Cathepsin S, a new pruritus biomarker in clinical dandruff/seborrhoeic dermatitis evaluation

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Abstract: Dandruff/seborrhoeic dermatitis (D/SD) is characterized by Malassezia colonization, impaired barrier function with subsequent inflammation, resulting in dandruff and itching. Histamine is one of the biomarkers of pruritus now widely used in treatment efficacy trials. The exact mechanism leading to histamine release and pruritus is not yet clear. However, it could involve cathepsin S, an activator of proteinase-activated receptor 2 (PAR2). The purpose of this study was to evaluate the levels of cathepsin S, PAR2 and histamine in patients with D/SD compared with healthy subjects through non-invasive sampling of the scalp and to correlate those markers with D/SD clinical parameters. A significant increase in the three biological markers was observed in the D/SD group versus healthy subjects, and those markers were correlated with clinical parameters. In conclusion, cathepsin S could be a potential marker of pruritus in D/SD and could help assessing the effect of treatments.

Key words: biomarkers – cathepsin S – dandruff – itch – pruritus – seborrhoeic dermatitis

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Background
Cathepsin S is a cysteine protease found in dendritic cells and macrophages, which plays a role in the major histocompatibility complex class II (1). This cysteine protease is very similar to mucunain (2), the active component isolated from spicules of Mucuna pruriens plants (also known as cowhage), and induces inflammation and itching. It is selectively upregulated in human keratinocytes under stimulation by gamma interferon, which suggests a potential role in inflammatory skin diseases (3). Besides, it was further demonstrated to activate the inflammatory modulating agent proteinase-activated receptor 2 (PAR2) (4) by cleaving its N-terminus-like serine proteases (5).

Questions addressed
Given that overexpression of cathepsin S can induce chronic atopic dermatitis in mice (6), we wondered if it could play a role, as other nociceptive molecules, in dandruff/seborrhoeic dermatitis (D/SD)-associated pruritus. To investigate the role of cathepsin S in pruritus, we compared the dosage of cathepsin S in patients with D/SD and in a control group. We also assayed the protein expression of PAR2 (7) and histamine to compare the results of two distinct pathways of pruritus induction.

Experimental design
Population
A group of 11 female or male patients aged between 18 and 60 years, with a clinically diagnosed D/SD characterized by scales, erythema and pruritus and a clinical global score assessed by the investigator as ≥20, and a control group of 10 healthy subjects (CGS = 0) were included. Patients who had used haircare such as permanent waves and colorations, systemic or topical treatments with antibiotics, antifungals, corticosteroids or retinoids or any topical treatment for SD or dandruff within 2 weeks before inclusion were ineligible.

Written informed consent was obtained, and the study was performed according to the Declaration of Helsinki.

Clinical scoring
The clinical global score ranging from 0 to 80, performed by the investigator, was calculated taking into account the area covered by SD lesions and the severity of the squamous condition, as described by Squire et al. (8). Pruritus was evaluated on a 4-point scale (0 = absent, 1 = mild, 2 = moderate, and 3 = severe) by self-assessment.

Sample collection
The last shampoo had to be made at least 48 h before sampling. The samples for biological markers analysis were swabbed on 2 scalp areas (4 cm² each) similar in all subjects with a cotton swab impregnated with a mixture of PBS buffer containing 0.1% Triton x100 except for histamine analysis (water) and stored at −80°C.

Proteins were recovered from the swab sample by vortexing with 400 µl additional PBS with protease inhibitors (complete protease inhibitor cocktail; Roche Diagnostics GmbH, Mannheim, Germany) for ELISA and water for HPLC.

Cathepsin S, PAR2 and histamine analysis
PAR2 and cathepsin S were measured by ELISA using specific kits (ref. E90852Hu for PAR2, USCN Life Science Inc., Wuhan, PR; ref. DY1183 for cathepsin S, R&D Systems, Minneapolis, MN, USA). Histamine was measured by HPLC as described by Kerr (9). Protein quantifications were performed by Bio-Rad Protein assay (Bio-Rad, Hercules, CA, USA). The total protein concentration was used to normalize biomarker concentrations. Data are expressed as mean values ± standard deviation.

Statistical analysis
The statistical analysis was performed using SigmaStat 3.5 (Systat Software Inc., San José, CA, USA). Differences between groups were analysed using the Mann–Whitney rank-sum test. The correlation between changes in clinical score and biomarkers was analysed using the Spearman rank-order correlation (r). All statistical tests were performed using a significance level of 0.05.
The average PAR2 protein concentration in the D/SD group was 0.667 ± 0.002 for 4/11 in the D/SD group (Fig. 1). Finally, histamine protein concentration was thrice higher in the D/SD group, with 157 ± 180 ng/mg vs 40 ± 36 ng/mg for the control group (P = 0.036; Fig. 1). The average value for self-assessment of pruritus was 2.36 ± 0.67 in the D/DS group.

Cathepsin S, PAR-2 and pruritus were well correlated, as well as clinical global score, with very significant to highly significant positive correlations between cathepsin S and clinical score and the other biomarkers (Table 1).

Conclusions

This study clearly showed an increase in cathepsin S, PAR2 and histamine protein levels in D/SD lesions. Moreover, our results showed that cathepsin S levels significantly correlated with the other biomarkers but also with clinical parameters linked to the severity of D/SD and itching. The trigger of the increased expression of cathepsin S in D/SD pathogenesis and the links between cathepsin S and itching remain to be determined. One study suggested that locally high concentrations of cathepsin S in macrophages could induce massive activation of nerve fibres by a PAR-2-dependent pathway in the colon (10).

In conclusion, this pilot study suggests that cathepsin S could be used as a biomarker to objectify itching sensations and to contribute to the quantitative evaluation of D/SD treatments. Complete validation of cathepsin S as a DS biomarker will require confirmation by further studies, involving more invasive sampling. Furthermore, cathepsin S could be a target for antipruritic treatments in D/SD.

Acknowledgements

All authors revised the paper critically and approved the submitted and final versions. We thank Dr E. Durbise, experienced certified dermatologist, for her selection of patients. We thank Marielle Romet and Françoise Nourrit-Poirette (Santé Active Edition) for editorial assistance.

Author contributions

CV designed the study, analysed the data and wrote the paper. OL and AR collected the clinical data, performed the assays and the data analysis. VT and VM participated to the conception of the study and have revised the article. We thank Dr E. Durbise, experienced certified dermatologist, for her selection of patients. We thank Marielle Romet and Françoise Nourrit-Poirette (Santé Active Edition) for editorial assistance.

Conflicts of interest

None.

Table 1. Correlations between cathepsin S, histamine, PAR2 and CGS and pruritus (n = 21)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Histamine</th>
<th>PAR2</th>
<th>CGS</th>
<th>Pruritus</th>
</tr>
</thead>
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<tr>
<td>Cathepsin S</td>
<td>0.538</td>
<td>0.654</td>
<td>0.627</td>
<td>0.644</td>
</tr>
<tr>
<td>P value</td>
<td>0.012</td>
<td>0.001</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Histamine</td>
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<td>0.499</td>
<td>0.534</td>
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<tr>
<td>P value</td>
<td>0.103</td>
<td>0.021</td>
<td>0.013</td>
<td></td>
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<tr>
<td>PAR2</td>
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<td>0.536</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cath S, cathepsin S; CGS, clinical global score; ρ, Spearman’s rank correlation coefficient.

Results

The global average clinical score for D/SD group was 38.2 ± 18 vs 0 in the healthy group. The level of cathepsin S in the D/SD group was 5 times higher than in the healthy group with an average of 3.53 ± 2.77 ng/mg protein vs 0.70 ± 0.63 (P = 0.002; Fig. 1). An antifungal treatment was shown to lower the level of cathepsin S to the level of the control group (data to be published). The average PAR2 protein concentration in the D/SD group was 0.667 ± 1.09 ng/mg, whereas it was below the detection limit for every subject (10/10) in the control group and for 4/11 in the D/DS group (Fig. 1). Finally, histamine protein concentration was thrice higher in the D/SD group, with 157 ± 180 ng/mg vs 40 ± 36 ng/mg for the control group (P = 0.036; Fig. 1). The average value for self-assessment of pruritus was 2.36 ± 0.67 in the D/DS group.

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