

Rapid Desensitization with Autologous Sweat in Cholinergic Urticaria

Takeshi Kozaru¹, Atsushi Fukunaga¹, Kumiko Taguchi¹, Kanako Ogura¹, Tohru Nagano¹, Masahiro Oka¹, Tatsuya Horikawa¹ and Chikako Nishigori¹

ABSTRACT

Background: The majority of patients with cholinergic urticaria presents with strong hypersensitivity to autologous sweat. Patients with severe cholinergic urticaria are frequently resistant to H₁ antagonists which are used in conventional therapies for various types of urticaria. It has been reported that desensitization using partially purified sweat antigen was effective in a patient with cholinergic urticaria.

Methods: The aim of this study is to determine the usefulness of rapid desensitization with autologous sweat in severe cholinergic urticaria, because rapid desensitization has proven to be a quick and effective immunotherapy for allergies to various allergens. Six patients with severe cholinergic urticaria who are resistant to H₁ antagonists and have sweat hypersensitivity were enrolled in a rapid desensitization protocol.

Results: In all six patients, the responses for skin tests with autologous sweat were attenuated after rapid desensitization with autologous sweat. Two of the three cholinergic urticaria patients showed reduced histamine release with autologous sweat after the rapid desensitization with autologous sweat. Further, the rapid desensitization and subsequent maintenance treatment reduced the symptoms in five of the six patients.

Conclusions: This study provides evidence that rapid desensitization with autologous sweat is beneficial for treating cholinergic urticaria patients resistant to conventional therapy who have sweat hypersensitivity.

KEY WORDS

cholinergic urticaria, desensitization, histamine release, skin test, sweat

INTRODUCTION

Cholinergic urticaria, first described by Duke¹ in 1924, is characterized by unique clinical features: pinpoint-sized, highly pruritic wheals with surrounding erythema that occur after sweating. Typically, this disorder occurs in young adults. The precise underlying mechanism(s) of the whealing response in cholinergic urticaria has not yet been clarified but it has been proposed that sweat hypersensitivity is involved in the pathogenesis.^{2,5} Published data suggests that about two-thirds of cholinergic urticaria patients have a strong allergy to autologous sweat.^{2,5} Moreover, it is conceivable that the antigens in sweat may be autologous proteins, but that has not yet been deter-

mined.⁴

Although it has been shown that the majority of patients with cholinergic urticaria respond well to antihistamines, some patients with severe cholinergic urticaria often resist the conventional antihistamine treatment. Alternative treatments such as scopolamine and danazol have been reported, but they are not generally effective for severe cholinergic urticaria. Recently, it was reported that omalizumab, a humanized monoclonal antibody against IgE, was effective for a patient with severe cholinergic urticaria, indicating that cholinergic urticaria is mediated by IgE.⁶ Further, desensitization with a partially purified sweat antigen was effective in a patient with cholinergic urticaria.⁷ Meanwhile, accelerated immunother-

¹Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, Hyogo, Japan.

Authors' contributions: AF had full access to all data in this study and takes responsibility for the integrity of the data and the accuracy. Study concept and design were made by AF and TH. TK, AF, KT and KO performed acquisition of the data. AF, TN, MO, TH, and CN made analysis and interpretation of the data. TK, AF, KT, TH and CN wrote a draft of the manuscript. Critical revision of the manuscript for important intellectual content was done by AF, TH and CN. AF and KT contributed to administrative, technical and

material support.

Correspondence: Atsushi Fukunaga, MD, Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan.

Email: atsushi@med.kobe-u.ac.jp

Received 16 September 2010. Accepted for publication 7 October 2010.

©0000 Japanese Society of Allergology

Table 1 Clinical characteristics of patients with cholinergic urticaria

Patient	Age (y)	Sex	History	Accompanying symptom	Total IgE (IU/mL)	Skin test with serum	Skin test with sweat	Period of maintenance therapy
1	27	Female	Rhinitis, Asthma	AD	3403	negative	positive (1/1000)	2 months
2	25	Female	None	AD, CU	2163	negative	positive (1/1000)	12 months
3	45	Female	Asthma, AD	None	4147	negative	positive (1/100)	2 months
4	21	Female	None	None	128	negative	positive (1/100)	3 months
5	17	Female	AD	CU	82	negative	positive (1/100)	5 months
6	32	Female	None	AD	1131	negative	positive (1/1000)	2 months

AD, Atopic Dermatitis; CU, Cold Urticaria.

apy build-up schedules in selected patients may provide a rapid alternative to conventional immunotherapy without a significant increase in risk.^{8,9}

In this study, we performed rapid desensitization with autologous sweat in patients with severe cholinergic urticaria who have strong hypersensitivity to their sweat. Efficacy was evaluated by clinical symptoms, intradermal tests with autologous sweat and histamine release from basophils with autologous sweat. All patients showed attenuation of skin tests with autologous sweat after the rapid desensitization. Two of the three patients with cholinergic urticaria tested showed reduced histamine release from their basophils with autologous sweat after the rapid desensitization. Further, the rapid desensitization and subsequent maintenance therapy attenuated symptoms in five of the six patients with cholinergic urticaria. This study demonstrates that rapid desensitization with autologous sweat can be an effective alternative therapy for treatment-resistant patients with cholinergic urticaria who have sweat hypersensitivity.

METHODS

Six female patients with moderate-to-severe cholinergic urticaria were enrolled in this study. Cholinergic urticaria was confirmed by the development of numerous small wheals after exercise until sweating. The characteristics of those patients are described in Table 1. Their ages ranged between 17 and 45 years (mean, 27.8 years). Three of the patients had a past history of atopic diseases (2 atopic dermatitis [AD], 2 asthma and 1 allergic rhinitis). Four of the patients were accompanied with cold urticaria or AD. This study was approved by the Ethics Committee and all subjects provided written informed consent for this study. Relevant drugs such as histamine H1-receptor antagonists were withdrawn for at least 24 hours before the examination and desensitization therapy.

Venous blood was taken into sterile glass tubes and sera were separated by centrifugation at 500 g for

20 minutes and were passed through a 0.45- μ m MILLEX HV membrane (Millipore, Molsheim, France). Sweat was collected from each patient's forearm after exercise, and was sterilized using a 0.45- μ m MILLEX HV membrane, and then preserved frozen until use.

Samples of autologous diluted sweat (1/100, 1/1000) (0.02 mL), autologous serum (0.05 mL), and 0.9% sterile saline (0.02 or 0.05 mL) were separately injected intradermally into the volar aspect of the forearm of each subject. The diameters of wheals and erythema were measured after 15 minutes. Because healthy controls occasionally have positive reactions after intradermal injection of 1/10 autologous sweat,² we evaluated patients with strong hypersensitivity to sweat if the diameters of wheals induced by 1/100 or 1/1000-diluted sweat were equal to or larger than 6 mm. Reactions were assessed as positive if the diameter of the wheal induced by serum was equal to or larger than 6 mm.

Patients with strong hypersensitivity to sweat were enrolled in the rapid desensitization protocol. The desensitization protocol involves the following steps. Two serial autologous sweat dilutions (1/100 and 1/1000) in 0.9% sterile saline were prepared. Starting with an intradermal injection of 0.02 mL diluted autologous sweat at the highest dilution (1/100 or 1/1000) that elicited positive reactions in skin tests, the amount of diluted sweat was increased 50% each time, from 0.02 mL to 0.2 mL every hour, as detailed in Table 2. After these rapid desensitization protocols, we re-evaluated the hypersensitivity to sweat by intradermal injection (0.02 mL) of the dilution of sweat that initially showed a positive reaction (Table 2). All patients continued intradermal injections with 0.1 mL of 1/100 diluted sweat once per week as maintenance therapy for several months (patient 1: 2 months, patient 2: 12 months, patient 3: 2 months, patient 4: 3 months, patient 5: 5 months, patient 6: 2 months) (Table 1).

Table 2 Summary of the rapid desensitization protocol

Patients who showed positive reaction for 1/1000 diluted sweat		
Step no.	Solution dilution	Volume
1	1/1000	0.02
2	1/1000	0.03
3	1/1000	0.05
4	1/1000	0.07
5	1/1000	0.1
6	1/1000	0.15
7	1/100	0.02
8	1/100	0.03
9	1/100	0.05
10	1/100	0.07
11	1/100	0.1
12	1/100	0.15
13	1/100	0.2
14	1/1000	0.02
Patients who showed positive reaction for 1/100 diluted sweat		
Step no.	Solution dilution	Volume
1	1/100	0.02
2	1/100	0.03
3	1/100	0.05
4	1/100	0.07
5	1/100	0.1
6	1/100	0.15
7	1/100	0.2
8	1/100	0.02

Histamine release tests were performed *in vitro* using HRT (Shionogi, Osaka, Japan) as previously described.² Values of percent histamine release from basophils of patients with diluted autologous sweat are shown in Table 3.

The symptom of patients with cholinergic urticaria was assessed by the patient questionnaire included a 10-point visual analogue scale of urticarial activity before desensitization and during the maintenance therapy.

RESULTS

All patients had a history of moderate-to-severe cholinergic urticaria, which was confirmed by the development of numerous small wheals after exercise until sweating. They had positive skin tests to their own 1/100 or 1/1000 diluted sweat and negative skin tests to their autologous serum (Table 1). These patients were assessed as cholinergic urticaria with strong hypersensitivity to sweat. Because they were resistant to conventional treatment such as histamine H1-receptor antagonists, they were enrolled for the rapid desensitization protocol (Table 2). Rapid desensitization with 1/1000 and 1/100 diluted sweat and

subsequent weekly desensitization with 0.1 ml of 1/100 diluted sweat were performed for patients 1, 2 and 6. Rapid desensitization and subsequent weekly desensitization with only 1/100 diluted sweat were performed in patients 3, 4 and 5. No patient developed anaphylaxis or systemic wheal reactions (except for itching at the injection site) during the rapid desensitization and subsequent maintenance therapy. Sweat hypersensitivity in these patients was evaluated *in vivo* by intradermal tests with autologous sweat and *ex vivo* by histamine release tests with autologous sweat at pre- and post- rapid desensitization. In all of them, skin tests with autologous sweat were attenuated at the post-desensitization compared to pre-desensitization (Table 3). Two of the three examined patients showed reduction of histamine release with autologous sweat after rapid desensitization compared to before it (Table 3). Moreover, five of the six patients had improved intensity and frequency of cholinergic urticaria's symptom during the rapid desensitization and subsequent maintenance therapy (Table 3). Only patient 3 discontinued the subsequent maintenance therapy because of the lack of efficacy in symptom's improvement. In particular, patient 4 had gradual but marked improvement in the severity and frequency of cholinergic urticaria according to her evaluation (Fig. 1).

DISCUSSION

Cholinergic urticaria can significantly impair quality of life, especially in relation to exercise, bathing and sexual activities, although the symptoms subside rapidly, usually within 1 hour. Zuberbier *et al.* suggested that the majority of patients with cholinergic urticaria respond well to antihistamines.¹⁰ However, patients with severe cholinergic urticaria are usually resistant to conventional antihistamines. For patients with severe cholinergic urticaria who resist therapy with H1 antagonists, treatment becomes more difficult. One study demonstrated the successful use of beta-blockers, scopolamine, danazol and prednisolone.¹¹ Recently, treatment with a humanized monoclonal IgG anti-IgE antibody, omalizumab, was shown to be beneficial for two cases with CU.^{6,11} However, omalizumab has side effects (including anaphylaxis and headache) and is expensive. In addition, one case with severe cholinergic urticaria was unresponsive to omalizumab as well as antihistamines and multiple other treatments,¹² indicating that omalizumab is not successful for all cases of treatment-resistant cholinergic urticaria.

We previously demonstrated that the majority of patients with cholinergic urticaria are highly sensitive to crude autologous sweat.² In patients with cholinergic urticaria and AD, semi-purified sweat antigen was released from basophils sensitized with their serum.^{5,13} The incidence of atopic diseases in patients with cholinergic urticaria tends to be higher in the

Table 3 Summary of skin tests, histamine release tests (HRT) with sweat and urticarial activity (VAS) in 6 cholinergic urticaria patients (The comparison between pre-desensitization and post-desensitization)

Pt	Skin test (wheal/flare; mm)		%HRT		Urticarial Activity (VAS)	
	before desensitization	after desensitization	before desensitization	after desensitization	before desensitization	during maintenance therapy
1	8 × 8/9 × 9 (1/1000) 2 × 2/5 × 6 (H)	7 × 5/9 × 8 (1/1000) 2 × 3/7 × 4 (H)	68.7 (1/1000) 50.0 (1/100 IgE)	20.4 (1/1000) 47.4 (1/100 IgE)	10	5
2	6 × 6/9 × 10 (1/1000) 5 × 5/7 × 7 (H)	4 × 4/4 × 4 (1/1000) 6 × 5/14 × 10 (H)	Not Done	Not Done	9	4
3	6 × 7/12 × 13 (1/100) 4 × 4/8 × 8 (H)	4 × 4/7 × 8 (1/100) 5 × 4/9 × 7 (H)	33 (1/100) 70.5 (1/10 IgE)	0 (1/100) 77.6 (1/10 IgE)	8	discontinuance
4	7 × 7/13 × 14 (1/100) 10 × 11/24 × 24 (H)	6 × 6/6 × 6 (1/100) Not done	31 (1/100) 27.1 (1/100 IgE)	32 (1/100) 25.4 (1/100 IgE)	10	2
5	6 × 6/15 × 8 (1/100) 7 × 5/12 × 8 (H)	4 × 4/8 × 6 (1/100) 5 × 6/10 × 9 (H)	Not Done	Not Done	10	4
6	8 × 8/9 × 9 (1/1000) 3 × 4/6 × 10 (H)	3 × 2/3 × 2 (1/1000) 4 × 5/7 × 8 (H)	Not Done	Not Done	10	3

H, histamine prick test; 1/10 or 1/100 IgE, 1/10 or 1/100 diluted anti-IgE antibody.

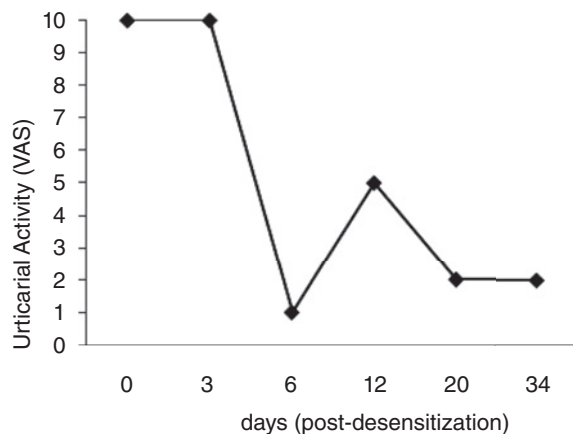


Fig. 1 The therapeutic effect by a desensitization therapy. The rapid desensitization with autologous sweat results in a gradual but marked improvement in symptoms of cholinergic urticaria. (Patient 4: a representative case)

Japanese population.^{2,5} These reports suggest that the possible pathogenesis of cholinergic urticaria as well as AD is involved in sweat hypersensitivity, which seems to be mediated via specific IgE for certain antigens in sweat. Tanaka *et al.* reported that desensitization with semi-purified sweat antigen is effective for a patient with cholinergic urticaria.⁷ Moreover, we found that conventional desensitization with crude autologous sweat is a safe and beneficial therapy for patients with AD who show hypersensitivity to sweat. However, the disadvantages of conventional immunotherapy include poor compliance, delayed efficacy and patient frustration.⁹ We therefore used a rapid desensitization protocol with autologous sweat for patients with severe cholinergic urticaria who had

hypersensitivity to sweat. The rapid desensitization therapy and subsequent maintenance therapy were clinically effective for five of the six patients with treatment-resistant cholinergic urticaria, and simultaneously, the hypersensitivity to sweat was diminished in most patients.

Our data raise some questions to be resolved in the future, the most important issue being to characterize the antigen in sweat. When that antigen is identified, it could be purified and used to desensitize patients with cholinergic urticaria. Alternative treatments for cases that do not respond to desensitization or do not have a strong hypersensitivity to sweat need to be developed. We assume that the mechanism of action of rapid desensitization with sweat involves the attenuation of hypersensitivity to autologous sweat including the reduction of reactivity of mast cells but not a decrease of sweat antigen because autologous sweat that was used for the desensitization and histamine release tests had already been collected before the treatment. However, the detail mechanism of action of rapid desensitization with sweat should be examined in the future. Since tolerance may be lost if patients stop immunotherapy at an early stage, follow-up studies are required to examine the duration and frequency of the maintenance therapy.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (to A.F.).

REFERENCES

1. WW D. Urticaria caused specifically by the action of physical agents. *J Am Med Assoc* 1924;**83**:3-9.

2. Fukunaga A, Bito T, Tsuru K *et al.* Responsiveness to autologous sweat and serum in cholinergic urticaria classifies its clinical subtypes. *J Allergy Clin Immunol* 2005; **116**:397-402.
3. Adachi J, Aoki T, Yamatodani A. Demonstration of sweat allergy in cholinergic urticaria. *J Dermatol Sci* 1994; **7**:142-9.
4. Horikawa T, Fukunaga A, Nishigori C. New concepts of hive formation in cholinergic urticaria. *Curr Allergy Asthma Rep* 2009; **9**:273-9.
5. Takahagi S, Tanaka T, Ishii K *et al.* Sweat antigen induces histamine release from basophils of patients with cholinergic urticaria associated with atopic diathesis. *Br J Dermatol* 2009; **160**:426-8.
6. Metz M, Bergmann P, Zuberbier T, Maurer M. Successful treatment of cholinergic urticaria with anti-immunoglobulin E therapy. *Allergy* 2008; **63**:247-9.
7. Tanaka T, Ishii K, Suzuki H, Kameyoshi Y, Hide M. [Cholinergic urticaria successfully treated by immunotherapy with partially purified sweat antigen]. *Arerugi* 2007; **56**:54-7 (in Japanese).
8. Cox L. Accelerated immunotherapy schedules: review of efficacy and safety. *Ann Allergy Asthma Immunol* 2006; **97**: 126-37; quiz 137-40, 202.
9. Smits WL, Giese JK, Letz KL, Inglefield JT, Schlie AR. Safety of rush immunotherapy using a modified schedule: a cumulative experience of 893 patients receiving multiple aeroallergens. *Allergy Asthma Proc* 2007; **28**:305-12.
10. Zuberbier T, Althaus C, Chantraine-Hess S, Czarnetzki BM. Prevalence of cholinergic urticaria in young adults. *J Am Acad Dermatol* 1994; **31**:978-81.
11. Otto HF, Calabria CW. A case of severe refractory chronic urticaria: a novel method for evaluation and treatment. *Allergy Asthma Proc* 2009; **30**:333-7.
12. Sabroe RA. Failure of omalizumab in cholinergic urticaria. *Clin Exp Dermatol* 2010; **35**:e127-9.
13. Tanaka A, Tanaka T, Suzuki H, Ishii K, Kameyoshi Y, Hide M. Semi-purification of the immunoglobulin E-sweat antigen acting on mast cells and basophils in atopic dermatitis. *Exp Dermatol* 2006; **15**:283-90.