Intrathecal cdk5 inhibitor, roscovitine, attenuates morphine antinociceptive tolerance in rats[1]

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ABSTRACT

AIM: To investigate the effect of cyclin-dependent kinase 5 (Cdk5) inhibitor roscovitine on the morphine antinociceptive tolerance development in rats. **METHODS:** Tail-flick test as pain threshold measurement and intrathecal injection techniques were used. **RESULTS**: Intrathecal roscovitine infusion alone produced an antinociceptive effect. Tolerance was induced by continuous intrathecal infusion of morphine 5 µg/h for 5 d. Co-administration of intrathecal roscovitine 1 µg/h for 5 d enhanced the morphine antinociceptive effect in tolerant rats. It also caused a shift in the morphine antinociceptive dose-response curve to the left when co-administered with morphine during tolerance induction, and caused a 67 % reduction in the increase in the ED_{50} of morphine (dose producing 50 % of the maximum response). **CONCLUSION**: Cdk5 modulation is involved in the antinociceptive tolerance of morphine. Intrathecal roscovitine administration could attenuate this tolerance development.

INTRODUCTION

The most efficacious drugs used to relieve pain are the opioid analgesics. However, chronic administration of these drugs leads to the development of tolerance and dependence, processes that were intimately related to opioid addiction. Tolerance is manifested as a decreased potency of the drug, so that progressively larger doses must be administered to achieve a given level of analgesia. The processes underlying opioid tolerance involve complex compensatory changes in many opioid and nonopioid neuronal circuits[1,2]. The cellular mechanism underlying the development of morphine tolerance remains controversial. It has been postulated that morphine tolerance is a result of (i) the up- or down-regulation of opioid receptors (µ, δ, or κ subtypes), (ii) the uncoupling or desensitization of G-proteins, (iii) altered intracellular signaling, including via adenyl cyclase, protein kinase C, or nitric oxide, and (iv) the involvement of post-receptor neural events, particularly those involving the *N*-methyl-*D*-aspartate (NMDA) receptor, γ-aminobutyric acid, and monoamine neurotransmitter[3]. Chronic morphine exposure up-regulates the transcription factors cAMP-response-element-binding protein (CREB) and deltaFosB, both of which appear to mediate an aspect of tolerance[4,5]. DeltaFosB is thought to be a sustained molecular switch for addiction[4]. Cyclin-dependent kinase 5 (Cdk5) was shown to be a downstream target gene of delta FosB, and to regulate the

effects of chronic cocaine exposure in mice[6]. Cdk5 is a member of the Cdk family of serine/threonine kinases and is so named because of its sequence homology to other Cdks[7,8]. It is activated by neuron-specific p35 proteins and exists as a large, multimeric complex associated with cytoskeletal proteins in the neurons, where it phosphorylates a wide variety of proteins, all of which have serine/threonine sites in (K/RT/SPXK)-type motifs[9]. Cdk5 and p35 are expressed predominantly in post-mitotic neurons, with essential roles in neuronal migration, neurite outgrowth, and the laminar configuration of the cerebral cortex[10-12]. Cdk5 has been shown to regulate NMDA receptor phosphorylation. Interestingly, Cdk5 is modulated by metabotropic glutamate receptors in neostriatal neurons[13]. Besides, opioid addiction was associated with hyperphosphorylation of neurofilament. Neuronal Cdk5 and its neuron-specific activator p35 play a major role in regulating the cytoskeleton dynamics[14]. These facts together imply a strong interaction between Cdk5 and morphine tolerance, which may be involved in the pain signal transduction.

Roscovitine is a potent and selective inhibitor of Cdk5, and competes for the ATP-binding site of the kinase. ED_{50} for Cdk5 is 0.16 µmol/L[15]. Roscovitine 5 µmol/L significantly inhibits NMDA-induced long-term potentiation in hippocampal CA1 neurons[16]. In the present study, the contribution of Cdk5 modulation to morphine antinociceptive tolerance was evaluated in tolerant rats.

METHODS AND MATERIALS

Animals and groups Male Sprague-Dawley rats (Academia Sinica, Taiwan, China), weighing 280-320 g, were kept two per cage for at least 5 d after their arrival. The rats housed in a room with a 12:12 h dark-light cycle, and a temperature of 22±0.5 ℃, with food (Lab Diet 5010; PMI Nutrition, Brentwood, MO, USA) and water *ad libitum*. The ethical guidelines specified by Chang-Gung Animal Ethics were followed throughout the study. Chronic intrathecal catheters were implanted under isoflurane anesthesia. Briefly, a polyethylene (PE-5) catheter, filled with 0.9 % saline, was advanced 8.5 cm caudally through an incision in the atlanto-occipital membrane, and its tip positioned at the level of the lumbar enlargement. The rostral tip of the catheter was passed subcutaneously, and connected to an Alzet osmotic pump (200 µL capacity, 1 µL/h pump rate; Model 2001, Alza Corporation, Mountain View, CA, USA). Pump function was confirmed by weighing the pump before placement and after the experiment. Rats

showing neurological deficits after implantation were excluded. Rats were randomly divided into three groups. Group I received continuous intrathecal roscovitine 1 µg/h infusion for 5 d. Group II received continuous intrathecal morphine (5 µg/h) infusion for 5 d. Group III received both continuous intrathecal roscovitine 1 µg/h and morphine 5 µg/h infusions for 5 d. Each group consisted of 10 rats. Dosage was chosen due to preliminary result.

Behavioral testing Tolerance to the antinociceptive effect of morphine was induced by continuous intrathecal infusion of morphine (5 µg/h) for 5 d. To investigate the effects of the Cdk5 inhibitor, roscovitine, on morphine tolerance, we calculate the maximum possible effect (MPE) for morphine antinociception after morphine tolerance was developed. The effects of roscovitine on the morphine antinociceptive dose-response curve were examined on the fifth day of tolerance induction. Nociceptive responsiveness was determined using the warm water (52 ℃) immersion tail-flick test. Latency to the first rapid tail flick represented the behavioral end-point and cut-off latency is 10s to avoid tissue damage. The tail-flick test was performed daily. After a 5-d infusion, the tail-flick response was converted from a defined latency to the MPE after by morphine challenge (1, 10, and 20 µg, intrathecal infusion) as follows:

MPE (%)=(maximum latency–baseline latency)× 100/cut-off latency (10 s)–C baseline latency.

Drugs Roscovitine (LC Laboratories, Woburn, MA, USA) was dissolved in dimethyl sulfoxide (Me$_2$SO), which has no significant effect on antinociception[17].

Statistical analysis Morphine antinociceptive dose-response latency was analyzed by computer-assisted linear regression (Cricket Graph 1.32; Islandia, NY, USA). The ED_{50} was defined as the morphine dose that inhibited 50 % MPE measured by the tail-flick test and was calculated using the linear regression equations. All data were presented as mean±SEM and subjected to analysis of variance and the Dunnett test, and $P<0.05$ was considered significant.

RESULTS

Roscovitine mediated anitnociception Intrathecal administration of roscovitine alone (group I) produced a prolonged tail-flick response compared with the baseline value ($P<0.05$ *vs* control). Our preliminary results showed that larger dosage (5 µg/h) would produce tail-flick response greater than cut-off latency

(10 s). The antinociceptive effect of roscovitine did not increase, nor did tolerance to roscovitine develop, during a 5-d infusion (Fig 1).
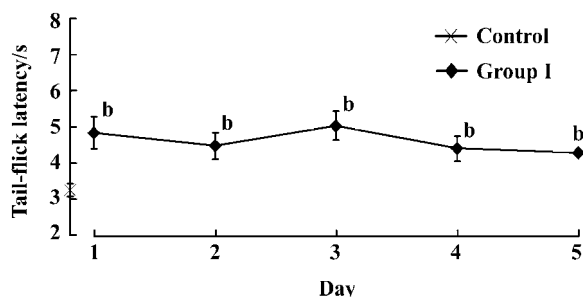

Fig 1. Daily thermal antinociceptive effects in the rats of roscovitine (1 μg/h) administered intrathecally (group I) for 5 d was determined using the tail-flick test. The cross (×) shows baseline tail-flick latency on d 0 (3.25±0.17 s). *n*=10. Mean±SEM. [b]*P*<0.05 *vs* control group.

Roscovitine attenuated morphine tolerance development The maximum antinociceptive effect of morphine (group II) was observed on d 2 during the induction of morphine tolerance. The tail-flick latency for roscovitine co-administered with morphine (group III) was higher than that for morphine alone. Morphine antinociceptive tolerance developed by d 3. Morphine maintained an antinociceptive effect when co-administered with roscovitine during the induction of morphine tolerance. On d 5, maximum tolerance was attained in the rats treated with morphine alone. Roscovitine co-administration attenuated morphine antinociceptive tolerance (*P*<0.05 *vs* group II on d 3, 4, 5) (Fig 2).

Roscovitine shifted the antinociception dose-response curve in tolerant rats On d 0, after the intrathecal administration of morphine (1, 10, and 20 μg), the MPE in control group were 26±2, 48±3, and 61±4 (%) respectively with ED_{50} estimated to be 10.63 μg. On d 5, the MPE in group I were 32±2, 57±5, and 77±5 (%) with ED_{50} to be 3.6 mg. In group II, the MPE were 17±2.5, 32±2.5, and 37±3 (%) with ED_{50} to be 41.09 mg. In group III, the MPE were 22±2, 41±2, and 52±3 (%) with ED_{50} to be 19.11 mg. There were statistical significances compared to the control group (*P*<0.05 in 1, 10, and 20 μg of group I, and 10, 20 μg of group II). The dose-response curve was shifted to the right in the group II, compared with the control group. The co-administration of roscovitine during induction of morphine tolerance reversed this rightward shift. It also caused a 67 % reduction in the increase in
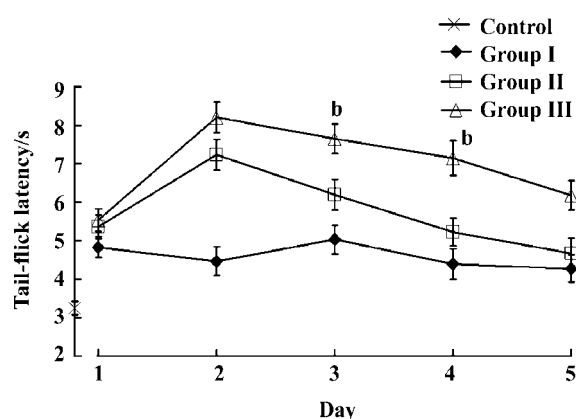

Fig 2. Effect of roscovitine on the development of morphine tolerance. The cross (×) shows the mean tail-flick latency of rats before the study. Group I=roscovitine (1 μg/h) infusion. Group II=morphine (5 μg/h) infusion. Group III=roscovitine (1 μg/h) and morphine (5 μg/h) infusions. *n*=10. Mean±SEM. [b]*P*<0.05 *vs* group II.

the ED_{50} of morphine. Interestingly, the dose¨Cresponse curve shifted to the left in the group I indicating the antinociceptive effect of roscovitine (Fig 3).
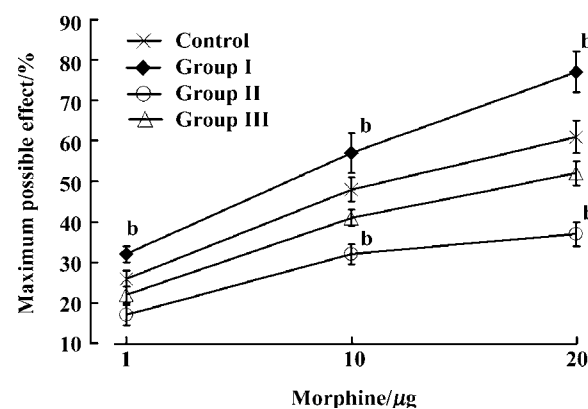

Fig 3. Effects of roscovitine on the dose-response relationship for morphine antinociception, measured by the tail-flick test, before morphine tolerance was developed. On d 5, the dose-response curve was shifted to the right in the group II. Co-administration of roscovitine for 5 d reversed this rightward shift. The curve was shifted to the left in the group treated with roscovitine (1 μg/h) for 5 d group. Group I=roscovitine (1 μg/h) infusion. Group II=morphine (5 μg/h) infusion. Group III=roscovitine (1 μg/h) and morphine (5 μg/h) infusion. Control=normal rats. *n*=10. Mean±SEM. [b]*P*<0.05 *vs* control group.

DISCUSSION

Tolerance is one of the behavioral adaptations to prolonged use of opioid drugs such as morphine, and is

defined as a reduced sensitivity to the drug effects, which generally refers to the attenuation of analgesic efficacy. The cellular mechanism underlying the development of morphine tolerance remains controversial. However, activation of the transcription factors CREB and deltaFosB appears to mediate aspects of tolerance and dependence[4]. Cdk5 is the downstream gene target of deltaFosB and regulates the effects of chronic cocaine exposure[6]. Furthermore, dysregulation of neuronal Cdk5/p35 are found in opioid addicts and opiate-treated rats[14]. The present study demonstrated that a potent Cdk5 selective inhibitor, roscovitine, attenuated the development of morphine antinociceptive tolerance. Potentiation of morphine antinociception was observed when roscovitine was co-administered before the development of morphine tolerance. Furthermore, the administration of roscovitine alone produced antinociceptive effects without the development of tolerance. These results suggest that Cdk5 modulation was not only involved in the development of morphine tolerance but also in pain signal transduction.

The modulation of Cdk5 activity was involved in both morphine tolerance and pain signal transduction[3,16]. Recently, it was found that acute treatment of rats with morphine will increase the density of Cdk5 in cerebral cortex[14]. It is logistical to deduce Cdk5 inhibition might attenuate morphine tolerance development in early days. However, in the same study, chronic morphine treatment will decrease Cdk5 and p35. The exact underlying mechanism is not known. It might be caused by the partial loss of the ability of attenuating morphine tolerance development (Fig 2, group III in d 5 *vs* d 4). But further study is warranted (intrathecal roscovitine infusion after five days rather than co-administraiton).

In conclusion, the current findings suggested that intrathecal roscovitine administration could prevent the development of morphine tolerance. Intrathecal roscovitine administration alone induced a significant antinociceptive effect without the development of tolerance. Hypothetically, co-administration of opioids with Cdk5 inhibitory drugs (such as roscovitine, olomoucine, or butyrolactone) could alter the opioid tolerance that develops with long-term use. Cdk5 modulation was associated with antinociception and the development of morphine tolerance.

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