Non-Histaminergic Itch Mediators Elevated in the Skin of a Porcine Model of Scabies and of Human Scabies Patients

Sanders, Kristen M; Nattkemper, Leigh A; Rosen, Jordan D; Andersen, Hjalte H; Hsiang, Jeremy; Romanelli, Paolo; Bernigaud, Charlotte; Guillot, Jacques; Chosidow, Olivier; Yosipovitch, Gil

Published in:
The Journal of Investigative Dermatology

DOI (link to publication from Publisher):
10.1016/j.jid.2018.09.032

Publication date:
2019

Document Version
Accepted author manuscript, peer reviewed version

Accepted Manuscript

Non-Histaminergic Itch Mediators Elevated in the Skin of a Porcine Model of Scabies and of Human Scabies Patients

Kristen M. Sanders, Leigh A. Nattkemper, Jordan D. Rosen, Hjalte H. Andersen, Jeremy Hsiang, Paolo Romanelli, Charlotte Bernigaud, Jacques Guillot, Olivier Chosidow, Gil Yosipovitch

PII: S0022-202X(18)32780-5

DOI: https://doi.org/10.1016/j.jid.2018.09.032

Reference: JID 1653

To appear in: *The Journal of Investigative Dermatology*

Received Date: 3 August 2018

Revised Date: 12 September 2018

Accepted Date: 27 September 2018

Please cite this article as: Sanders KM, Nattkemper LA, Rosen JD, Andersen HH, Hsiang J, Romanelli P, Bernigaud C, Guillot J, Chosidow O, Yosipovitch G, Non-Histaminergic Itch Mediators Elevated in the Skin of a Porcine Model of Scabies and of Human Scabies Patients, *The Journal of Investigative Dermatology* (2018), doi: https://doi.org/10.1016/j.jid.2018.09.032.

TITLE: Non-Histaminergic Itch Mediators Elevated in the Skin of a Porcine Model of Scabies and of Human Scabies Patients

AUTHORS: Kristen M. Sanders[1,2], Leigh A. Nattkemper[1,2], Jordan D. Rosen[1,2], Hjalte H. Andersen[3], Jeremy Hsiang[1,2], Paolo Romanelli[1], Charlotte Bernigaud[4-5], Jacques Guillot[5], Olivier Chosidow[4], and Gil Yosipovitch[1,2*]

[1]Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, FL, USA
[2]Miami Itch Center, University of Miami Miller School of Medicine, Miami, FL, USA
[3]Department of Health Science and Technology, Center of Sensory-Motor Interaction, Aalborg University, Aalborg, Denmark
[4]Service de Dermatologie, Hôpital Henri Mondor, AP-HP, Université Paris-Est, Créteil, France
[5]Research group Dynamyc, EA 7380, Université Paris-Est, Ecole nationale vétérinaire d'Alfort, Maisons-Alfort & Créteil, France

ORCIDs:
Kristen M. Sanders: https://orcid.org/0000-0002-9326-427X
Leigh A. Nattkemper: https://orcid.org/0000-0003-0895-7985
Jordan D. Rosen: https://orcid.org/0000-0001-9648-1467
Hjalte H. Andersen: https://orcid.org/0000-0002-7596-7764
Jeremy Hsiang: https://orcid.org/0000-0001-9648-1467
Paolo Romanelli: https://orcid.org/0000-0002-6390-0511
Charlotte Bernigaud: https://orcid.org/0000-0002-4563-8927
Jacques Guillot: https://orcid.org/0000-0003-3577-6979
Olivier Chosidow: https://orcid.org/0000-0002-8400-4310
Gil Yosipovitch: https://orcid.org/0000-0001-6303-1822

[*]**Corresponding Author**:
Gil Yosipovitch
1600 NW 10th Ave
Rosenstiel Medical Science Building 2023
University of Miami Miller School of Medicine
Miami, FL 33136
Telephone: 305-243-4472
Fax: 305-243-4081
Email: gyosipovitch@med.miami.edu

SHORT TITLE: Itch mediators elevated in scabies skin

ABBREVIATIONS USED: TRPV1, transient receptor potential vanilloid 1; TRPA1, transient receptor potential ankyrin 1, PAR-2, protease-activated receptor 2; ENFD, epidermal nerve fiber density

LETTER

To the Editor:

Scabies, caused by infestation of the *Sarcoptes scabiei* mite, has been estimated to have a prevalence of 146 million people worldwide (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017). A hallmark of scabies is intense itch that worsens at night (Jannic et al., 2018). In a study of children and adults with scabies, 94.5% of patients reported pruritus (Boralevi et al., 2014). A questionnaire study found an average itch intensity of 7.2 out of 10 on the visual analogue scale for scabies patients (Shin et al., 2017). In another study, 36.3% of patients described their itch as “intense” or “severe” (Worth et al., 2012). Itch can continue for up to four weeks after scabies treatment (Chosidow, 2006) and has important consequences for quality of life (Shin et al., 2017, Worth et al., 2012). Additionally, scratching can lead to secondary bacterial infection of the skin (impetigo), which can have life-threatening complications (Heukelbach and Feldmeier, 2006). However, the molecular mechanisms of scabies itch are largely unknown.

Though histamine is considered the classical pruritogen, most types of chronic itch fail to respond to antihistamine treatment. Recent studies have emphasized the importance of nonhistaminergic and neural mechanisms in chronic itch conditions (Sanders et al., 2016). The ion channels transient receptor potential (TRP) vanilloid 1 (TRPV1) and TRP ankyrin 1 (TRPA1) are expressed on itch-signaling primary afferent neurons. Tryptase, expressed by mast cells, and its receptor protease-activated receptor 2 (PAR-2) are major nonhistaminergic itch mediators in diseases like atopic dermatitis. We hypothesized that these mediators may also play a role in scabies-related itch. Additionally, numerous dermatoses characterized by itch are known to alter epidermal innervation, and such changes may underlie persistent itch following

treatment. For this reason, we examined whether epidermal nerve fiber density (ENFD) is altered in scabies. An increase in ENFD would indicate that scabies induces epidermal hyperinnervation.

To investigate the potential mechanisms of scabies itch, we collected samples from scabies-infested and healthy control skin of pigs and humans. The porcine model of scabies was induced as previously described (Bernigaud et al., 2016, Mounsey et al., 2010); see Supplementary Materials. Three female *Sus scrofa domesticus* "large white" pigs were used for the experiment. Mite-infested skin crusts were directly transplanted into the ear canal of the naive pigs in order to induce a scabies infestation. Biopsies were taken from areas of lesional, mite-infested skin and non-lesional (clinically normal in appearance), mite-free skin along the back and neck.

Human samples included de-identified skin biopsies from 6 scabies patients (3 male/3 female, age 65.00 ± 4.58) and 4 healthy control volunteers (2 male/2 female, age 63.33 ± 10.21). Samples were obtained from the University of Miami Department of Dermatology & Cutaneous Surgery. Biopsies were taken from the abdomen. For scabies patients, diagnosis was confirmed by visualization of mites via microscopy of lesional skin adjacent to the tissue used for this study. Use of de-identified samples was considered non-human research by the University of Miami Institutional Review Board and did not require patient consent.

Using immunohistochemistry, we quantified the expression of the itch mediators TRPV1, TRPA1, PAR-2, tryptase, and histamine in both pig and human samples (see Supplementary Materials). We also quantified expression of β-tubulin in order to identify changes in ENFD.

In the porcine model (Figure 1), epidermal expression of TRPV1, TRPA1, and PAR-2 was increased in tissue infested with scabies ($p=0.031$, $p<0.0001$, and $p=0.002$, respectively). The number of tryptase+ cells near the dermal-epidermal junction was increased in porcine tissue

infested with scabies (p=0.021). The number of histamine+ cells did not differ between scabies-infested and control skin (p=0.73). ENFD did not differ between scabies and control tissue (p=0.059).

In human samples (Figure 2), epidermal expression of TRPV1, TRPA1, and PAR-2 was elevated in scabies-infested tissue compared to healthy control (p=0.002, p=0.027, and p=0.010, respectively). Furthermore, the number of tryptase+ cells near the dermal-epidermal junction was increased in human tissue infested with scabies (p=0.043). However, the number of histamine+ cells near the dermal-epidermal junction was decreased in scabies-infested tissue (p=0.003). ENFD was elevated in human skin infested with scabies (p=0.037).

In conclusion, we found significant elevations of nonhistaminergic itch mediators in the skin of a pig model experimentally infested with scabies and of human scabies patients. In both species, scabies infestation induced statistically significant elevations in expression of TRPV1, TRPA1, and PAR-2 in the epidermis and in the number of tryptase+ cells near the dermal-epidermal junction. For these results, the pig model showed remarkable similarities to the human patients with scabies, suggesting that this model is well suited to study scabies-related itch. The slight differences between the pig and human samples (number of histamine+ cells and ENFD) may be explained by several possible factors, such as biopsy site, duration of scabies, scratching of the skin, or general species differences. Future work will be needed to confirm these results in a larger sample size.

Though antihistamines have been suggested as an adjunct therapy to treat scabies itch (Hengge et al., 2006, Mounsey and McCarthy, 2013, Salavastru et al., 2017), our results suggest that antihistamine treatment is unlikely to reduce scabies itch. The nonhistaminergic mediators

we identified are commonly overexpressed in other chronic itch diseases and may provide targets for treatment of this severe and bothersome symptom.

CONFLICT OF INTEREST

KMS, LAN, JDR, HHA, JH, PR, JG, and OC state no conflict of interest. CB received research support from Bioderma Laboratoire Dermatologique and Codexial Dermatologie to assist with the establishment and development of the scabies experimental porcine model in France. GY is a consultant and member of advisory board of Menlo, TREVI, Sienna, Galderma, Novartis, Sanofi, Pfizer and is funded by Sun Pharma and Pfizer.

REFERENCES

Bernigaud C, Fang F, Fischer K, Lespine A, Aho LS, Dreau D, et al. Preclinical Study of Single-Dose Moxidectin, a New Oral Treatment for Scabies: Efficacy, Safety, and Pharmacokinetics Compared to Two-Dose Ivermectin in a Porcine Model. PLoS Negl Trop Dis 2016;10(10):e0005030.

Boralevi F, Diallo A, Miquel J, Guerin-Moreau M, Bessis D, Chiaverini C, et al. Clinical phenotype of scabies by age. Pediatrics 2014;133(4):e910-6.

Chosidow O. Clinical practices. Scabies. N Engl J Med 2006;354(16):1718-27.

GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390(10100):1211-59.

Hengge UR, Currie BJ, Jäger G, Lupi O, Schwartz RA. Scabies: a ubiquitous neglected skin disease. The Lancet Infectious Diseases 2006;6(12):769-79.

Heukelbach J, Feldmeier H. Scabies. The Lancet 2006;367(9524):1767-74.

Jannic A, Bernigaud C, Brenaut E, Chosidow O. Scabies Itch. Dermatol Clin 2018;36(3):301-8.

Mounsey K, Ho MF, Kelly A, Willis C, Pasay C, Kemp DJ, et al. A tractable experimental model for study of human and animal scabies. PLoS Negl Trop Dis 2010;4(7):e756.

Mounsey KE, McCarthy JS. Treatment and control of scabies. Curr Opin Infect Dis 2013;26(2):133-9.

Salavastru CM, Chosidow O, Boffa MJ, Janier M, Tiplica GS. European guideline for the management of scabies. J Eur Acad Dermatol Venereol 2017;31(8):1248-53.

Sanders KM, Nattkemper LA, Yosipovitch G. Advances in understanding itching and scratching: a new era of targeted treatments. F1000Res 2016;5.

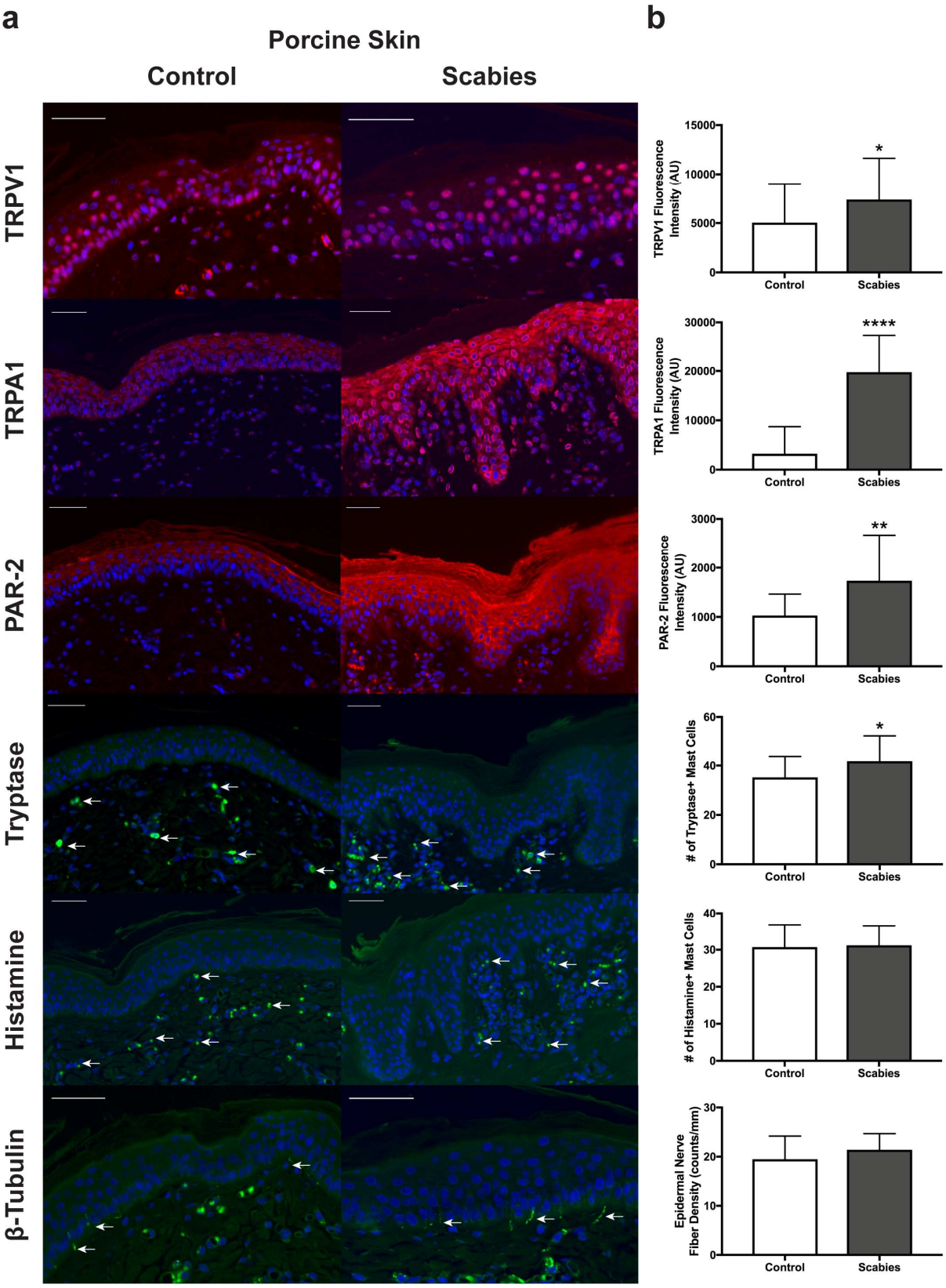
Shin K, Jin H, You HS, Kim JM, Shim WH, Kim GW, et al. Clinical characteristics of pruritus in scabies. Indian J Dermatol Venereol Leprol 2017;83(4):492-3.

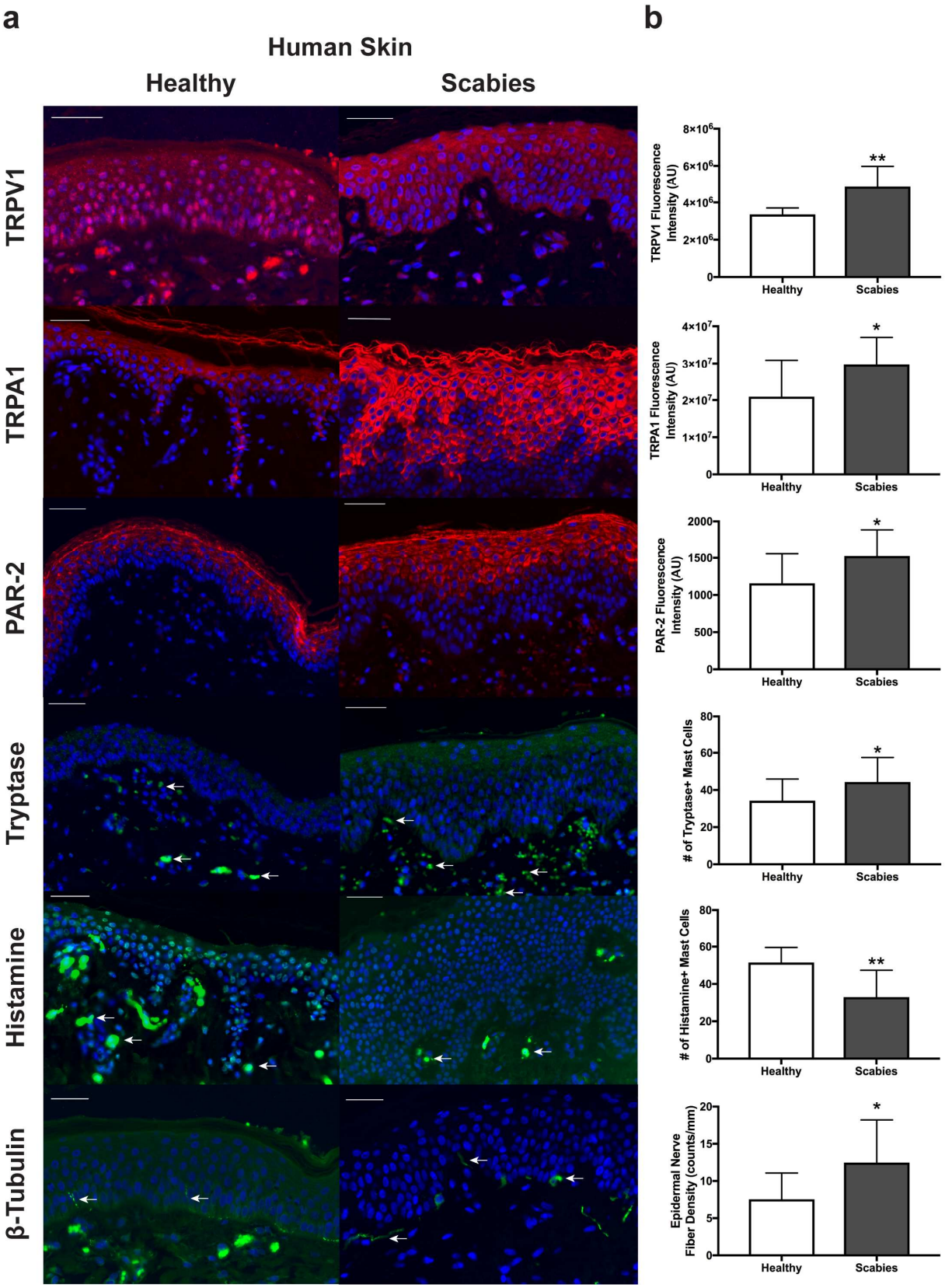
Worth C, Heukelbach J, Fengler G, Walter B, Liesenfeld O, Hengge U, et al. Acute morbidity associated with scabies and other ectoparasitoses rapidly improves after treatment with ivermectin. Pediatr Dermatol 2012;29(4):430-6.

FIGURE LEGENDS

Figure 1. Non-histaminergic itch mediators were elevated in porcine skin with scabies. (A) Representative images of control (left) or scabies-infested (right) porcine skin immunostained for TRPV1, TRPA1, PAR-2, tryptase, histamine, and β-tubulin (n=3). Scale bar = 50 μm. Arrows indicate examples of positively stained cells. (B) Quantification of immunostaining. Epidermal expression of TRPV1, TRPA1, and PAR-2 was increased in tissue infested with scabies. The number of tryptase+ cells was increased in porcine tissue infested with scabies. The number of histamine+ cells did not differ between scabies-infested and control skin. ENFD, visualized by β-tubulin staining, did not differ between scabies and control tissue. * = $p<0.05$, ** = $p<0.01$, **** = $p<0.001$, unpaired t-test scabies vs control. AU = arbitrary units.

Figure 2. Non-histaminergic itch mediators were elevated in the skin of human patients with scabies. (A) Representative images of healthy (left) or scabies-infested (right) human skin immunostained for TRPV1, TRPA1, PAR-2, tryptase, histamine, and β-tubulin (n=4-6). Scale bar = 50 μm. Arrows indicate examples of positively stained cells. (B) Quantification of immunostaining. Epidermal expression of TRPV1, TRPA1, and PAR-2 was elevated in scabies-infested tissue compared to healthy control. The number of tryptase+ cells was increased in human tissue infested with scabies. The number of histamine+ cells was decreased in scabies-infested tissue. ENFD, visualized by β-tubulin staining, was elevated in human skin infested with scabies. * = $p<0.05$, ** = $p<0.01$, unpaired t-test scabies vs control. AU = arbitrary units.


a
Porcine Skin
Control
Scabies
TRPV1
TRPA1
PAR-2
Tryptase
Histamine
β-Tubulin
b
TRPV1 Fluorescence Intensity (AU)
15000
10000
5000
0
*
Control
Scabies
TRPA1 Fluorescence Intensity (AU)
30000
20000
10000
0
****
Control
Scabies
PAR-2 Fluorescence Intensity (AU)
3000
2000
1000
0
**
Control
Scabies
# of Tryptase+ Mast Cells
60
40
20
0
*
Control
Scabies
# of Histamine+ Mast Cells
40
30
20
10
0
Control
Scabies
Epidermal Nerve Fiber Density (counts/mm)
30
20
10
0
Control
Scabies


a
Human Skin
Healthy
Scabies
TRPV1
TRPA1
PAR-2
Tryptase
Histamine
β-Tubulin
b
TRPV1 Fluorescence Intensity (AU)
8×10^6
6×10^6
4×10^6
2×10^6
0
**
Healthy
Scabies
TRPA1 Fluorescence Intensity (AU)
4×10^7
3×10^7
2×10^7
1×10^7
0
*
Healthy
Scabies
PAR-2 Fluorescence Intensity (AU)
2000
1500
1000
500
0
*
Healthy
Scabies
# of Tryptase+ Mast Cells
80
60
40
20
0
*
Healthy
Scabies
# of Histamine+ Mast Cells
80
60
40
20
0
**
Healthy
Scabies
Epidermal Nerve Fiber Density (counts/mm)
20
15
10
5
0
*
Healthy
Scabies