. Further, it is well known that nociceptive assays of pain in experimental animals are analogous to different pain conditions in humans. Thus, the present study was designed to investigate pharmacodynamic interactions in a rat model of carrageenan-induced hyperalgesia. The accumulating evidence for the presence of the COX enzymes both in the periphery and in the spinal cord and analgesic efficacy of COX inhibitors and gabapentin in relieving acute inflammatory pain prompted to carry out pharmacodynamic interaction between NSAIDs and gabapentin to assess any beneficial interactions, if any, existed in these acute models of nociception.

10.2.2. MATERIALS AND METHODS

10.2.2.1. Experimental animals

As per 1.2.1.

Each animal was used for a single treatment and each group consisted of 6 or 8 animals.

10.2.2.2. Carrageenan-induced mechanical hyperalgesia

Mechanical hyperalgesia (decreased threshold to mechanical stimuli) were evaluated in ipsilateral paw of rats. The withdrawal threshold was evaluated using an analgesymeter (Ugo Basile, Italy) by applying noxious pressure to hind paw and expressed in grams as per 1.2.2.3b.
10.2.2.3. Drugs and treatment schedule

The drugs used in the study were indomethacin, naproxen, valdecoxib, gabapentin (all from Panacea Biotec Ltd., Lalru, India).

The solutions of all the drugs used alone or in combination for per oral administration were freshly prepared by solubilizing them in distilled water with one or two drops of Tween 80 except gabapentin, which was dissolved in distilled water only. All the drug(s) solutions were administered in a constant volume of 1 ml/100 g body weight 2 h after carrageenan injection into the hindpaw. Control animals received equivalent vehicle orally.

10.2.2.4. Experimental design

10.2.2.4.1. Dose-response studies for NSAIDs and gabapentin

At least four doses were used to determine a dose-response curve and each group consisted of six to eight animals. Dose-response curves were constructed to assess antihyperalgesic activity of NSAIDs and gabapentin administered orally in carrageenan-induced hyperalgesia. Several doses of naproxen (1, 3, 10 or 30 mg/kg, p.o.), valdecoxib (0.3, 1, 3 or 10 mg/kg, p.o.), and gabapentin (3, 10, 30 or 100 mg/kg, p.o.) were administered to plot dose-response curve in carrageenan-induced hyperalgesia. ED$_{50}$ (effective dose estimated to produce 50%MPE or reversal of hyperalgesia) with its 95% confidence intervals (CI) using standard linear regression analysis of log dose-response curve was calculated for each drug.

10.2.2.4.2. Drug-interaction studies

Isobolographic analysis was used to assess drug-interaction studies as per 9.1.2.4.2. The theoretical and experimental ED$_{50}$ values of the studied combination were also contrasted by calculating the interaction index ($\gamma$) as described in 9.2.2.4.2.

10.2.2.4.3. Measurement of gastrointestinal side effects

NSAID-induced gastric damage in rats was evaluated as described in 10.1.4.3. Gabapentin (100 mg/kg) and its combination with naproxen (100 mg/kg + 30 mg/kg, respectively) and valdecoxib (100 mg/kg + 10 mg/kg, respectively) were administered orally at the same time to several groups (six to eight rats in each) of fasted rats.

10.2.2.5. Statistical analysis
Chapter 10

As per 2.1.2.5 and 9.1.2.5.

10.2.3. RESULTS

10.2.3.1. Dose-response studies for NSAIDs and gabapentin

Carrageenan administration into the left hindpaw produced significant edema associated with hyperalgesia, as shown by a decreased paw withdrawal threshold in response to mechanical pressure 2 h after injection. The dose-dependent reversal of carrageenan-induced mechanical hyperalgesia by naproxen (1.0 – 30 mg/kg, p.o.) and valdecoxib (0.3 – 10 mg/kg, p.o.) are shown in Fig. 9.2.6 & 9.2.8A and Fig. 9.2.7 & 9.2.8B respectively. In this assay, valdecoxib was approximately 4 times more potent than naproxen (Table 10.2.1). Gabapentin (3 – 100 mg/kg, p.o.) also produced dose-dependent reversal of hyperalgesia with peak reversal of hyperalgesia (82%) was observed 2 h after drug administration at dose 100 mg/kg (Fig. 10.2.1). The corresponding ED$_{50}$ with its 95% confidence intervals for each NSAID and gabapentin after oral administration in this assay were calculated 2 h after drug administration and are shown in Table 10.2.1.

10.2.3.2. Drug-interaction studies

Interaction studies between gabapentin and NSAIDs on carrageenan-induced hyperalgesic response showed a dose-dependent antihyperalgesic activity after oral administration (Fig. 10.2.2A and 2B). The corresponding ED$_{50}$ with its 95% confidence intervals for such combinations in this assay were calculated 2 h after drug mixture administration (Table 10.2.1). Isobolographic analysis, using fixed ratio (1:1) ED$_{50}$ fractions revealed a significant supra-additive or synergistic interaction between naproxen or valdecoxib and gabapentin after oral administration in this noxious assay (Fig. 10.2.3A and 3B). The points for experimental ED$_{50}$ for these combinations were obtained markedly below the theoretical additive line indicating possible synergistic interaction. Further, the experimental ED$_{50}$ doses were significantly less ($p < 0.05$) than the calculated additive ED$_{50}$ doses thereby demonstrating a synergistic interaction. The interaction index ($\gamma$) for these combinations was far below 1 indicating supra-additive interaction only (Table 10.2.1).

The calculated theoretical ED$_{50}$ with 95% CI and the corresponding experimental ED$_{50}$ with 95% CI obtained for each agent and their combinations and interaction index for such combinations are given in Table 10.2.1.
10.2.3.3. Measurement of gastrointestinal side effects

The administration of gabapentin (100 mg/kg, p.o.) did not produce ulcers or erosions. Its adverse effects were similar to those of vehicle. Indomethacin (20 mg/kg, p.o.) produced severe gastric lesions with prominent pointed erosions and frank hemorrhagic ulcers. However, naproxen (30 mg/kg, p.o.) generated a less number of petechiae and erosions as compared to indomethacin, which was considered to be the most disadvantageous in terms of the number and severity of the lesions caused in the stomach (i.e., ulcers or erosions) (100%). In contrast, valdecoxib (10 mg/kg, p.o.) did not produce any gastric erosions and ulcers. The combination of naproxen with gabapentin generated less number of erosions and ulcers as compared to indomethacin. However, there is no significant difference in ulcer severity in the combination of naproxen with gabapentin as compared to naproxen alone. The combination of valdecoxib with gabapentin did not produce any gastric erosion and the effect was similar to those of vehicle or each drug alone (Fig. 10.2.4).

Fig. 10.2.1. Dose-dependent antinociceptive effect of orally administered gabapentin (3 – 100 mg/kg) against carrageenan-induced hyperalgesia in rats. The drug was administered was 2 h after subplantar injection of carrageenan (100 μg per paw). Values are mean ± S.E.M.
Fig. 10.2.2. Dose-dependent reversal of orally administered (A) naproxen (Nap; 1 – 30 mg/kg), gabapentin (Gab; 3 – 100 mg/kg), and their combination (Nap+Gab; 3.81 – 30.51 mg/kg), (B) valdecoxib (0.3 – 10 mg/kg), gabapentin (Gab; 3 – 100 mg/kg), and their combination (Val+Gab; 3.25 – 26.02 mg/kg) against carrageenan-induced hyperalgesia in rats. The percent reversal of hyperalgesia was calculated 4 h after carrageenan administration by taking the values in the control group as 0% reversal. Values are mean ± S.E.M. *P < 0.05 vs vehicle control.
Fig. 10.2.3. Isobologram for interaction between orally administered (A) naproxen and gabapentin (1:1 of their $ED_{50}$ fractions) and (B) valdecoxib and gabapentin (1:1 of their $ED_{50}$ fractions) in the carrageenan-induced hyperalgesia in rats. The straight solid line is the theoretical additive line and the open point corresponds to theoretical $ED_{50} \pm SEM$ and the filled point corresponds to experimental $ED_{50} \pm SEM$ of the mixture.
Fig. 10.2.4. Percent of gastric erosions and ulcers produced by two orally administered doses of vehicle (Veh), indomethacin (Ind; 20 mg/kg), naproxen (Nap; 30 mg/kg), valdecoxib (Val; 10 mg/kg), gabapentin (100 mg/kg), a combination of naproxen and gabapentin (Nap+Gab; 30+100 mg/kg), and valdecoxib and gabapentin (Val+Gab; 10+100 mg/kg) in rats. Indomethacin and vehicle were taken as positive and negative control, which represent the 100% and 0%, respectively. Each bar represents mean ± SEM of percent of gastric ulcer severity. *\(P < 0.05\) as compared to vehicle control, a\(P < 0.05\) as compared to indomethacin-treated group, \(n = six\) animals per group.

Table 10.2.1. Theoretical and experimental ED\(_{50}\) values with 95% confidence intervals (CI) and interaction index for the antinociceptive effect of naproxen, valdecoxib, gabapentin alone and their combination with gabapentin.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>ED(_{50}) (mg/kg; 95% CI)</th>
<th>Carrageenan-induced hyperalgesia</th>
<th>Interaction index ((\gamma))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Theoretical</td>
<td>Experimental</td>
<td></td>
</tr>
<tr>
<td>Naproxen</td>
<td>----</td>
<td>6.03 (4.3 – 8.46)</td>
<td>----</td>
</tr>
<tr>
<td>Valdecoxib</td>
<td>----</td>
<td>1.54 (0.96 – 2.46)</td>
<td>----</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>----</td>
<td>24.48 (15.79 – 38.58)</td>
<td>----</td>
</tr>
<tr>
<td>Naproxen + Gabapentin</td>
<td>(13.67 – 17.81)</td>
<td>15.26</td>
<td>7.76</td>
</tr>
<tr>
<td>Valdecoxib + Gabapentin</td>
<td>(10.88 – 15.56)</td>
<td>13.01</td>
<td>8.05</td>
</tr>
</tbody>
</table>

The drugs were administered orally for antinociceptive interaction in carrageenan-induced hyperalgesia. *\(\gamma\) values are far below 1 indicating supra-additive interaction.
10.2.4. DISCUSSION

The present study examined the possible antinociceptive interactions between COX inhibitors and gabapentin in rat model of inflammatory pain. Oral administration of COX inhibitors or gabapentin alone resulted in the dose-dependent antihyperalgesic effect. Oral co-administration of naproxen or valdecoxib with gabapentin showed dose-dependent synergistic antinociceptive activity that was confirmed by isobolographic analysis and interaction index. In addition, the combination of COX inhibitors with gabapentin is further benefited by the absence of severe gastrointestinal side effects. Therefore, the beneficial, but differential interaction between NSAIDs and gabapentin in this model of inflammatory pain suggesting that the PGs derived from COX-1 and/or COX-2 invariably participate along with antinociceptive mechanisms of gabapentin and play an important role in this beneficial interaction.

Role of inhibition of COX isoforms in synergistic interaction

It is well known that intraplantar administration of carrageenan causes inflammation and tissue injury, which evokes persistent afferent traffic that initiates a spinal sensitization. Accumulating data indicates for the presence of both the COX mRNA and protein constitutively and COX-2 mRNA is up-regulated 3 – 6 h following peripheral inflammation (Beiche et al., 1996; Hay et al., 1997; Itchitani et al., 1997). It has been reported that the nonselective NSAID ketorolac, the selective COX-2 inhibitor SC-58365 or anti-PGE\(_2\) monoclonal antibody administered intravenously 1 – 3 h after carrageenan injection, eliminated established hyperalgesia, suggesting that continuous production of PGE\(_2\) was needed to sustain the hyperalgesic response (Zhang et al., 1997). These results suggest that the induction of COX-2 mRNA by carrageenan may play a crucial role in induction and maintenance of hyperalgesia, both in the periphery and in the spinal cord. It is well reported that both COX-1 and COX-2 inhibitors could prevent carrageenan-induced PGE\(_2\) production in the rat footpad. In addition, carrageenan-induced edema elicited upregulation of microsomal PGE\(_2\) synthase-1 and COX-2 in brain, spinal cord, and ipsilateral paw along with elevated levels of PGE\(_2\), 6-keto-PGF\(_{2\alpha}\), PGD\(_2\), and TXB\(_2\) in the early phase and PGE\(_2\) during the late phase (Guay et al., 2004).

In contrast to the results in the formalin test, the selective COX-2 inhibitor SC-58125, but not the selective COX-1 inhibitor SC-560, reduced the level of PGE\(_2\) in

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cerebrospinal fluid after carrageenan injection into rat hind paw and prevented the development of hyperalgesia (Yaksh et al., 2001). During tissue injury COX generates PGs, potent sensitizing agents, which are able to modulate multiple sites in the nociceptive pathway enhancing transduction (peripheral sensitizing effect) and transmission (central sensitizing effect) of nociceptive information. PGE$_2$ is thought to be a principle mediator of the hypersensitivity (Daher and Tonussi, 2003) but several prostanoids show similar effects (Willingale et al., 1997; Minami et al., 2001). The main mechanism involved in the hyperalgesic action of prostaglandins is traditionally considered to be due to a sensitizing effect on primary afferent nerves (Davies et al., 1984). Indeed, PGE$_2$ is released within the spinal cord after peripheral nociceptive stimulation (Ebersberger et al., 1999; Guhring et al., 2000; Samad et al., 2001). These increases in the release of PGE$_2$ have functional relevance since they are typically blocked by the intrathecal and systemic administration of COX inhibitors. These data suggest that COX-2 activity in the CNS might play an important role in the induction and maintenance of the hyperalgesic state (Seibert et al., 1994; Smith et al., 1998; Yaksh et al., 2001).

**Role of gabapentin in synergistic interaction**

Several studies demonstrated the efficacy of gabapentin in a variety of pain syndromes (Field et al., 1997; Hunter et al., 1998; Gustafsson et al., 2003). Accumulating data indicates that gabapentin has high affinity for binding to $\alpha_2\delta$ subunit of high voltage-dependent Ca$^{2+}$ channels (VDCC) that could explain the inhibitory actions of this agent on voltage-gated Ca$^{2+}$ channels and on Ca$^{2+}$ influx into synaptosomes. However, gabapentin affinity for VDCC and its significance for Ca$^{2+}$ channel function are under constant investigation (Gee et al., 1996; Stefani et al., 1998; Fink et al., 2000). Bayer et al. (2004) reported that selective blockade of N-type Ca$^{2+}$ channels dramatically increased whereas blockade of P/Q-type Ca$^{2+}$ channels strongly attenuated increased inhibition of gabapentin evoked synaptic transmission suggest that gabapentin affects both excitatory and inhibitory spinal neurotransmission via a presynaptic mechanism which preferentially involves various types of Ca$^{2+}$ channels.

Although, gabapentin was originally designed as structural analogue of GABA, however, it is not an agonist at both GABA$_A$ and GABA$_B$ receptors (Suman Chauhan et al., 1999; Taylor et al., 1998). Despite this fact, few studies have found
that gabapentin acutely increases GABA synthesis and release in brain regions, indirectly activates GABA\textsubscript{B} receptors (Loscher et al., 1991; Bertrand et al., 2001). Further, it has also been suggested that gabapentin action could result from its interaction with synthesis, degradation, or the reuptake of neurotransmitters (Kocsis and Honmou 1991; Silverman et al., 1991). However, the mechanisms in particular of its analgesic action are still unknown. It has been reported that gabapentin did not alter tactile withdrawal thresholds in naïve animals, however, it showed antiallodynic and antihyperalgesic effects in conditions of hypersensitivity indicates that injury-induced neuronal plasticity of the nervous system is essential for the action of gabapentin (Lu and Westlund, 1999; Gustafson et al., 2003). Interestingly, gabapentin decreased c-fiber evoked responses after inflammation (Stanfa et al., 2001). In the present study, systemic administration of gabapentin alone resulted in the dose-dependent antinociception in carrageenan-induced hyperalgesia indicating that gabapentin could inhibit and/or block mechanisms involved in direct stimulation of peripheral nociceptors and subsequent central sensitization. The results are in consistence with previous reports where gabapentin decreased central sensitization and hyperalgesia due to peripheral inflammatory response (Stanfa et al., 1997; Field et al., 1996, 1997; Hunter et al., 1998).

The advantages of combining two agents in balanced and beneficial analgesia are not only for synergism and dose reduction but also to avoid or decrease adverse effects without loss of efficacy (Kehlet and Dahl, 1993; Raffa, 2001). Therefore, present study also examined the presence of gastric injuries at doses of naproxen (30 mg/kg), valdecoxb (10 mg/kg, p.o.), gabapentin (100 mg/kg), and their combination at fixed doses that produced the maximum antinociceptive effect in carrageenan-induced hyperalgesia. The results showed that the adverse effects could be reduced; that is, the incidence of gastrointestinal adverse events (erosions) was absent with the combination of valdecoxib and gabapentin than with or indomethacin alone, whereas the combination of naproxen and gabapentin was also able to generate ulcers (low percent); these adverse effects were similar to that produced by naproxen alone. This finding is particularly important because the combination did not produce a higher incidence of side effects than that produced by each drug alone; instead, the results of synergistic antinociceptive interaction without adverse events reflect a potentiation type of interaction.
The mechanisms responsible for supra-additive interactions are poorly understood, despite a number of hypotheses have been proposed to describe such effects (Yaksh and Malmberg, 1994). These include the pharmacokinetic interaction where one agent increases the systemic levels of the other agent by altering observed and derived pharmacokinetic parameters and pharmacodynamic interaction in which the agents act at different sites, modulate a common pathway and/or enhance activity of the other agent. The interaction may also be physiologic where a different endogenous substance is released by the combination. A number of synergistic combinations have been reported, however, the mechanisms for synergistic action are generally not known (Tallarida et al., 2001). In the present study, the synergy between NSAIDs and gabapentin involved pharmacodynamic interaction since the equieffective doses when combined showed marked synergism with concomitant decrease in the dose and adverse effects. Although the present study did not examine the drug concentration(s), the pharmacokinetic interactions between NSAIDs and gabapentin cannot be precluded as the synergy reflects pharmacokinetic and/or pharmacodynamic interactions. However, the lack of change in duration of action of combination of these agents at equieffective doses suggests a pharmacodynamic rather than pharmacokinetic interaction.

The other common or uncommon mechanisms between these agents could also be involved in synergistic interaction. Central sensitization following inflammation is mediated in part by the action of spinal glutamate at NMDA receptors that results in increased Ca^{2+} influx, neuronal excitability and spinal PGs release (Vetter et al., 2001; Svensson et al., 2003). It is well reported that the high affinity of gabapentin to α₂δ subunit of high voltage-gated Ca^{2+} channels binding site play an important role in the spinal nociceptive processing. Recently, it has been demonstrated that gabapentin is capable of inhibiting spinal release of glutamate (Coderre et al., 2005). Moreover, its efficacy has been well established in chronic pain situations like neuropathic pain in animals and humans (Gee et al., 1996; Luo et al., 2001; Coderre et al., 2005). Thus, it is possible that gabapentin may decrease Ca^{2+} influx and thereby spinal PGs release. Adding to this, COX inhibitors with gabapentin synergize these effects by further inhibiting PGs release. Numerous studies have demonstrated that there is an increase in the release of SP and CGRP from small-diameter sensory neurons during inflammation (Donnerer et al., 1992; Garry and
Hargreaves, 1992). An action of PGE$_2$ on prostanoid receptors at the presynaptic membrane may cause enhancement of nociception by facilitating the spinal release of the excitatory neurotransmitter glutamate (Nishihara et al., 1995) and neuropeptides (Nicol et al., 1992; Hingtgen and Vasko, 1994) from primary afferent C-fibre terminals, as has been demonstrated in DRG cells and isolated spinal cord tissue. These effects are mediated by an increase in the inward calcium current. At the post-synaptic level, PGE$_2$ can directly activate deep neurons in the dorsal horn via EP$_2$-like receptors, thus enhancing the transmission of nociceptive responses (Baba et al., 2001). In fact, COX inhibitors attenuated PGs induced release of SP and CGRP (Vasko, 1995; Southhall et al., 1998). It has been reported that gabapentin attenuated hyperalgesia arising from intrathecal administration of SP or NMDA (Patridge et al., 1998). Furthermore, gabapentin did not affect the stimulated release of neuropeptides from spinal cord slices taken from contralateral to inflammation. On the contrary, it significantly attenuated the stimulated release of neuropeptides from spinal cord slices taken from ipsilateral to inflammation (Fehrenbacher et al., 2003). Thus, it is also be possible that the synergism between NSAIDs and gabapentin might involve simultaneous inhibition of neuropeptides release.

In summary, the present study quantified the antinociceptive synergy between NSAIDs and gabapentin in the acute inflammatory pain in the rat. Furthermore, the synergistic antinociception during inflammatory pain resulted in less severe gastric side effects. Thus, the combination of the NSAIDs used in the present study with gabapentin may provide better analgesia with reduced adverse drug effects and the analgesic dose requirement.