Korean J Pain 2020;33(4):326-334
https://doi.org/10.3344/kjp.2020.33.4.326
pISSN 2005-9159 eISSN 2093-0569

KJP

Experimental Research Article

Check for updates

# Effect of intraperitoneally administered propentofylline in a rat model of postoperative pain

**Geun Joo Choi[1], Hyun Kang[1], Jun Mo Lee[1], Chong Wha Baek[1], Yong Hun Jung[1], Young Cheol Woo[1], Jae Hyuk Do[2], and Jin Soo Ko[3]**

[1]*Department of Anesthesiology and Pain Medicine, Chung-Ang University College of Medicine, Seoul, Korea*
[2]*Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, Korea*
[3]*Department of Plastic Surgery, National Police Hospital, Seoul, Korea*

**Received** July 8, 2020
**Revised** August 8, 2020
**Accepted** August 9, 2020

**Handling Editor:** Jong Yeon Park

**Correspondence**
Hyun Kang
Department of Anesthesiology and Pain Medicine, Chung-Ang University College of Medicine, 84 Heukseok-ro, Dongjak-gu, Seoul 06974, Korea
Tel: +82-2-6299-2571, 2579, 2586
Fax: +82-2-6299-2585
E-mail: roman00@naver.com

**Background:** In this study, we sought to evaluate whether systemic propentofylline (PPF) has antiallodynic effects in a rat model of postoperative pain, and to assess the mechanism involved.
**Methods:** After plantar incision, rats were intraperitoneally injected with various doses of PPF to evaluate its antiallodynic effect. To investigate the involved mechanism, rats were intraperitoneally injected with yohimbine, dexmedetomidine, prazosin, naloxone, atropine or mecamylamine, following the incision of the rat hind paws, and then PPF was administered intraperitoneally. The mechanical withdrawal threshold (MWT) was evaluated using von Frey filaments at various time points and serum levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 were measured to determine the inflammatory response level.
**Results:** MWT was significantly increased after intraperitoneal injection of 30 mg/kg of PPF when compared with the control group. Injection of PPF and yohimbine, atropine or mecamylamine showed significant decreases in the MWT, while injection of PPF and dexmedetomidine showed a significant increase. Systemic administration of PPF inhibited the post-incisional increase in serum level of TNF-α and IL-1β.
**Conclusions:** Systemic administration of PPF following surgery presented antiallodynic effects in a rat model of postoperative pain. The antiallodynic effects against mechanical allodynia could be mediated by α-adrenergic and cholinergic receptors.

**Key Words:** Acute pain; Animals; Hyperalgesia; Injections, Intraperitoneal; Pain; Pain Management; Pain, Postoperative; Propentofylline; Rats.

## INTRODUCTION

Postoperative pain control is a major issue for clinicians in terms of patient management after surgery. Considering that more than 80% of patients have an experience of postoperative pain that is moderate to severe [1,2], the management of acute pain following surgery is crucial. Besides, more than 50% of patients complain of inadequate pain control after surgery or other procedures [1], which suggest the development of chronic pain. Currently, multimodal therapeutic approaches through various kinds of medications and techniques are recommended as suggesting a more effective and synergistic effect on pain control compared to single modality approaches. Pharmacological



**Author contributions:** Geun Joo Choi: Writing/manuscript preparation; Hyun Kang: Study conception; Jun Mo Lee: Writing/manuscript preparation; Chong Wha Baek: Writing/manuscript preparation; Yong Hun Jung: Writing/manuscript preparation; Young Cheol Woo: Writing/manuscript preparation; Jae Hyuk Do: Study conception; Jin Soo Ko: Study conception.

regimens for pain management are also continuously emphasizing moving toward reducing opioid use [3]. Thus, it has become necessary to determine candidate approaches for the management of postoperative pain that contribute to the reduction of opioid use.

Propentofylline (PPF) is a unique methylxanthine derivative with clear phosphodiesterase, cyclic adenosine monophosphate, and adenosine actions [4]. Many researchers have reported that PPF shows anti-proliferative and neuroprotective effects on stroke, chronic pain, and opioid tolerance [4,5]. It is associated with modulating spinal glial activity and proliferation, which consequently reduces the expression of chemokines and neuronal activity [5,6]. There have been many studies regarding the beneficial effect of PPF on chronic pain, including neuropathic pain caused by peripheral nerve injury and spinal cord injury [6-9]. Based on previous research on its use for chronic pain, PPF can be beneficial for acute episodes such as postoperative pain, and is a promising candidate for postoperative pain management.

We hypothesized that PPF is effective for postoperative pain. In order to identify the relationship between PPF and postoperative pain, we used an incisional pain model in rats applying intraperitoneal administration of PPF [10]. The primary endpoint was to evaluate the antiallodynic effect of PPF. The secondary endpoint was to assess the potential mechanism associated with the antiallodynic effect. The effect of PPF on the inflammatory response was also evaluated through measuring serum concentrations of pro-inflammatory cytokines.

## MATERIALS AND METHODS

The present study was performed in accordance with the Animal Research: Reporting In Vivo Experiment (ARRIVE) statement [11].

### 1. Study animals

The experiment was approved by the Institutional Animal Care and Use Committee at Chung-Ang University (No. 2015-00063). All experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Adult male Sprague–Dawley rats (250–300 g; Coretec, Seoul, Korea) were single-housed in cages in a temperature-controlled room (22℃), and fed a standard laboratory diet and tap water. They were kept under a 12-hour light/dark cycle (lights on from 8:00 a.m. to 8:00 p.m.) and acclimated to the housing facilities for one week prior to the experimental procedures. Rats showing any abnormalities were excluded.

### 2. Surgical procedure

All surgical procedures were performed under sterile conditions by one investigator who was unaware of the group allocation. General anaesthesia was induced with 6% isoflurane in 100% oxygen inside a sealed clear plastic chamber until the rats became immobile. They were then maintained on a non-rebreathing anaesthetic circuit mask using 1% to 2% isoflurane in 100% oxygen. Cefazolin (20 mg/kg; Chong Kun Dang Pharmaceutical Co., Seoul, Korea) was administered subcutaneously prior to incision. The plantar surface of the left hind paw of each rat was prepared aseptically for surgery. The incisional pain model was created as previously described [10]. In brief, a 1 cm longitudinal skin incision extending towards the digits was made with a blade at a point approximately 0.5 cm distal to the tibiotarsal joint on the plantar surface of the left hind paw. The plantaris muscle was isolated, elevated slightly, and then incised longitudinally. The incision was closed with two interrupted horizontal mattress sutures of 5-0 nylon.

### 3. Drug preparation and administration

All the medications used in the present experiment were purchased from Sigma-Aldrich (St. Louis, MO). PPF was dissolved in 2 mL sterile endotoxin-free isotonic saline. The control groups were prepared with syringes containing 2 mL normal saline. The syringes were covered with opaque tape and numbered sequentially according to the randomization list of a respective experiment for allocation concealment. Computer-generated randomization was performed using PASS$^{TM}$ 11 software (NCSS, Kaysville, UT). Drugs in prepared syringes were intraperitoneally administered according to the study protocol. All experimental procedures were conducted with operatives blinded to the group allocation.

### 4. Evaluation of the antiallodynic effect of PPF administered after incision (POST-PPF)

Evaluation of the anti-nociceptive effect of intraperitoneally administered PPF against mechanical allodynia was performed after plantar incision. Thirty-two rats were randomly assigned to one of four groups of eight: the control group and POST-PPF 3 mg/kg, 10 mg/kg and 30 mg/kg groups according to the administered dose of PPF. Either normal saline (the control group) or various doses of PPF (the experimental groups) were injected intraperitoneally 2 hours after plantar incision. The dose level of PPF was based on the amount used in a previous experimental study [7].

## 5. Evaluation of the antiallodynic effect of PPF administered before incision (PRE-PPF)

Evaluation of the anti-nociceptive effect of intraperitoneally administered PPF before plantar incision was performed. Thirty-two rats were randomly assigned to one of four groups of eight rats: the control group; and PRE-PPF 3 mg/kg, 10 mg/kg, 30 mg/kg groups by administered doses of PPF. Normal saline or various doses of PPF were injected intraperitoneally 30 minutes before plantar incision.

## 6. Elucidation of mediated mechanism in PPF-induced antiallodynia

The observed effects of PPF against mechanical allodynia induced by plantar incision were examined in order to determine whether they are mediated by the following receptors: alpha (1 and 2) adrenergic, cholinergic (nicotinic and muscarinic), and opioid. Forty-two rats were randomly assigned to one of seven groups of 6: a PPF-only group as the control, and PPF with one of the study drugs: yohimbine (2 mg/kg), dexmedetomidine (50 μg/kg), prazosin (1 mg/kg), atropine (5 mg/kg), mecamylamine (1 mg/kg), or naloxone (5 mg/kg)) for the other groups. The study drug or normal saline was intraperitoneally injected 2 hours after the plantar incision, and 10 minutes after that, 30 mg/kg of PPF was injected intraperitoneally.

## 7. Behavioural measurements

We tested the behavioural responses to mechanical stimuli in order to evaluate the antiallodynic effect and possible mediated mechanisms of PPF, respectively. Individual rats were placed on an elevated plastic mesh floor (8 × 8 mm perforations) under an overturned clear plastic cage (21 × 27 × 15 cm) and allowed to acclimatise for 15 minutes. The rats were then tested to determine their mechanical withdrawal thresholds (MWTs) to stimuli using von Frey filaments (Stoelting Co., Wood Dale, IL). Filaments with bending forces of 4, 9, 20, 59, 78, 98, 147, and 254 mN were applied vertically to the plantar aspect of the hind paw by administering sufficient pressure to gently bend them until either the hind paw was withdrawn or a bending force of 254 mN (the cut-off value) was reached. Each filament was applied three times at intervals of 3 minutes. The lowest bending force that caused paw withdrawal after application of the filament was used to determine the MWT of the hind paw. After a response was observed, filaments with higher and lower bending forces were applied to confirm the MWT level.

For evaluation of the POST-PPF groups, the MWT was assessed according to the following schedule: 1 day before incision (BL); 2 hours after plantar incision (*i.e.*, immediately before PPF administration) (AI); and 15, 30, 45, 60, 80, 100, and 120 minutes; 24 and 48 hours; and 7 days after the injection of 0.9% saline or PPF. For evaluation of the PRE-PPF groups, the MWT was assessed according to the following schedule: 1 day before incision; 2 hours after plantar incision; and 15, 30, 45, 60, 80, 100, and 120 minutes; 24 and 48 hours; and 7 days after the first measurement of MWT.

## 8. Assessment of motor impairment

In order to identify the effect of PPF on motor function (the sedative effect of PPF), we used an accelerating Rotarod treadmill (Jeung Do Bio & Plant Co., Ltd., Seoul, Korea). Twelve rats were randomly assigned to one of two groups of six rats: the PPF and control groups. The rats were injected intraperitoneally with 30 mg/kg PPF or normal saline 2 hours after the plantar incision. The Rotarod test was performed before injection of PPF or normal saline, and at 2 and 24 hours after the injection. Specifically, the rats were placed on the treadmill running at a speed with a gradual increase from 1 to 18 rotations per minute (rpm) for 120 seconds and maintained for another 30 seconds at 18 rpm [12]. The time at which the rat fell off the treadmill was noted.

## 9. Pro-inflammatory cytokine assay

The rats were injected intraperitoneally with 30 mg/kg of PPF or normal saline 2 hours after plantar incision. At 1 and 48 hours after PPF or normal saline injection, blood samples were collected from the lateral tail vein of the rats into a chilled, sterile tube containing EDTA (EDTA vacutainer, Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 2,000 g for 15 minutes. The plasma was harvested and stored at -80℃ until it was assayed for tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6; their plasma concentrations were assessed with commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). Individual experimental procedures were performed according to the manufacturer's instructions.

## 10. Comparison of PPF with ketorolac as a positive control

Ketorolac was used as the reference analgesic in order to compare the PPF groups with a positive control group [13]. Ketorolac (30 mg/kg) was intraperitoneally injected 2 hours after plantar incision in six rats before behavioural measurements. The area under curve (AUC) of the MWT

over time, between the time points of AI and 120 minutes for each rat, was calculated for this experiment.

### 11. Statistical analysis

The primary outcome measure of this study was the MWT to stimulation using von Frey filaments. In order to estimate the group size for a study assessing the pre-emptive or treatment antiallodynic activity of PPF, a pilot study was conducted for measuring MWTs to von Frey filament stimulation in eight allodynia-induced rats. The average MWT at the baseline (BL); immediately after incision (AI); and at 15, 30, 45, 60, 80, 100, and 120 minutes; 24 and 48 hours; and 1-week post-operation were 78.4, 17.5, 17.5, 12.9, 12.9, 13.7, 12.9, 12.9, 12.5, 9.7, 9.7, and 38.0 mN, respectively, with standard deviations ranging from 0.9 to 11.5 mN, and an autocorrelation between adjacent measurements on the same individual of 0.6 mN. For the power calculation, we assumed that first-order autocorrelation adequately represented the autocorrelation pattern. In order to compare between-group differences, we used the Geisser-Greenhouse Corrected F-test for a repeated-measures analysis of variance (rANOVA). We wanted to detect 10%, 20%, and 30% increases in the MWT in the PPF 3 mg/kg, PPF 10 mg/kg, and PPF 30 mg/kg groups, compared with allodynia-induced rats. The standard deviation was 3.60 mN, and the actual effect standard deviation was 2.31 mN, thus the effect size was 0.64 mN. Therefore, eight rats per group were needed for $\alpha = 0.05$ and a power of 80%. The PASS 11™ software (NCSS) was used to calculate the sample size.

The Shapiro–Wilk test was used to test for the normality of the variables. IL-1β and IL-6, and the AUC of the MWT over time between AI and 120 minutes passed the normality test, but TNF-α and the MWT did not. We additionally checked q-q plots for TNF-α and the MWT, which did not show marked deviation from linearity. Therefore, we assumed that the normal distribution requirement for parametric testing had not been violated, and so decided that rANOVA was applicable. Because IL-1β, IL-6, TNF-α, and the Rotarod test passed sphericity testing, they were compared using rANOVA, followed by *t*-tests with Bonferroni correction ($\alpha = 0.05/2 = 0.025$ or $\alpha = 0.05/3 = 0.017$). Because applying Mauchly's sphericity test indicated that the assumption of sphericity had been violated in the pre-emptive study ($\chi^2$ (65) = 302.23, $P < 0.001$), the treatment study ($\chi^2$ [65] = 468.16, $P < 0.001$), the mechanism study ($\chi^2$ [65] = 303.09, $P < 0.001$), and the positive control study ($\chi^2$ [65] = 302.13, $P < 0.001$), we used a one-way Wilk's lambda multivariate analysis of variance (MANOVA), a generalized form of univariate analysis of variance (ANOVA), comparing two or more dependent variables. Moreover, univariate ANOVA with Bonferroni correction ($\alpha = 0.05/12 = 0.0042$) was used to compare the MWT at each time point.

When the homoscedasticity requirement, using Levene's test for homogeneity of variances, was not met using ANOVA, we used Welch's corrected ANOVA. Tukey's or Tamhane's T2 post-hoc test was used when ANOVA or Welch's corrected ANOVA was significant in identifying the groups with statistically significant mean differences.

Individual measurements were expressed as the mean ± standard deviation and analysed with SPSS 23.0 (IBM Co., Armonk, NY). A *P* value of 0.05 or less was considered statistically significant.

## RESULTS

### 1. Study animals

A total of 124 rats completed the present study without follow up loss. Throughout the experimental period, the rats remained well-groomed and appeared to ingest normal amounts of food and water. Except for impaired weight-bearing on the area of the incision, their gaits appeared unaffected. None of the rats developed complications in the surgical wound.

### 2. Effects of the PPF on mechanical allodynia

**Fig. 1** shows the effect of PPF on mechanical allodynia administered after plantar incision. The results of MANOVA showed statistically significant difference among the groups (F[36.0, 56.96] = 2.532, $P = 0.001$: Wilk's lambda = 0.048, partial $\eta^2 = 0.636$). The MWT values at 45, 60, 80, 100, and 120 minutes for the PPF 30 mg/kg group, and at 60

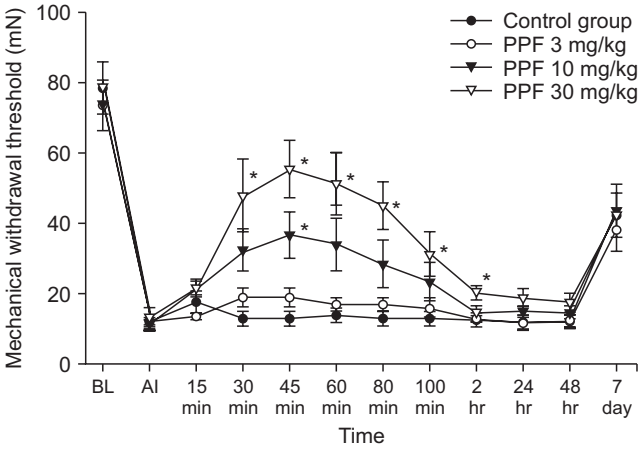


**Fig. 1.** Antiallodynic effect of post-incisional-administered propentofylline (PPF). BL: baseline, AI: after incision. *$P < 0.05$ compared with the control group.

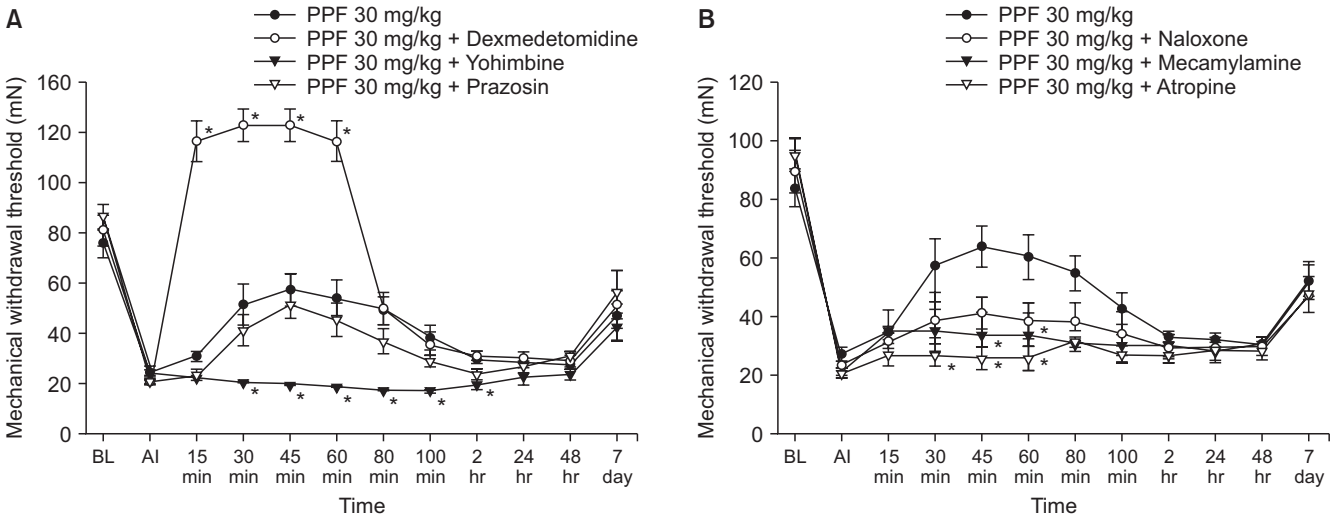


**Fig. 2.** Antiallodynic mechanisms of propentofylline (PPF) with (A) dexmedetomidine, yohimbine or prazosin and (B) naloxone, mecamylamine or atropine. BL: baseline, AI: after incision. *$P < 0.05$ compared with the PPF 30 mg/kg group.

minutes for the PPF 10 mg/kg group, were significantly increased compared to the control group. The MWT values at 45, 60, and 80 minutes in the PPF 30 mg/kg group were significantly increased compared to the PPF 10 mg/kg group. On the other hand, PPF administered before plantar incision showed no significant change in the MWT (data not shown). The MANOVA results show no statistically significant differences among the groups ($F[33.0, 36.06] = 0.769$, $P = 0.776$: Wilk's lambda = 0.208, partial $\eta^2 = 0.407$).

## 3. Possible mechanisms mediated in PPF-induced antiallodynia

**Fig. 2** show the possible mechanism mediated in PPF-induced analgesia. The MANOVA results show statistically significant differences among the groups ($F[33.0, 36.06] = 0.769$, $P = 0.776$: Wilk's lambda = 0.208, partial $\eta^2 = 0.407$). Compared with group PPF 30 mg/kg as the control, the MWT values at 15, 30, 45, and 60 minutes for the PPF 30 mg/kg group with dexmedetomidine were significantly increased, those at 30, 45, 60, 80, 100, and 120 minutes for the PPF 30 mg/kg group with yohimbine were significantly decreased (**Fig. 2A**). Similarly, compared with group PPF 30 mg/kg as the control, the MWT values at 30, 45, 60, and 80 minutes for group PPF 30 mg/kg with atropine and those at 45 and 80 minutes for group PPF 30 mg/kg with mecamylamine were significantly decreased (**Fig. 2B**).

## 4. Effect of PPF on the Rotarod test

Intraperitoneal injection of 30 mg/kg PPF did not have a significant effect on the motor performance of the treatment group rats compared to those injected with the

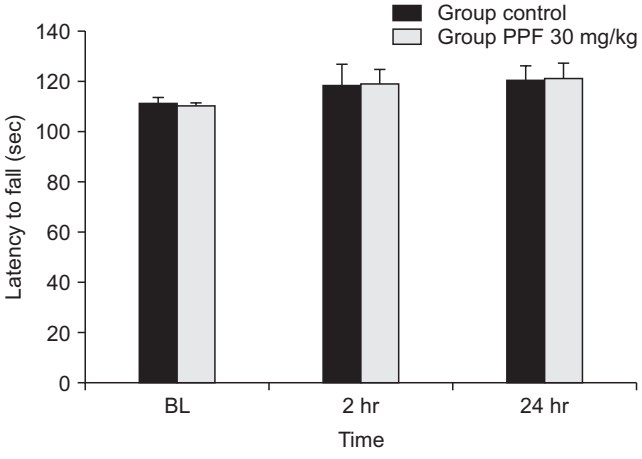


**Fig. 3.** Effects of propentofylline (PPF) on Rotarod testing. BL: baseline.

control vehicle. **Fig. 3** shows that there was no difference among the groups with respect to latency before falling off the Rotarod ($F[1, 0.222] = 0.002$, $P = 0.964$, partial $\eta^2 = 0.001$).

## 5. Effects of the PPF on inflammatory responses

There was a statistically significant difference between the PPF 30 mg/kg and control groups in the serum level of TNF-α ($F[1, 13973.15] = 56.87$, $P < 0.001$, partial $\eta^2 = 0.850$), IL-1β ($F[1, 11443.76] = 11.99$, $P = 0.006$, partial $\eta^2 = 0.545$) and IL-6 ($F[1, 2610.40]$, $P = 0.042$, partial $\eta^2 = 0.353$). The serum level of TNF-α was significantly reduced 1 hour after injection of PPF 30 mg/kg compared to that in the control group (**Fig. 4A**). The serum level of IL-1β was significantly reduced 48 hours after injection of PPF 30 mg/kg compared to that in the control group (**Fig. 4B**). However, there was no difference in the serum levels of IL-6 between the


https://doi.org/10.3344/kjp.2020.33.4.326

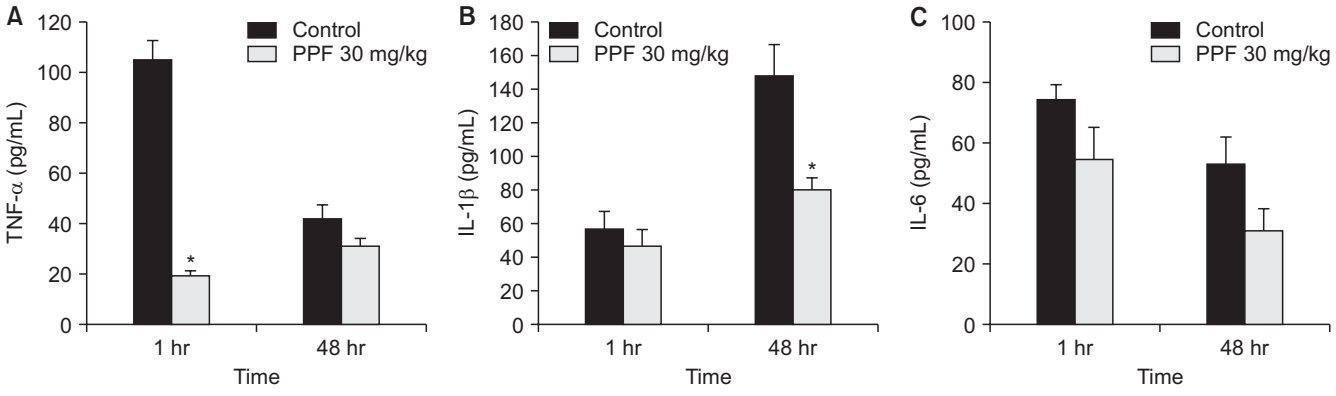


**Fig. 4.** Effect of propentofylline (PPF) on the plasma concentration of (A) tumor necrosis factor (TNF)-α, (B) interleukin (IL)-1β (C) and IL-6. *$P$ < 0.05 compared with control group.

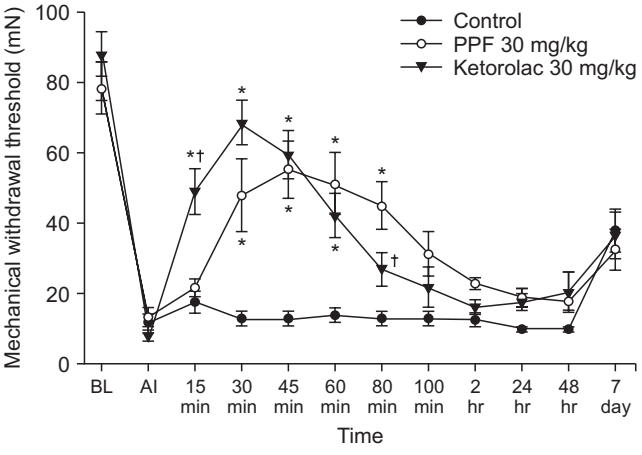


**Fig. 5.** Antiallodynic effect of propentofylline (PPF) 30 mg/kg compared with ketorolac 30 mg/kg and control group. BL: baseline, AI: after incision. *$P$ < 0.05 compared with control group, †$P$ < 0.05 compared with PPF 30 mg/kg group.

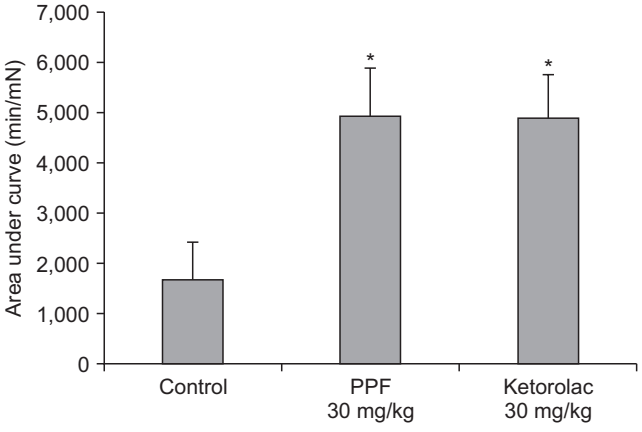


**Fig. 6.** Area under curve for mechanical withdrawal threshold for the comparison of propentofylline (PPF) 30 mg/kg, ketorolac 30 mg/kg and control group. *$P$ < 0.05 compared with control group.

two groups at any particular time point (**Fig. 4C**).

## 6. Comparison of the PPF and positive control

**Fig. 5** shows a comparison of the control, the PPF 30 mg/kg, and the ketorolac 30 mg/kg groups. The results of MANOVA showed statistically significant difference among the groups (F[24.0,20.0] = 5.985, $P$ < 0.001, partial $\eta^2$ = 0.878). The MWT values at 30, 45, 60, and 80 minutes for the PPF 30 mg/kg group and at 15, 30, 45, and 60 minutes for the ketorolac 30 mg/kg group were significantly increased compared to the control group. The MWT values for the ketorolac 30 mg/kg group were significantly increased at 15 minutes and significantly decreased at 80 minutes compared to the PPF 30 mg/kg group.

**Fig. 6** shows the AUC for the comparison of the three groups. There was a significant difference in the AUC of the MWT over time between AI and 120 minutes (F[2.21] = 17.594, $P$ < 0.001). The AUCs of the MWT over time between AI and 120 minutes were significantly lower in the control group than those in groups PPF 30 mg/kg and ketorolac 30 mg/kg ($P$ < 0.001 and $P$ < 0.001, respectively), but there was no evidence of difference between groups PPF 30 mg/kg and ketorolac 30 mg/kg ($P$ = 0.999).

# DISCUSSION

This study is the first to show the effect of systemic administration of PPF in a rat model of postoperative pain. Our findings present that PPF administered intraperitoneally following surgery showed an antiallodynic effect in a dose-dependent manner, whereas PPF before surgery did not show any significant change in the MWT. The antiallodynic property of PPF was antagonized by yohimbine, mecamylamine, and atropine, which indicates the involvement of $\alpha_2$- adrenergic and cholinergic receptors.

Systemic PPF attenuated mechanical allodynia, that was estimated by von Frey filaments following the incision of

the rat hind paws. This result will contribute beneficially to the strategy of postoperative pain management in clinical practice by enabling opioid-sparing multimodal approaches. Although postoperative pain management per se is a critical issue, there is the possibility that inappropriate pain management during the perioperative period can progress to chronic pain. Repetition of nociceptive stimuli during the perioperative period can cause changes in the nervous system including central sensitization, which is linked with persistent nervous system changes. Chronic post-surgical pain is one of the most common and significant complications following surgery [14], and usually has a neuropathic pain component. Many researchers have reported the effectiveness of PPF on chronic pain, especially neuropathic pain [4,6-9]. Systemic or intrathecal administration of PPF following peripheral nerve injury in rodents showed a treatment effect on neuropathic pain-related behaviour and decreased astrocyte reactivity and spinal cord microglia [6-9].

In the present study, PPF administered systemically was effective at reducing acute pain following surgery. It reduced mechanical allodynia for up to 2 hours after surgery, which can be explained by the half-life of PPF and its active metabolite being around 1 hour [15]. Besides, PPF presents its beneficial effect as a glial modulator of neuropathic pain. Taken together, PPF is a promising analgesic for both acute and chronic phases of postoperative pain management, which may prove to be a successful strategy for improving clinical pain control after surgery.

The anti-inflammatory effect of PPF contributed to the relief of postoperative pain in this study. Since PPF was administered systemically, we recognize that the action of PPF may involve the glia as well as other cell types, such as resident peripheral immune cells [16]. The inflammatory response associated with pain at the site of surgical incision can cause peripheral and further central sensitization related to pain augmentation [17,18]. PPF augments the production of anti-inflammatory cytokine, which consequently downregulates the production of pro-inflammatory cytokine. The TNF-$\alpha$ and IL-1$\beta$ pro-inflammatory cytokines, which cause the noxious escalation of pathological glial activation, from microglia to astrocytes, not only leads to secondary neuronal damage but is also essential in the development of pain behaviour and central sensitization [19-21]. PPF may be beneficial regarding the response to postoperative inflammation by blocking glial activation as well as the synthesis and secretion of pro-inflammatory cytokines [15]. TNF-$\alpha$, first produced in response to inflammation, is an important cytokine regarding the starting of the inflammatory process. IL-1$\beta$ is an influential mediator during the process of inflammatory reaction [22,23]. Indeed, we found that TNF-$\alpha$ and IL-1$\beta$ were significantly reduced at 1 hour and 48 hours following surgery, respectively.

Although the mechanisms of postoperative pain have not yet been properly elucidated, the connection between the $\alpha$-adrenergic and cholinergic systems has been investigated in a variety of pain pathways [24-26]. The $\alpha_2$-adrenergic receptors are associated with pain reduction [24,25]. This is correlated with current findings that dexmedetomidine, an $\alpha_2$ agonist, augmented the antiallodynic effect, while yohimbine, an $\alpha_2$ antagonist, reversed it. Especially, it is remarkable that PPF with dexmedetomidine reduced post-incisional pain to a considerable extent. Dexmedetomidine is a potent $\alpha_2$-adrenergic agonist with analgesic and sedative properties, which is widely used in clinical settings [27]. The combination of PPF and dexmedetomidine exerted an additive effect on decreasing postoperative pain. Although we used dexmedetomidine in order to evaluate the mechanism of PPF's antiallodynic effect on postoperative pain, the combination of PPF and dexmedetomidine suggests its clinical viability as a novel strategy for anaesthesia and analgesia. It would be also beneficial if isobolgramic study to identify the synergistic effect of PPF and dexmedetomidine could be performed in the future.

The antiallodynic effect of PPF on postoperative pain was comparable with that of ketorolac as the reference analgesic. Ketorolac is a non-steroidal anti-inflammatory drug that is widely employed for clinical use and well recognized as a prevalent analgesic [28]. Moreover, many preclinical studies have examined its analgesic efficacy in rodents [29,30]. Hence, our finding that the comparable analgesia of PPF and ketorolac support the viability of the clinical use of PPF.

There are some limitations to this study. First, we evaluated the effect of PPF over a short period of time. Given the previous reports of the effectiveness of PPF for chronic pain, a longer period of MWT evaluation is necessary. Second, the pain model used in our study does not reflect all types of surgical procedure. Further study developing different pain models, especially for abdominal and pelvic surgery, is necessary for clinical application, because these types of surgery can cause postoperative pain as even more severe. Despite these limitations, this study and the rigorous methodology indicated substantial strength as the first experimental study regarding the effect of systemic PPF in a rat model of postoperative pain.

In conclusion, systemic PPF showed antiallodynic effect along with a reduction of pro-inflammatory cytokines in a rat model of postoperative pain. Its antiallodynic effect may be associated with $\alpha_2$-adrenergic and cholinergic receptors. Given that PPF is an effective modulator of acute pain, it could be used as part of a beneficial strategy for a

multimodal analgesic approach for pain control after surgery.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

## FUNDING

This Research was supported by the Chung-Ang University Research Grants in 2019. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (grant no. 2018R1A2A2A05021467).

## ORCID

Geun Joo Choi, https://orcid.org/0000-0002-4653-4193
Hyun Kang, https://orcid.org/0000-0003-2844-5880
Jun Mo Lee, https://orcid.org/0000-0001-5607-7232
Chong Wha Baek, https://orcid.org/0000-0001-9917-8738
Yong Hun Jung, https://orcid.org/0000-0001-8531-7039
Young Cheol Woo, https://orcid.org/0000-0001-7318-4814
Jae Hyuk Do, https://orcid.org/0000-0002-2229-0024
Jin Soo Ko, https://orcid.org/0000-0002-6037-3226

## REFERENCES

1. Apfelbaum JL, Chen C, Mehta SS, Gan TJ. Postoperative pain experience: results from a national survey suggest postoperative pain continues to be undermanaged. Anesth Analg 2003; 97: 534-40.
2. Gan TJ, Habib AS, Miller TE, White W, Apfelbaum JL. Incidence, patient satisfaction, and perceptions of post-surgical pain: results from a US national survey. Curr Med Res Opin 2014; 30: 149-60.
3. Chou R, Gordon DB, de Leon-Casasola OA, Rosenberg JM, Bickler S, Brennan T, et al. Management of postoperative pain: a clinical practice guideline from the American Pain Society, the American Society of Regional Anesthesia and Pain Medicine, and the American Society of Anesthesiologists' Committee on Regional Anesthesia, Executive Committee, and Administrative Council. J Pain 2016; 17: 131-57.
4. Sweitzer S, De Leo J. Propentofylline: glial modulation, neuroprotection, and alleviation of chronic pain. Handb Exp Pharmacol 2011; (200): 235-50.
5. Raghavendra V, Tanga F, Rutkowski MD, DeLeo JA. Antihyperalgesic and morphine-sparing actions of propentofylline following peripheral nerve injury in rats: mechanistic implications of spinal glia and proinflammatory cytokines. Pain 2003; 104: 655-64.
6. Tawfik VL, Regan MR, Haenggeli C, Lacroix-Fralish ML, Nutile-McMenemy N, Perez N, et al. Propentofylline-induced astrocyte modulation leads to alterations in glial glutamate promoter activation following spinal nerve transection. Neuroscience 2008; 152: 1086-92.
7. Ellis A, Wieseler J, Favret J, Johnson KW, Rice KC, Maier SF, et al. Systemic administration of propentofylline, ibudilast, and (+)-naltrexone each reverses mechanical allodynia in a novel rat model of central neuropathic pain. J Pain 2014; 15: 407-21.
8. Gwak YS, Crown ED, Unabia GC, Hulsebosch CE. Propentofylline attenuates allodynia, glial activation and modulates GABAergic tone after spinal cord injury in the rat. Pain 2008; 138: 410-22.
9. Zhang J, Wu D, Xie C, Wang H, Wang W, Zhang H, et al. Tramadol and propentofylline coadministration exerted synergistic effects on rat spinal nerve ligation-induced neuropathic pain. PLoS One 2013; 8: e72943.
10. Brennan TJ, Vandermeulen EP, Gebhart GF. Characterization of a rat model of incisional pain. Pain 1996; 64: 493-501.
11. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol 2010; 8: e1000412.
12. Sluka KA, Kalra A, Moore SA. Unilateral intramuscular injections of acidic saline produce a bilateral, long-lasting hyperalgesia. Muscle Nerve 2001; 24: 37-46.
13. Kim TK, Kim YS, Yoon JR, Han IS, Kim JS, Lee CW. The effect of an intraperitoneal injection of ketamine and ketorolac on mechanical allodynia in rats with spinal nerve ligation. Korean J Anesthesiol 2004; 46: 719-23.
14. Macrae WA. Chronic pain after surgery. Br J Anaesth 2001; 87: 88-98.
15. Sweitzer SM, Schubert P, DeLeo JA. Propentofylline, a glial modulating agent, exhibits antiallodynic properties in a rat model of neuropathic pain. J Pharmacol Exp Ther 2001; 297: 1210-7.
16. Jung S, Donhauser T, Toyka KV, Hartung HP. Propentofylline and iloprost suppress the production of TNF-alpha by macrophages but fail to ameliorate experimental autoimmune encephalomyelitis in Lewis rats. J Autoimmun 1997; 10: 519-29.
17. Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci Lett 2004; 361: 184-7.
18. Watkins LR, Maier SF, Goehler LE. Immune activation: the

role of pro-inflammatory cytokines in inflammation, illness responses and pathological pain states. Pain 1995; 63: 289-302.
19. Colburn RW, DeLeo JA, Rickman AJ, Yeager MP, Kwon P, Hickey WF. Dissociation of microglial activation and neuropathic pain behaviors following peripheral nerve injury in the rat. J Neuroimmunol 1997; 79: 163-75.
20. Schubert P, Morino T, Miyazaki H, Ogata T, Nakamura Y, Marchini C, et al. Cascading glia reactions: a common pathomechanism and its differentiated control by cyclic nucleotide signaling. Ann N Y Acad Sci 2000; 903: 24-33.
21. Schubert P, Ogata T, Rudolphi K, Marchini C, McRae A, Ferroni S. Support of homeostatic glial cell signaling: a novel therapeutic approach by propentofylline. Ann N Y Acad Sci 1997; 826: 337-47.
22. Clark IA. How TNF was recognized as a key mechanism of disease. Cytokine Growth Factor Rev 2007; 18: 335-43.
23. Luo G, Hershko DD, Robb BW, Wray CJ, Hasselgren PO. IL-1beta stimulates IL-6 production in cultured skeletal muscle cells through activation of MAP kinase signaling pathway and NF-kappa B. Am J Physiol Regul Integr Comp Physiol 2003; 284: R1249-54.
24. Crassous PA, Denis C, Paris H, Sénard JM. Interest of alpha2-adrenergic agonists and antagonists in clinical practice: background, facts and perspectives. Curr Top Med Chem 2007; 7: 187-94.
25. Lavand'homme PM, Eisenach JC. Perioperative administration of the alpha2-adrenoceptor agonist clonidine at the site of nerve injury reduces the development of mechanical hypersensitivity and modulates local cytokine expression. Pain 2003; 105: 247-54.
26. Schechtmann G, Song Z, Ultenius C, Meyerson BA, Linderoth B. Cholinergic mechanisms involved in the pain relieving effect of spinal cord stimulation in a model of neuropathy. Pain 2008; 139: 136-45.
27. Jessen Lundorf L, Korvenius Nedergaard H, Møller AM. Perioperative dexmedetomidine for acute pain after abdominal surgery in adults. Cochrane Database Syst Rev 2016; 2: CD010358.
28. Boyer KC, McDonald P, Zoetis T. A novel formulation of ketorolac tromethamine for intranasal administration: preclinical safety evaluation. Int J Toxicol 2010; 29: 467-78.
29. Chellman GJ, Lollini LO, Dorr AE, DePass LR. Comparison of ketorolac tromethamine with other injectable nonsteroidal anti-inflammatory drugs for pain-on-injection and muscle damage in the rat. Hum Exp Toxicol 1994; 13: 111-7.
30. Malmberg AB, Yaksh TL. Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the formalin test in the rat. J Pharmacol Exp Ther 1992; 263: 136-46.