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INVESTIGATIVE REPORT

Pruritic and Vascular Responses Induced by Serotonin in Patients with Atopic Dermatitis and in Healthy Controls

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Atopic dermatitis (AD) is a chronic inflammatory skin disease with often severe itch. The aim of this study was to determine the pruritogenic and vascular effect of serotonin (5-hydroxytryptamine; 5-HT) in patients with AD and in healthy controls. A 50 µg dose of 5-HT was injected intradermally into non-lesional skin of 25 patients with AD and 25 healthy control individuals, and the effect compared with 0.2 µg histamine as a positive control, and buffer as a negative control. Pruritus was recorded by the subjects, using a computerized visual analogue scale, while flare and wheal were recorded by the investigator. There was no qualitative or quantitative difference in 5-HT-induced itch between patients and control subjects, or between males and females. However, reduced flare and wheal were found in the patient group for 5-HT. There were no correlations between clinical findings (i.e. eczema severity, clinical pruritus) and recorded experimental itch, or flare or wheal responses for 5-HT, in the patients with AD. In both groups a shorter itch latency was found for 5-HT compared with histamine. Through the use of intradermal injections, making it possible to calculate the dose of substance delivered, a lower vascular response to 5-HT was shown in patients with AD compared with healthy controls. In addition to confirming a pruritogenic role of 5-HT in both patients with AD and healthy controls, we found a shorter itch latency for 5-HT compared with histamine in both groups. The short itch latency time may indicate a direct effect of 5-HT on itch receptors. ***Key words: atopic dermatitis; histamine; itch; serotonin; vascular response.***

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Atopic dermatitis (AD) is a chronic inflammatory, severely itching, skin disease, which affects up to 20% of Swedish children up to the age of 15 years, and 2–3% of the adult population. It is characterized by pruritus, dry skin, excoriations, and a typical distribution of the skin lesions, often worsening during winter and times of stress.

The mediator(s) causing itching in AD is (are) unknown. Histamine has so far not been considered a major candidate, since the patients do not obtain relief from the use of H1 antihistamines (1). Interleukin (IL)-31 (2) has been suggested to be an important itch mediator, as well as many other factors (for review see e.g. (3)).

Serotonin (5-hydroxytryptamine; 5-HT) is a neuromediator with important central and peripheral effects. 5-HT may have a role in skin inflammation (for review see e.g. (4)). Patients with AD have been reported to have a higher level of 5-HT in plasma compared with patients with psoriasis and healthy controls (5).

5-HT has been shown to cause pruritus in normal individuals (6–11) and, in one study, in patients with AD (11). In the latter study (11), Hosogi et al. using iontophoresis, reported that 5-HT can be a potent histamine-independent pruritogen in lesional skin of AD. They also showed induction of flare, but not wheal, with 5-HT. Weisshaar et al. (8) induced itch and vasocutaneous reactions by application of 5-HT into healthy volunteers, also using iontophoresis. Thomsen et al. (9) found that intradermally injected 5-HT elicited itch only in normal, but not in experimentally induced eczematous, skin, in non-atopic volunteers. Thus, studies of cutaneous responses to 5-HT are rather heterogeneous in their design and performance and limited regarding patients with AD.

The aim of the present study was to explore the pruritogenic and vascular effects of 5-HT in patients with AD compared with healthy control subjects.

MATERIALS AND METHODS

Subjects

A total of 25 patients with AD, 14 females and 11 males, mean age 31.1 ± 7.8 years (age range 19–46 years) (Table I) was recruited from patients with ongoing AD and itching (in the past 3 days and with a wide individual itch range), who were referred by their family doctors to our clinical department.

The patients were diagnosed as having AD using the criteria of Williams et al. (12). The scoring of AD using objective SCORAD (SCORing Atopic Dermatitis) was 32.8 ± 12.3, the clinical itch for the previous 3 days according to the visual analogue scale (VAS) was 3.9 ± 2.1 cm, and a Patient-Oriented Eczema Measure (POEM) was 12.1 ± 6.3 (13). The patients should not have had systemic therapy (including phototherapy and antihistamines) in the month prior to the study. They were allowed to use topical therapy (glucocorticoids, calcineurin

Table I. *Demographic data for patients with atopic dermatitis (AD) and healthy controls (HC)*

Characteristics	AD ($n=25$)	HC ($n=25$)
Age, years, range	19–46	22–41
Age, years, mean ± SD	31.1 ± 7.8	30.4 ± 6.2
Sex, F:M, *n*	14:11	17:8
Objective SCORAD (0–83), mean ± SD	32.8 ± 12.3	NA
Clinical SCORAD pruritus (0–10 cm), mean ± SD	3.9 ± 2.1	NA
POEM (0–28), mean ± SD	12.1 ± 6.3	NA

NA: not applicable; SD: standard deviation; SCORAD: SCORing Atopic Dermatitis; POEM: Patient-Oriented Eczema Measure.

inhibitors) on lesional skin of the arm to be injected, but not on non-lesional skin (the site of injection).

A total of 25 healthy control individuals, mainly staff and students, 17 females and 8 males, mean age 30.4 ± 6.2 years (age range 22–41 years), with no history of previous or present hay fever, asthma or AD/eczema, was also recruited.

The experiment was performed during the period March to May 2011. Ethical permission was obtained from the local ethics board.

Substances

All substances were dissolved in sterile, pyrogen-free, physiological saline containing 10% (v/v) Sörensen phosphate buffer ($Na_2HPO_4 + KH_2PO_4$ 67 mmol/l; pH 7.4). 5-HT was used at a concentration of 2.5 mg/ml, based on the results of a pilot study. Histamine dihydrochloride, 10 µg/ml (Sigma-Aldrich, Stockholm, Sweden), was used as a positive control and Sörensen's buffered saline (see above) as a negative control. The histamine dosage is one of the standard dosages that we, as well as others, have used in studies of experimental itch with histamine (see, e.g. 14–16)). The substances were injected intradermally and separately in random order in volumes of 20 µl. Injections were made in non-lesional areas, with no visible or palpable lesion within a 5 cm area, on the lateral part of the upper arms of the subjects. No injections were made in lesional areas, because of difficulties standardizing such lesions and measuring vascular effects.

The substances had been coded in vials with different colours by a laboratory technician. De-coding was carried out after completion of the whole study. Thus, the experiment was performed under double-blind conditions. To maintain experimental consistency, the same individual (A.R.) performed the experiment, including the injections and supervision of recordings in all subjects.

Recording experimental pruritus

The duration and intensity of pruritus were recorded simultaneously, using the VAS linked to a computer (Somedic, Hörby, Sweden), whereas the areas of the flare and wheal reactions were measured with a ruler after each injection. Subjects were asked to rate their itch by moving a knob on a 100-mm VAS, graded from "no itch" (0 mm) to "maximum itch" (100 mm). All subjects were instructed to adjust the position of the knob continuously so that the position always reflected the present itch. The investigator also regularly reminded subjects about this during the experiment. Subjects always used their dominant hand to ensure the best motoric precision of the rating. In addition, the localization of the different injection sites was randomized to avoid systematic error depending on localization.

The time intervals between each injection and the initiation and termination of pruritus were recorded. Thus, calculations of itch latency (s), itch duration (s), maximum itch intensity (mm) and area under the curve (AUC) (mm×s) were automatically entered into the computer. Such methods for measuring experimental pruritus have been used extensively and were validated in earlier studies (15, 17, 18). The areas of flare and wheal reactions were recorded after 5 min and outlined on a transparent plastic film. We considered 5 min to be the most appropriate time period, based on earlier studies (e.g. 8, 11, 19), and on the results of a pilot study in the present investigation, which showed fairly constant values for wheal, while flare increased slightly over time (0–15 min). The maximum perpendicular diameters for the flare were measured in mm and the surface calculated in mm^2 (20), while, for the wheal the maximum diameter in mm was recorded. The wheal was recorded by measuring only one diameter, because pilot tests with the dosages used showed that the reactions were always round or oval, and symmetrical with no pseudo-pods, making it necessary to measure a perpendicular diameter. The reactions to all injected substances were followed for 15 min regardless of whether the subject experienced itch.

Statistical analysis

Statistical analysis was performed using Stata version 8 (Stata Corporation, College Station, USA). To detect the difference in medians between variables, Student's *t*-test, Wilcoxon signed-rank test for dependant samples, and Mann-Whitney *U* test for independent samples were used. Spearman's rank-order test was used to determine the correlation between the variables. A *p*-value < 0.05 (two-tailed) was considered statistically significant.

RESULTS

The quality of sensation of the experimentally induced pruritus varied among participants.

Some described pruritus as burning, others as painful. However, there was no difference between the qualitative descriptions reported by patients with AD and by healthy controls, regardless of the substance injected.

Table II. *Subjective rating of experimental itch in patients with atopic dermatitis (AD) and healthy controls (HC)*

	Itch latency (s)		Itch duration (s)		Itch MAX (mm)		AUC (mm×s)	
Substances	AD Mean ± SD	HC Mean ± SD	AD Mean ± SD	HC Mean ± SD	AD Mean ± SD	HC Mean ± SD	AD Mean ± SD	HC Mean ± SD
5-HT	18.2 ± 16.7*	17.5 ± 24.0*	611.3 ± 302.4	521.2 ± 352.1	27.7 ± 20.9	27.6 ± 24.6	10,593 ± 13,513	9,564 ± 12
Histamine	57.8 ± 65.3	25.5 ± 24.4	519.6 ± 269.2	533.4 ± 307.0	26.7 ± 22.1	39.0 ± 26.6	9,739 ± 9,764	15,043 ± 15
Buffer	73.0 ± 77.9	140.5 ± 184.6	228.0 ± 287.6	126.2 ± 271.3	7.1 ± 9.3	4.6 ± 14.4	1,461 ± 2,688	1,423 ± 52

If the substance did not provoke itch, there was no itch latency, and itch duration, itch MAX and AUC were 0.
*Significantly shorter than for histamine.
5-HT: 5-hydroxytryptamine; AUC: area under the curve; MAX: maximum intensity.

5-HT, histamine and buffer induced itch in 23, 22 and 14 patients with AD and 21, 23 and 9 healthy controls, respectively. For the different itch variables there was no significant difference between patients with AD and healthy controls (Table II). However, itch latency differed significantly between the substances, being shorter for 5-HT compared with histamine, both in patients with AD ($p = 0.001$) and in healthy controls ($p < 0.05$).

5-HT, histamine and buffer induced flare and wheal in all 25 patients with AD and in all 25 healthy controls, respectively. However, flare and wheal differed between patients with AD and controls, with lower values in patients with AD for 5-HT ($p < 0.01$ and $p < 0.05$, respectively) as well as for histamine ($p < 0.001$ and $p < 0.01$, respectively) (Table III).

There was no difference between the sexes for itch, flare or wheal results.

There was a correlation between the objective SCORAD and the clinical SCORAD pruritus ($p < 0.001$) and POEM ($p < 0.01$), respectively. There were no correlations between the clinical findings (i.e. clinical SCORAD pruritus, POEM), and the recorded experimental itch, nor flare or wheal responses for 5-HT in patients with AD. There was, however, a negative correlation ($p < 0.05$) for histamine, between clinical SCORAD pruritus and wheal, as well as between POEM and wheal, in patients with AD, i.e. the higher the SCORAD pruritus or POEM score, the smaller the wheal.

DISCUSSION

In the present study there was a similar itch response in non-lesional skin of patients with AD and healthy controls, at the group level, to 5-HT and to histamine. On the other hand, 5-HT, analogous to histamine, gave smaller flare and wheal responses in patients with AD compared with healthy controls.

It is well known that patients with AD have an abnormal vascular response with a tendency to vasoconstriction (21). This may explain the different vascular effects induced by 5-HT and histamine in our patients with AD and healthy controls.

Hosogi et al. (11), using iontophoresis, found a lower itch response to 5-HT in non-lesional compared with lesional skin of patients with AD, whereas the itch response of healthy controls was in the range of the lesional skin.

Table III. *Vascular responses (flare and wheal) in patients with atopic dermatitis (AD) and healthy controls (HC)*

	Flare (mm^2)		Wheal (mm)	
Substances	AD Mean ± SD	HC Mean ± SD	AD Mean ± SD	HC Mean ± SD
5-HT	10.6 ± 6.6*	16.8 ± 7.7	7.1 ± 1.4*	8.2 ± 2.1
Histamine	7.8 ± 6.1*	13.6 ± 5.2	7.3 ± 1.9*	8.7 ± 1.4
Buffer	1.5 ± 2.2	0.5 ± 0.4	4.0 ± 1.7	4.2 ± 1.4

*Significantly smaller than in HC.
5-HT: 5-hydroxytryptamine.

The means of administration of substances and the locally reached tissue concentration might be responsible for different results at the group level in our study and earlier studies. We chose the injection technique rather than iontophoresis, as it has the advantage that the dosage can be calculated from the injection volume and substance concentration. A concentration of 2.5 mg/ml was used because it gave a reliable itch reaction in our pilot study. This corresponds to an absolute dosage of 50 µg, which is similar to 5-HT doses used by Fjellner & Hägermark (6). We have previously found the concentration of 5-HT to be 9.85 ng/g in healthy skin and 24.25 ng/g in eczematous skin (22). Our dosage might not be physiological; however, during inflammation high concentrations in local tissue may be achieved.

It is not surprising that iontophoretically applied pruritogens induce a more pronounced itch response in lesional skin, with its damaged skin barrier, than in non-lesional skin. The question is whether this reflects only the delivery of a higher dosage through the damaged skin or increased sensitivity to these pruritogens in the lesional skin. We did consider injecting into lesional skin, but refrained for reasons mentioned above (see Methods).

There was a difference in sex proportion between patients with AD and healthy controls, yet we could not observe a difference between the sexes either for the itch or the flare or wheal results. This is in accordance with no difference between men and women for itch responses elicited experimentally with histamine, compound 40/80 or wool (1).

5-HT also induced pruritus in normal individuals. This is in line with previous studies (6–11). The question is how 5-HT causes a pruritic response. A possible mechanism is via activation of keratinocytes, e.g. transient receptor potential vanilloid (TRPV) receptors (23), which, in turn, activate sensory nerves. Sensory nerve fibres may also be triggered, either directly, via 5-HT receptors (24) or indirectly via effect on inflammatory cells, which like keratinocytes, have receptors for 5-HT (4). Another possible mechanism is via an effect on the vessel wall. The fact that the pruritic effect did not differ significantly between patients with AD and controls, while at the same time, the vascular response did, indicates that the latter is not the primary cause of pruritus. This is supported by a study in which vasoregulation at the site of 5-HT injection occurred in the absence of scratching reflexes (25). This difference between pruritic and vascular effects of 5-HT was studied earlier by Yamaguchi et al. (24) using rodents. In this case it was also stated that the vascular response was of less importance in relation to pruritus due to 5-HT. Moreover, the lower itch latency for 5-HT compared

with histamine might support a direct effect of 5-HT on itch receptors on sensory nerves.

Warmer skin temperatures may evoke itch due to a decrease in threshold for 5-HT-evoked itch signalling. TRPV4, a warmth-sensitive cation-channel expressed in skin cells and sensory neurones, plays an important role in the enhancement of 5-HT-evoked itch by skin warming (26). This may be of particular importance in patients with AD, who are sensitive to heat. In our study the room temperature was kept constant for patients with AD and healthy controls.

In conclusion, this study confirms a pruritogenic role of 5-HT, both in patients with AD and in healthy controls, and shows a lower vascular response of 5-HT in patients with AD compared with healthy controls. In addition, the short itch latency time might indicate a direct effect of 5-HT on itch receptors.

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