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Central centrifugal cicatricial alopecia severity is associated with cowhage-induced itch

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Summary

Background—Patients with central centrifugal cicatricial alopecia (CCCA) often suffer from varying degrees of itch, pain and burning sensations. However, the neural component of these skin sensations has not been assessed.

Objective—To conduct a comprehensive analysis of C nerve fibre function relating to itch and pain perception in patients with CCCA using thermosensory testing and experimental itch models.

Methods—Fifteen healthy African-American women and 16 African-American female patients with CCCA participated in the study and underwent quantitative computerized thermosensory testing to assess warmth and heat pain thresholds. Itch was induced using histamine iontophoresis and application of cowhage spicules, and the intensity of each itch was assessed. The association between itch intensity and CCCA severity score was examined.

Results—A positive correlation between CCCA severity score and peak itch ratings of cowhage on the lesional scalp (crown) was observed ($P = 0{\cdot}023$, $r = 0{\cdot}562$). Notably, the histamine peak itch rating was not found to have a significant correlation with CCCA severity score ($P = 0{\cdot}913$). The crown also had significantly higher warmth and pain thresholds than the occiput in both healthy subjects and patients with CCCA.

Conclusions—Our results suggest a putative role for the protease-activated receptor (PAR)-2, which is activated by cowhage, in the pathogenesis of CCCA. Future studies should examine PAR-2-directed therapeutics for patients with CCCA. Examining for itch and other dysaesthesias in patients with CCCA is of vital importance to dermatologists in assessing disease severity.

Central centrifugal cicatricial alopecia (CCCA) is the most common cause of scarring hair loss in African-American women, although it also affects men and other ethnic groups.[1] CCCA is characterized by scarring hair loss, mainly over the crown, which spreads centrifugally. These patients often suffer from varying degrees of itch, pain, burning,

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Conflicts of interest
None declared.

stinging and tenderness.[1,2] These complaints have anecdotally been associated with the extent of alopecia and active inflammation.[3] Despite recent advances in our understanding of the scarring alopecias, the mechanism behind the unique features of itch in this alopecia is poorly understood.

Our group recently showed that the healthy scalp has a significant insensitivity of small C nerve fibres to thermal, heat pain, itch and neurogenic inflammation compared with forearm skin.[4] The goal of the current study was to expand our understanding of the function of C nerve fibres in CCCA. A comprehensive analysis of C nerve fibre function relating to itch and pain perception was performed using quantitative thermosensory testing as well as cowhage- and histamine-induced experimental models for itch. Furthermore, the present study allowed us to assess the validity of cowhage as an experimental model for scalp itch and contrast it with the classic itch-inducer histamine.

Materials and methods

Design overview

An investigator assessment of the severity of the alopecia was performed using the central scalp alopecia photographic scale in African-American women.[5] The intensity of baseline itch was assessed. The experiment was carried out on two different locations on the midline scalp (crown and occiput) and the volar aspect of the forearm. Hair parting was made in a cross shape to expose the scalp and minimize the influence of the hair barrier. Quantitative thermosensory testing was used to assess warmth and heat pain thresholds. Itch was induced using histamine iontophoresis and, subsequently, cowhage spicules at the same zone but in two separate spots 10 cm apart. The intensity of each induced itch was assessed. Itch-induction experiments were separated by a break of 10 min.

Subjects

Fifteen healthy African-American women and 16 female African- American patients with CCCA participated in this study. The healthy group had an average age of 38·9 ± 13·2 years (range 20–59); the CCCA group had an average age of 43·8 ± 10·4 years (range 29–64). All procedures were approved by the Wake Forest University Health Sciences Institutional Review Board. All volunteers provided written informed consent and were free to withdraw from the study at any time.

Central scalp alopecia photographic scale in African- American women

Investigators scored the subjects' hair loss using a 6-point scale that includes graduations in hair loss from normal (pattern 0) to bald scalp (pattern 5).[5] Subjects with CCCA who enrolled in our study had the following scores: pattern 1 in two subjects; pattern 2 in seven subjects; pattern 3 in four subjects; pattern 4 in one subject; and pattern 5 in two subjects.

Psychophysical quantitation of itch intensity

Subjects rated their itch intensity every minute for a duration of 10 min using a numerical visual analogue scale (VAS) from 0 (no itch) to 10 (maximum unbearable itch).

Histamine itch induction

Itch was evoked using a round iontophoresis electrode, 14 mm in diameter, which was filled with a 1% solution of histamine, dissolved in 2% methylcellulose gel (Sigma, St Louis, MO, U.S.A.) and administered with a current of 200 μA for a duration of 30 s (Perimed PF 3826 Perilont Power device; Perimed, Stockholm, Sweden).

Cowhage itch induction

Itch was evoked by the application of 40–45 cowhage spicules, which were counted under a magnifying lens and then were rubbed into the subject's skin by a study investigator. The spicules were rubbed gently in a circular motion within a 4 cm^2 area of skin, for 45 s. After itch-intensity assessment, the spicules were removed using adhesive tape (Scotch brand; 3M, St Paul, MN, U.S.A.), which led to the quick cessation of itching.

Quantitative thermosensory testing

Thermal stimuli were delivered using the TSA-II Neurosensory Analyzer (Medoc Ltd., Ramat-Yishai, Israel). The thermode (probe) contact area is 12 cm^2 and has a baseline temperature of 32 °C. The thermode warms the skin surface at a linear rate of 0·4 °C s^{-1} to a maximum of 50 °C. At 50 °C the stimulus automatically terminates. Thermal thresholds were measured first as warmth sensation thresholds and then as heat pain detection thresholds. For each thermal modality at these sites, thermal thresholds were determined three times by the ascending method of limits, and subjects were instructed to press a response button upon detection of a thermal stimulus. These values were used to compute the mean threshold. The investigator who assessed the sensory thresholds was blinded to the hair loss severity score.

Statistical analysis

All statistical analyses were performed using PASW 18.0 software (SAS, Chicago, IL, U.S.A.) with statistical significance set at $P < 0{\cdot}05$. Paired *t*-tests were performed to compare warmth and heat pain thresholds, and mean and peak VAS ratings of the itch induced, between healthy subjects and patients with CCCA and among the different sites: forearm, crown and occiput. A Spearman correlation analysis was performed between baseline itch rating, CCCA severity score, thermal thresholds and the experimentally induced itch intensity ratings.

Results

Warmth and heat pain thresholds

There were no significant differences in warmth and heat pain thresholds between healthy subjects and patients with CCCA in all the three tested sites (Table 1). No effect was found between CCCA severity score and thermal thresholds.

Variability in itch perception

Cowhage-induced itch—Itch was induced by cowhage on the crown in 12 out of the 15 healthy subjects (80%), and in all the 16 subjects with CCCA. All 31 subjects had itch induced by cowhage on the occiput and forearm. No statistically significant difference was noted between the healthy subjects and patients with CCCA in cowhage-induced itch ratings on the scalp. However, the lesional scalp (crown) in subjects with CCCA had higher average and peak cowhage-itch ratings than the healthy crowns, although this did not reach statistical significance ($P = 0{\cdot}093$ and 0·15, respectively). The average and peak itch intensity induced by cowhage on the forearm, occiput and crown revealed that average and peak itch ratings of cowhage on the crown and occiput of healthy subjects were markedly lower than on their forearms (for average itch intensity: $P = 0{\cdot}013$ for crown vs. forearm and $P = 0{\cdot}009$ for occiput vs. forearm; for peak itch intensity: $P = 0{\cdot}006$ for crown vs. forearm; and $P = 0{\cdot}083$ for occiput vs. forearm). Similarly, in patients with CCCA the average and peak itch ratings of cowhage on the crown and occiput were lower than the forearm (for average itch intensity: $P = 0{\cdot}289$ for crown vs. forearm; and $P = 0{\cdot}004$ for occiput vs. forearm; for peak itch intensity: $P = 0{\cdot}005$ for crown vs. forearm; and $P = 0{\cdot}002$ for occiput vs. forearm). In

addition, the peak itch rating of cowhage on the occiput was statistically higher than on the crown ($P = 0{\cdot}018$) in healthy subjects only. Means of average and peak itch intensity induced by cowhage are shown in Table 2.

Histamine-induced itch—In 10 out of the 15 healthy subjects (67%) and in 10 out of the 16 subjects with CCCA (62%) histamine induced itch perception on the crown. Similarly, in 11 out of 15 healthy subjects (73%) and in 13 out of 16 subjects with CCCA (81%) histamine induced itch perception on the occiput. All 31 subjects had itch induced by histamine on the forearm. No statistically significant differences were noted between the healthy subjects and patients with CCCA in histamine-induced itch ratings in all the three tested sites, However, nonlesional scalp (occiput) in subjects with CCCA had higher average itch ratings than the healthy occiput but it did not reach statistical significance ($P = 0{\cdot}183$).

Average and peak histamine-induced itch ratings on the crown and occiput were statistically lower than on the forearm in both healthy subjects and patients with CCCA (in healthy subjects, for average: $P = 0{\cdot}003$ for crown vs. forearm and $P = 0{\cdot}001$ for occiput vs. forearm; for peak: $P = 0{\cdot}008$ for crown vs. forearm and $P = 0{\cdot}003$ for occiput vs. forearm; in subjects with CCCA, for average: $P < 0{\cdot}001$ for crown vs. forearm and $P = 0{\cdot}015$ for occiput vs. forearm; for peak: $P = 0{\cdot}001$ for crown vs. forearm and $P = 0{\cdot}034$ for occiput vs. forearm). In addition, in subjects with CCCA, the crown had lower average and peak histamine-induced itch ratings compared with the occiput (average $P = 0{\cdot}05$, peak $P = 0{\cdot}04$). However, no statistically significant difference was noted between the crown and occiput in histamine-induced itch ratings for healthy individuals. Means of average and peak histamine itch measured by the VAS are shown in Table 3.

Cowhage- vs. histamine-induced itch intensity—On the forearm, peak itch ratings were higher following cowhage application vs. histamine application in both healthy subjects and patients with CCCA ($P = 0{\cdot}003$ and $P < 0{\cdot}001$, respectively); however, there were no statistically significant differences in average itch ratings between cowhage and histamine in healthy and CCCA forearms ($P = 0{\cdot}971$ and $P = 0{\cdot}57$, respectively).

On the occiput (nonlesional scalp), peak itch ratings were higher following cowhage application vs. histamine application in both healthy subjects and patients with CCCA ($P < 0{\cdot}001$, $P = 0{\cdot}003$, respectively). However, average itch ratings of the occiput were statistically significantly higher following cowhage application vs. histamine application in healthy subjects only (healthy $P < 0{\cdot}001$; CCCA $P = 0{\cdot}111$). On the crown (lesional scalp), both peak and average itch ratings following cowhage application were higher than with histamine application in both healthy subjects and subjects with CCCA (peak: $P = 0{\cdot}012$ and $P < 0{\cdot}001$, respectively; average $P = 0{\cdot}042$ and $P = 0{\cdot}002$, respectively).

CCCA severity score and itch intensity

We noted a positive correlation between CCCA severity score and peak itch ratings of cowhage on the lesional scalp (crown) (peak cowhage itch rating $P = 0{\cdot}023$, $r = 0{\cdot}562$). Notably, histamine itch rating was not found to have a significant correlation with CCCA severity score (peak histamine itch rating, $P = 0{\cdot}913$). Baseline itch intensity did not have an effect on scalp itch intensity induced by histamine or cowhage, or on thermal thresholds.

Discussion

Pruritus, tenderness and burning sensation are common symptoms in CCCA.[6,7] These symptoms were previously suggested to be associated with the extent of alopecia and active inflammation in early presentations of CCCA.[3] The present study demonstrates that the severity of cowhage-induced itch, but not of histamine-induced itch, correlates to CCCA

severity ratings. In addition, itch ratings were higher following cowhage vs. histamine application in both healthy subjects and those with CCCA.

These results highlight a role for cowhage as an experimental model for scalp itch. While histamine has been used as the standard experimental pruritogen in the past decades, it is not the primary pruritogenic mediator in many chronic pruritic disorders, such as atopic dermatitis.[8] Similarly, the results of the present study suggest that cowhage, which induces a robust itch response on lesional scalp compared with histamine, is a more appropriate experimental model for scalp itch in patients with CCCA. Indeed, CCCA itch is often accompanied by burning, tenderness and/or pricking sensations[1,2] that appear to correspond to sensations accompanying cowhage-induced itch.[9,10]

Cowhage spicules elicit itch by stimulating protease-activated receptor (PAR)-2 in the skin. PAR-2 is a well-known mediator involved in chronic pruritus.[2] Thus, the results of the present study may suggest a putative role for PAR-2 in the pathogenesis of CCCA. This is also supported by the observation that antihistamines are not very effective in relieving CCCA itch. As a bridge to CCCA, patients with human skin fibrosis and scleroderma were also found to have increased expression of PAR-2.[11] This finding is significant, as CCCA is a scarring alopecia.

The above results may also provide an explanation for why tetracycline has been shown to be effective in treating CCCA.[1] Interestingly, tetracycline attenuates the effect of PAR-2 and PAR-2-mediated downstream signalling.[12] Therapies targeting PAR-2 should be investigated in the future for the treatment of CCCA.

We believe that examining for itch and other dysaesthesias in patients with CCCA is of vital importance to dermatologists in assessing the disease progression and response to treatment.

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What's already known about this topic?

- Patients with central centrifugal cicatricial alopecia (CCCA) often complain of intense itch, pain and burning sensations. However, the neural component of these skin sensations has not been assessed.

What does this study add?

- This study demonstrates a significant correlation between cowhage-induced itch intensity and the severity of CCCA.
- As cowhage signals through the protease-activated receptor (PAR)-2, these results suggest a putative role for the PAR-2 pathway in CCCA-associated skin sensation. Examining for itch and other dysaesthesias in patients with CCCA is of vital importance to dermatologists in assessing disease severity.

Table 1

Thermal thresholds reported in healthy subjects and patients with central centrifugal cicatricial alopecia (CCCA) at the three tested sites

	Warmth threshold (°C)			Pain threshold (°C)		
	Crown	**Occiput**	**Forearm**	**Crown**	**Occiput**	**Forearm**
Healthy subjects	47·6 ± 3·8	42·5 ± 4·1	34·8 ± 1·2	49·9 ± 0·4	49·1 ± 2·5	44 ± 3
Subjects with CCCA	47·8 ± 2·4	41·95 ± 4·3	34·1 ± 1·1	49·8 ± 0·7	48·9 ± 1·3	43·3 ± 3·7

Data are mean ± SD.

Table 2

Average and peak cowhage-induced itch intensity in healthy subjects and patients with central centrifugal cicatricial alopecia (CCCA) reported at the three tested sites measured on a numerical visual analogue scale (VAS) scale of 0–10

	Forearm	Occiput	Crown
VAS of itch induced in healthy subjects			
Average	2·86 ± 1·24	2·02 ± 1·08	1·7 ± 1·55
Peak	7·27 ± 1·94	6·0 ± 2·95	4·47 ± 3·3
VAS of itch induced in patients with CCCA			
Average	3·11 ± 0·82	2·15 ± 1·1	2·69 ± 1·89
Peak	8·5 ± 1·86	6·0 ± 2·85	6·19 ± 3·02

Data are mean ± SD.

Table 3

Average and peak histamine-induced itch intensity in healthy subjects and patients with central centrifugal cicatricial alopecia (CCCA) reported at the three tested sites measured on a numerical visual analogue scale (VAS) scale of 0–10

	Forearm	Occiput	Crown
VAS of itch induced in healthy subjects			
Average	2·88 ± 1·89	0·74 ± 0·98	0·75 ± 1·08
Peak	4·8 ± 2·48	2·2 ± 2·93	1·93 ± 2·74
VAS of itch induced in subjects with CCCA			
Average	2·81 ± 1·89	1·59 ± 1·57	0·86 ± 1·17
Peak	5·13 ± 2·7	3·44 ± 3·16	1·69 ± 1·74

Data are mean ± SD.