

## Original Article

# Effects of propofol on CB1 receptor expression in spinal cord of rats with intrathecal morphine-induced pruritus

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**Abstract:** *Backgrounds:* It has been demonstrated that cannabinoid-1 (CB1) receptors in anterior cingulate cortex (ACC) may play a key role in propofol-mediated relief of the intrathecal morphine-induced pruritus in rats. *Objective:* To explore the effect of propofol on the CB1 receptor expression of rat spinal cord with intrathecal morphine-induced pruritus. *Materials and methods:* A total of 108 rats were randomly divided into 6 groups: control, normal saline, propofol, intralipid, SR141716A + propofol and Tween 80 + propofol groups. Rats were intrathecally injected with 10  $\mu$ l normal saline or 40  $\mu$ g/kg morphine. 10 min after the intrathecal administration, 80  $\mu$ l/kg normal saline, 80  $\mu$ l/kg normal saline, 0.8 mg/kg propofol and 80  $\mu$ l/kg intralipid were administered via the jugular vein to rats in control, normal saline, propofol, and intralipid groups, respectively; while in the SR141716A + propofol and Tween 80 + propofol groups, rats were administered with SR141716A (1 mg/kg) or Tween 80 (Tween 80/isotonic saline = 1/9) 1 mg/kg by the jugular vein respectively, followed by venous injection of 0.8 mg/kg propofol to both groups. *Results:* The scratching response was significantly attenuated in propofol and Tween 80 + propofol groups while significantly increased in SR141716A + propofol group ( $P < 0.001$ ). The expression of CB1 receptor in spinal cord of propofol and Tween 80 + propofol groups were significant increased at 8 min after the administration and remarkably lower in SR141716A + propofol group at 16 min after the administration. *Conclusion:* Increased expression of CB1 receptors in spinal cord may contribute to the propofol reversal of intrathecal morphine-induced scratching.

**Keywords:** Propofol, cannabinoid receptor 1, intrathecal morphine-induced pruritus

## Introduction

Pruritus is one of the most common adverse events, which are noted in the post-duration of spinally administered morphine [1]. Although naloxone is an effective drug to prevent the pruritus, it could reduce the required analgesic effect [2]. Clinical studies have indicated that sub-hypnotic dosage (10-20 mg) of propofol is effective for the treatment of the pruritus without disrupting intrathecal morphine-induced analgesia [3-5]. However, the exact mechanism remains unclear.

In a previous study [6], we have demonstrated that morphine-induced pruritus is effectively prevented by propofol and that the protein expression level of the cannabinoid receptor type 1 (CB1) in anterior cingulate cortex (ACC) is increased, indicating that CB1 may play a key role in propofol-mediated relief of the intrathecal morphine-induced pruritus in rats.

CB1 receptors are located primarily in the central and peripheral nervous system, with hind-brain and spinal cord more concentrated [7, 8]. Since propofol could inhibit the information transmission in the spinal dorsal horn [9] and the spinal lamina I mediates the intrathecal morphine-induced pruritus [10], we hypothesized that in addition to the brain, CB1 receptors in spinal cord may play a role in propofol-mediated prevention of the pruritus. In this study, we investigated the expression level of CB1 receptors in spinal cord in rats with intrathecal morphine-induced pruritus after the treatment of propofol.

## Materials and methods

### Animal model

Ethical approval for this study (Ethical Committee 2012-35) was provided by the Medical

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Ethical Committee on the Use and Care of Animals of the 2<sup>nd</sup> Affiliated Hospital of Wenzhou Medical University, Wenzhou, China (Chairperson Prof. Qingquan Lian) on 10<sup>th</sup> October 2012. Adult male Sprague Dawley rats (250-300 g) were used according to the protocol, and the rats were housed in an air-conditioned room with controlled temperatures ( $24 \pm 2^\circ\text{C}$ ) and lights (lights on from 8:00 to 20:00). Animals can access food and water *ad libitum*.

The following drugs were purchased: chloral hydrate (5% solution, 350 mg/kg) and lidocaine (2%) from Zhejiang Chengyi Pharmaceutical Co. Ltd (China), morphine sulfate (NO. 120403-1) from Yichang Humanwell Pharmaceutical Co. Ltd (China), propofol (2%) from Fresenius Kabi (Germany), Intralipid® (10%) from Huarui Pharmaceutical Co. Ltd (China), and CB1 antagonist SR141716A from R&D Systems Co. Ltd (America). All chemicals were dissolved in sterile isotonic saline except SR141716A, which was dissolved in 10% Tween 80 (Beijing Solarbio Science & Technology Co. Ltd, China) and 90% isotonic saline.

Pruritus animal model was established as described previously [6]. In brief, animals were operated for laminectomy at the L3-L4 level to expose the spinal cord under anaesthesia, then the PE-10 (OD: 0.5 mm, ID: 0.25 mm, AniLab Software and Instruments Co. Ltd, China) was inserted into the intrathecal space. A PE-50 tube (OD: 0.96 mm, ID: 0.58 mm, AniLab Software and Instruments Co. Ltd, China) was inserted into the right jugular vein as well. Both catheters were fixed in the posterior cervical area through subcutaneous tunnelling. After the rats were completely recovered from anaesthesia, the motor function was checked. Only those that had normal motor function were chosen for further observation. Three days after the surgery, 10  $\mu\text{l}$  of 2% lidocaine was injected through the intrathecal catheter to confirm the right catheter position, and the rats showing lower limb paralysis were selected for further observation.

Seven days after the operation, a scratching response to intrathecal morphine was observed and the number of hindlimb scratching episodes was recorded by camera. The rat was placed in an individual transparent chamber (24  $\times$  20  $\times$  40 cm) and was observed for two hours before intrathecal morphine injection.

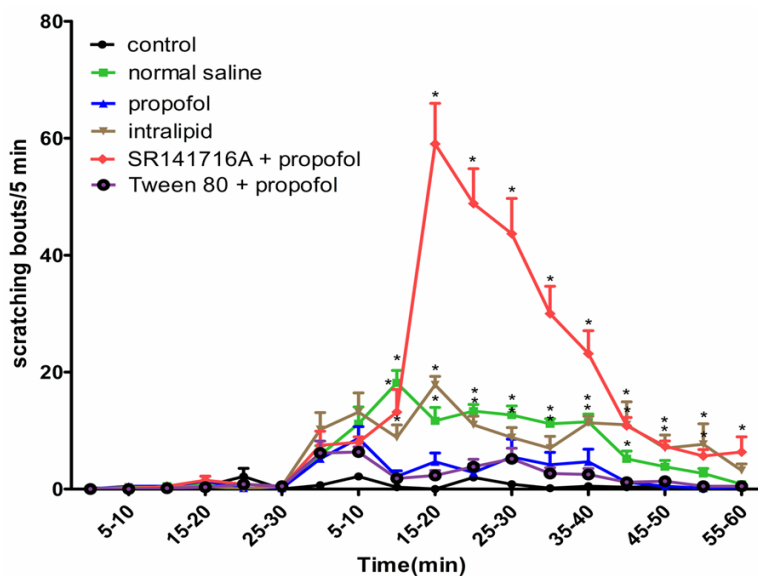
The scratching response was recorded from 30 min before the intrathecal injection to 60 min after the injection. After videotaping started, all investigators left the room. The scratching response was scored by an investigator who blind to the study. Hindpaw scratching behaviour was recorded every 5 minutes. Only the rats with more than two scratching times at the first 5 min after the intrathecal morphine injection would be continued for further observation, otherwise the rat would be excluded [11].

### Experimental design

The first part of this study was to create an intrathecal morphine-induced pruritus model in rats to evaluate the effect of propofol on intrathecal morphine-induced pruritus. A total of 180 rats were enrolled. Of them, 117 were successfully created morphine-induced pruritus. Then 108 rats were randomly divided into 6 groups using the random number generator in Microsoft Excel: control, normal saline, propofol, intralipid, SR141716A + propofol and Tween 80 + propofol groups. In the control group, rats were intrathecally injected with 10  $\mu\text{l}$  normal saline, while in normal saline, propofol, intralipid, SR141716A + propofol, and Tween 80 + propofol groups, rats were intrathecally injected by 40  $\mu\text{g}/\text{kg}$  morphine to successfully create the pruritus model. An additional 10  $\mu\text{l}$  of normal saline was administered to flush the catheter. 10 min after the intrathecal administration of normal saline or morphine, 80  $\mu\text{l}/\text{kg}$  normal saline, 80  $\mu\text{l}/\text{kg}$  normal saline, 0.8 mg/kg propofol and 80  $\mu\text{l}/\text{kg}$  intralipid were administered via the jugular vein to rats of control, normal saline, propofol, intralipid groups respectively; while in the SR141716A + propofol and Tween 80 + propofol groups, rats were administered SR141716A (1 mg/kg) [12, 13], Tween 80 (Tween 80/isotonic saline = 1/9) 1 mg/kg by the jugular vein, respectively, then 1 min later, 0.8 mg/kg propofol were administered to both groups by the jugular vein. All intravenous injections were completed within 10 s.

The second part of this study was to observe pruritus behaviour. Six rats in each group were randomly selected for pruritus behaviour observation. The scratching responses of the rat were recorded from 30 min before the intrathecal injection to 60 min after the injection. The

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**Figure 1.** Scratching reaction of rats after intrathecal 10  $\mu$ l normal saline or 40  $\mu$ g/kg of morphine to intravenous normal saline, normal saline, propofol, intralipid, SR141716A + propofol and Tween 80 + propofol administration. Compared with the propofol group, \* $P < 0.05$ .

remaining rats in each group were sacrificed after 4, 8, 12, and 16 min of the jugular vein administration to collect the lumbar enlargement for the next part of the study (timepoints were based on the results of pilot study), the concentration of CB1 receptor in lumbar enlargement was determined by Western blot analysis ( $n = 3$ ).

Samples of tissue were collected from rats' lumbar enlargement and flash frozen in liquid nitrogen, and then stored at  $-80^{\circ}\text{C}$  until the measurement. The frozen tissues were rapidly thawed and homogenized at  $4^{\circ}\text{C}$  in 200  $\mu$ l cold radio immunoprecipitation assay (RIPA, Beyotime) and sonicated to dissolve the tissue completely. The homogenates were centrifuged at  $12000 \times g$  for 10 min at  $4^{\circ}\text{C}$  and the supernatants were collected. Protein concentration was determined by bicinchoninic acid assay (BCA) kit (Beyotime). A total of 30  $\mu$ g proteins from each sample were loaded per lane for SDS-PAGE (10% SDS gel). Protein samples were denatured and resolved in 10% SDS-PAGE then transferred to a Millipore (Bedford MA) Immobilon-P polyvinylidene fluoride (PVDF) membrane at  $4^{\circ}\text{C}$ , 300 mA for 70 min. The transfer buffer contained 20% methanol, 48 mM Tris Ph9.2, and 39 mM glycine. The membranes were blocked with 5% nonfat milk in TTBS (20 mmol/L Tris-Cl, PH 7.5, containing

0.15 mol/L NaCl, and 0.1% Tween-20) for 2 h, and then incubated overnight at  $4^{\circ}\text{C}$  with primary rabbit polyclonal antibodies against CB1 (1:200 dilution, Abcam, USA) and mouse monoclonal antibody against  $\beta$ -actin (1:1000 dilution, ZSGB-BIO). On the following day, membranes were washed and exposed to horseradish peroxidase (HRP) conjugated goat anti-rabbit (1:5000 dilution, Jackson ImmunoResearch Laboratories, Inc, USA) or mouse (1:10000 dilution, Jackson ImmunoResearch Laboratories, Inc, USA) IgG as second antibodies at room temperature for 1 h. The intensities of bands obtained from Western blot were estimated with ImageQuant LAS-4000 mini.

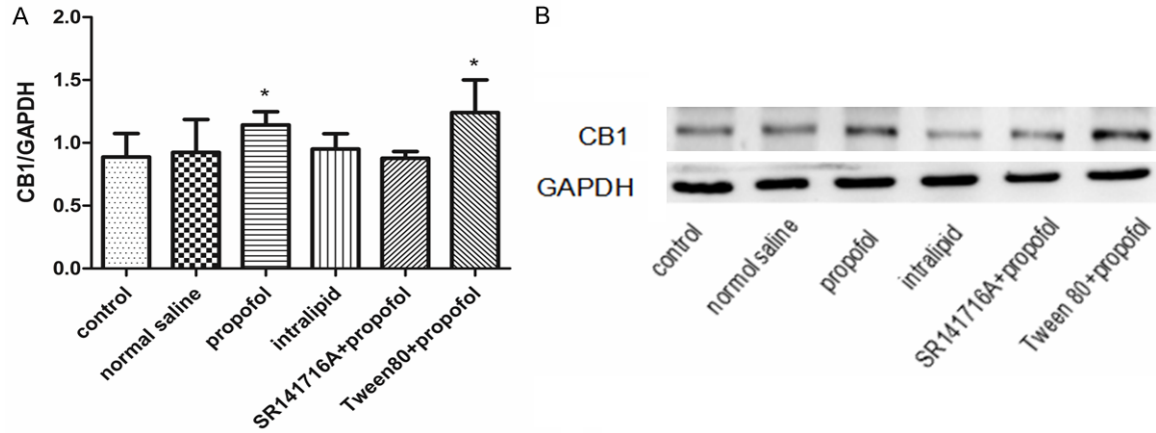
### Statistical analysis

Values were presented as mean  $\pm$  standard deviation (SD) or number as appropriate. Behaviour data were made by repeated measures 2-way analysis of variance (RM-ANOVA) with group and time as variables and Dunnett multiple (post hoc) comparisons (SPSS 17.0, SPSS Inc, Chicago, IL). The results of Western blot were compared using ANOVA. The post hoc comparisons were performed by Newman-Keuls tests and Bonferonni corrections. A  $P$  value of less than 0.05 was considered statistically significant.

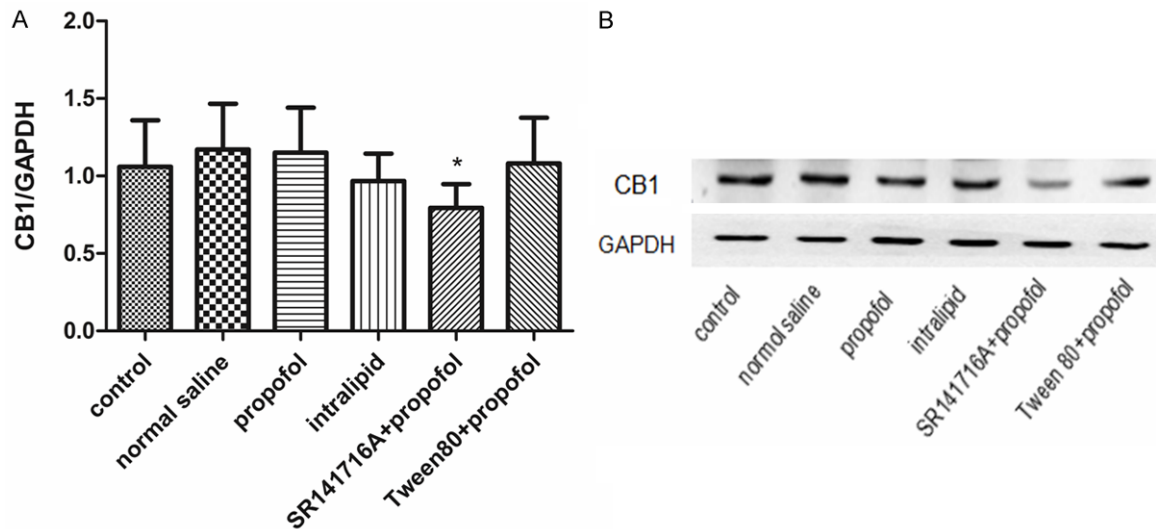
### Results

RM-ANOVA revealed a significant effect of propofol and group  $\times$  time interaction [ $F(5, 30) = 78.10, P < 0.001$ ;  $F(55, 330) = 13.74, P < 0.001$ ]. Compared with intralipid and normal saline groups, the scratching behaviour was significantly attenuated in the propofol and Tween 80 + propofol groups, CB1 receptor antagonists could cause intense scratching responses ( $P < 0.001$ , Dunnett's test) (Figure 1). The continuous data of scratching times were not normally distributed (Kolmogorov-Smirnov test of normality). There were no signs of sedation or motor impairment (e.g., eyes closing, erratic movements, general behavioural depression), all rats retained their righting

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**Figure 2.** The expression of CB(1) receptor in spinal cord at 8 min after administration of the drugs. A. The expression of CB(1) receptor in spinal cord was higher in propofol and Tween 80 + propofol groups, compared with control, normal saline, intralipid and SR141716A + propofol groups,  $*P < 0.05$ . B. Densitometric analysis of the CB(1) receptor in spinal cord; data were normalized by GAPDH (lower band).



**Figure 3.** The expression of CB(1) receptor in spinal cord at 16 min after administration of the drugs. A. The expression of CB(1) receptor in spinal cord was lower in SR group, compared with control, normal saline, propofol, intralipid and Tween 80 + propofol groups,  $*P < 0.05$ . B. Densitometric analysis of the CB(1) receptor in spinal cord; data were normalized by GAPDH (lower band).

reflex in the propofol and Tween 80 + propofol groups.

Compared with the control, normal saline, intralipid, and SR141716A + propofol groups, the scratching response was significantly attenuated in propofol and Tween 80 + propofol groups, and the scratching response was significantly increased in SR141716A + propofol group ( $P < 0.001$ ). At 8 min after the administration timepoints, compared with the control, normal saline, intralipid, and SR141716A +

propofol groups, the CB1 receptor expression levels of spinal cord in propofol and Tween 80 + propofol groups were significant increased ( $0.92 \pm 0.21$ ,  $0.87 \pm 0.78$ ,  $0.95 \pm 0.12$ ,  $0.88 \pm 0.05$  vs.  $1.17 \pm 0.47$ ,  $1.07 \pm 0.12$ , respectively,  $P < 0.05$ ); the expression of CB1 receptor was no significant difference among the control, normal saline, intralipid, and SR141716A + propofol groups (**Figure 2**). At 16 min after the administration time points, the CB1 receptor expression level of spinal cord in SR141716A + propofol group was remarkably lower than

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those in control, normal saline, propofol, intralipid and Tween 80 + propofol groups ( $0.79 \pm 0.15$  vs.  $1.06 \pm 0.30$ ,  $1.17 \pm 0.29$ ,  $1.15 \pm 0.29$ ,  $0.97 \pm 0.18$ ,  $1.08 \pm 0.30$ , respectively,  $P < 0.05$ ); the expression of CB1 receptor was no significant difference among the control, normal saline, propofol, intralipid and Tween 80 + propofol groups (**Figure 3**). The expression of CB1 receptor was no significant difference among the control, normal saline, propofol, intralipid, SR141716A + propofol and Tween 80 + propofol groups at 4 and 8 min after the administration time points.

### Discussion

In this study, the results presented that the expression of CB1 receptor, which has been reported as an important factor for propofol to relieve intrathecal morphine-induced pruritus in the previous study [6], also increased in the spinal cord.

The selected doses of intrathecal morphine in this study were based on previous studies [6, 14, 15], which found that 40  $\mu\text{g}/\text{kg}$  of morphine was an effective dosage and no sedative effect. The anaesthetic dose of propofol in rats is 8 mg/kg, one-tenth anaesthetic dose (0.8 mg/kg) of propofol was applied in this study according to the clinical pruritus treating dose (10-20 mg). From our pilot study, we tested and found that 0.8 mg/kg of propofol injection didn't have the effect on rats' normal righting reflex. In our study, the maximal scratching response caused by intrathecal administration of morphine occurred at 10-15 min after the injection, this finding was consistent with the previous study [6]. It has been reported that the whole-brain content of anandamide, including CB1 receptor, was increased 8 min after administration of propofol and returned to baseline at 40 min after the administration [16], combined with the finding of our pilot study, then we chose 4, 8, 12, and 16 min after the jugular vein propofol administration to detect the expression of CB1 receptor in the spinal cord.

Many studies have shown that spinal cord, especially lumbar enlargement, is an important part of the pruritus information transferring [17-19]. The exact mechanism of neuraxial opioid-induced pruritus is not fully understood, studies have reported that it may be a manifesta-

tion of local, segmental excitation by opioids within the spinal cord itself [2, 20]. It also has been reported that propofol could produce marked depression of posterior horn transmission in the spinal cord and probably exerted its antipruritic action through this inhibition [3, 21]. The study [22] has shown that intrathecal administration of CB1 antagonist rimonabant in mice could cause severe scratching response, however, intracisternal administration did not have this reaction, it means that CB1 receptors antagonist-mediated pruritus may not directly activate the brain itching signalling pathway, but firstly act on the spinal cord or the peripheral nervous system. What's more, it has been reported that substantial levels of CB1 receptor immunoreactivity in smaller DRG cells and widespread labelling of the dorsal root [23] and that may be an important key for the ability of cannabinoids to suppress C-fibre mediated spinal response. In addition, it has been demonstrated that the  $\mu$ -opioid receptor subtype MOR1D and gastrin releasing peptide receptor (GRPR) played an important role in intrathecal morphine induced itching [19], which are mainly located in the spinal cord Lamina. These are consistent with the conclusions of this study, which implies the spinal cord is the important information delivery site of opioid-induced pruritus.

As the results showed, low-dose propofol could significantly alleviate intrathecal injection of morphine-induced scratching behaviour, CB1 receptor antagonists could cause intense scratching reaction, and the expression of CB1 receptor in the spinal cord of propofol group was significantly increased at 8 minutes after the administration while the SR141716A + propofol group was lower than that in propofol group, we considered that the CB1 antagonists could inhibit the enhancement of propofol on the expression of CB1, which indicates that propofol plays its effect on CB1 receptors. In addition, compared with other groups, the expression of CB1 receptors in SR141716A + propofol group was lower at 8 min after the administration, the reason could due to the agonistic effect of propofol weakened while the antagonists effect of SR141716A still was at a higher level. As shown in the results, with the efficacy of propofol and SR141716A declined gradually, behavioural response was no significantly difference between groups at 30-40 min

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after the injection. From this study and previous reporting [6], it showed that the changes of CB1 receptors expression in spinal cord and ACC were produced at the same time, so there was no judgment of propofol firstly exciting CB1 receptors in spinal cord or in ACC. As the inhibitor of fatty acid amide hydrolase (FAAH), propofol could increase the AEA concentration and indirectly stimulate CB1 receptor [16, 24]. However, in addition to the CB1 receptor, whether propofol also acts on other receptors, and if those receptors have crosstalk with CB1 receptors or not, is pending as of yet.

The main limitation of this study was that the AEA concentration in ACC and spinal cord was not determined.

In summary, low-dose propofol can significantly alleviate intrathecal injection of morphine-induced scratching behaviour, and increased expression of CB1 receptors in the spinal cord may contribute to the propofol reversal of intrathecal morphine-induced scratching.

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### Disclosure of conflict of interest

None.

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