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□ 원 저 □

Effect of Celecoxib, a Cyclooxygenase-2-specific Inhibitor, has no Effect on Chronically Maintained Neuropathic Pain in Rats

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Abstract

장기간 유지된 신경병증성 통증 흰쥐에서 선택적 COX2 억제제인 Celecoxib의 진통효과

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배경: 신경병증성 통증은 스테로이드, 아편유사제 등의 진통제에 잘 반응하지 않는다. 하지만 염증성 매개물질들이 신경병증성 통증의 발생에 관여한다는 보고가 있다. 특히 선택적 COX2 억제제인 celecoxib의 신경병증성 통증에 대한 효과에 관해서 상반된 연구결과가 존재한다. 본 연구는 신경병증성 통증 모델인 척추신경 결찰모델을 이용 기계적, 냉각 이질통 및 온도감각 과민현상의 발현에 celecoxib이 미치는 영향을 관찰하여 celecoxib의 항통각효과를 확인하고자 하였다.

방법: 30마리의 쥐를 이용 척추신경을 결찰하여 신경병증성 통증을 유도하였다 celecoxib (1, 10, 100, and 300 mg/kg)을 경구 투여하였고 총 30마리 중 12마리의 쥐에서 열, 기계적자극에 대해서 통각과민, 냉각자극에 의해 이질통이 발생하였다. 약물 투여 후 30, 60, 120, 180분 후 von Frey, 냉각자극검사, Hargreaves검사를 시행하여 쥐의 행동변화를 관찰하였다.

결과: 신경결찰 후 5일 후에 celecoxib의 용량에 관계없이 열, 기계적 자극에 의한 통각과민, 냉각자극에 대한 이질통을 감소시키지 않았다($P > 0.05$). 또한 celecoxib투여에 의한 장기간의 항 통각효과는 관찰되지 않았다($P > 0.05$).

결론: celecoxib을 경구로 투여하였을 때 장기간 유지된 신경병증성 통증 흰쥐에서 약의 투여용량, 투여기간에 따른 항 통각작용은 관찰되지 않았다. 따라서 조직 손상후 발생된 장기간의 신경병증성 통증에 있어서 celecoxib은 효과가 없는 것으로 사료된다. **(JKDSA 2008; 8: 29～34)**

핵심용어: 이질통, Celecoxib, Cyclooxygenase-2, 통각과민, 척추신경결찰

INTRODUCTION

Neuropathic pain, characterized by spontaneous pain, hyperalgesia and allodynia, includes a multiplicity of causes (Jensen et al, 2001). To date, a lot of researches have been carried out to develop the useful animal models that can dependably give rise to a neuropathic pain-like syndrome in rodents and indicate an analgesic efficacy in human clinical approaches. For examples, chronic constriction injury (Bennett and Xie, 1988), partial sciatic nerve ligation (Seltzer et al, 1990), spinal nerve ligation (Kang et al, 2005), and lately the spared

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nerve injury (Bourquin et al, 2006; Decosterd and Woolf, 2000), have been developed.

Among diverse theories that describe the mechanisms of neuropathic pain, the close correlation between inflammatory responses and neuropathic pain states has been suggested (Bingham et al, 2005; Broom et al, 2004; Frisen et al, 1993; MacPherson, 2002; Tracey and Walker, 1995). Especially, local up-regulation of cyclooxygenase 2 (COX2) at the lesion of peripheral nerve injury and the following release of prostaglandin E2 (PGE2) is closely related toperipheral sensitization, acting to change the threshold and excitability of the nociceptor peripheral terminal (McCleskey and Gold, 1999; O'Banion, 1999). On the other hand, COX2 mRNA expression is noticeably increased in the spinal cord after the injection of peripheral inflammatory stimuli such as complete Freund's adjuvant (CFA) (Hay et al, 1997) and carrageenan (Ichitani et al, 1997), which means that the increase of COX2 mRNA in the spinal cord is involved in inflammatory allodynia and hyperalgesia and implies that some of the analgesic effect of COX2 inhibitors are not peripheral but central (Hay et al, 1997; Samad et al, 2001; Tegeder et al, 2001; Yaksh et al, 2001; Yamamoto and Nozaki-Taguchi, 1997). Also, COX2 protein levels in the dorsal spinal cord and thalamus (but not in the ventral spinal cord, cingulate cortex and locus coeruleus) increased significantly one day after nerve ligation, compared with those in the sham animals (Zhao et al, 2000).

There have been lots of researches showing that COX2 plays a pivotal role in producing inflammatory pain. However, when it comes to in neuropathic pain models, the role of COX2 is still unclear because of various experimental settings. Therefore, the present study was carried out to evaluate the effectiveness of the selective COX2 inhibitor, celecoxib (Celebrex®), on the long maintained neuropathic nociception evoked by spinal nerve ligation using behavioral tests including von Frey, cold, and Hargreaves' test, together.

MATERIALS AND METHODS

1. Animals

Thirty male Sprague-Dawley rats, 150–200 g (Central Lab. Animal Inc., Seoul, South Korea), were maintained in a climate-controlled room on a 12-h light/dark cycle with lights on at 07 : 00 h and food and water *ad libitum*. All procedures were performed in accordance with the guidelines specified in the NIH Guide for the Care and Use of Laboratory Animals (NIH publication No. 86–23, revised 1985) and the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals (Zimmermann, 1983). Every effort was conducted to minimize the number of animals used and their discomfort.

2. Spinal Nerve Ligation (SNL) model of neuropathic pain

The SNL model was performed as previously described (Kang et al, 2005) in 18 out of 30 rats showing no pain behaviors before surgery. Briefly, rats were anesthetized using isoflurane (3% for induction followed by 2% for maintenance). After surgical preparation, the removal of the transverse process of left 6^{th} lumbar vertebra was made to uncover the 4^{th} and 5^{th} lumbar (L4 and L5) spinal nerves running just underneath the process. The L4 and L5 spinal nerves need to be separated in some animals to make the L5 spinal nerve accessible for ligation. Once enough length of the L5 spinal nerve is freed from the adjacent structure, a piece of 6-0 silk thread is placed around the L5 spinal nerve and the nerve is tightly ligated to interrupt all axons in the nerve. The incision was closed and rats were allowed to recover for at least 5 days.

3. Behavioral testing and drug treatment

All animals were allowed to adapt to the behavioral testing apparatus for at least three habituation sessions. Following habituation, at least two baseline measures were obtained for each of the behavioral tests in two

separate sessions within the week before surgery. After completion of the surgical procedures, the behavioral tests were performed at appropriate intervals.

Mechanical allodynia: Rats were placed on a metal mesh grid covered by individual transparent plastic boxes (8 × 8 × 18 cm^3). After 20 min acclimatization, a hindpaw withdrawal in response to normally innocuous mechanical stimuli was measured using von Frey monoflilaments (0.2−15 g). The base of the third and fourth toes was stimulated for threshold testing after the spinal nerve ligation. The mechanical threshold was determined by the up-down method (Dixon, 1980).

Cold allodynia: Rats were placed in individual plastic boxes with a mesh floor and allowed to explore and groom until they settled. A drop of acetone was applied to the plantar hindpaw using a feeding tube attached to a syringe without touching the skin. The duration of the withdrawal response was recorded with an arbitrary minimal value of 0.5 s and a maximum of 20 s (Choi et al, 1994).

Thermal hyperalgesia: Rats were placed in Perspex boxes and the lateral plantar surface was exposed to a beam of radiant heat through a transparent Perspex surface (Hugo Basile Inc., Italy) (Hargreaves et al, 1988). The withdrawal latency was measured with a minimal value of 0.5 s and a maximum of 20 s. The heat stimulation was repeated 3 times at an interval of 5 ± 10 min for each paw and the mean calculated.

Drug treatment: At the 50th day after SNL operation, groups of three rats each received p.o. administration of either 1, 10, 100, or 300 mg/kg of celecoxib (Celebrex®, Pharmacia, South Korea) in 5% carboxymethylcellulose sodium salt (CMC). After administration, von Frey, cold, and Hargreaves' tests were done at different time points (30, 60, 120, and 180 min).

4. Statistics

Animals were randomly assigned to each treatment group. Results are presented as mean ± SEM. The data was analyzed using one-way ANOVA with post-hoc Bonferroni's multiple comparison methods (SPSS v. 10.0, SPSS Inc. Chicago, Illinois). The significance level was set at $P < 0.05$.

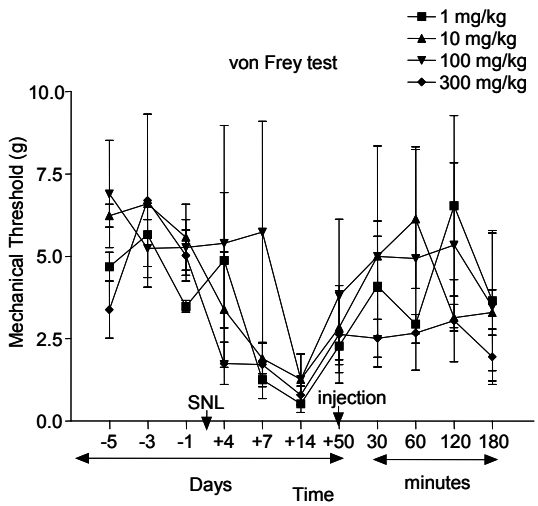

Fig. 1. The effect of celecoxib on mechanical allodynia 50 days after spinal nerve ligation. After establishment of baseline behavior, 1, 10, 100, and 300 mg/kg celecoxib were p.o. administered followed by mechanical allodynia testing was performed at different time courses (30, 60, 120, and 180 min). There were no significant differences among groups ($P > 0.05$). Data represent the mean ± SEM.

RESULTS

Two weeks after SNL, the threshold decreased significantly, which was consistent with the time course of the development of mechanical allodynia (Kang et al, 2005). At the 50th day after SNL, the definite maintenance phase of pain, the antinociceptive effect of oral celecoxib on mechanical allodynia was evaluated depending on drug dosages (1, 10, 100, and 300 mg/kg) and time (30, 60, 120, and 180 min) after the administration. All treated dosages of oral celecoxib failed to attenuate significantly the allodynic condition in response to application of von Frey filaments ($P > 0.05$, Fig. 1). Also, there was no significant difference in the effect of the drug at the

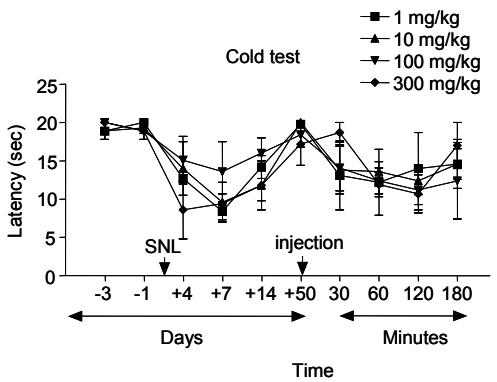

Fig. 2. The effect of celecoxib on cold allodynia 50 days after spinal nerve ligation. The withdrawal response duration (in seconds) after cold stimulation (acetone) was not significantly increased after celecoxib p.o. administration ($P > 0.05$). Data represent the mean ± SEM.

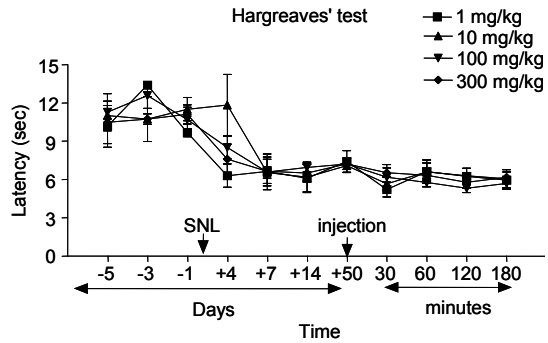

Fig. 3. The effect of celecoxib on thermal hyperalgesia 50 days after spinal nerve ligation. In all groups, the withdrawal response latency (in seconds) to nociceptive heat stimulation (Hargreaves' test) was not significantly increased after celecoxib p.o. administration ($P > 0.05$). Data represent the mean ± SEM.

different time points ($P > 0.05$, Fig. 1). Furthermore, oral celecoxib also failed to show an antinociceptive effect on withdrawal latency following acetone application ($P > 0.05$, Fig. 2) or radiant heat exposure ($P > 0.05$, Fig. 3).

DISCUSSION

Neuropathic pain, which is difficult to treat with conventional analgesics such as NSAIDs and opioids, has continued to be challenge for clinicians and preclinical researchers so far. In addition, the mechanisms associated with neuropathic pain are still not clear. Unfortunately, alternative medications have not proven definitely effective. On the other hand, the role of spinal COX in nociception has been implicated in several studies (Uda et al, 1990; Yaksh and Malmberg, 1993). COX is the key enzyme in the synthesis of prostaglandins (PGs) from arachidonic acids. COX has two isoforms, COX1 and COX2. COX1, the constitutive isoform, is expressed in most tissues and thought to mediate physiological actions (Dubois et al, 1998; Feng et al, 1993). However, the prominent inducibility of COX2 in neurons by synaptic stimuli suggests that it may play a important role in the development of neuroplasticity associated with neuropathic pain (Yamagata et al, 1993).

In the present study, SNL model was used for neuropathic pain-like behavior and celecoxib, one of NSAIDs, was orally administered with different dosages 1, 10, 100, and 300 mg/kg at the 50th day after SNL nerve injury for dealing with chronically maintained neuropathic pain-like behavior. Celecoxib, a selective COX2 inhibitor, failed to relieve allodynia and hypersensitivity in SNL model of neuropathic pain, which suggests that at least COX2 may not be involved in the maintenance of allodynia and hypersensitivity caused by SNL. Although the drug or neuropathic pain model or drug administration route of our study is different from ones of several previous studies, our behavioral results are somewhat consistent with ones of them. For example, first, intrathecal injection of 100 μg of indomethacin immediately or one day after nerve ligation attenuated the development of tactile allodynia but had no effect on established allodynia 14 days after ligation (Zhao et al, 2000). Zhao et al. (Zhao et al, 2000), also, studied that COX2 protein levels in the dorsal spinal cord and thalamus increased

significantly one and 14 days after nerve ligation, compared with those in the sham rats. Therefore, Zhao et al. suggested that spinal COX2 probably plays a critical role in the early development, but not in the maintenance, of tactile allodynia caused by the nerve injury in SNL model of neuropathic pain. On the basis of Zhao et al.'s results, we can, also, implicate when celecoxib is applied long period after nerve injury, it has no effect in lessening allodynia and hyperalgesia produced by SNL despite the very high dose administration of celecoxib. Secondly, recently, it was suggested that rofecoxib treatment (1 and 3.2 mg/kg i.p. for 5 and 3 days respectively starting on the day of SNI surgery) failed to modify the initiation of allodynia and hyperalgesia in the SNI model (Broom et al, 2004) However, Broom et al. found that rofecoxib significantly reduced inflammatory hypersensitivity evoked by injection of CFA into one hindpaw (Broom et al, 2004). Therefore, Broom et al. suggested that COX2 is not related to dorsal horn neurotransmission after SNI and is not important for any central sensitization that may occur in SNI model. Furthermore, they insisted that COX2 does not appear to be involved either in the development or long term maintenance of allodynia and hyperalgesia following SNI because rofecoxib, the selective COX2 inhibitor, failed to ameliorate neuropathic pain-like behaviors in SNI model (Broom et al, 2004).

In summary, even though the drug or neuropathic pain model used in studies was different each other, it is still controversial whether COX2 is important or involved in neuropathic pain or not. However, we can conclude that celecoxib has no the antinociceptive effect, at least, on chronically long-term sustained thermal hyperalgesia, and mechanical as well as thermal allodynia caused by SNL in rats. Therefore, it may be thought that it is not satisfactory to treat the long-term established neuropathic pain with celecoxib.

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