CHAPTER 4:

ROLE OF CYCLOOXYGENASE ISOFORMS ON HYPERSENSITIVITY IN SPARED NERVE INJURY: AN ANIMAL MODEL OF NEUROPATHIC PAIN
4.1. INTRODUCTION

Pathophysiological changes occurring within damaged nerves as a result of injury can contribute to their injury-induced activation and induce a state of prolonged neuronal hyperexcitability within the dorsal horn of the spinal cord (Zimmerman, 2001). The associated behavioral hypersensitivity to noxious (hyperalgesia) and non-noxious (allodynia) stimuli can be difficult to treat with conventional analgesics (Sindrup and Jenssen, 1999), pre-empting the need to identify the drugs with alternative mechanisms of action, which can prove adequate pain relief.

Intrathecal administration of PGE$_2$ and PGF$_{2\alpha}$ results in spontaneous agitation, hyperalgesia and tactile allodynia in mice and rats (Minami et al., 1994; Turnbach et al., 2002). Further, COX inhibitors significantly attenuated the PGE$_2$ and PGF$_{2\alpha}$-induced hyperalgesia and allodynia (Taiwo and Levine, 1988; Park et al., 2000). It has been reported that COX-1 immunoreactive profile in spinal cord is up-regulated early (4 days and 2 weeks) following spinal nerve ligation and partial sciatic nerve transaction (Zhu and Eisenach, 2003). On the other hand, increased COX-2 expression without any change in COX-1 expression in the spinal cord is observed from 2 weeks onwards following peripheral nerve injury (Ma and Eisenach, 2002, 2003a). On the contrary, it has also been reported that spinal COX-2 expression is unaltered 7 and 14 days after nerve injury (Zhao et al., 2000; Broom et al., 2004). Despite numerous studies on the possible involvement of PGs in the hyperexcitability, central sensitization and neuropathic pain, the relative role of COX isoforms contributing to the pathophysiology of neuropathic pain has not been fully understood.

It has been reported that the efficacy of various agents in experimental neuropathic pain is dependent on the type of nerve injury and time and route of drug administration (Tal and Bennett 1994; Martin and Eisenach 2001). Indeed, a number of animal models of neuropathic pain have been developed and characterized over the past two decades. There are a number of shortcomings with these animal models, which provide important clues in understating the underlying pathophysiology of neuropathic pain in humans. The Bennett and Xie model (Bennett and Xie, 1988) involves some kind of injury to the sciatic nerve, however, leave some degree of intact peripheral nerve fibers, which allows for the preservation of the sensory transmission into the central nervous system, and behavioral analysis of the response
of the animals to different sensory stimulation (Hao et al., 2000). The spared nerve injury model is a new model of peripheral nerve damage that involves axotomy and ligation of the tibial and common peroneal nerves leaving the sural nerve intact, which enables investigation of the changes in both injured primary sensory neurons and neighboring sensory neurons, and their contribution to the pathophysiology of neuropathic pain (Decosterd and Woolf, 2000). Further, SNI is a well characterized model of peripheral neuropathy and showed highest general sensitivity to various classes of drugs (Erichson and Blackburn-Munro, 2002).

Therefore, the present study was undertaken to determine the effect of acute as well as chronic systemic treatment with selective COX-1, non-selective COX-1/COX-2 or selective COX-2 inhibitors on attenuation of hypersensitivity following SNI in rats. Further, intrathecal administration of COX inhibitors was also employed to study spinal mechanisms in the manifestation of neuropathic pain.

4.2. MATERIALS AND METHODS

4.2.1. Experimental animals

As per 1.2.1.

Male Wistar rats (bred in Central Animal House of Panjab University, Chandigarh) weighing 130-170 g at the start of the surgery were randomly divided into various groups consisting of 6 - 8 animals per group. Following surgery, the animals were housed per cage with food and water ad libitum.

4.2.2. Spared nerve injury (SNI)

The SNI was produced according to the method described by Decosterd and Woolf (2000). Briefly, the rats were anaesthetized using 40 mg/kg sodium pentobarbital intraperitoneally and the skin on the lateral left thigh was incised. The cranial and caudal parts of the biceps femoris muscle were separated and held apart by a retractor to expose the sciatic nerve and its three terminal branches (sural, common peroneal, and tibial nerves). The tibial and common peroneal were tightly ligated with 4/0 silk and 2-3 mm of the nerve distal to the ligation was removed. Any stretching or contact with the intact sural nerve was avoided. The muscle and skin were closed in two layers. In sham-operated rats, the same surgical procedure was followed, the connective tissue was freed, and no ligatures were applied. After surgery, all animals received gentamicin (5 mg/kg, i.p.) to prevent any sepsis.

4.2.3. Assessment of neuropathic pain
Allodynia (heightened response to normally non-noxious stimuli) and hyperalgesia (decreased threshold to noxious stimuli) were evaluated in sham and sciatic nerve injured rats.

4.2.3.1. Cold allodynia
Cold sensitivity was quantified by measuring the duration of paw withdrawal in response to acetone application. An acetone drop was formed at the end of a piece of a small polyethylene tube, which was connected with a syringe. The drop was gently applied to the plantar surface of the hind paws. The duration of time in seconds that the animal spent lifting, shaking, or licking the acetone applied hind paw was recorded during a 2 min period that started immediately after acetone application.

4.2.3.2. Mechanical hyperalgesia
The withdrawal threshold of the ipsilateral (nerve injured) and contralateral (nerve uninjured) paw was measured using an analgesymeter (Ugo Basile, Italy) by applying noxious pressure to hind paw and expressed in grams as per 1.2.2.3b.

4.2.4. Drugs and treatment schedule
Resveratrol (Archer Chem, India), naproxen, and rofecoxib (both from Panacea Biotec Ltd., India) were used in the present study.

4.2.4.1. Systemic administration
As per 3.1.2.4.1.

4.2.4.2. Spinal administration
As per 3.1.2.4.2.

4.2.4.3. Treatment schedule
All animals were acclimatized to laboratory environment for at least 2 h before testing. All the rats were subjected to these two pain tests on day 0 before performing surgery. To evaluate the effect of COX inhibition during the development of neuropathic state and on the existing hypersensitivity following nerve injury, two sets of rats were administered with single dose of vehicle (saline/tween mixture), resveratrol, a selective COX-1 inhibitor (10 or 30 mg/kg, i.p.), naproxen, a non-selective COX inhibitors (10 or 30 mg/kg, i.p.) or rofecoxib, a selective COX-2 inhibitors (3 or 10 mg/kg, i.p.) on day 4 and 14 following SNI, respectively and the response to behavioral tests in ipsilateral paws was tested 2 h before, 0.5, 1, 2, 4, and 24 h after drug administration.

To evaluate the effect of chronic administration of COX inhibitors on the development of neuropathic pain, vehicle (saline/tween mixture), resveratrol,
naproxen (each 3 or 10 mg/kg, i.p.) or rofecoxib (1 or 3 mg/kg, i.p.) were administered 2 h before surgery and continued once daily for 7 days post-nerve injury.

Similarly, the same doses of the drugs were also administered to separate groups of rats on day 7 and continued once daily through day 14 post-nerve injury to determine the effect of chronic administration of COX inhibitors on the maintenance of hypersensitivity. The ipsilateral paw withdrawal response to acetone application and mechanical stimulation was tested on day 7 or 14 following 2 h after treatment and once a week thereafter for 4 weeks after nerve injury.

Further, separate groups of rats were intrathecally (i.t.) administered with 70% DMSO, resveratrol, naproxen (each 100 or 300 µg i.t.) or rofecoxib (30 or 100 µg i.t.) and tested for behavioral response of ipsilateral paws at 0.5, 1, 2, 4 and 24 h after treatment on day 4 or 14 following SNI in rats. Motor function was evaluated by the placing/stepping reflexes 15 min after intrathecal administration in rats.

4.2.5. Statistical analysis
As per 3.1.2.5.

4.3. RESULTS

4.3.1. General behavior of sham-operated and SNI animals
In the present series of experiments, the baseline paw withdrawal response in each test obtained on day 0 for each rat was relatively stable and showed no significant variation. Before the SNI, application of an innocuous cold stimulus (acetone drop) to the hind paws evoked no flexor response. Starting on day 1 post-SNI the rats developed a marked cold hypersensitivity of the ipsilateral paw reflected in an increased duration of paw withdrawal (7.66 ± 2.33 sec) in response to acetone application. The duration of paw withdrawal in response to acetone stimulation increased (16.11 ± 2.86 sec) significantly until day 14 and the cold hypersensitivity was maintained until day 28 post-injury when application of acetone continued to elicit a sustained flexor response indicative of an abnormal responsiveness to a normally innocuous stimulus. The ipsilateral and contralateral mean paw withdrawal threshold to pressure was 176.5 ± 17.45 g and 179 ± 12.88 g, respectively, on day 0 before performing surgery. The ipsilateral paw withdrawal responses of all the SNI rats were significantly less than that of sham-operated rats on day 4 onwards and reached steady state between days 7 and 28 after surgery indicating the development and maintenance of allodynia and hyperalgesia in a time-dependent manner (Fig.
4.1A and 1B). However, the paws contralateral to the nerve injury side evoked no flexor response or the response was shorter than 0.5 sec following acetone stimuli applied to the hind paws. Similarly, there was no significant change in the contralateral paw withdrawal responses to mechanical stimulation as compared to basal threshold values throughout the observation period. The body weights and general health of the animals were closely monitored. Many of the animals had slightly flexed nerve injured paws resting lightly and often elevated above the cage floor, but otherwise appeared healthy, exhibited normal grooming, feeding behavior, and gained weight normally.

4.3.2. Effect of COX inhibition during the development of hypersensitivity following SNI

There was no significant difference in ipsilateral paw withdrawal responses between various groups of animals except sham-operated animals on day 4 before drug administration. Systemic administration of single dose of resveratrol (10 or 30 mg/kg, i.p.) on day 4 following SNI did not show any significant difference in the ipsilateral paw withdrawal responses to thermal (Fig. 4.2A) and mechanical (Fig. 4.2B) stimulation as compared to vehicle treatment. Similarly, administration of single dose of naproxen (10 or 30 mg/kg, i.p.) or rofecoxib (3 or 10 mg/kg, i.p.) on day 4 following SNI did not reverse cold allodynia (Fig. 4.3A and 4.4A) and mechanical hyperalgesia (Fig. 4.3B and 4.4B) as compared to vehicle treatment.

4.3.3. Effect of COX inhibition on the existing hypersensitivity following SNI

There was no significant difference in ipsilateral paw withdrawal responses between various groups of animals except sham-operated animals on day 14 before drug administration. Acute systemic administration of resveratrol (10 or 30 mg/kg, i.p.), naproxen (10 or 30 mg/kg, i.p.) or rofecoxib (3 or 10 mg/kg, i.p.) on day 14 following SNI had no effect on the existing hypersensitivity as compare to saline/tween mixture treatment throughout the observation period (cold allodynia: Fig. 4.5A, 4.6A, 4.7A; mechanical hyperalgesia: 4.5B, 4.6B, and 4.7B, respectively).

4.3.4. Chronic systemic administration of COX inhibitors on the development of neuropathic pain

Chronic treatment with resveratrol (3 or 10 mg/kg, i.p., initiated 2 h before surgery and continued once daily for 7 days post-nerve injury) in SNI rats had no effect on cold allodynia (Fig. 4.8A) and mechanical hyperalgesia (Fig. 4.8B) throughout the study period. Similarly, preemptive treatment with naproxen (3 or 10 mg/kg, i.p.) or
rofecoxib (1 or 3 mg/kg, i.p.,) in SNI rats did not show any significant difference in the ipsilateral paw withdrawal responses to thermal (Fig. 4.9A and 4.10A) and mechanical (Fig. 4.9B and 4.10B) stimulation as compared to vehicle treatment till day 28 following SNI. Thus, preemptive COX inhibition had no effect on the development of hypersensitivity in SNI rats.

4.3.5. Chronic systemic administration of COX inhibitors on the maintenance of neuropathic pain

In a separate group of rats, the development and maintenance of pain related behavior was monitored and rats received drug treatment once daily starting on day 7 when the steady state of hypersensitivity reached and continued for one week following SNI. There was no significant difference in the ipsilateral paw cold hypersensitivity and mechanical hyperalgesia of vehicle-treated rats and rats treated with any of the COX inhibitor on the last of day of drug administration and throughout the remaining observation period (cold alldynia: Fig. 4.11A, 4.12A, 4.13A; mechanical hyperalgesia: 4.11B, 4.12B, 4.13B, respectively). Thus, postoperative treatment with a selective COX-1, COX-2, or nonselective COX inhibitor had no effect on the maintenance of hypersensitivity following SNI.

4.3.6. Intrathecal administration of COX inhibitors on neuropathic pain

Intrathecal administration of 70% DMSO caused no detectable motor weakness as judged by placing/stepping reflexes. The mean paw withdrawal responses to cold and mechanical stimulation in SNI rats administered intrathecally (100 or 300 µg/rat) with resveratrol or naproxen were not significantly different from those of 70% DMSO control animals on day 4 following SNI (cold alldynia: Fig. 4.14A and 4.15A; mechanical hyperalgesia: 4.14A and 4.15B). The intrathecal treatment of rofecoxib (30 or 100 µg/rat) did not reverse cold (Fig. 4.16A) and mechanical (Fig. 4.16B) hypersensitivity during the observation period on the day of experimentation in SNI rats.

There was no significant difference in the ipsilateral paw cold alldynia and mechanical hyperalgesia of DMSO-treated rats and rats intrathecally treated with any of the COX inhibitor on day 14 following SNI (cold alldynia: Fig. 4.17A, 4.18A, and 4.19A; mechanical hyperalgesia: 4.17B, 4.18B, and 4.19B). Thus, there is no involvement of spinal COX isoforms on existing hypersensitivity following SNI.
Fig. 4.1. Spared nerve injury produced hypersensitivity in the ipsilateral paw. Ipsilateral and contralateral paw withdrawal responses to (A) acetone application and (cold allodynia) (B) mechanical stimulation (hyperalgesia) assessed prior to, on day 1, day 4 and thereafter once a week for 4 weeks following sham or spared nerve injury (SNI). Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.2. Effect of intraperitoneally administered resveratrol (Res; 10 or 30 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia on day 4 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.3. Effect of intraperitoneally administered naproxen (Nap; 10 or 30 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia on day 4 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4. Effect of intraperitoneally administered rofecoxib (Rof; 10 or 30 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia on day 4 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.5. Effect of intraperitoneally administered resveratrol (Res; 10 or 30 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia on day 14 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.6. Effect of intraperitoneally administered naproxen (Nap; 10 or 30 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia on day 14 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4. Effect of intraperitoneally administered rofecoxib (Rof; 3 or 10 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia on day 14 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.8. Effect of intraperitoneally administered resveratrol (Res; 3 or 10 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia in nerve injured rats. Treatment was initiated after behavioral assessment on day 0 and continued once daily till day 7 following nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.9. Effect of intraperitoneally administered naproxen (Nap; 3 or 10 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia in nerve injured rats. Treatment was initiated after behavioral assessment on day 0 and continued once daily till day 7 following nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4. 10. Effect of intraperitoneally administered rofecoxib (Rof; 1 or 3 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia in nerve injured rats. Treatment was initiated after behavioral assessment on day 0 and continued once daily till day 7 following nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.11. Effect of intraperitoneally administered resveratrol (Res; 3 or 10 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia in nerve injured rats. Treatment was initiated after behavioral assessment on day 7 and continued once daily till day 14 following nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group, n = 6-8 in each group.
Fig. 4.12. Effect of intraperitoneally administered naproxen (Nap; 3 or 10 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia in nerve injured rats. Treatment was initiated after behavioral assessment on day 7 and continued once daily till day 14 following nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group, n = 6-8 in each group.
Fig. 4.13. Effect of intraperitoneally administered rofecoxib (Rof; 1 or 3 mg/kg) on (A) cold allodynia and (B) mechanical hyperalgesia in nerve injured rats. Treatment was initiated after behavioral assessment on day 7 and continued once daily till day 14 following nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.14. Effect of intrathecally administered resveratrol (Res; 100 or 300 µg/rat) on (A) cold allodynia and (B) mechanical hyperalgesia on day 4 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4. 15. Effect of intrathecally administered naproxen (Nap; 100 or 300 μg/rat) on (A) cold allodynia and (B) mechanical hyperalgesia on day 4 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.16. Effect of intrathecally administered rofecoxib (Rof; 30 or 100 μg/rat) on (A) cold allodynia and (B) mechanical hyperalgesia on day 4 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.17. Effect of intrathecally administered resveratrol (Res; 100 or 300 µg/rat) on (A) cold allodynia and (B) mechanical hyperalgesia on day 14 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Chapter 4

Fig. 4.18. Effect of intrathecally administered naproxen (Nap; 100 or 300 μg/rat) on (A) cold allodynia and (B) mechanical hyperalgesia on day 14 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Fig. 4.19. Effect of intrathecally administered rofecoxib (Rof; 30 or 100 µg/rat) on (A) cold allodynia and (B) mechanical hyperalgesia on day 14 following nerve injury in rats. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean ± S.E.M. *P < 0.05 vs sham-operated group. n = 6-8 in each group.
Chapter 4

4.4. DISCUSSION

In the present study, SNI procedure resulted in long lasting behavioral hypersensitivity to cold and mechanical stimulation. Treatment with resveratrol, a selective COX-1 inhibitor, naproxen, a nonselective COX inhibitor or rofecoxib, a selective COX-2 inhibitor did not affect allodynia and hyperalgesia when single doses of the drugs were administered systemically at the time of development of hypersensitivity or when steady state pain related behaviors are existing due to nerve injury indicating that there is no involvement of both COX isoforms in the initiation and on the existing hypersensitivity in SNI.

It has been previously reported that continuous administration of systemic or epidural NSAIDs relieved neuropathic pain in cancer patients indicating that PGs are continuously produced for longer periods in neuropathic pain (Ripamonti et al., 1996; Lauretti et al., 1998). Moreover, daily intraplantar injections of PGE$_2$ in rats for 14 days produced persistent mechanical nociceptor hypersensitivity state that lasted for more than 30 days which was significantly attenuated by chronic treatment with indomethacin (Ferreira et al., 1990). Recent evidence suggested that COX-2 expression is not static, but changes in a time-dependent manner after peripheral nerve injury (Ma and Eisenach, 2002, 2003a, b). It has also been reported that COX-1 expression in spinal cord is up-regulated 4 days and 2 weeks after spinal nerve ligation and partial sciatic nerve transection. Various studies demonstrated antiallodynic and antihyperalgesic effects of systemically administered selective COX-2 inhibitors in models of post-operative pain and cancer neuropathic pain (Yamamoto et al., 2000; Fox et al., 2004). In view of this, COX inhibitors were administered for longer periods during development and maintenance of hypersensitivity. However, both preemptive and postoperative administration of COX inhibitors failed to attenuate the hypersensitivity. Recently it has been reported that there is no change in COX-2 expression following nerve injury and systemic administration of COX inhibitors failed to attenuate the hypersensitivity (Zhao et al., 2000; Broom et al., 2004; Schafers et al., 2004). These data along with the present results suggesting that there is no role of isoforms of COX-1 and/or COX-2 in the development and maintenance of neuropathic pain due to SNI.

It is well known that spinal and peripheral PGs are involved in sensitization of central and peripheral afferent fibers, respectively, that contribute to the hypersensitivity (Ferreira et al., 1990; Taiwo and Levine, 1990; Willingale et al.,
Chapter 4

Further, direct administration of PGE$_2$ and PGF$_{2\alpha}$ into spinal cord results in spontaneous agitation, hyperalgesia and tactile allodynia, which were significantly attenuated by COX inhibitors in mice and rats (Taiwo and Levine, 1988; Minami et al., 1994; Park et al., 2000; Turnbach et al., 2002). Therefore in the present study, the COX inhibitors were also administered intrathecally to study the spinal mechanisms contributing to the manifestation of neuropathic pain. Interestingly, intrathecally administered COX inhibitors also did not reverse hypersensitivity on the day of experimentation. Our findings are in general agreement with the previous reports in which spinally administered COX inhibitor had no effect on hypersensitivity (Lashbrook et al., 1999; Hefferan et al., 2003). The present results clearly indicating that PGs produced by COX isoforms might not be involved in the hypersensitivity following SNI.

Some studies along with the present study found that systemic and spinal administration of COX inhibitors were ineffective (Lashbrook et al., 1999; Syriatowicz et al., 1999; Hefferan et al., 2003), while others detected significant antiallodynic and antihyperalgesic effects in alleviating neuropathic pain in various experimental models (Ma and Eisenach, 2002, 2003a,b). Few clinical trials reported the analgesic effects of NSAIDs in the treatment of neuropathic pain, however, the data on the use and efficacy of NSAIDs for the treatment of neuropathic pain is controversial. Recently, it has been reported that there are phenotypic changes in the expression of neurotransmitters, and the development of ectopic activity in the injured and/or uninjured neurons and satellite glial cells (Fukuoka et al., 2001; Tsuzuki et al., 2001; Obata et al., 2003, 2004). Thus, the diverse effects of COX inhibitors in neuropathic models support the idea that different modes of nerve damage and phenotypic changes underlie the differential effects of these agents in the separate animal models of neuropathic pain.

The present lack of efficacy of these agents can most likely not be ascribed to inappropriate dose selection, availability of drug at nerve injury, and poor penetration into spinal cord. Rofecoxib and resveratrol are non-polar and readily cross lipophilic barriers whereas naproxen is weakly non-polar, thus it may be misinterpreted for its less efficacy in alleviating hypersensitivity to nerve injury (Abdel-Halim et al., 1978; Merck and Co. Vioxx information, issue 2000; Wang et al., 2002; Dembo et al., 2005). However, the doses of these agents employed were in the same range that
showed centrally mediated effects following systemic administration (Chan et al., 1999; Josa et al., 2001; Wang et al., 2002). Thus, it is unlikely to interpret that these agents are poorly distributed to the spinal cord. So, the relatively less efficacy of these drugs is not due to sufficient drug concentration at the vicinity of spinal cord, but it may be due to absence of adequate levels of COX-1 and/or COX-2 expression to release PGs or ineffective participation of COX-1 and/or COX-2. It is further supported by the absence of effects of resveratrol and rofecoxib, a selective COX-1 and COX-2 inhibitor, respectively, in attenuating neuropathic pain. These results suggesting that both COX isoforms are not contributing to the hypersensitivity in SNI model of neuropathic pain.

In summary, the results of the present study demonstrated the lack of effectiveness of the selective COX-1 and/or COX-2 inhibition in attenuating peripheral neuropathic pain in this model of nerve injury and indicated that the development and maintenance of hypersensitivity in SNI model is not dependent on both isoforms of COX.