

Topical non-peptide antagonists of sensory neurotransmitters substance P and CGRP do not modify patch test and prick test reactions: a vehicle-controlled, double-blind pilot study

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Abstract Immunologic responses in the skin can be modulated by such neurotransmitters of sensory nerve fibers as substance P (SP) and calcitonin gene-related peptide (CGRP). The first-generation receptor antagonists were peptides with large molecules and had to be injected intracutaneously. The aim of this study was to examine the topical effects of non-peptide antagonists to substance P (aprepitant) and CGRP (telcagepant), respectively, on delayed and immediate reactions in the skin and on associated pruritus. A lipophilic formulation of aprepitant 5 % and a hydrophilic formulation of telcagepant 1 % were developed. Their effect on the skin barrier was measured in terms of transepidermal water loss (TEWL) while permeation was calculated using permeation coefficients. Patch tests in patients allergic to nickel and prick test reactions to histamine were used as models. None of the treatments increased TEWL, suggesting there to be no impairment of the skin barrier. Permeation coefficients indicated moderate permeation. Histamine prick tests induced a flare with a mean area of $662 \pm 275 \text{ mm}^2$ and a weal with a mean volume of $49 \pm 11 \text{ mm}^3$. These reactions as well as histamine-induced pruritus were not affected significantly by any of the treatments. Treatment with aprepitant and its vehicle alone resulted in a potentiating of the infiltration of nickel reactions compared with test reactions obtained after

no treatment ($1147 \pm 423 \text{ mm}^3$ and $1427 \pm 566 \text{ mm}^3$ vs $683 \pm 202 \text{ mm}^3$) ($p = 0.03$). Telcagepant induced vasoconstriction in the skin but did not change the infiltration of nickel reactions. None of the treatments influenced the nickel patch test induced pruritus. The data suggest that the topical application of non-peptide antagonists penetrates the skin but does not inhibit contact dermatitis or pruritus.

Keywords Neuropeptides · Substance P · Calcitonin Gene-Related Peptide · Aprepitant · Telcagepant · Contact dermatitis

Introduction

Immunologic responses in the skin can be modulated by neurotransmitters of sensory nerve fibers, such as substance P (SP) and calcitonin gene-related peptide (CGRP) and by their antagonists [14]. The SP-antagonist Spantide I targeting the NK1 receptor, as well as CGRP/8-37/in low dose, targeting the CGRP receptor, was shown to attenuate contact dermatitis [13, 15]. Aprepitant (534.5 g/mol), the NK1 receptor antagonist, licensed against nausea, reduces pruritus in lymphoma patients [3]. In an open-label study on 20 dermatologic patients with therapy resistant pruritus, peroral treatment with aprepitant for 1 week reduced pruritus significantly (from 8.4 to 4.9 on VAS) [13]. The authors speculated that the antipruritic effect of aprepitant is mainly mediated in the skin, but not in the CNS [13].

The first-generation neuropeptide receptor antagonists (Spantide I and CGRP/8-37/) were of high molecular weight (1497.8 g and 3125.6 g, respectively) and had to be injected intracutaneously [13, 15]. Non-peptide receptor antagonists, of lower molecular weight, are better candidates for topical administration. In a previous vehicle-

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controlled, double-blind study, we found no significant effect of topical aprepitant on clinical pruritus [17].

A new non-peptide CGRP receptor antagonist, telcagepant (566.5 g/mol), has been used in clinical trials for treating migraine [4]. This pain-relieving effect may be mediated by vasoconstriction induced by telcagepant as well as by inhibition of neuronal circuits. Thus, we found it interesting to examine its local effect on experimental pruritus.

The purpose of this study was to examine the topical effects of aprepitant and telcagepant on patch test and prick test reactions in the skin as well as on pruritus associated with these skin reactions.

Materials and methods

Description of the aprepitant formulation

Aprepitant, a moderately lipophilic molecule, is available commercially as capsular Emend[®] (Merck & Co., Inc.) containing 125 mg aprepitant as well as absorption-enhancers: saccharose, cellulose, hydroxipropyl cellulose and sodium lauryl sulfate [12]. It was blended in a concentration of 5 % aprepitant in a lipophilic vehicle containing glycerine, by APL (Umeå, Sweden) in accordance with the EU GMP (Good manufacturing practice) rules [5]. To study the permeation of aprepitant, the skin permeation coefficient was calculated using three different models of Frasch, Potts and Robinson [6, 9, 19].

Description of the telcagepant formulation

Telcagepant, MK-0974 (C₂₆H₂₇F₅N₆O₃, having the CAS Registry Number 781649-09-0), is a hydrophilic substance. It was dissolved in a hydrogel (containing 20 % propylene glycol) in a concentration of 1 %.

To study the effects of the preparations on the skin barrier, transepidermal water loss (TEWL) was measured using a closed chamber device (VapoMeter) [8, 20].

Study design for the urticarial reaction

Thirteen healthy non-atopic volunteers (11 women, mean age 44 years, age range 23–69 years) participated. Aprepitant (150 mg), telcagepant (100 µg) and their respective vehicles were applied to an area of 4 cm x 4 cm, each, of the volar surface of the forearms and the vehicle to the other forearm, in accordance with a blind, right-left protocol.

The tests were covered by a hydrocolloid film (Tegaderm-film, 1622 W, 3 M Health Care) and removed after 30 min. Then, the skin was wiped free of the creams and TEWL was measured. Thereafter, histamine was pricked on the pre-treated areas and on control areas of the skin.

The distances between the prick tests were at least 6 cm. The area of flare and the intensity of itch (VAS) were evaluated after 5 min and the volume of the weal after 15 min [16].

Study design for the patch tests

A letter was sent to 42 patients having patch test verified contact allergy to nickel (+ + or + + + reactions). Eleven patients (ten females, mean age 43 years, age range 19–69 years) agreed to participate [7].

Patch tests with 5 % nickel sulfate in petrolatum were performed on five locations on the dorsal part of the upper arms using 50 mg in Finn chambers (11 mm) for subjects with + + reactions and 20 mg in Finn chambers (8 mm) for subjects with + + + reactions. The distance between the Finn chambers was 2.6–3.2 cm.

After 48 h the patch tests were removed and evaluated. Four test areas were then covered in accordance with a randomised schedule, employing a blind right-left protocol, using Finn chambers (11 mm). The treatment patches employed in each case approximately 50 mg of 5 % aprepitant, 50 mg of lipophilic vehicle alone, 50 µl telcagepant, and 50 µl of hydrogel alone. The patients returned 24 h later, the patch tests were removed and reactions were scored clinically and the infiltration of the volume was calculated [7, 15]. The patients scored pruritus using VAS [10].

Statistical analysis was performed using Student's *t* test for paired parametric data.

The study was approved by the Ethics Committee of the University of Lund, all participants giving their written informed consent.

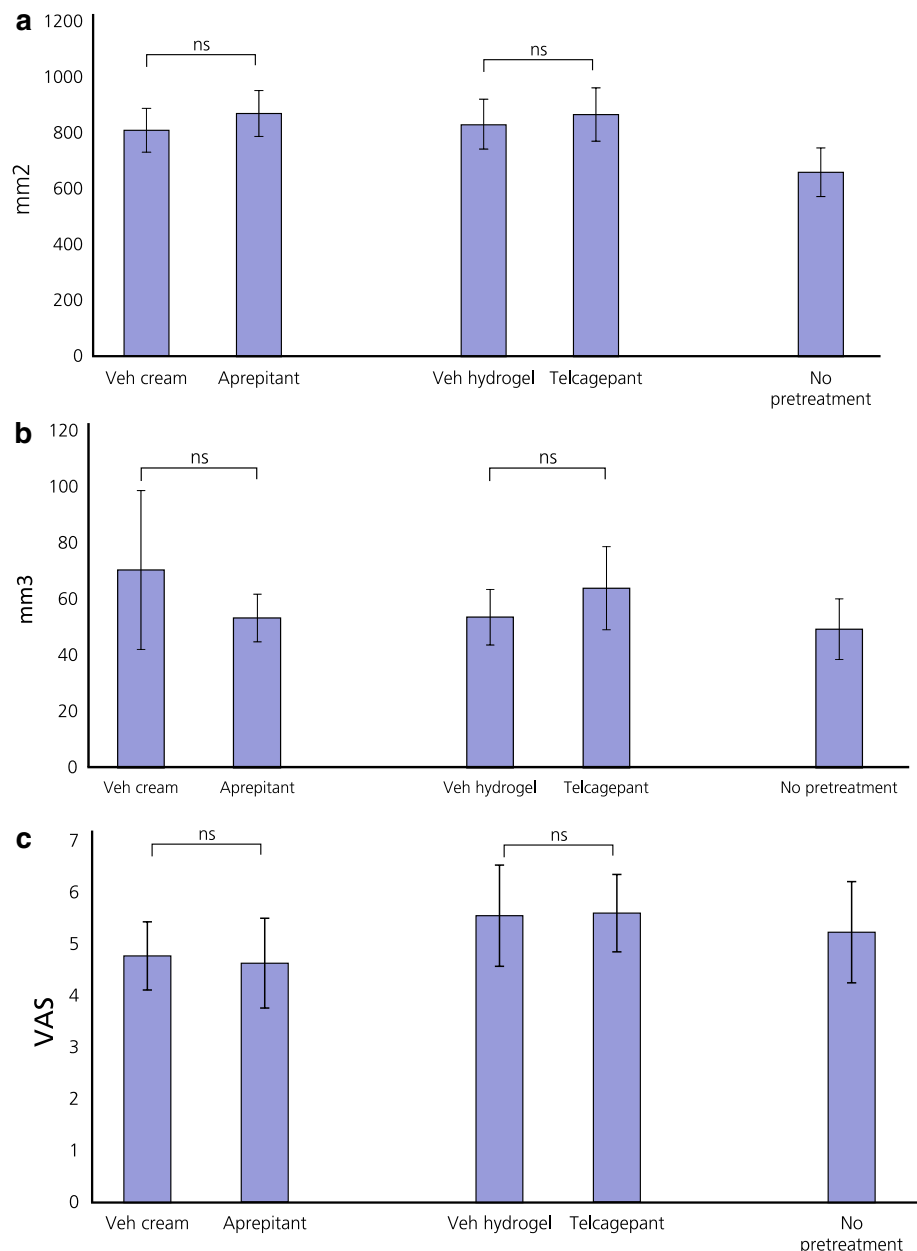
Results

The aprepitant and its vehicle alone improved the skin barrier, TEWL being reduced: 4.0 ± 1.7 g/m²h after use of aprepitant and 5.1 ± 1.8 g/m²h after use of the vehicle alone vs the baseline value of 10.4 ± 1.3 g/m²h (*p* = 0.001).

With input of molecular weight = 534.427 g/mol and log*P* = 4.994, the permeation coefficient (*k_p*) was calculated to 4,23E – 03 cm/hour with the Frasch model, to 3,10E – 03 cm/hour with the Potts and Guy model and to 3,48E – 03 cm/hour with the modified Robinson model [6, 9, 19].

The telcagepant did not affect the skin barrier, TEWL being 10.4 ± 1.9 g/m²h after use of telcagepant and 8.9 ± 0.7 g/m²h after use of the vehicle alone vs the baseline value of 10.4 ± 1.3 g/m²h (*p* = 0.53). In four of the subjects, telcagepant induced transient bleaching of the skin, visible with the naked eye (Fig 2a).

Fig. 1 a. Histamine-induced flare after pretreatment with aprepitant, telcagepant, their vehicles and a control. b. Histamine-induced weal after pretreatment with aprepitant, telcagepant, their vehicles and a control. c. Histamine-induced pruritus after pretreatment with aprepitant, telcagepant, their vehicles and a control



The flare and weal induced by histamine prick tests ($662 \pm 87 \text{ mm}^2$ and $49 \pm 11 \text{ mm}^3$, respectively) were not significantly affected by any of the pre-treatments (Fig. 1 a-b). The pruritus was also not affected by any of the pre-treatments; the values obtained ranging from $4.6 \pm 0.9 \%$ to $5.6 \pm 0.8 \%$ (Fig. 1c).

In the experiments with nickel, no patients reported loosening of patch tests. Treatment with aprepitant and its vehicle alone resulted in a potentiating of the infiltration of nickel reactions compared with no treatment ($1147 \pm 423 \text{ mm}^3$ and $1427 \pm 566 \text{ mm}^3$ vs $683 \pm 202 \text{ mm}^3$) ($p = 0.03$), suggesting that the vehicle cream potentiated the volume of infiltration (Fig. 2a-b). The scores of nickel reactions were not altered by the treatments except for the

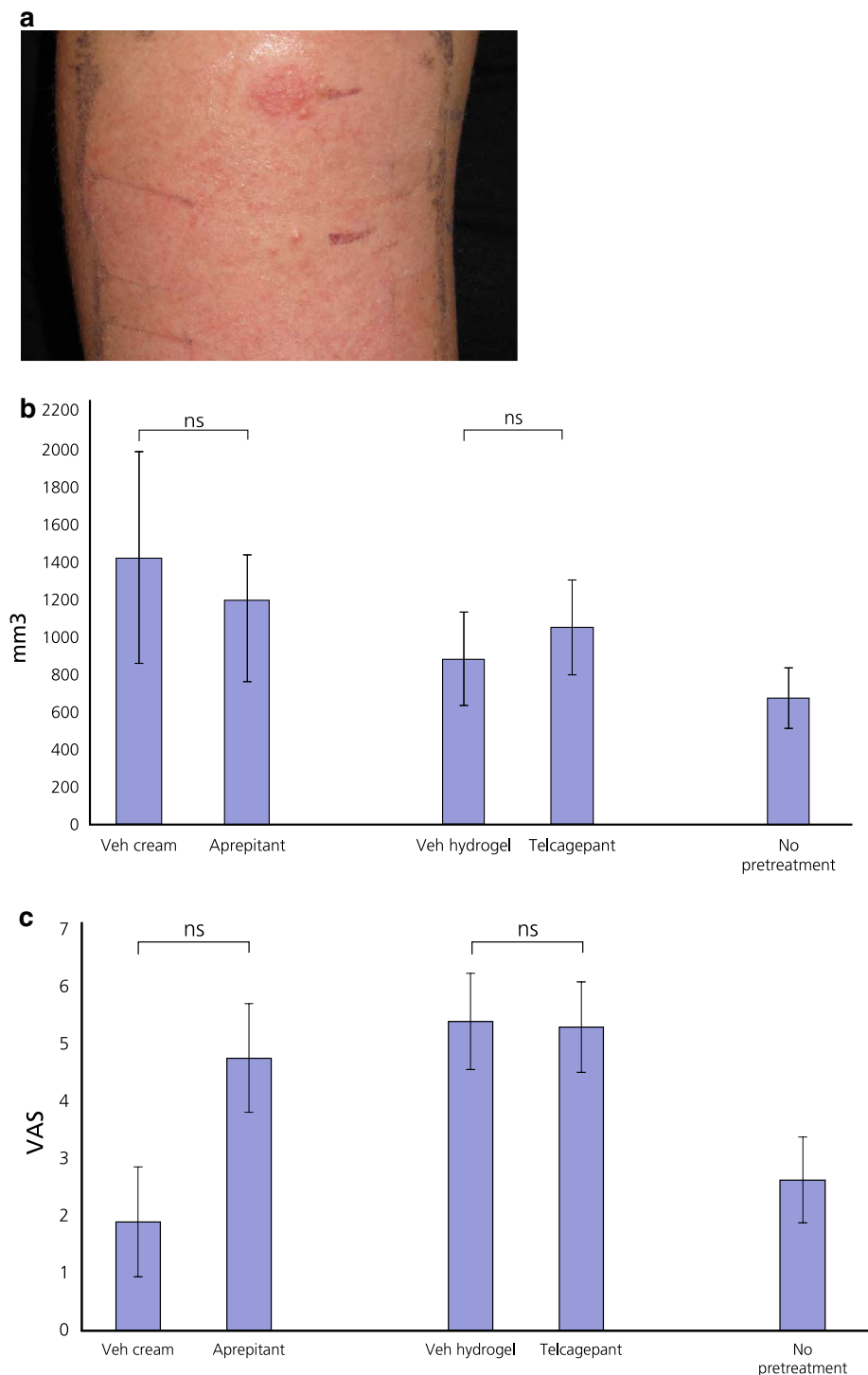
lipophilic vehicle, the mean score being 2.5 ± 0.8 vs control 2.0 ± 0.3 ($p = 0.02$) (Table 1).

Treatment with telcagepant and its vehicle alone did not affect patch test reactions significantly, resulting in a mean infiltration volume of $1058 \pm 316 \text{ mm}^3$ and $877 \pm 312 \text{ mm}^3$, respectively (Fig. 2a-b, Table 1). The pruritus was not affected by any of the treatments (Fig. 2c).

Discussion

The results of this pilot study have to be interpreted with caution, as we only had 11–13 individuals in each setting. Topical application of the aprepitant and the telcagepant in

Fig. 2 a. Nickel patch tests on one upper arm of subject no 7, where a difference was obvious; after treatment with aprepitant (+++), telcagepant (0). b. Nickel patch tests after treatment with aprepitant, telcagepant, their vehicles and a control. c. Pruritus induced by nickel patch tests after treatment with aprepitant, telcagepant, their vehicles and a control



the doses tested, failed to attenuate either patch test results, prick test reactions or associated pruritus. We found none of the treatments to cause irritation of the skin, since TEWL did not increase with the treatments given as compared with the baseline. This finding is in line with previous observations of aprepitant having no effect on erythroderma in eight patients with T- cell lymphoma [2, 3]. The disappointing effect of the topical application of

aprepitant on experimental itch is in line with our previous vehicle-controlled, double-blind clinical pilot study of 13 patients with pruritus [17]. It could be explained in terms of insufficient permeation of the skin by our formulation. However, the permeation coefficients calculated in our study will classify aprepitant as a moderate permeant according to Barber et al. [1]. In addition to occlusion (hydrocolloid film), lipophilic vehicle and hydroxipropyl

Table 1 Nickel patch tests evaluated after 72 h using a score system where + denotes palpable erythema, ++ infiltration and +++ vesicular reactions

Patient	No treatment	Aprepitant	Vehicle cream	Telcagepant	Vehicle hydrogel
1	+++	+++	+++	+++	+++
2	++	++	+++	+++	++
3	++	++	++	++	++
4	+++	+++	+++	+++	+++
5	++	+++	+++	+++	+++
6	+++	+++	+++	+++	+++
7	+	+++	+++	0	+
8	+	+	++	+	++
9	0	+	+	+	0
10	+++	+++	+++	+++	+++
11	+++	+++	+++	+++	+++

cellulosa and sodium lauryl sulfate (included in capsule Emend[®] to increase peroral absorption) were used to enhance skin permeability and should facilitate permeation further. Thus, it appears that the aprepitant should indeed penetrate into the skin adequately. In four of the eleven subjects, telcagepant induced transient bleaching of the skin, visible with the naked eye. It suggests that telcagepant finds its way into the dermis and is available to target cell in a pharmacologically active form.

Another explanation for none effect on patch tests would be the rather high concentration of the allergen. It was chosen to avoid negative patch tests and to make it possible to observe an inhibition. However, the reactions might have been too strong to be modified by the antagonists. Yet another interesting study would be to apply both antagonists as substance P and CGRP together, as they co-exist in the same nerve fibers [15]. However, it may be difficult to find a common vehicle for both compounds, since aprepitant is lipophilic and telcagepant is hydrophilic.

The discrepancy between the antipruritic effect of aprepitant reported by Ständer et al. and our results may be due to the mode of administration [13, 18]. Peroral administration of aprepitant inhibits not only peripheral but also central tachykinin receptors and may exert its effects mainly by modifying the perception of itch.

In conclusion, the topical application of 5 % aprepitant or 1 % telcagepant was not found to attenuate either patch tests, prick tests or associated pruritus in the present vehicle-controlled pilot study. Efforts should be made to develop skin permeating vehicles for the delivery of peptide antagonists to SP and CGRP [11].

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Conflict of interest None.

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