Abnormal Axon Reflex-Mediated Sweating Correlates with High State of Anxiety in Atopic Dermatitis

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ABSTRACT
Background: Sweating plays a key role in skin homeostasis, including antimicrobial and moisturizing effects, and regulation of skin surface pH. Impaired axon reflex-mediated (AXR) sweating has been observed in patients with atopic dermatitis (AD). However, the mechanism of such abnormal sudomotor axon reflex remains to be revealed.

Methods: To investigate this mechanism, sudomotor function was analyzed using a quantitative sudomotor axon reflex test (acetylcholine iontophoresis) in patients with AD (n = 26) and healthy volunteers (n = 12). Correlation between sudomotor function and certain background factors, including Spielberger State Trait Anxiety Inventory score, Severity Scoring of Atopic Dermatitis (SCORAD) score, number of circulating eosinophils, and serum concentrations of thymus and activation-regulated chemokine and immunoglobulin E radioimmunosorbent test, was validated.

Results: Latency time was significantly prolonged in AD (p = 0.0352), and AXR sweating volume (mg/0-5 min) was significantly lower in AD patients than in healthy controls (p = 0.0441). Direct sweating volume (mg/0-5 min) was comparable in AD patients and healthy controls. A significant correlation between the evaluation results of quantitative sudomotor axon reflex tests and certain background factors was not observed. The latency time in non-lesioned and lesioned areas for AD patients versus continuous anxiety value in the Spielberger State Trait Anxiety Inventory and the AXR versus SCORAD showed significant correlations (p = 0.0424, p = 0.0169, and p = 0.0523, respectively).

Conclusions: Although the number of study subjects was little, abnormal AXR sweating in patients with AD was observed. Correlative analysis suggests possible involvement of continuous anxiety and the immune system in such abnormal sudomotor function.

KEY WORDS
acetylcholine, anxiety, atopic dermatitis, axon reflex, sweating

INTRODUCTION
Sweating plays a key role in skin homeostasis, with antimicrobial1,2 and moisturizing effects,3 and in the regulation of skin surface pH.4 Certain data also suggest that impaired sweating contributes to the pathogenesis of atopic dermatitis (AD). It has been reported that showering at school reduces the severity of AD.5 Since it is known that the pHs of both sweat and the skin surface increase with time,6 old sweat might cause skin barrier dysfunction and promote infection, which are regarded as exacerbating factors for AD.4,7 In contrast, decreased sweating might cause dry skin and exacerbate skin symptoms in AD. In fact, acetylcholine (ACh)-mediated sweating is impaired in patients with AD.3 Furthermore, a significant increase in ACh tissue content8 and reduced ACh receptor expression levels9 in lesioned skin of AD patients suggest that the cholinergic system of the skin is modulated in AD. This belief assumes that...
a modulated ACh system might affect sudation in AD; however, ACh-induced direct sweat volume does not appear to be affected. Looking at an altered ACh system in the local skin alone cannot explain abnormal sweating responses in AD patients.

To assess the reproducibility of sudomotor function in AD patients, a quantitative sudomotor axon reflex test (QSART) was performed. To elucidate the mechanisms of altered ACh-induced sweating, we evaluated the correlation between the measurements of sweating and certain clinical factors, including Spielberger State Trait Anxiety Inventory (STAI) score, serum concentrations of immunoglobulin E radioimmunosorbent test (IgE-RIST) and thymus and activation-regulated chemokine (TARC), and the number of circulating eosinophils.

**METHODS**

**SUBJECTS**

**Quantitative Sudomotor Axon Reflex Test (QSART)**

Examination was performed based on the method established by Lee et al. Briefly, the subjects were asked to remain quiet for 60 min before undergoing QSART in a hospital outpatient clinic at constant temperature (20°C) and humidity (60%). The multicompartamental sweat capsule used in QSART consists of two independent compartments (Fig. 1A). ACh (100 mg/ml) iontophoretically applied to the skin from the outer compartment stimulates the underlying sweat glands directly (DIR sweating), while the glands of the skin in the central compartment of the capsule are activated indirectly via an axon reflex (AXR sweating; Fig. 1B-D). The central compartment of the capsule serves as the site for AXR sweat volume measurement during the 5 min of iontophoresis. Data for DIR sweating were obtained over the subsequent 5 min.

Sweat onset time, that is, the latency period for sweating after current loading (latency time), and sweat volume over 5 min were measured, and the area under the sweating curve was calculated from 0-5 min for AXR sweating and from 6-11 min for DIR sweating. As for the measurement of DIR sweating volume, a few cases with immeasurable volumes were omitted from the Table 1.

**Study Design**

This study was approved by the Institutional Review Board of Osaka University Hospital. Measurements of serum TARC and total IgE (radioimmunoassay) concentrations and eosinophil number were performed on all study subjects after they signed an informed consent form and provided written and oral information to the study physicians. Patients with AD fulfilled diagnostic criteria by both Hanifin and Rajka and the Japanese Dermatological Association. As a result, 26 adult patients with AD [male : female; 11 : 15, 43.58 ± 15.3 years old; mean ± standard deviation (SD)] and 12 healthy volunteers (male : female; 5 : 7, 32 ± 5.8 years old; mean ± SD) were admitted into the study. The severity of AD was assessed with Severity Scoring of Atopic Dermatitis (SCORAD). STAI, designed and standardized by Spielberger et al., was used to evaluate anxiety.

**Laboratory Methods**

Fasting blood samples were drawn from the subjects. After serum separation, levels of TARC and IgE and eosinophil number were measured by enzyme-linked immunosorbent assay, radioimmunoassay, and visual evaluation, respectively.
Sweating and High Anxiety in Atopic Dermatitis

**RESULTS**

**ACH-INDUCED SWEATING VOLUME IN HEALTHY AND AD SUBJECTS**

ACh-induced DIR and AXR mediated sweating volume were measured in healthy and AD subjects. First, comparison of AXR or DIR-mediated sweating volume between healthy and AD subjects (non-lesioned area) was performed (Fig. 2A). AXR-mediated sweating volume in AD patients (non-lesioned area) was significantly lower than that in healthy subjects. On the one hand, DIR-mediated sweating volume in AD patients was comparable to that of healthy subjects (Fig. 2A). Next, comparison between lesioned and non-lesioned skin in subjects with AD was performed (Fig. 2B). AXR-mediated sweating volume in lesioned skin was significantly lower than that in non-lesioned skin. These results indicate that AXR-, but not DIR-mediated sweating, is attenuated in AD subjects (Fig. 2C).

**LATENCY TIME FOR ACh-INDUCED SWEATING IN HEALTHY AND AD SUBJECTS**

Time to onset of sweating after the start of ACh iontophoresis (latency time) was measured (Fig. 3). Latency time in subjects with AD (non-lesioned skin) was significantly prolonged compared with healthy subjects ($p = 0.0352$, unpaired t test) (Fig. 3A). Unexpectedly, there was no difference between non-lesioned and lesioned skin in AD subjects (Fig. 3B), suggesting that abnormal ACh-induced sweating responses are commonly found in AD subjects regardless of the presence or absence of dermatitis.

**RELATED BACKGROUND FACTORS TO ALTERED SUDOMOTOR FUNCTION**

To explore the mechanisms of abnormal ACh-induced sweating in AD subjects, the relationships between measurements of sweating and certain clinical factors, such as the STAI score, SCORAD score, number of circulating eosinophils, and serum concentrations of TARC and IgE-RIST, were verified. As shown in the Table 1, trait anxiety vs. latency time (both non-lesioned and lesioned skin) and SCORAD vs. AXR-mediated sweating volume in non-lesioned skin showed significant positive correlations. Other parameters did not have a significant correlation with measurements of sweating.

**DISCUSSION**

The involvement of sweating in the pathogenesis of AD has become a major topic of discussion. As mentioned above, recent studies have organized the pathogenic involvement of sweat to include not only a barrier function but also the exacerbation of AD.1,3,5,9 However, the cause and mechanism of abnormal ACh-induced axon reflex sweating in AD patients remain unclear.3 Here, we validated the reproducibility of QSART in AD subjects. As a result, decreased axon reflex-mediated sweating volume and prolonged latency time in subjects with AD were confirmed. It was noteworthy that these findings were also observed in non-lesioned skin of AD patients. This phenomenon implies that local inflammation alone cannot explain the impaired sudomotor function in AD. Although there is no direct evidence supporting this

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**Table 1** Correlation between clinical data and altered sudomotor function

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<th>STAI (state anxiety)</th>
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NL, non-lesional; L, lesioned.

Case number (n), Pearson r (r), and p value (two-tailed) (p).
hypothosis, ACh has been reported to accumulate in lesional skin in AD patients.8 Thus, it might be supposed that ACh receptor down-regulation in the skin of those with AD might occur via ACh saturation. To explore the cause of this abnormity, this report focused on certain clinical parameters.

Interestingly, trait anxiety was positively correlated to latency time in both non-lesioned and lesional areas of AD. Although, there was few evidence supporting the relationship between trait anxiety and ACh, Sklán et al. reported that the higher the trait anxiety, the lower the enzyme activity of acetylcholinesterase (AChE).15 Furthermore, previous studies had suggested that AD is a skin disease associated with increased anxiety levels.16,17 Though this might be just a mere coincidence, if enzymatic activity of AChE decreased in AD, the dysregulation might cause the anxious mood and the accumulation of ACh, confirming past findings.8,16,17 Thus, the enzymatic activity of AChE in AD was considered worthy of attention. In a measurement of sweat volume, despite marked reduction in AXR-mediated sweating volume in AD subjects, DIR-mediated sweating volume was preserved. Therefore, it could be proposed that malfunction of sudomotor nerve might lead to decrease in the AXR-mediated sweating response in AD (Fig. 4). Such an anomaly in the sweating response in AD patients might lead to the development of dry skin (Fig. 4).

The other positive correlation was found between SCORAD and AXR-mediated sweating volume in non-lesional areas (Table 1). It is difficult to explain why these factors correlate with each other, despite no definite correlation between SCORAD and AXR-mediated sweating volume in lesional areas. However, we treated increased AXR-mediated sweating volume in non-lesional area the same as in cases of compensatory hyperhidrosis, which is occasionally observed in cases of invasive treatments of hyperhidrosis.18 Thus, it might be speculated that damaged sympathetic nerves in lesional skin had relatively increased the sweating volume in surrounding non-lesional areas.

Regarding the correlation between SCORAD and trait or state anxiety level, there was no correlation (data not shown), similar to the previous report.17 This result led us to think that high anxiety level was not just result of disease severity. Yet, in this study, there were limitations in the statistical strategy that had assessed the relation between a variety of primary endpoints. Thus, we deemed it necessary to consider that poor statistical correlation in evaluative
consequences did not mean no relation to each other. In conclusion, this study revealed abnormal sweat responses in subjects with AD, and the higher the trait anxiety, the slower the sweat response. In contrast, previous report indicated rinsing old sweat on skin surface reduced severity of AD. Put it all together, although there are arguments both for and against the involvement of “sweat” in the exacerbation of AD, our results suggest that studies of “sweat” should be divided into “sweating response” and “after sweating.” From this point of view, we should give AD patients psychosomatic treatments and lifestyle guidance to stimulate adequate sweating. Additionally, we must endorse the importance of washing away sweat daily.5

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REFERENCES