

# The false-positive responses of analgesic drugs to the intradermal serotonin- and compound 48/80-induced scratches as an animal model of itch

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Intradermal injection of pruritogens such as serotonin, histamine and compound 48/80 into the skin and then, the evaluation of the scratching behavior is the commonly used animal model to advance pruritic research and drug development. However, predictive validity of this model is poorly documented. There is a close interaction between itch and pain sensations with regard to mediation through an anatomically and functionally identical neuronal pathway. One approach is whether the existing animal model of itch differentiates itch or pain to show efficacy of clinically effective analgesic drugs as a back translation. In this study, we explored the effects of different group of analgesic drugs on serotonin and compound 48/80-induced scratching behavior in Balb-C mice. Serotonin (25 µg) and compound 48/80 (100 µg) was injected intradermally in a volume of 50 µl into the rostral part of skin on the back of male mice and scratches were counted for a 30-min observation period. Morphine (1, 3, 10 mg/kg), tramadol (20, 40, 80 mg/kg), cannabinoid agonist CP 55,940 (0.1, 0.3, 1 mg/kg), paracetamol (100, 200, 300 mg/kg) and diclofenac (50, 100, 200 mg/kg) were given intraperitoneally 30 min prior to pruritogen injection. The analgesic drugs dose dependently blocked serotonin and compound 48/80-induced scratching behavior with exerting complete inhibition at certain doses. Our data suggests that intradermal pruritogen-induced scratching models may not discriminate pain and itch sensations and give false positive results when standard analgesic drugs are used.

Key words: analgesic drugs, itch, scratching, pruritogen, neck model, false positive

## INTRODUCTION

Itch, an unpleasant sensation associated with the desire to scratch, is a common symptom of many diseases, including those affecting the skin as well as other organ systems. In recent years, pruritus has been gained more increased interest in medicine due to its higher prevalence than thought, serious impact on quality of life such as impaired sleep quality, more depressive symptoms and higher levels of anxiety (Zachariae et al. 2012) and limited efficacy of current antipruritic agents (Carstens and Akiyama 2014). The tremendous advances have been made to understand the molecular and cellular mechanisms in the transmission of itch sensation from periphery to central nervous system (Han and Dong 2014). Animal models of itch served as the

major tool in these advances, from identification of novel targets and mechanisms of new therapeutics (Shimada and LaMotte 2008, LaMotte et al. 2011). It is well known that animal models relevant to human disease or symptom of disease is essential for the development of new therapeutics and innovative ways to treat and cure the diseases (Denayer et al. 2014). Thus, an animal model of itch must fulfill some criteria of validity such as predictive validity which refers to how specific and sensitive the model is to detect pharmacological effects, avoiding false positive or false negative results (Gobira et al. 2013).

Although pain and itch are clearly different protective sensation, there is a close interaction between them in respect of mediation through an anatomically and functionally identical neuronal pathway (Davidson and Giesler 2010,

Ringkamp and Meyer 2014). An ideal animal model of itch should discriminate pain and itch. Behaviorally, in contrast to withdrawal reflex to painful stimuli, the most characteristic responses to pruritic stimuli is scratching reflexes (Paus et al. 2006). While the significant progress has been performed in the development of animal models for itch by changing from acute injection models to a disease-relevant approach, intradermal injection of pruritogens such as serotonin, histamine and compound 48/80 into skin and then, the evaluation of the scratching behavior are still the most commonly used animal model in research and development of pruritus (Akiyama et al. 2010, Kremer et al. 2010, LaMotte et al. 2011). Several criticism have been raised against the hindlimb scratching behavior as an only indicator of the sensation of itch after pruritic stimuli applied to the nape of the neck in these standard animal models of itch (Shimada and LaMotte 2008, Hachisuka and Ross 2014). One approach is whether the existing animal model of itch differentiates itch or pain to show efficacy of clinically effective analgesic drugs as a back translation. Due to our knowledge, there are no comprehensive researches, including multiple dose response studies to evaluate effects of analgesic drugs on the scratching behaviors of intradermally injected pruritogens into the nape of the neck. In this study, we aimed to explore the dose response effects of opioid analgesics morphine and tramadol, a mixed cannabinoid CB1 and CB2 receptor agonist CP 55, 940, paracetamol and diclofenac sodium, the nonsteroidal anti-inflammatory drugs (NSAIDs), on intradermal compound 48/80 and serotonin induced scratching behavior in mice.

## MATERIALS AND METHODS

### Animals

Approximately, 6 weeks old male Balb-C mice (23–28 g) were used. They were housed in groups of six under controlled temperature (22±1°C) and humidity (55±10%). The room was lighted from 7:00 a.m. to 7:00 p.m. Food and water were available ad libitum. The animals were randomly divided. The study was approved by the Committee for Animal Experiments of Ondokuz Mayıs University and Gülhane Military Medical Academy.

### Drugs and chemicals

Serotonin hydrochloride (Sigma-Aldrich, St Louis, MO, USA) and compound 48/80 (Sigma-Aldrich, St Louis, MO, USA) were dissolved in 0.9% saline. Paracetamol and CP 55,940 (Sigma-Aldrich, St Louis, MO, USA; respectively) were dissolved in a vehicle solution containing 20:1:1:78 (v/v/v/v) mixture of DMSO:ethanol:Tween 80:0.9% saline. Diclofenac

sodium (Sigma-Aldrich Co., USA) and morphine sulphate (Galen, Istanbul, Turkey) were dissolved in 0.9% saline. Tramadol (Contromal amp, 100 mg/kg, Abdi İbrahim, Turkey) was diluted in 0.9% saline. All drugs were given into the mice by an intraperitoneal (i.p.) route in a volume of 10 ml/kg.

### Behavioral experiments

Serotonin (25 µg) and compound 48/80 (100 µg/site) were injected intradermally in a volume of 50 µl into the neck, rostral part of the mice. After injections, the animals were put in a plexiglass cylinder (20×20×40 cm) and one observer calculated the animal behaviour movements only for 30 minutes in this area. The scratching counting was performed in parallel to the experiments. Time course and number of scratches through injection site via hindpaws of mice were counted by an observer during each 10-min interval of the entire 30 min observation period, according to the previous studies (0–10, 10–20 and 20–30 min) (Andoh and Kuraishi 1998, Togashi et al. 2002).

Different doses morphine (1, 3, 10 mg/kg), tramadol (20, 40, 80 mg/kg), CP 55,940 (0.1, 0.3, 1 mg/kg), paracetamol (100, 200, 300 mg/kg) and diclofenac (50, 100, 200 mg/kg) were given i.p. 30 min prior to intradermal pruritogen injections. All of drug doses were selected according to their antinociceptive doses obtained in various studies for morphine, tramadol, CP 55,940, paracetamol and diclofenac; respectively (Jin et al. 2014, Aydin et al. 2012, Miller et al. 2012, Roca-Vinardell et al. 2003, Hossain et al. 2013). Control mice received saline (physiologic % 0.9 sodium chloride solution) or vehicle solution, were intraperitoneally (i.p.) administered. To compare the statistical significance of serotonin and compound 48/80 on itch behavior, saline and vehicle solution also were given into the neck of mice.

### Statistical analysis

All data are expressed as means ±SEMs. Groups of 6–15 mice were used. The significance of any difference was assessed using one-way ANOVA or two-way repeated-measures ANOVA followed by the Bonferroni *post hoc* test. Statistical data analysis was performed using Graph Pad Prism version 4.0 software (Graph Pad, San Diego, CA).

## RESULTS

### The effects of analgesic drugs on intradermal compound 48/80-induced scratches

Intradermal 0.9% saline, and vehicle in which paracetamol and CP 55,940 were dissolved, injected into the

rostral back, elicited a total of  $2 \pm 0.54$  and  $1.16 \pm 0.57$  scratches, respectively, in 30 min. Repeated measure of two way ANOVA showed that intradermal administration of compound 48/80 resulted in a significant increase in the number of scratches as compared to saline and vehicle treated control groups (Figs 11A and 11B). Scratching elicited by compound 48/80 administrations was significantly increased at 10, 20 and 30 min compared to saline and vehicle groups, ( $F_{1,121}=38.71$ ,  $p<0.001$ ) and ( $F=1.63$ ,  $p<0.001$ ), respectively. Total number of scratches to compound 48/80 (100  $\mu$ g) was found to be  $145.15 \pm 15.8$  within 30 min. Morphine dose dependently and significantly blocked the number of scratches elicited by compound 48/80 ( $F_{3,56}=16.97$ ,  $p<0.001$ ,  $n=7$ ) (Figs 1A and 1B). While there was no significant change in the number of scratches when the morphine at the dose of 1 mg/kg was administered ( $148 \pm 7.8$ ) (Figs 1A and 1B), however a peak effect with the fully resolving of scratches was obtained after the morphine at the dose of 10 mg/kg, (Fig. 1B). Similar to morphine, tramadol also dose dependently inhibited compound 48/80 induced scratches at 10, 20 and 30 min ( $F_{3,48}=46.39$ ,  $p<0.001$ ,  $n=7$ ) (Fig. 2A). The maximal dose of tramadol (80 mg/kg) lead to totally inhibition of compound 48/80 induced scratches during the 30 min observation period (Fig. 2B).

The cannabinod drug, CP 55,940 elicited dose dependent antiscratching effect at 10, 20 and 30 min after compound

48/80 administration ( $F_{3,40}=21.19$ ,  $p<0.001$ ,  $n=6$ ) (Fig. 3A). While the lowest dose of CP 55,940 (0.1 mg/kg) lead to  $67 \pm 11$  scratches within 30 min, its highest dose (1 mg/kg) totally abolished scratch bouts after compound 48/80 injection (Fig. 3B).

The widely used analgesic drug, paracetamol, dose dependently reduced scratches bouts induced by compound 48/80 ( $F_{3,40}=28.14$ ,  $p<0.001$ ,  $n=6$ ) (Fig. 4A). The total number of scratches within 30 min after compound 48/80 administration was  $4 \pm 0.84$  following the highest dose of paracetamol (300 mg/kg) (Fig. 4B). The NSAID, diclofenac, also dose dependently and significantly blocked compound 48/80 induced scratches ( $F_{3,40}=18.42$ ,  $p<0.001$ ,  $n=6$ ) (Fig. 5A). While the lowest dose of diclofenac (50 mg/kg) was ineffective, its highest dose (200 mg/kg) fully inhibited scratches elicited by the compound 48/80 within 30 min after its injection (Fig. 5B).

### The effects of analgesic drugs on intradermal serotonin-induced scratches

Intradermal injection of serotonin into the nape of the neck elicited a significant increase in the number of scratches at 10, 20 and 30 min after injection when compared to saline and vehicle treated control groups (Figs

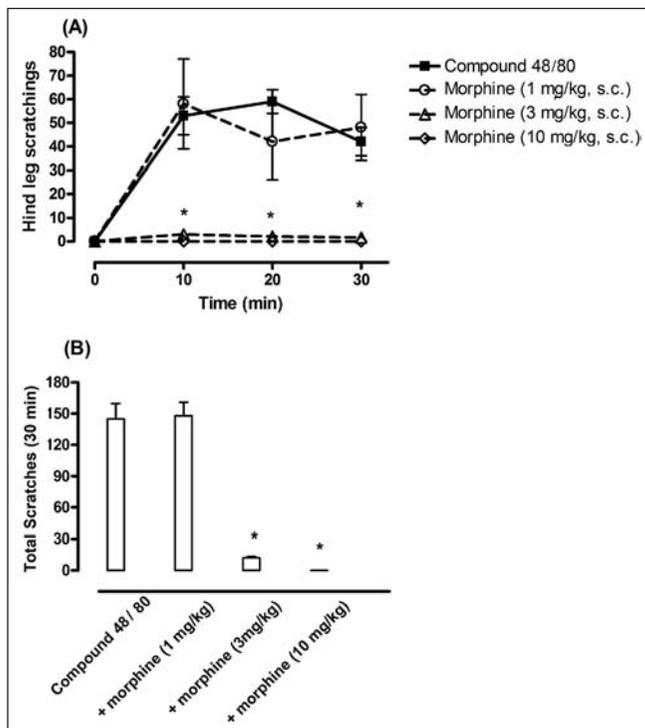


Fig. 1. (A) Morphine was given i.p. 30 min prior to intradermal compound 48–80 injection and the number of bouts of scratching behavior was measured between 0–10, 10–20 and 20–30 min after the injection. (B) Total number of scratches for 30 min were compared in each morphine dose group against control group. \* $p<0.001$ , compared to control groups.

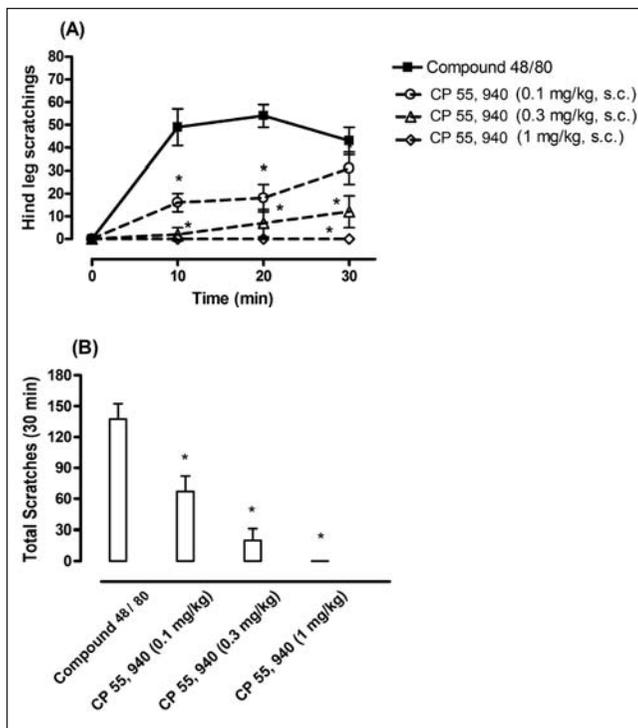


Fig. 2. (A) CP 55,940 was given i.p. 30 min prior to intradermal compound 48–80 injection and the number of bouts of scratching behavior was measured between 0–10, 10–20 and 20–30 min after the injection. (B) Total number of scratches for 30 min were compared in each CP 55,940 dose group against control group. \* $p<0.001$ , compared to control groups.

11A and 11B). Total number of scratches for 30 min after serotonin injection was found to be  $28.5 \pm 1.6$  (Figs 11A and 11B). Morphine dose dependently and significantly blocked serotonin induced scratching behavior ( $F_{3,40}=29.9$   $p<0.001$ ,  $n=6$ ) (Fig. 6A). At the dose of 10 mg/kg morphine decreased serotonin-induced scratches to  $0.8 \pm 0.4$  within 30 min (Fig. 6B). Similar to morphine, tramadol dose dependently and significantly blocked serotonin induced scratching behavior at 10, 20 and 30 min ( $F_{3,40}=125.5$   $p<0.001$ ,  $n=6$ ) (Figs 7A and 7B). The highest dose of tramadol (80 mg/kg) decreased the serotonin induced scratches from  $30.17 \pm 2.52$  to  $0.5 \pm 0.34$  within 30 min (Fig. 7B).

CP 55,940 produced a dose-related antiscratching effect at 10, 20 and 30 min after serotonin injection ( $F_{3,40}=75.75$ ,  $p<0.001$ ,  $n=6$ ) (Fig. 8A). While the lowest dose of CP 55,940 (0.1 mg/kg) elicited  $5.5 \pm 1.87$  scratches, its highest dose (1 mg/kg) effectively diminished scratches to  $0.5 \pm 0.22$  s within 30 min after serotonin injection (Fig. 8B). Paracetamol elicited a significant effect on serotonin-induced scratches ( $F_{3,40}=7.24$ ,  $p<0.001$ ,  $n=6$ ) (Fig. 9A). While the lowest dose of paracetamol (100 mg/kg) was ineffective, its highest dose (300 mg/kg) blocked scratches at 10 and 20 min after serotonin injection (Fig. 9B). The highest dose of paracetamol (300 mg/kg) decreased serotonin induced scratches from  $28.5 \pm 1.67$  to  $3.5 \pm 1.46$  within 30 min (Fig. 9B). Diclofenac also dose-dependently and significantly blocked

serotonin-induced scratching behavior ( $F_{3,40}=18.42$ ,  $p<0.001$ ,  $n=6$ ) (Fig. 10A). The lowest dose of diclofenac (50 mg/kg) did not alter the number of scratches induced by serotonin, whereas its highest dose (200 mg/kg) totally inhibited scratches induced by serotonin within 30 min (Fig. 10B).

## DISCUSSION

In this study, we evaluated the effects of analgesic drugs on intradermally applied pruritic agents, compound 48/80 and serotonin-induced scratching behavior, which suggested the most characteristic response to itching in animals. The doses of the analgesic drugs used in our study are selected according to their potent analgesic effects in various pain models in mice (Jin et al. 2014, Aydin et al. 2012, Miller et al. 2012, Roca-Vinardell et al. 2003, Hossain et al. 2013). Our observation showed that all of the analgesic drugs effectively blocked intradermal pruritogen-induced scratches when applied into the nape of the neck of mice.

Most of the previous studies performed on the basis of application of the some pruritive substances into the neck of rodents (Kuraishi et al. 1995, Inagaki et al. 2002, Green et al. 2006, Shimada et al. 2006). While serotonin acts as a neurotransmitter in the central nervous system

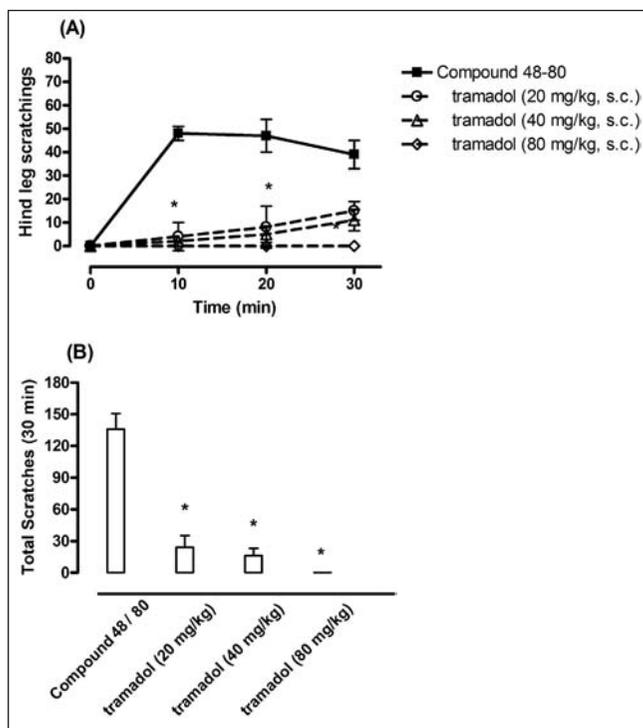


Fig. 3. (A) Tramadol was given i.p. 30 min prior to intradermal compound 48–80 injection and the number of bouts of scratching behavior was measured between 0–10, 10–20 and 20–30 min after the injection. (B) Total number of scratches for 30 min were compared in each tramadol dose group against control group. \* $p<0.001$ , compared to control groups.

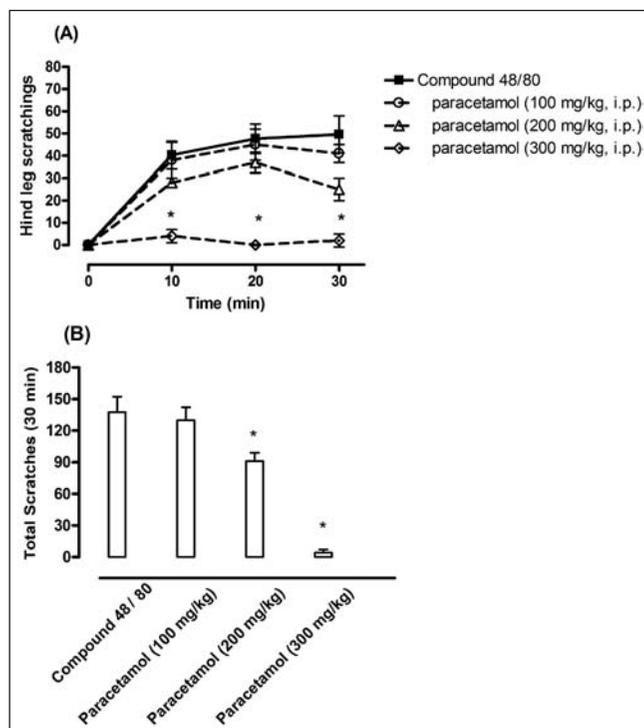


Fig. 4. (A) Paracetamol was given i.p. 30 min prior to intradermal compound 48–80 injection and the number of bouts of scratching behavior was measured between 0–10, 10–20 and 20–30 min after the injection. (B) Total number of scratches for 30 min were compared in each paracetamol dose group against control group. \* $p<0.001$ , compared to control groups.

(Budzinska et al. 2014), it elicits a clear itch when applied in rodents peripherally (Yamaguchi et al. 1999, Jinks and Carstens 2002). Also, compound 48/80 is a well known mast cell degranulator agent and causes itch via liberation of histamine, furthermore it was reported that the pruritic effect compound 48/80 occurs via mast cell-independent pathway (Inagaki et al. 2002). However, currently it should be questioned that how much these agents lead to real itch behavior when applied into the neck of rodents. According to our results, all of analgesic drugs used in our study completely blocked scratching. Thus, we should face whether the application of the putative pruritogens may cause real pain rather than pruritus and than analgesics really block pain.

Some previous studies have proposed that substances induce scratching behavior in some situation may reflect pain rather than itch in humans depending on the application of the site. For example, capsaicin has been shown to produce itch when applied in a punctiform manner into the skin (Sikand et al. 2009). Additionally, it was indicated that when capsaicin was applied topically, it caused itch more than pain (Green 1990, Wang et al. 2010). On the contrary, it has also been reported that intradermal injections of capsaicin causes pain by leading burning or stinging (Byas-Smith et al. 1999, Simone et al. 1989). For this reason, to differentiate the pain and itch behavior by

applying the putative pruritogens in animals, Shimada and LaMotte (2008) proposed mouse cheek model, with hind limb scratches and forelimb wiping behavior. According to the results performed by Shimada and LaMotte (2008), both histamine and capsaicin caused scratching behaviour when applied into the nape of neck and one type of behaviour was only seen described as hind limb scratching (Shimada and LaMotte 2008). However, in the cheek models, capsaicin have been shown to cause wiping when applied into the cheek, rather than itching, as an indicator of pain. Akiyama and others (2010) reported that systemically applied morphine couldn't inhibit intradermal serotonin and histamine induced itch when applied to the cheek, and morphine has been shown to reduce wiping caused by capsaicin. For this reason, some authors have advised that wiping and scratching are distinguished in relation to the inhibition of the effects of the putative algescogenic capsaicin and putative pruritogens histamine and serotonin, so wiping shows pain, and scratching shows itch, in cheek model (Shimada and LaMotte 2008, Akiyama et al. 2010). Moreover, intracisternal application morphine has been shown to potentiate the intradermally injected serotonin induced scratch in eye-wiping test in rats (Moser and Giesler 2014). Clinically opioid induced itch was well reported in many studies as an adverse effect (Cousins and Mather 1984, Ballantyne et al. 1988). Opioid antagonists, mainly naloxone

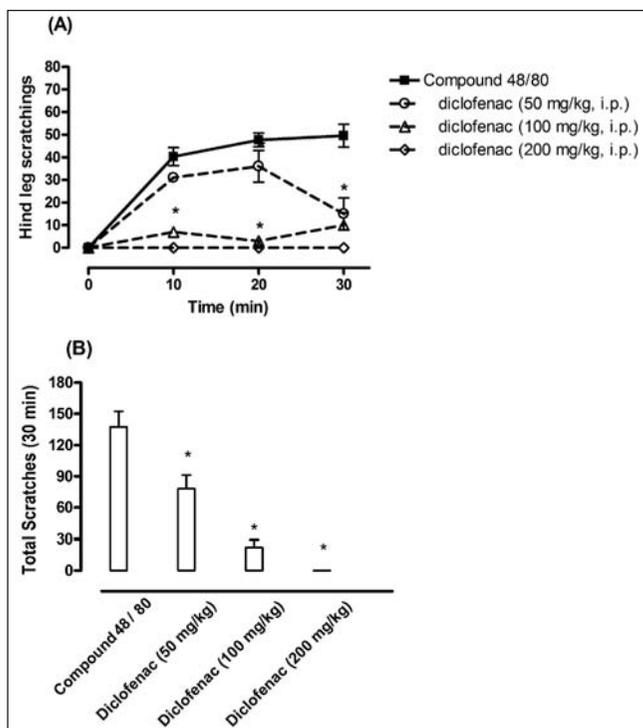


Fig. 5. (A) Diclofenac was given i.p. 30 min prior to intradermal compound 48-80 injection and the number of bouts of scratching behavior was measured between 0-10, 10-20 and 20-30 min after the injection. (B) Total number of scratches for 30 min were compared in each diclofenac dose group against control group. \*p<0.001, compared to control groups.

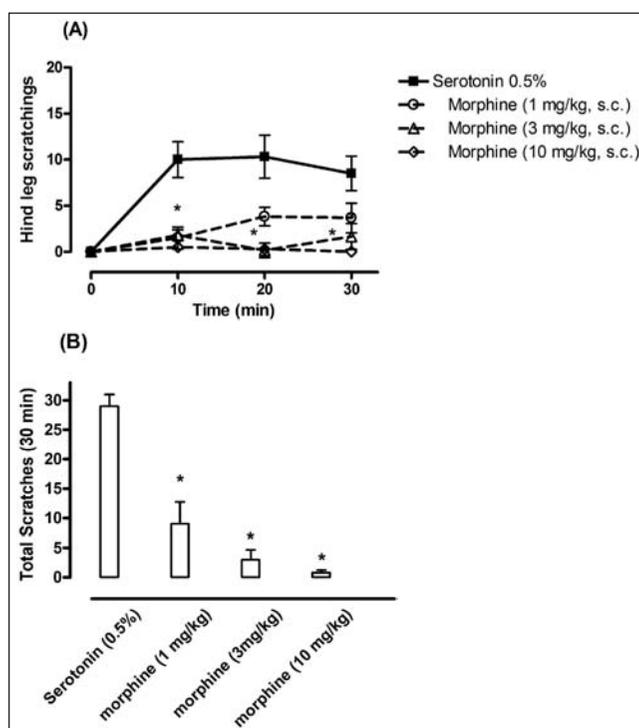


Fig. 6. (A) Morphine was given i.p. 30 min prior to intradermal serotonin injection and the number of bouts of scratching behavior was measured between 0-10, 10-20 and 20-30 min after the injection. (B) Total number of scratches for 30 min were compared in each morphine dose group against control group. \*p<0.001, compared to control groups.

is used in the treatment of opioid-induced itch (Bergasa et al. 1995, Brune et al. 2004). However, in our study, morphine (at the dose of 3 and 10 mg/kg, i.p.) and tramadol, and also other analgesics, clearly diminished the serotonin and compound 48/80 induced scratches when applied into the neck in a dose dependent manner. Morphine at the dose of 10 mg/kg, i.p. may be sedative, however dose of 3 mg/kg, i.p. is not sedative (Patti et al. 2005) and give similar significance compared to the dose of 10 mg/kg, i.p. used in our study. These results may support our claim, the lack of effect of morphine on scratching in cheek model performed by Akiyama et al. shows that putative pruritogens lead to more itch behaviour in cheek model in comparison to the neck model. Furthermore, scratching behavior in arthritic rats induced by inoculation with mycobacterium butyricum was inhibited by morphine and acetylsalicylate, suggesting that the scratching reflected pain rather than itch (De Castro-Costa et al. 1987).

In addition to their analgesic effect, in certain human studies it was shown that cannabinoids were effective in the treatment of pruritus, i.e. cholestatic pruritus (Neff et al. 2002) and the synthetic cannabinoid agonist HU-211 also suppressed pruritus when applied locally (Dvorak et al. 2003). In experimental studies, it was reported that an exogenous cannabinoid  $\Delta$  (9)-tetrahydrocannabinol and fatty acid amide hydrolase (FAAH) enzyme inhibitor

URB597 reduced the itching behavior induced by compound 48/80 (Schlosburg et al. 2009). Furthermore, S-777469, a novel cannabinoid type 2 receptor agonist, in mice has been shown to reduce the scratches (Haruna et al. 2016). These findings support the antipruritic effect of various cannabinoidergic drugs or substances depends on the neck model (Haruna et al. 2016, Schlosburg et al. 2009). However, Spradley and others (2012) proposed that the inhibition of 5-HT induced scratching in the rostral back (but not in the cheek) by the previously administration of degrading enzymes for the endocannabinoids anandamide (URB597) or 2-arachidonoylglycerol (JZL184) may depend the differences of the pain and itch arising from trigeminally-innervated skin of the face or scalp. This finding elicited by the Spradley and others (2012) supports our finding that the cannabinoid receptor agonist CP 55,940 inhibited itch responses in neck model with all the doses used. Nonetheless, we don't fully claim that cannabinoidergic drugs don't exert any antipruritic effect, further studies must be performed via using both neck and cheek models to research the exact mechanism of the cannabinoidergic substances for itch.

It was shown that itch is blocked by certain NSAIDs such as tenoxicam and diclofenac, in humans (Colbert et al. 1999, Chang et al. 2013). The effect of prostaglandins (PGs) on pruritus has also been researched in further studies

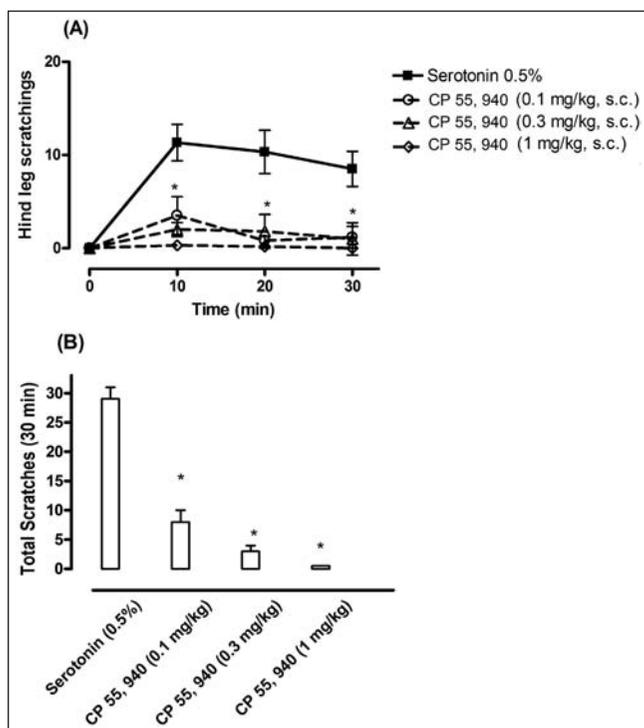


Fig. 7. (A) CP 55,940 was given i.p. 30 min prior to intradermal serotonin injection and the number of bouts of scratching behavior was measured between 0–10, 10–20 and 20–30 min after the injection. (B) Total number of scratches for 30 min were compared in each CP 55,940 dose group against control group. \* $p < 0.001$ , compared to control groups.

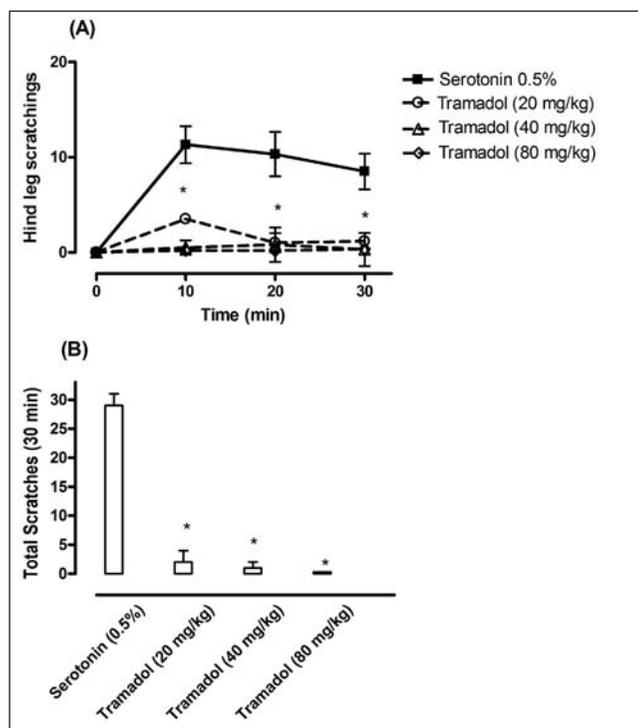


Fig. 8. (A) Tramadol was given i.p. 30 min prior to intradermal serotonin injection and the number of bouts of scratching behavior was measured between 0–10, 10–20 and 20–30 min after the injection. (B) Total number of scratches for 30 min were compared in each tramadol dose group against control group. \* $p < 0.001$ , compared to control groups.

including both human (Neisius et al. 2002) and rodents (Andoh and Kuraishi 1998, Takaoka et al. 2007). The importance of the arachidonic acid cascade in substance P-induced itch and the inhibition of the production of leukotriene B4 for the inhibitory effect of dexamethasone on itch-associated response has been pointed out (Andoh and Kuraishi 1998). Histamine release from mast cells induced by fibrinogen degradation product was reversed by anti-inflammatory drugs (Wojtecka-Lukasik et al. 1988). Thus, blocking the certain mediators by the analgesic NSAIDs may support the inhibition of itch. However, these experimental studies research the role of prostaglandins depend also neck models (Andoh and Kuraishi 1998, Takaoka et al. 2007). In our study, potent and weak PG synthesis inhibitors diclofenac and paracetamol, respectively, inhibited the scratching behavior. Our finding suggest that NSAIDs may inhibit pain behavior rather than itch. Further studies must be done to differentiate pain and itch behavior by using cheek cheek and neck models. Despite this, also in neck models some conflicting results have been declared regarding the effect of certain types of NSAIDs on itch behavior. For instance, Andoh and Kuraishi (1998) showed that NSAIDs indomethacin and diclofenac have been shown to exert no effect on substance P-induced scratching in mice in neck model. This discrepancy may

arise from the different pruritogen used (substance-P) or pharmacokinetic profile or both. In that study, the dose of diclofenac and indomethacin were 3–10 and 1–10 mg/kg, respectively and were given by peroral route. However, in our study diclofenac was administered at the dose of 50–200 mg/kg, i.p. Moreover, indomethacin has been shown to have no effect on scratching behavior induced by compound 48/80 in neck model (Inagaki et al. 2002). In that study, indomethacin was also given at the dose of 1–10 mg/kg, but we don't think this effect has most likely arised from pharmacokinetic profile, because the dose 10 mg/kg of indomethacin is an analgesic dose used in most of the pain studies (Lavich et al. 2005, Zhao et al. 2014). In our study, also the low dose of indomethacin; 50 mg/kg, i.p., exerted a significant reduction on scratching behavior at the 30th minute. Thus, the lack of effect of indomethacin may be clarified by further studies. Taken together, the inhibitor effect of diclofenac and paracetamol may depend on their pain reducing effects.

Regarding the close interaction between pain and itch, the phenomena of allokinesis has been described (Simone et al. 1991, Heyer et al. 1995). As seen in the mechanism of allodynia development (Ran et al. 2014) persistent stimulation of primary afferents (pruritis receptors), causes allokinesis explained on the basis of central

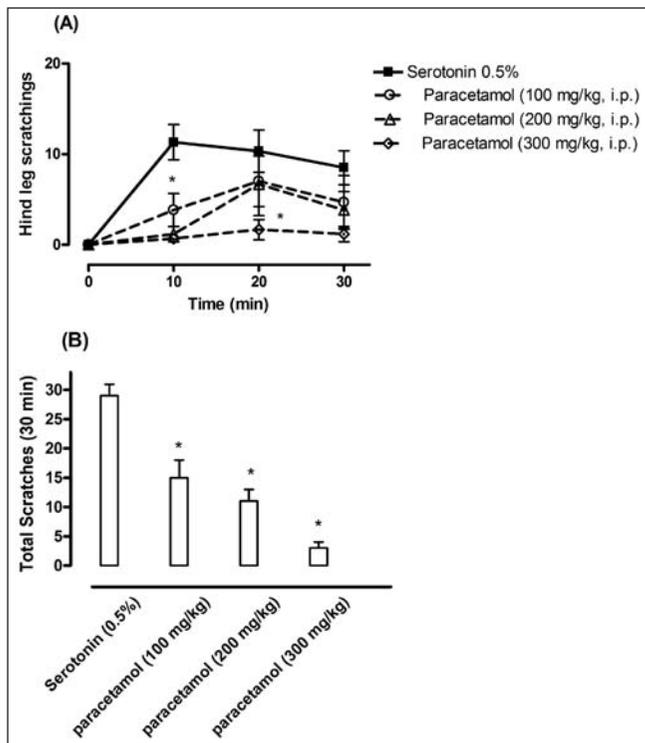


Fig. 9. (A) Paracetamol was given i.p. 30 min prior to intradermal serotonin injection and the number of bouts of scratching behavior was measured between 0–10, 10–20 and 20–30 min after the injection. (B) Total number of scratches for 30 min were compared in each paracetamol dose group against control group. \*p<0.001, compared to control groups.

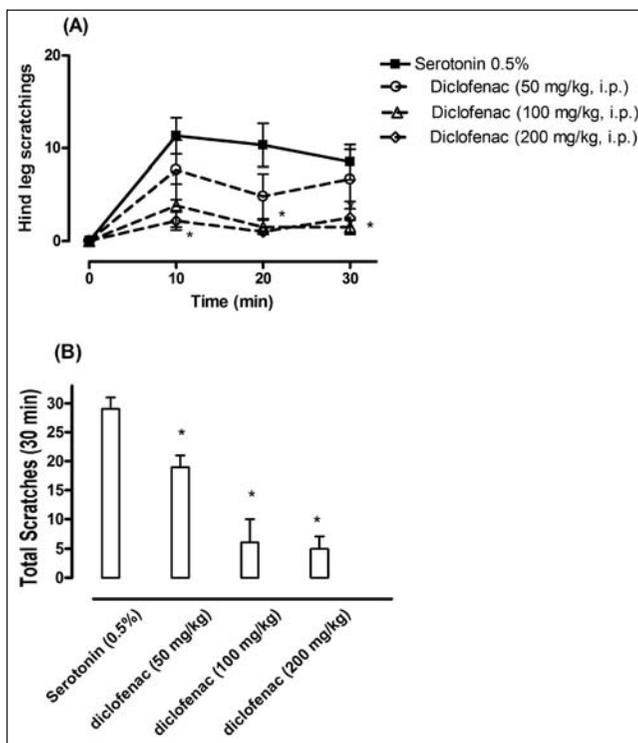


Fig. 10. (A) Diclofenac was given i.p. 30 min prior to intradermal serotonin 48–80 injection and the number of bouts of scratching behavior was measured between 0–10, 10–20 and 20–30 min after the injection. (B) Total number of scratches for 30 min were compared in each diclofenac dose group against control group. \*p<0.001, compared to control groups.

sensitization in chronic itch and elicited by low-threshold mechanoreceptors, A $\beta$  fibers induced by touching. In this phenomena, a normal touching that does not lead to any itching behavior, however, leads to itching in the patient whom allodynia developed. Furthermore, application of pinprick causes itch sensations in the surroundings of itching skin areas, though normally pinprick is a painful stimuli, also called hyperkinesia, have been reported following histamine iontophoresis in healthy volunteers (Atanassoff et al. 1999). In hyperkinesia, it has also been suggested that normally painful electrical stimuli were perceived as itching in the patients with atopic dermatitis (Nilsson and Schouenborg 1999). According to our data, we claim that the antipruritic effect of analgesic drugs might have been less likely arisen from the phenomena of allodynia or hyperkinesia because of the evaluating the acute situations. It is well known that both allodynia and hyperkinesia are the phenomena seen in the chronic processes, such as atopic dermatitis (Heyer et al. 1995, Groene et al. 2001). For instance in the study performed by Groene and others (2001), intradermally injected of acetylcholine, which normally provokes pain, provokes itch in patients with atopic dermatitis. Thus, it was suggested that in patients with chronic itch, a stimuli normally cause pain may be perceived as itch according

to the central processing of pruritus. However, in our study we didn't mimic the chronic itch models such as atopic dermatitis in rats and we have not claimed that the blocking itch behaviour effect of antinociceptive agents used in our study might have been arisen from the phenomena of allodynia.

Despite the potential separative effect of cheek model, also some uncertainties are seen in this model. For example, interestingly, in the cheek model, Gomes et al. suggested that endothelin-1 (ET-1) produces both pain and itch and the scratch and pain behaviours can not be distinguished (Gomes et al. 2012). This finding shows that itch and pain behavior may also alter according to the applied chemical agent as well as the application site. For example, morphine and the other analgesics used in our study completely blocked the pruritic effect of compound 48/80 and serotonin, which are putative pruritogenic agents. However, in one study, Liang et al., proposed that ET-1 induced scratching behavior was not blocked by systemically applied morphine in the neck model (Liang et al. 2011). This finding may arise from the administration of a different route, a different pruritogen (ET-1) or the relatively low dose of subcutaneously (s.c.) applied morphine, 1 mg/kg, s.c., similarly, in our study while the 1 mg/kg, i.p. dose of morphine couldn't reduce the effect of compound 48/80 induced scratching, the higher doses were significantly effective. According to these findings, we suggest that putative pruritic effect may also depend on the type of the chemical, in this regard. As a result, the neck model may give false positive results, however, as discussed before, the lack of effect of morphine on ET-1 (even may arise from the low dose) in the neck model shows that there is no perfect itch model that separate pain and itch behavior well, it may change according to the applied pruritogens and the application site.

As proposed above, the difference between the itch and pain may also arise from the applied site. Kuraishi and others (1995) suggested that compound 48/80 and substance P, but not capsaicin and histamine, caused pruritus when given by subcutaneous route in mice. In that study, we propose that the ineffectiveness of capsaicin and histamine on itch behavior may be arisen from the different injection site, because Kuraishi et al. applied these agents subcutaneously, not intradermally to the neck. This finding shows that itch behavior induced by putative pruritogens may alter according to the different application site, such as surface of skin (topical), intradermal and subcutaneous. Currently, to differentiate pain and itch receptors, it has been shown that MrgprA3 receptors are specifically responsible from itch transmission. According to the study performed by Han and others (2013), when mice TRPV1 receptors in MrgprA3(+) expressing neurons were excited by injection of capsaicin, a well-established mediator thought to be

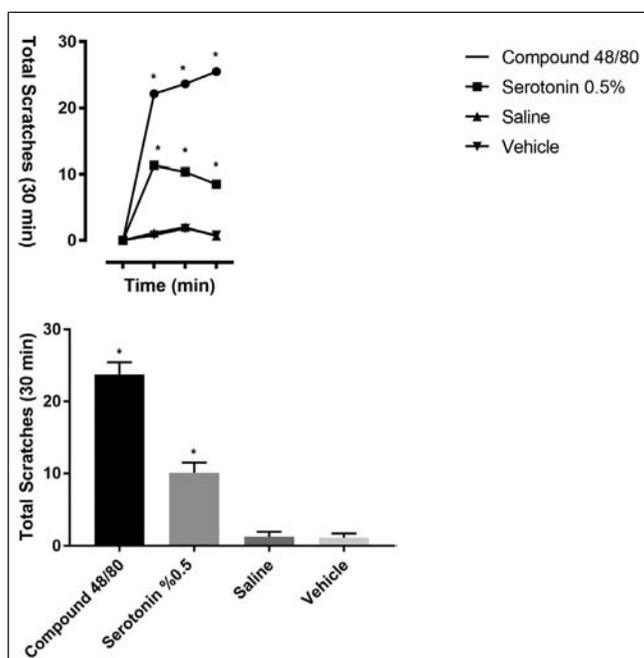


Fig. 11. (A) Intraperitoneal administered saline was given i.p. 30 min prior to intradermal saline, vehicle, serotonin and compound 48/80, and the number of bouts of scratching behavior was measured between 0–10, 10–20 and 20–30 min after the injection. (B) Total number of scratches for 30 min were compared in each saline (i.p.) group against control group. \* $p < 0.001$ , compared to control groups (intradermally injected saline and vehicle).

Note that all control groups are the pruritogens given only alone.

algesiogenic, the mice exerted itch behavior rather than pain. This finding support that pruritogens are criticism of itch models, chemicals which may not be always spesific to itch behavior and may be variable according to the application site even induced by the same pruritogen.

In conclusion, establishing a better model of itch is urgently needed. In the current models, intradermal application of the pruritogens into the rostral part of the neck may not ideal model for itch and may give false positive results with analgesic drugs, hence we claim that the neck model is inefficient for the pure determination of pain and itch for the drug effects on itch and pain. Because of the similarities and differences between pain and itch, we believe that new animal itch models have to be established to evaluate the real potency of the drugs used for anti-itch therapy. At least, currently, both neck and cheek models may be used in a comparative manner.

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