Neurokinin 1 receptor antagonists exhibit peripheral effects in prurigo nodularis including reduced ERK1/2 activation

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Abstract
Background Aprepitant is a neurokinin 1 receptor (NK1R) antagonist used for its antipruritic properties in dermatoses and systemic diseases. The mode of action is still unclear. A peripheral effect is assumed as aprepitant shows efficacy in inflammatory skin diseases including prurigo nodularis (PN).
Objectives To investigate the peripheral effects of NK1R antagonism in PN and cell culture models.
Methods Subjects with PN received an aprepitant treatment. Clinical, morphological and immunohistochemical changes were investigated in skin biopsies before and after treatment. Expression of NK1R was analysed by immunohistochemistry and for downstream pathways ((p)ERK1/2) by Western blotting in PN patients and matched healthy volunteers. Effects of NK1R blocking were analysed in cell cultures of primary keratinocytes by Western blotting for (p)ERK1/2 and by qPCR for NK1R, interleukin (IL)-1beta, IL-6, IL-8 and TNFalpha.
Results Aprepitant treatment showed significant reduction in pruritus intensity (P < 0.05) in PN and relevant immunohistochemical changes (down: CD5, CD25, up: CD79a, IL4). NK1R expression was higher in keratinocytes of PN patients compared to healthy controls. After treatment, epidermal NK1R expression increased while expression and activation of ERK1/2 decreased. In vitro, receptor up-regulation and reduced expression and activation of ERK1/2 were confirmed and reduced IL-expression shown when blocking NK1R.
Conclusion Our data confirm that NK1R antagonists such as aprepitant exhibit effects in the skin. Epidermal receptor expression, epidermal inflammatory ILs, ERK1/2 MAPK signalling and cutaneous inflammatory infiltrate were targets of NK1R antagonism. This may explain partly the antipruritic effect of NK1R antagonists next to its role in the central nervous system.

Conflict of interest
The authors have no conflict of interest.

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Introduction
Chronic prurigo (CPG) is defined by chronic pruritus, a prolonged scratching behaviour and by the presence of pruriginous hyperkeratotic lesions. According to the most predominant type of lesion, CPG is classified into papular, nodular, plaque-type, umbilicated or linear subtype. This article focuses on CPG of nodular subtype – prurigo nodularis (PN). The pathogenesis of CPG, and thus PN, is still largely unknown but finally continuing scratching in the context of chronic pruritic diseases leads to numerous, up to hundreds of severely itchy nodules. In general, CPG induces a high negative burden in patients and still constitutes a great therapeutic challenge. Actual therapies include emollients, systemic antihistamines, topical corticosteroids, gabapentinoids, antidepressants and immunosuppressants but little information is available on the best therapy and course of healing during effective therapy of CPG. Novel therapeutic targets have been identified and address, e.g. the neuropeptide substance P (SP), which is discussed to be a key cutaneous itch mediator in several skin diseases including PN. SP is a member of the tachykinin family and binds to several receptors including the neurokinin 1 receptor (NK1R) which is expressed in various cell types in the skin including keratinocytes, mast cells and fibroblasts. Sensory neurons release SP directly into peripheral tissues

The paper presents partial results of the doctor thesis of FR and JD.

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and initiates several inflammatory reactions,\textsuperscript{10,11} including mast cell activation and cutaneous neurogenic inflammation. In human keratinocytes, SP up-regulates the production of pro-inflammatory cytokines, such as interleukin (IL)-1a, IL-1b, IL-6, IL-8, the expression of TNFa\textsuperscript{12,13} and nerve growth factor (NGF).\textsuperscript{14,15} Furthermore, NK1R may activate the MAP kinase signal pathway member ERK1/2, a known inducer of pro-inflammatory ILs.\textsuperscript{13,16} Blocking the binding of SP by systemic application of the NK1R antagonist aprepitant showed a marked reduction of itch in large series of patients with chronic pruritus including PN,\textsuperscript{17,18} but failed in a recent randomized placebo-controlled clinical trial.\textsuperscript{19} However, another clinical trial with the novel NK1R antagonist serlopitant demonstrated high antipruritic effects in PN patients.\textsuperscript{20,21} Up to now it is unclear if the antipruritic effects of NK1R antagonists result from receptor binding in the skin or the central nervous system. This study aims to investigate if there are any changes in cutaneous NK1 receptor expression, IL expression or signal cascade activation after NK1R therapy as a parameter of peripheral effects in the skin.

\section*{Material and methods}

\subsection*{Participants and treatments}
A total of 35 participants were included in the study. Twenty-five patients suffered from PN (age range: 42.7–77 years; mean age: 61.9 ± 10.3 years), and 10 were healthy controls (21.7–71.2 years, 48.3 ± 13.7 years). All participants received skin biopsies from lesional (PN patients; not-excoriated nodules) or non-lesional (healthy, non-pruritic) skin.

Twelve of the patients with PN were followed over 4 weeks during routine therapy and received oral aprepitant 80 mg/day according to the current guidelines for the treatment of chronic pruritus.\textsuperscript{22,23} An 8 mm punch biopsy was obtained localization matched from lesional skin before and after treatment. The intensity of pruritus was assessed using the visual analogue scale (VAS) for pruritus.\textsuperscript{24} PN lesions were monitored before and after treatment. To allow inter-individual comparison, a scratch symptom score (SSS)\textsuperscript{25} was used and clinical photos were taken.

For assessment of cutaneous NK1R expression and for analysis of ERK1/2 expression and activation 13 additional PN patients and 10 matched healthy controls were included.

The study was approved by the local ethics committee (No. 2007-113-f-S, 2008-235-f-S), and written informed consent was obtained from each subject before inclusion into the study. The study was registered in the DRKS (No. DRKS00008006) and conducted according to the declaration of Helsinki.

\subsection*{Histology and immunohistochemistry}
Formalin fixed PN biopsies obtained before and after therapy were paraffin embedded and processed for H&E, PAS, Giemsa and Elastica staining. Serial sections were immunostained (Table S1) to detect marker expression of the inflammatory infiltrate and to provide an immunophenotype of PN. To determine differences in the expression pattern of NK1R between PN (n = 13) and normal skin (n = 10), cryosections were stained overnight against NK1R. Detailed protocols are provided in the supplemental data.

\subsection*{Western blotting}
Expression and activation of ERK1/2 was analysed by means of Western blotting. Primary antibodies targeting ERK1/2 or phospho-ERK1/2 were incubated overnight, and detection was carried out using a horseradish peroxidase-conjugated secondary antibody and ECL Plus detection on x-ray films. Signal intensity of unsaturated bands was analysed with ImageJ and normalized against CybB.

\subsection*{qPCR}
For expression analysis of NK1R, NK2R, IL-1beta, IL-6, IL-8 and TNFalpha by SYBR green based qPCR cDNA was generated from total RNA using the RevertAid First Strand cDNA Synthesis Kit. Detailed protocols and primer sequences are provided in the supplemental data.

\subsection*{Cell culture and stimulations}
Pooled primary normal human epidermal keratinocytes (NHEK) were obtained from PromoCell and cultured in Keratinocyte Growth Medium 2 (PromoCell, Heidelberg, Germany). Prior stimulation cells were starved for 24 h in medium lacking EGF. NK1R activation was done by stimulation with 1 \textmu mol/L SP (S6883; Sigma Aldrich, Munich, Germany) with or without prior blocking of the receptor for 1 h with 2.5 \textmu mol/L aprepitant (Sigma Aldrich) or 20 \textmu mol/L casopitant (NK1 receptor antagonist, kindly provided by NeRRe Therapeutics, Hertfordshire, UK). Cells were harvested after 6 h of stimulation for assessment of ERK1/2 activation and after 24 h for qPCR analyses.

\subsection*{Statistical analysis}
Statistical analyses were done with SPSS 24.0 (IBM, Armonk, NY, USA). A P-value of <0.05 was considered to be statistically significant. Semi-quantitative expression densities of immunohistochemical data were compared item wise for each patient. Normality of distribution was calculated using the Shapiro–Wilk test, and statistical significance was determined by paired Student’s t-test, Wilcoxon signed-rank sum test or Dunn’s test following a Kruskal–Wallis test.

\section*{Results}

\subsection*{Effects of blocking of NK1R in vivo}

\textit{Pruritus intensity and scratch symptoms} Prurigo nodularis lesions improved slightly after aprepitant treatment (Fig. 1).
Whereas the SSS (Table 1, Fig. 1) measured relative stable scratch related lesions from mean 4.4 ± 1.8 (median: 4.2) to 4.1 ± 2.2 (median: 3.45), a decrease in average VAS (before: mean 6.3 ± 1.24, median 6.0; after: mean 4.5 ± 2.8, median 4.5; \( P = 0.02 \)) was obtained after treatment (Table 1, Fig. 1).

**Histology and immunohistochemistry** Biopsies were taken before and after therapy from lesional nodules. Accordingly, the comparison of specimen (Tables S2 and S3, Fig. 1) revealed no significant histological changes. All specimens proved the diagnosis of PN, except of one biopsy after therapy, which showed healed lesions. Upon H&E and S-100 staining, nerve thickening could be found incidentally before and after therapy in specimens of three (25%) patients, independent of any correlation to the treatment (data not shown).

All analysed markers were found to be expressed in the inflammatory infiltrate of untreated PN lesions (Tables 2 and S4). The T-cell marker CD45 and CD45RO were most abundantly expressed (60–80% of cells positive) followed by CD3, CD4, CD5 and CD68 expressed in 40–60% cells of the inflammatory infiltrate. Twenty to Forty percentage of inflammatory cells were found to express IL-4, CD25, RORc, CD69, IL-17 and FoxP3. Low expression (up to 20% positive cells) was observed for CD8, INF-gamma, the B-cell marker CD20 and CD79a and CD45RA.

Therapy with aprepitant resulted in a decrease of positive immunostaining in 58.3% of the patients for CD25 (before therapy: 2.58 ± 1.17, after: 1.92 ± 0.67; \( P = 0.039 \)). A reduction in 41.7% of the patients was observed for CD4 (Fig. 2) but semi-quantitative analysis showed no significant decrease (before: 3.08, after: 3). Expression of another T- and B-cells marker, CD5, showed a trend towards decreased expression after therapy (before: 3.83 ± 0.94, after: 3 ± 1.35; \( P = 0.054 \)). Increased expression could be observed for CD79a in 58.3% (before: 0.92 ± 0.29, after: 1.67 ± 0.65; \( P = 0.005 \)) and for IL4 in 66.7% (before: 2.08 ± 1, after: 3 ± 1.35; \( P = 0.034 \)).

**NK1R and (p)ERK1/2 expression** Immunofluorescence staining in both normal skin of healthy controls and lesional skin of PN patients revealed abundant epidermal intercellular staining for NK1R, while dermal cells showed only incidental staining (Fig. 3). In PN, primarily the basal and suprabasal intercellular layers (spinous layer up to its middle and upper third) were positive for NK1R. Compared to normal skin of healthy controls, a more distinct signal of NK1R was observed in all patients with PN. This was confirmed in the quantitative analysis of fluorescence intensity using CTCF which showed an up-regulation of NK1R in the epidermis of patients with PN compared to healthy controls, though these results were not statistically significant (\( P = 0.087 \)). Negative controls showed no immunostaining (Fig. S1). In the skin of aprepitant treated PN patients, an intercellular up-regulation of epidermal NK1R expression was observed in comparison with specimens before therapy (\( P = 0.02 \)).

Of interest, irrespective of NK1R expression, both ERK1/2 expression and activation were reduced after therapy (Fig. 4).
Table 1 Patient characteristics of 12 patients and therapeutic effects after treatment with aprepitant

| Medication | Aprepitant n = 12 |
|------------|------------------|
| Gender (f/m) | 9/3 |
| Age range (years) (Mean ± SD; Median) | 43–77 (57.9 ± 9.4; 59.7) |
| Duration of pruritus (years) (Mean ± SD; Median) | 0.6–31.2 (10.7 ± 9.9; 9.5) |
| Underlying origin of pruritus | Atopic predisposition only 3; Systemic disease 2; Multifactorial aetiologies 6; Unknown origin 1 |
| Atopic predisposition | 10/12 (83.3%) |
| VAS (Mean ± SD; Median) Before therapy | 6.33 ± 1.3; 6.0 |
| After therapy | 4.46 ± 2.8; 4.5* |
| Scratch symptom score (Mean ± SD; Median) Before therapy | 4.32 ± 1.85; 4.2 |
| After therapy | 4.1 ± 3.33; 3.45 |

*P < 0.05, Student’s paired t-test.

Table 2 Change of cell surface expression markers before and after a therapy with aprepitant

| | Aprepitant (mean/SD) | | |
|---|---|---|---|
| | Before | After | Heat |
| CD3 | 3.92/0.79 | 3.5/1 | 0.241 |
| CD4 | 3.08 | 3/ | 0.83 |
| CD5 | 3.83/0.94 | 3/1.35 | 0.054 |
| CD8 | 1.75/0.62 | 1.42/0.79 | 0.266 |
| CD20 | 1.17/0.39 | 1.42/0.79 | 0.389 |
| CD25 | 2.58/1.17 | 1.92/0.67 | 0.039 |
| CD45 RA | 4/0.6 | 4.17/0.94 | 0.658 |
| CD45 RO | 1.42/0.9 | 1.67/0.65 | 0.389 |
| CD68 | 4.83/0.389 | 4.75/0.45 | 0.586 |
| CD69 | 3.42/1.44 | 3.08/0.67 | 0.457 |
| CD79a | 2.75/1.14 | 2.42/1.24 | 0.54 |
| IFNg | 0.92/0.29 | 1.67/0.65 | 0.005 |
| IL4 | 0.75/0.75 | 1.17/0.84 | 0.137 |
| IL17 | 2.08/1 | 3/1.35 | 0.034 |
| FoxP3 | 2.58/0.79 | 2.33/0.89 | 0.339 |
| RORe | 2.17/1.53 | 1.83/1.03 | 0.54 |
| RORe | 2.36/1.12 | 2.36/1.29 | 1 |

Percentual expression was determined before and after therapy by semi-quantitative analysis of immunohistochemistry. (1: >0–20% stained cells reflecting very low density; 2: >20–40%, low density; 3: >40–60%, intermediate density; 4: >60–80%, high density; 5: >80–100%, very high density). Statistically significant values in bold.

Effects of blocking NK1R in vitro

Expression of NK1R, ERK1/2 and cytokines Substance P induced ERK1/2 phosphorylation (Fig. 4) and expression of the pro-inflammatory cytokines TNFα, IL-1β, IL-6 and IL-8 (Fig. 5)

Discussion

The antipruritic efficacy of NK1R antagonism by aprepitant or serlopitant is well documented but not yet understood. Superficial spinal dorsal horn neurons that give rise to ascending somatosensory projections, express NK1R. Blocking of NK1R in these neurons leads to suppression of chronic itch in mice and is thus speculated to represent the mechanism underlying the antipruritic effect of aprepitant. Interestingly, according to the clinical experience, patients with PN, who express higher levels of SP in the skin respond much better to NK1R antagonism than those with systemic origin of chronic pruritus, suggesting a cutaneous component in the effect of aprepitant. Aprepitant was frequently supposed to mediate effects in the skin.
also reported to be insufficient for successful treatment in other studies.\textsuperscript{19,30–32}

On the other hand, NK1R antagonism with serlopitant revealed high antipruritic effects in PN patients in a recent clinical trial.\textsuperscript{21} Within our study, we aimed to identify putative cutaneous effects of NK1R antagonists in PN patients and in vitro in a cell culture model. The 4 weeks treatment with aprepitant led to significant reduction of itch intensity. This is in line with other therapies, like cyclosporine treatment, where intensity reduction can be achieved within 2–4 weeks.\textsuperscript{33} But, as expected, complete healing of PN lesions was not achieved. This would necessitate long-term treatment of several months.\textsuperscript{34}

Thus, we took skin biopsies from still preserved nodules and analysed receptor expression, IL levels, MAP kinase activation and inflammatory infiltrate pattern. This design had the advantage that relevant changes in the inflammatory infiltrate in the nodules could be detected. But, it was also the main limitation of our study, as we could neither comment on the course of healing, nor provide histological and immunohistochemical data on healed PN lesions. A longer therapy for months or even years with subsequent biopsies would provide a more comprehensive understanding of PN course. However, despite that, such a design necessitates more patients and centres; the duration of aprepitant therapy is limited due to side-effects and drug–drug

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{(a) Expression levels of neurokinin 1 receptor (NK1R) in the epidermis of healthy controls (HC, $n = 10$) and patients with prurigo nodularis (PN, $n = 13$). PN patients showed higher NK1R expression compared to HC but significance was not reached ($t$-test; $P = 0.087$). (b) Expression levels of neurokinin 1 receptor (NK1R) in the epidermis of patients with PN ($n = 11$) before and after treatment with 80 mg aprepitant for 4 weeks. NK1R level was increased after therapy ($P = 0.02$). For statistical analysis, the fluorescence intensity per unit area of epidermal NK1R was calculated (CTCF) in each group. *$P < 0.05$, central box spans the interquartile range, line inside the box shows the median, whiskers from minimum to maximum, dots show outliers.}
\end{figure}
interactions, respectively. Therefore, we decided in this study for a 4-week frame to get as much information as possible in a reasonable time.

The histological investigation confirmed the presence of an interstitial dermal inflammation and, upon comparison of biopsies before and after therapy, the relative stability of the infiltrate. As topical therapies are not used during the 4 weeks, our data suggest that aprepitant therapy led to some relevant changes in inflammatory markers as shown by immunohistochemistry suggesting peripheral effects in the skin. CD5 (positive upon strong stimulus for activation of cells) and CD25 (chain of IL-2 receptor, late activation marker of T and B cells) were down-regulated, while CD79a B cells were significantly up-regulated during the aprepitant therapy. These findings are still puzzling and their relevance in terms of an antipruritic effect yet unclear. Another interesting finding was the presence of Th17 cells [reflected by presence of IL-17, RORc and FoxP3 positive regulatory T cells (Tregs)] in PN skin, and, though not significant, the drop by 41.7% of IL-17 positive cells after aprepitant therapy. It can be speculated that this might be directly linked to NK1R antagonism. SP may impact the differentiation of naïve and memory T cells what results in the up-regulation of IL-17 through NK1R in the presence of monocytes.35 As it is well-known that SP levels are increased in PN,17 the high expression of IL-17 and drop after therapy with aprepitant in PN may be related to SP. Previously, a significant up-regulation of IL-17 in the skin of PN patients in comparison with healthy skin was described on mRNA level.36 To the best of our knowledge, this is the first report to confirm a relevant IL-17 presence in PN lesions on protein level. IL-17 is produced by Th17 and dermal γδ cells which are a subset of T cells.72 Park et al.36 demonstrated in the peripheral blood of PN patients significantly elevated IL-17, CD3, CD4 and CD8 cells. This suggests that Th17 cells are the source of IL-17 in PN what is supported by our results. IL-17

Figure 4 Blocking of NK1R reduces ERK1/2 activation in vivo and in vitro. (a) Prurigo nodularis patients (n = 8) showed reduced expression and activation of ERK1/2 after aprepitant therapy (4 weeks, 80 mg daily aprepitant). Shown are the blots of two exemplary patients. (b) In vitro data confirmed this finding. Primary keratinocytes showed increased ERK1/2 activation (n.s.) after stimulation with substance P (6 h, 1 μmol/L). This activation was completely blocked by prior (1 h) targeting of NK1R with aprepitant (2.5 μmol/L, P < 0.05) or casopitant (20 μmol/L, P < 0.01). Statistical analysis was done by Wilcoxon signed-rank test (a) or Dunn’s test following Kruskal–Wallis test (b) using SPSS 24 (* P < 0.05).
receptors are expressed on keratinocytes, which is relevant for IL-17 mediated epidermal hyperplasia, production of various pro-inflammatory cytokines, and attraction of monocytes and neutrophils into the tissue as known from psoriasis. It might be speculated that IL-17 has a similar role in PN as a hyperplastic epidermis and chronic persistent inflammatory infiltrate including neutrophils and abundant macrophages are a hallmark of PN.

Using immunohistochemical staining, the NK1R expression was further analysed as indicator for aprepitant-mediated cutaneous effects. In fact, a significant up-regulation of the epidermal NK1R expression after therapy was observed underlining further a peripheral effect of aprepitant. Interestingly, we found that the NK1R is up-regulated in PN even before the therapy compared to healthy controls. This is in line with published data were chronic pruritus patients show higher NK1R expression compared to healthy controls in peripheral blood but has not yet been shown to be relevant in the skin of these patient, too. On the one hand, this supports the important role of the receptor in pruritic diseases such as PN but, on the other hand, does not explain the antipruritic effect by itself. Especially in consideration of the up-regulation of NK1R after therapy as observed in this study, the role of NK1R in the epidermis seems unclear. A possible explanation of these findings could be the well-known slow dissociation of aprepitant, a competitive antagonist, from the human NK1R which could thereby act in a quasi-irreversible or non-competitive manner. Thus, aprepitant blocks the influence of the NK1R-signalling in keratinocytes for a long period. Comparable receptor up-regulation has been already described in settings with irreversible or long-lasting blockade in other receptor systems including pruritus relevant ones like the opioid receptors but not for NK1R, so far.

We could confirm this NK1R up-regulation in vitro when blocking the receptor in primary keratinocyte cell cultures. Furthermore, blocking of NK1R in primary keratinocytes reduced significantly the activation of ERK1/2 and the expression of pro-inflammatory cytokines like TNFα, IL-6 and IL-8 that are regulated by ERK1/2. We could confirm reduced activation of ERK1/2 in PN patients after aprepitant therapy. This is most likely due to reduced transactivation of the epidermal growth factor receptor by SP/NK1R (data not shown) that has also been reported by others. Our data are in line with previous reports as the importance of ERK1/2 activation in the spinal cord for itch sensations has been already demonstrated. But, to the best of our knowledge we are the first to show that it may be an important factor for itch perception in the skin, too. Taken together, our data suggest that systemic administration of a NK1R antagonist leads to effects of the substance in the skin and that the therapeutic effect could be a mixed effect of central and peripheral blocking of ERK1/2 signalling.

In addition to these results, we can provide a comprehensive analysis of markers of PN. Histological criteria of PN were described in 2010 by Weigelt et al. All investigated markers were found to be present in the infiltrate of PN nodules before the therapy. Leucocytes, T-cells and macrophage markers were most prominently present, whereas cytotoxic T cells, NK1 cells...
and B cells were rarely present. INF-gamma, reflecting Th1 cells, were also rare; IL-4 pointing to Th2 cells were found to be positive in up to 40% of inflammatory infiltrate cells, which is in line with the atopic predisposition found in the majority of our patients. Abundant presence of IL-4 mRNA was previously demonstrated in PN patients, which might be an important factor for chronicity of the disease. The presence of FoxP3 positive Tregs was not reported before in PN. Tregs are most likely involved in the maintenance of skin homeostasis and in regulation of cutaneous immune responses. In inflammatory skin diseases such as atopic dermatitis and psoriasis, the final role of Tregs has yet not properly defined. Tregs are discussed to have an essential function in autoimmune diseases and to play a role in the central nervous system is likely, too. This and the role of epidermal ERK1/2 in these processes have to be investigated in more depth and may provide new strategies for therapeutic approaches of CPG.

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References

1 Pereira MP, Steinke S, Zeidler C et al. European academy of dermatology and venereology European prurigo project: expert consensus on the definition, classification and terminology of chronic prurigo. J Eur Acad Dermatol Venereol 2018; 32: 1059–1065.
2 Sacó M, Cohen G. Prurigo nodularis: picking the right treatment. J Fam Pract 2013; 64: 221–226.
3 Bobko S, Zeidler C, Osada N et al. Intraepidermal nerve fibre density is decreased in lesional and inter-lesional prurigo nodularis and reconstitutes on healing of lesions. Acta Derm Venereol 2016; 96: 404–406.
4 Zeidler C, Ständer S. The pathogenesis of Prurigo nodularis – ‘Super-Itch’ in exploration. Eur J Pain 2016; 20: 37–40.
5 Scholzen T, Armstrong CA, Bunnett NW, Luger TA, Olerud JE, Ansel JC. Neuropeptides in the skin: interactions between the neuroendocrine and the skin immune systems. Exp Dermatol 1998; 7: 81–96.
6 Ständer S, Luger TA. NK-1 antagonists and itch. Handb Exp Pharmacol 2015; 226: 237–255.
7 Liu JY, Hu JH, Zhu QG, Li FQ, Sun HJ. Substance P receptor expression in human skin keratinocytes and fibroblasts. Br J Dermatol 2006; 155: 657–662.
8 Budai D, Larson AA. Role of substance P in the modulation of C-fiber-evoked responses of spinal dorsal horn neurons. Brain Res 1996; 710: 197–203.
9 Klein CM, Coggleshall RE, Carlton SM, Sorkin LS. The effects of A- and C-fiber stimulation on patterns of neuropeptide immunostaining in the rat superficial dorsal horn. Brain Res 1992; 580: 121–128.
10 Ansel JC, Brown JR, Payan DG, Brown MA. Substance P selectively activates TNF-alpha gene expression in murine mast cells. J Immunol 1993; 150: 4478–4485.

Columbo M, Horowitz EM, Kagey-Sobotka A, Lichtenstein LM. Substance P activates the release of histamine from human skin mast cells through a pertussis toxin-sensitive and protein kinase C-dependent mechanism. Clin Immunol Immunopathol 1996; 81: 68–73.
11 Viaz I, Guenniche A, Doutrremepuis JD, Reichert U, Caudy A, Schmitt D. Substance P and keratinocyte activation markers: an in vitro approach. Arch Dermatol Res 1996; 288: 85–90.
12 Shi X, Wang L, Clark JD, Kingery WS. Keratinocytes express cytokines and nerve growth factor in response to neuropeptide activation of the ERK1/2 and JNK MAPK transcription pathways. Regul Pept 2013; 186: 92–103.
13 Burbach GJ, Kim KH, Zivony AS et al. The neurosensory tachykinins substance P and neurokinin A directly induce keratinocyte nerve growth factor. J Invest Dermatol 2001; 117: 1075–1082.
14 Dallos A, Kiss M, Polyanka H, Dobozy A, Kemény L, Huu S. Effects of the neuropeptides substance P, calcitonin gene-related peptide, vasoactive intestinal polypeptide and galanin on the production of nerve growth factor and inflammatory cytokines in cultured human keratinocytes. Neuropeptides 2006; 40: 251–263.
15 Kepler CK, Markova DZ, Koerner JD et al. Receptor antagonist suppresses inflammatory cytokine expression in human disc cells. Spine (Phila Pa 1976) 2015; 40: 1261–1269.
16 Ständer S, Siepmann D, Herrgott I, Sunderkötter C, Luger TA. Targeting the neurokinin receptor 1 with aprepitant: a novel antipruritic strategy. PLoS ONE 2010; 5: e10968.
17 Santini D, Vincenzi B, Guida FM et al. Aprepitant for management of severe pruritus related to biological cancer treatments: a pilot study. Lancet Oncol 2012; 13: 1020–1024.
18 Tsianakas A, Zeidler C, Riepe C et al. Aprepitant in anti-histamine-refractory chronic nodular prurigo: a multicentre, randomized, double-blind, placebo-controlled, cross-over, phase-II trial (APREPRU). Acta Derm Venereol 2019; 99: 379–385.
19 Ständer S, Kwon P, Luger TA. Randomized, Double-Blind, Placebo-Controlled, Study of the Neurokinin-1 Receptor (NK1-R) Antagonist Serlopitant in Subjects with Prurigo Nodularis (PN). Paper presented at: Annual Meeting of the American Association of Dermatology, 2017.
20 Yoshiovitch G, Ständer S, Kerby MB et al. Serlopitant for the treatment of chronic pruritus: results of a randomized, multicenter, placebo-controlled phase 2 clinical trial. J Am Acad Dermatol 2018; 78: 882–891.
21 Ständer S, Darsow U, Mettang T et al. S2k guideline–chronic pruritus. J Dtsch Dermatol Ges 2012; 10(Suppl 4): S1–S27.
22 Weißhaar E, Szpejteowski JG, Darsow U et al. European guideline on chronic pruritus. Acta Derm Venereol 2012; 92: 563–581.
23 Phan NQ, Blome C, Fritz F et al. Assessment of pruritus intensity: prospective study on validity and reliability of the visual analogue scale, numerical rating scale and verbal rating scale in 471 patients with chronic pruritus. Acta Derm Venereol 2012; 92: 502–507.
24 Ständer S, Augustin M, Reich A et al. Pruritus assessment in clinical trials: consensus recommendations from the International Forum for the Study of Itch (IFSI) Special Interest Group Scoring Itch in Clinical Trials. Acta Derm Venereol 2013; 93: 509–514.
Peripheral effects of NK1R antagonism in PN

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Immunohistochemical analysis of the NK1R expression in the skin.

Figure S2. Expression levels of neurokinin 1 receptor (NK1R) and neurokinin 2 receptor (NK2R) in primary keratinocytes (NHEKs) following NK1R antagonism.

Table S1. Antibodies, their targets and technical details.

Table S2. Results of histological investigation of the epidermis in 12 patients (H&E, PAS, Giesma staining).

Table S3. Results of histological investigation of the dermis in 12 patients (H&E, PAS, Giesma staining).

Table S4. Percentual change of cell surface expression markers before and after a therapy with aprepitant (n=12).

Appendix S1. Material and Methods.