Roles of Serotonergic and Adrenergic Receptors in the Antinociception of Selective Cyclooxygenase-2 Inhibitor in the Rat Spinal Cord

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Background:
The analgesic mechanisms of cyclooxygenase (COX)-2 inhibitors have been explained mainly on the basis of the inhibition of prostaglandin biosynthesis. However, several lines of evidence suggest that their analgesic effects are mediated through serotonergic or adrenergic transmissions. We investigated the roles of these neurotransmitters in the antinociception of a selective COX-2 inhibitor at the spinal level.

Methods:
DUP-697, a selective COX-2 inhibitor, was delivered through an intrathecal catheter to male Sprague-Dawley rats to examine its effect on the flinching responses evoked by formalin injection into the hindpaw. Subsequently, the effects of intrathecal pretreatment with dihydroergocristine, prazosin, and yohimbine, which are serotonergic, α1 adrenergic and α2 adrenergic receptor antagonists, respectively, on the analgesia induced by DUP-697 were assessed.

Results:
Intrathecal DUP-697 reduced the flinching response evoked by formalin injection during phase 1 and 2. But, intrathecal dihydroergocristine, prazosin, and yohimbine had little effect on the antinociception of intrathecal DUP-697 during both phases of the formalin test.

Conclusions:
Intrathecal DUP-697, a selective COX-2 inhibitor, effectively relieved inflammatory pain in rats. Either the serotonergic or adrenergic transmissions might not be involved in the analgesic activity of COX-2 inhibitors at the spinal level. (Korean J Pain 2011; 24: 179-184)

Key Words:
analgesia, COX-2 inhibitor, inflammatory pain, mechanism, spinal cord.
INTRODUCTION

Cyclooxygenase (COX)–2 inhibitors are one of the most commonly used types of analgesics. Inhibition of COX–2, which is increased in the spinal cord after peripheral inflammation [1], and the consequent blockade of prostaglandin biosynthesis, have been widely accepted as the mechanisms underlying the analgesic action of this group of drugs. However, several lines of evidence suggest that their analgesic effects are also exerted by a variety of peripheral and central mechanisms including endo–cannabinoids, nitric oxide, and the monoaminergic, cholinergic, and opioid systems [2,3].

Among them, monoamines such as serotonin (5–hydroxytryptamine, 5-HT) and norepinephrine (NE), and their corresponding receptors, were shown to be present within the spinal cord [4,5] and to play an important role in the modulation of nociceptive transmission [6]. The involvement of 5-HT and NE in the antinociceptive effects of COX–2 inhibitors has already been documented in other reports with animal models. Orally administered rofecoxib increased 5-HT levels in the rat frontal cortex, and the analgesic activity of this COX–2 inhibitor was significantly decreased by depletion of central 5-HT [7]. Additionally, destruction of bulbospinal noradrenergic projection neurons by intracerebroventricular injection of 6-hydroxydopamine was shown to eliminate the effect of nonsteroidal anti-inflamatory drugs [8]. Taken together, these data indicate that there is an interaction of COX–2 inhibitors with the central serotonergic and adrenergic systems. However, at the spinal level, these interactions are not clearly defined.

The aim of this study was to clarify the roles of 5-HT and NE on the analgesic activity of COX–2 inhibitors at the spinal level. Therefore, 5-HT receptor antagonists and α1 adrenergic and α2 adrenergic receptor antagonists were intrathecally administered to investigate their ability to reverse the antinociception produced by COX–2 inhibitors in a rat model of inflammatory pain.

MATERIALS AND METHODS

All procedures were carried out with the approval of the Institutional Animal Care Committee, Research Institute of Medical Science, Male Sprague–Dawley rats weighing 250–300 g were used in these experiments. The rats were housed in a vivarium maintained at 20–23°C with a 12 h light/dark cycle, and were given food and water ad libitum. A polyethylene tube (PE–10) was catheterized and inserted into the subarachnoid space in sevoflurane–anesthetized rats as described previously [9,10]. The rats were closely monitored and, if motor abnormalities appeared, they were euthanized through a volatile anesthetic overdose. Normal rats were kept in individual cages, and a period of not less than 5 days was allowed for each rat to recover from intrathecal catheterization. Rats displaying apparently normal behavior and weight gain were then assigned to the experiment.

The following drugs were used in this study: DUP–697

| Drug & chemical name | Subtype affinity (pA2)* | Elimination half-life (hour) | Dose (μg) |
|----------------------|-------------------------|-----------------------------|-----------|
| Dihydroergocristine: | Non-selective 5-HT receptor antagonist | 13.6 | 3 |
| (5α,10β)-9,10-Dihydro-12-hydroxy-2′-(1-methylethyl)-5′-(phenylmethyl)-ergotaman-3′,6′,18-trione mesylate | α1 (vs noradrenalin) | 8.18 ± 0.11 | 5.94 ± 0.10 |
| α2 (vs clonidine) | 0.006 | 1.9 |
| α2/α1 † | | |
| Prazosin: | | | |
| 1-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)piperazine hydrochloride | | | |
| Yohimbine: | | | |
| 17α-Hydroxyyohimbic-16α-carboxylic acid methyl ester hydrochloride | | | |

Data are expressed as mean ± SEM. *Log[concentration of antagonist which necessitates doubling the concentration of agonist]⁻¹. †Calculated from the antilogarithm of the difference between the pA2 values obtained at α2 and α1 adrenergic receptors.
(5-Bromo-2-(4-fluorophenyl)-3-[4-(methylsulfonyl)phenyl]-thiophene), dihydroergocristine mesylate (Research Biochemical Internationals, Natick, MA, USA), and prazosin hydrochloride and yohimbine hydrochloride (Sigma Aldrich Co., St. Louis, MO, USA). All drugs were dissolved in 100% dimethylsulfoxide (DMSO) and intrathecally administered using a hand-driven, gear-operated syringe pump. The drugs were delivered in a volume of 10 μl solution, followed by an additional 10 μl of saline to flush the catheter.

On experiment days, the rats were placed in a re-straining cylinder and held for 20 min for adaptation. To investigate the effect of COX-2 inhibitor in the formalin test, rats were treated intrathecally with vehicle or DUP-697 (10, 30, 100, 300 μg), administered 10 min before the formalin test (n = 7 in each group). Doses of DUP-697 were determined based on previous studies [2]. Next, rats were pretreated with dihydroergocristine (5-HT receptor antagonist, 3 μg), prazosin (α1 adrenergic receptor antagonist, 3 μg), or yohimbine (α2 adrenergic receptor antagonist, 10 μg), in order to determine the roles of these agents in the activity of DUP-697 (n = 7 in each group). Pharmacological characteristics of these antagonists are presented in Table 1 [11-15]. Doses of the drugs were chosen based on previous experiments, in which the maximum dosage that did not affect the control formalin response or cause side effects such as motor impairment was de-
Fig. 2. The effects of intrathecal dihydroergocristine (3 μg), prazosin (3 μg), and yohimbine (10 μg) on the antinociception of intrathecal DUP−697 (300 μg) during phase 1 (A) and phase 2 (B) in the formalin test. Dihydroergocristine, prazosin, and yohimbine were administered 10 min before the delivery of DUP−697, and the formalin test was done 10 min later. None of these antagonists affected the antinociception of DUP−697 during both phases of the formalin test. Data are presented as the percentage of control. Each bar represents mean ± SEM of 7 rats.
of the prazosin-pretreated group during phases 1 and 2 was 30% and 40%, respectively ($P > 0.05$, Fig. 2), and that of the yohimbine-pretreated group was 32% and 40%, respectively ($P > 0.05$, Fig. 2). Therefore, intrathecal pre-
treatment with dihydroergocristine, prazosin, and yo-
himbine did not reverse the flinching response during both
phases of the formalin test. There was no apparent abnor-
mal behavior in the rats following the administration of the
experimental drugs.

DISCUSSION

Formalin-induced nociception consists of two different
nociceptive states. The first is acute nociception (phase I),
which is followed by the facilitated state (phase 2). The
phase 1 response appears to result from an immediate and
intense increase in the primary afferent activity. On the
other hand, the phase 2 response mirrors the activation
of wide dynamic range neurons of dorsal horn with a con-
tinuous low level of activity in the primary afferent.
Therefore, phase 2 reflects a facilitated state which ap-
ppears to be a prominent and intensified state of pain in
spite of a reduced level of afferent input [17]. This pain
model may serve as a tool for observing the effects of var-
ious analgesic agents on these two pain types at once.

In this study, intrathecal DUP-697 reduced the flinching
response evoked by formalin injection during both
phases of nociception, indicating that this selective COX–2
inhibitor possesses a central mechanism of action at the
spinal level, a finding consistent with previous reports
[2,18]. On the other hand, pretreatment with 5-HT re-
ceptor and $\alpha_1$ and $\alpha_2$ adrenergic receptor antagonists did
not antagonize the effect of intrathecally administered
DUP–697. These findings suggest that the analgesic
mechanisms of COX–2 inhibitor might not be associated
with either the serotoninergic or adrenergic systems, at least
in the spinal cord.

Nevertheless, as documented earlier, previous reports
support the involvement of central monoaminergic trans-
misions in the antinociceptive activity of COX inhibiting
agents [7,8,19–22]. The discrepancy between our data and
these previous reports may result from methodological dif-
ferences in the experiments, such as the types of stimuli
utilized, the types and doses of drugs administered, the
relative affinities or selectivities of the drugs used, and the
routes of drug delivery. However, several reports support
our results in terms of the routes of drug delivery. Intra-
peritoneally administered acetylsalicylic acid and acet-
aminophen significantly increased 5-HT and NE content in
the brain [19–21], but such an elevation was not observed
in the spinal cord [21]. In addition, the antinociceptive ef-
fect of orally administered paracetamol was reversed by a
5-HT$_{1A}$ receptor antagonist [22]. However, when the same
drug was delivered intrathecally, a 5-HT$_{1A}$ receptor antagon-
ontist did not inhibit its analgesic action [22]. An agent ad-
ministered systemically can reach supraspinal sites to
stimulate descending serotonergic pathways, which may
participate in the antinociception produced by intra-
peritoneally or orally administered COX inhibiting agents.
On the other hand, intrathecal delivery in the volume used
in the current study (20 $\mu$L) may not spread more proximally
than the basal cistern and would be confined to the spinal
cord [23,24]. Therefore, the analgesic action induced by
intrathecal injection of COX–2 inhibitor in the current
study might not have activated serotoninergic pathways.

Systemic administration of adrenergic receptor ago-
nists with diclofenac or ketoprofen resulted in a synergistic
antinociceptive effect, while intrathecal combinations of
the same drugs exhibited an additive rather than syner-
gistic interaction [25,26]. Similarly, systemic, but not in-
trathecal, coadministration of metamizol, nimesulide, ace-
taminophen, piroxicam or naproxen with clonidine showed
superadditivity [27]. These data indicate that COX inhibi-
ting agents may activate supraspinal mechanisms to inter-
act with the noradrenergic system. Taken together with the
results of the current investigation, these findings suggest
that the antinociceptive effects of COX–2 inhibitors might
involve not spinal but instead mainly supraspinal mono-
aminergic transmissions.

There are some limitations to the current study. First,
we evaluated the roles of 5-HT and $\alpha_1$ and $\alpha_2$ adrenergic
receptor antagonists only at the spinal level. The supra-
spinal system may also play an important role, and the two
systems may interact with each other in the nociception.
Second, there are numerous subtypes of 5-HT, $\alpha_1$ and $\alpha_2$
adrenergic receptors, and the analgesic mechanism of
COX–2 inhibitors might be associated with specific sub-
types of those receptors. Therefore, further research will
be needed to establish the properties of supraspinal path-
ways in relation to serotonergic and noradrenergic trans-
mision, and the differential roles of their receptor sub-
types in COX–2 inhibitor analgesia.
In conclusion, intrathecal DUP-697, a selective COX–2 inhibitor, effectively relieved inflammatory pain in rats. The 5–HT and NE systems might not be involved in the analgesic activity of COX–2 inhibitors on the facilitated pain state as well as on acute pain in the rat spinal cord.

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