Inhibitory Effect of the Repeated Treatment with Unsei-in on Substance P-Induced Itch-Associated Responses through the Downregulation of the Expression of NK₁ Tachykinin Receptor in Mice  

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Unsei-in, a traditional medicine, is prescribed against pruritic cutaneous diseases, but the mechanisms of antipruritic action are still unclear. In the present study, we examined the antipruritic effects of Unsei-in in mice. Single administration of Unsei-in did not inhibit substance P-induced itch-associated response (scratching) in mice. However, repeated treatment with Unsei-in for 7 d significantly inhibited substance P-induced scratching. The same repeated treatment with Unsei-in suppressed the expression of NK₁ tachykinin receptors in the skin. These results suggest that Unsei-in inhibits substance P-associated itching and that the inhibition is at least partly due to the suppression of the expression of NK₁ tachykinin receptors in the skin.  

Key words Unsei-in; substance P; itch-associated response  

Unsei-in is a traditional medicine which is composed of Rehmanniae Radix, Paeoniae Radix, Cnidii Rhizoma, Angelicae Radix, Scutellariae Radix, Phellodendric Cortex, Coptidis Rhizoma and Gardeniae Fructus. It is used to treat pruritic cutaneous diseases such as eczema and skin eruptions. However, the antipruritic activity of Unsei-in and its mechanisms are unclear.  

Substance P (SP) is a potent pruritogenic peptide and speculated to be involved in pruritic diseases, such as hemodialysis-associated pruritus, atopic dermatitis and psoriasis. SP elicits scratching behavior, mediated by NK₁ tachykinin receptors, in mice. This response is suppressed by pretreatment with naloxone, capsaicin, and compound 48/80. Similarly, several kinds of human itching are alleviated by treatment with naloxone and capsaicin. SP-induced itching is inhibited by pretreatment with compound 48/80 in humans. These similarities between murine scratching and human itching suggest that SP-induced scratching behavior of the mouse is an itch-associated response. In the present study, we examined whether Unsei-in could inhibit itch-associated response induced by SP in mice and whether NK₁ tachykinin receptors would be involved in the action of Unsei-in.  

MATERIALS AND METHODS  

Animals We used male ICR mice (Japan SLC, Shizuoka) of 4 weeks of age at the start of experiment. They were housed under controlled temperature (23—25 °C) and light conditions (lights on from 08:00 to 20:00). Food and water were freely available. Procedures for the animal experiments were approved by the Committee for Animals Experiments in Toyama Medical and Pharmaceutical University.  

Drugs SP (Peptide Institute, Minoh) was dissolved in physiologic saline and injected intradermally (i.d.) at a dose of 100 nmol/site to the rostral part of the back. The extract of Unsei-in, a gift from Kanebo (Tokyo), was suspended in 5% arabic gum and administered perorally once a day for 7 d. SP was injected 30 min after the single or the last injection of Unsei-in.  

Behavioral Experiments The hair was clipped over the rostral part of the murine back the day before the injection of pruritogen. Before behavioral recording, the mice (four animals per observation) were put into an acrylic cage composed of four cells (13×9×30 cm) for at least 1 h for acclimation. Immediately after an i.d. injection, the animals were kept out of the observation room. Playing back of the video served for counting scratching behavior. The mouse generally scratches several times for about 1 s and a series of these movements was counted as one bout of scratching.  

Western Blotting The protein from the skin of mouse rostral back was extracted with the lysis buffer [20 mM Tris—HCl (pH 7.5), 137 mM NaCl, 1% NP-40, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml leupeptin]. The protein (20 μg) was separated by electrophoresis using 10% SDS-polyacrylamide gel and then transferred to a polyvinylidene difluoride membrane. After the blocking with 5% skim milk solution for 1 h, the membrane was reacted with anti-NK₁ receptor or anti-β-actin overnight at 4 °C. Subsequently, it was incubated with horseradish peroxidase-conjugated IgG for 1 h at room temperature. A band was monitored using chemiluminescent reagents (Amersham Bioscience, Piscataway, NJ, U.S.A.). Chemiluminescent signals were detected using X-ray film and analyzed using the NIH Image program. The data was normalized with β-actin.  

Statistical Analysis All data are presented as mean and S.E.M. Statistical significance was analyzed using t-test; p<0.05 was considered significant.  

RESULTS  

Effects of Single and Repeated Administration of Unsei-in on SP-Induced Scratching An i.d. injection of SP (100 nmol/site) elicited scratching of the skin around the
injected site by the hind paws in mice. The effect peaked in
the initial 5-min period and almost subsided by 30 min (Fig.
1a). When applied orally 30 min before SP injection, Unsei-
in (300, 1000 mg/kg) did not inhibit SP-induced scratching
(Figs. 1a, b).

Repeated administration of Unsei-in (300 mg/kg) for 7 d
significantly inhibited SP-induced scratching (Figs. 2a, b).

DISCUSSION

Although single administration of Unsei-in (300, 1000 mg/kg)
was without effects, the repeated administration inhibited
SP-induced itch-associated response in mice. The
clinical dose of Unsei-in is 2400 mg per day. Given that body
weight is 60 kg, the dose is 40 mg/kg. Thus, the effective
dose in mice is 7.5-fold higher than that in humans. However,
the effective dose of Unsei-in in the present experiments was
similar to that in other experiments in mice, such as to picryl
chloride-induced delayed-type hypersensitivity,15) ovalbumin-
induced hypersensitivity,16) carrageenan-induced oedema and
acetic acid-induced abdominal constriction.17) Differences in
effective dose between humans and mice might be at least
due to differences in the absorption and/or metabolism of
this medicine.

The inhibitory effect of Unsei-in at a dose of 1000 mg/kg
was similar to that of the dose of 300 mg/kg (n=8; data not
shown).

Expression Level of NK1 Tachykinin Receptors in the
Skin

The protein was extracted from the skin in Unsei-in
(300 mg/kg) or vehicle (5% Arabic gum)-treated mice. The
expression of NK1 receptor was significantly inhibited by re-
peated treatment of Unsei-in (Fig. 3).

Fig. 1. Effects of Single Administration of Unsei-in on Substance P-Induced
Scratching in Mice

Unsei-in (USN; 300, 1000 mg/kg) and vehicle (VEH: 5% arabic gum) were adminis-
tered orally 30 min before substance P (100 nmol/site) injection. (a) The time course of
scratching following SP injection. (b) The number of scratch bouts for 30 min. Values
are the means and S.E.M. for eight animals.

Fig. 2. Effect of Repeated Administration of Unsei-in on SP-Induced
Scratching in Mice

Unsei-in (USN: 300 mg/kg) and vehicle (VEH: 5% arabic gum) were administered
orally once a day for 7 d. Substance P (100 nmol/site) was injected intradermally 30 min
after the last administration of Unsei-in. (a) The time course of scratching following
substance P injection. (b) The number of scratch bouts for 30 min. Values are the means
and S.E.M. for eight animals. *p<0.05.

The single administration of Unsei-in did not inhibit SP-
induced itch-associated response in mice. Since the scratch-
inducing action of SP is mediated by NK1, but not NK2 and
NK3, tachykinin receptors.8) Therefore, the results suggest
that Unsei-in does not block NK1 tachykinin receptors. On
the other hand, repeated administration of Unsei-in at the
dose which inhibited SP-induced scratching reduced the ex-
pression level of NK1 tachykinin receptors in the skin. The
results suggest that the inhibition of the expression NK1
tachykinin receptors is at least partly involved in the inhibi-
tion of SP-induced itch-associated response. In our prelimi-
nary experiments, repeated administration of Unsei-in
(1000 mg/kg) did not inhibit scratching induced by serotonin
(100 nmol/site) in mice. Scratch-inducing mechanisms may
be different between SP and serotonin.8,18,19) Therefore, if
Unsei-in produces the inhibition of primary afferents or cen-
tral nervous system, it might affect serotonin-induced scratching. Thus, the action of Unsei-in may not be mediated by non-specific inhibition of primary afferents and central nervous system.

In the skin, there are several NK<sub>1</sub> receptor-expressing cells, including keratinocytes, Langerhans cells, endothelial cells, macrophages, the sensory nerve terminal, and mast cell. It is worth to examine which cells would be affected by repeated administration of Unsei-in.

In summary, Unsei-in inhibited SP-induced itch-associated response through the suppression of the expression of NK<sub>1</sub> tachykinin receptors. Unsei-in may be effective against SP-associated itching.

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REFERENCES