

Occupational asthma caused by African maple (Obeche) and Ramin: evidence of cross reactivity between these two woods

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Summary

Four patients with occupational asthma were studied. All patients were exposed to African maple dust and two of them were also exposed to Ramin dust. Skin tests and bronchial provocation tests with African maple extracts revealed an immediate response in all patients, and the same tests performed with Ramin extracts again revealed an immediate reaction in either exposed or non-exposed patients to Ramin wood dust. Specific IgE antibodies against African maple extracts were demonstrated in all patients as measured by a reverse enzyme immunoassay. Cross reactivity between both woods was demonstrated by a reverse enzyme immunoassay inhibition study. Unexposed persons and exposed asymptomatic workers did not exhibit reactivity to both woods in any of the tests listed above. To the best of our knowledge, this is the first article in which occupational asthma due to Ramin is defined, and cross sensitivity between two different woods is demonstrated.

Introduction

Occupational asthma due to the inhalation of a variety of wood dusts has been widely recognized. The pattern of response may be either an immediate asthmatic reaction, a delayed reaction or a combination of the two. A syndrome resembling extrinsic allergic alveolitis, but not bronchial asthma, has been reported in a woodworker exposed to Ramin [1]. The underlying pathogenesis remains unclear in many cases and immunologic and non-immunologic mechanisms may be involved [2]. Recently, two cases of IgE-mediated occupational asthma due to African maple have been described [3]. Cross sensitivity among the different species of responsible woods has not been reported at present and each species appeared to exert a specific effect.

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The purpose of this article is to present the clinical features, immunologic evaluation and results of provocation challenges in four additional woodworkers exposed to the dust of African maple (*Triplochiton scleroxylon*) and two of them also exposed to Ramin (*Gonystilus bancanus*) dust. A type I IgE-mediated immunologic mechanism was demonstrated in all patients. In addition, the existence of cross sensitivity between these two woods was also demonstrated.

Materials and methods

Patients

Four patients with features of bronchial asthma were studied. All were exposed to African maple dust in their occupations and two of them were also exposed to Ramin dust. They were all admitted to the Centro Ramon y Cajal for study after having been away from work at least 2 weeks.

Wood extracts

Wood extracts for either *in vivo* and *in vitro* tests were done as previously described [3] with the following wood dusts: African maple, Ramin, Western red cedar, *Mansonia* and Elondo.

Skin tests

Skin-prick tests were done with all the wood extracts beginning with a concentration of 0.001% w/v and were progressively tenfold until a positive reaction was obtained. A battery of commercially available wood extracts (Western red cedar, iroko, oak and Cedar of Lebanon) (Bencard Laboratories, Betchworth, Surrey, U.K.) and common inhalant allergens were also tested. Ten asymptomatic individuals exposed to African maple and Ramin, ten atopic non-exposed subjects with seasonal allergic rhinitis and/or asthma and ten normal persons were also tested as a control.

Precipitin tests

Patient sera and control sera were tested for precipitating antibodies to different African maple and Ramin dilutions by an agar gel diffusion method [4].

Specific IgE determination to African maple

(a) Reverse enzyme immunoassay (REIA). This assay was performed as previously described [3] without any modifications. Briefly, the method detects the presence of specific IgE by isolating the IgE from the sera in a first step. The specific IgE is revealed by means of peroxidase-labelled allergen [5-7] in a second step. Basically, the method has five steps: (a) Coating of microtiter plates with a monospecific anti-human IgE; (b) incubation with the sera; (c) incubation with the peroxidase-labelled allergen; (d) incubation with the substrate; (e) arresting of the reaction and reading at 492 nm.

(b) REIA inhibition assay. This method is based on the inhibition induced in the REIA values when the unlabelled allergen is mixed with the peroxidase-labelled allergen and then simultaneously added in step C continuing the assay as described above. 0.2 ml (0.5 μ g peroxidase per well) containing decreased amounts of the following unlabelled antigens (African maple, Ramin, *Mansonia* and Elondo crude extracts) were incubated for 1 hr while shaking. Steps A, B, and D were performed without modifications. In step E the substrate reaction was arrested, in some cases, in the first 5-10 min instead of the standard time (30 min), in order to achieve OD₄₉₂ lower than 2.

Bronchial provocation test

Patients had inhalation tests with African maple and Ramin during asymptomatic periods as previously described [8,9] with the following modifications: aerosols were generated in all instances by a De Vilbiss 646 nebulizer with an output of 0.280 ml/min, delivered into a mask held loosely over the subject's mouth and clipped nose and inhaled by a tidal breathing for 2 min. FEV₁ and FVC were measured before and at intervals of 5 min following each inhalation for the first half hour and then at intervals of 1 hr for 8 hr and again at 24 hr. Only one extract was used for provocation on any 1 day.

Inhalations were discontinued when there was a fall in FEV₁ equal to or more than 20%. Challenges with wood extracts were preceded by a control inhalation with PBS, pH 7.3 solution, which caused no reaction. Challenges with African maple and Ramin were conducted beginning with a concentration of 0.0001% and 0.001% w/v respectively, and were increased tenfold until a positive response was obtained. Two asthmatic non-exposed subjects and four African maple- and Ramin-exposed asymptomatic persons were directly challenged with 10% w/v African maple and Ramin extracts after obtaining informed consent.

The inhibitory effect of disodium cromoglycate on the inhalation reactions was also studied in patients 3 and 4. Disodium cromoglycate (40 mg) was given by inhalation using a spinhaler 30 min before the provocation tests.

Results

Clinical features

The clinical details of the patients are presented in Table 1; all were men between 34 and 49 years old. Patients 1 and 2 were sawmill workers and were exposed to Ramin and African maple dusts. Patient 3 was a woodcarver occasionally exposed to African maple and patient 4 was a carpenter who usually worked with African maple. The two latter patients had never before worked with Ramin and therefore had never been exposed to the dust of this wood. Respiratory symptoms developed after a period of 1–5 years of relatively steady exposure to the wood dusts. Initial symptoms consisted of nasal itching, sneezing and rhinorrhea at work. Several weeks later they began to notice non-productive cough, wheezes and shortness of breath. A significant relief of symptoms occurred on weekends and during vacations. Avoidance of work markedly decreased the severity of symptoms until finally the patients remained asymptomatic. There was no history of atopy in the patients' families and only one patient had a past history of atopy. Chest X-ray films showed no abnormalities in any of the patients.

Skin tests

Skin test results are presented in Table 2. All patients had an immediate reaction after skin-prick testing with African maple and Ramin extracts. Skin tests with the remaining wood extracts were negative. Control subjects did not react to the 10% w/v dilution of both woods. Neither the patients nor the control subjects had positive reactions to the commercial common allergen battery except patient 1 and control atopic individuals who had positive responses to several grass pollens.

Precipitin tests

Precipitins with any of the different dilutions of African maple and Ramin extracts were not found in the patients' sera.

Table 1. Clinical features

Patient no.	Occupation	Age (years)	Duration of exposure to African maple dusts (years)	Duration of exposure to Ramin dusts (years)	Duration of symptoms (years)	Associated rhinitis	Smoking history		Total IgE (IU/ml)	Family history of atopy	History of other allergies
							past	present			
1	Sawmill worker	36	11	11	8	Yes	No	No	650	No	Hay fever
2	Sawmill worker	49	12	11	7	Yes	Yes	No	180	No	No
3	Woodcarver	34	8*	NE	1	Yes	No	No	160	No	No
4	Carpenter	42	4	NE	3	Yes	Yes	Yes	840	No	No

* Occasionally exposed.

NE= Non-exposed.

Table 2. Skin-prick tests

African maple			Ramin		
Patient no.	Extract concentration (w/v)	Weal size (mm)	Patient no.	Extract concentration (w/v)	Weal size (mm)
1	1%	16 by 7	1	1%	6 by 4
2	1%	12 by 7	2	10%	7 by 6
3	0.1%	11 by 8	3	10%	7 by 7
4	0.1%	14 by 7	4	10%	8 by 4

REIA and REIA inhibition assays

Table 3 shows the OD₄₉₂ obtained in the four patients as compared with sixty non-exposed atopic sera. As can be seen in Fig. 1, the REIA values can be inhibited by unlabelled African maple as well as by Ramin. The African maple wood extract seems to contain 100 times more antigenic activity than the Ramin wood extract, both having similar protein contents as measured by Bradford [10].

It is noteworthy that both inhibition assays show similar shapes in the four patients studied. This fact suggests that both wood extracts contain the same antigens. On the contrary, *Mansonia* and *Elondo* wood extracts do not induce inhibition in the African maple REIA even at the highest doses tested (data not shown).

Bronchial provocation test

The results of inhalation tests are presented in Table 4. A positive immediate reaction was observed in all patients after challenge with African maple and Ramin extracts. No late reactions were observed. Threshold concentration challenges with African maple extracts were necessary to obtain a positive reaction between 1 and 10% w/v. Preinhalation of DSCG markedly inhibited the reaction in patients 3 and 4, either after African maple or Ramin extract challenges (Figs 2 and 3). Unexposed asthmatic subjects and exposed asymptomatic individuals did not react to inhalation challenges with either wood extract.

Discussion

African maple (*Triplochiton scleroxylon*) is a West African tree belonging to the Sterculiaceae family. The leaves and fruits are maple-like and therefore it is an easily recognizable tree. The principal producer and exporter countries are the Ivory Coast, Cameroun, the Congo, Nigeria, Liberia and Central Republic of Africa. Nowadays, this timber constitutes the most commercially exploited and exported African wood. The stock of African maple in the mentioned countries is estimated at more than 200 million m³ [11]. It is widely used for all those purposes for which durability and strength are not so important. Their uses include: mouldings, framings, model-making, boxes

Table 3. Specific IgE determination against African maple by REIA

Patient no.	1	2	3	4	60 control sera	Blank
REIA value (OD ₄₉₂)	0.975	0.900	8.600	0.560	0.080 ± 0.020	0.070 ± 0.010

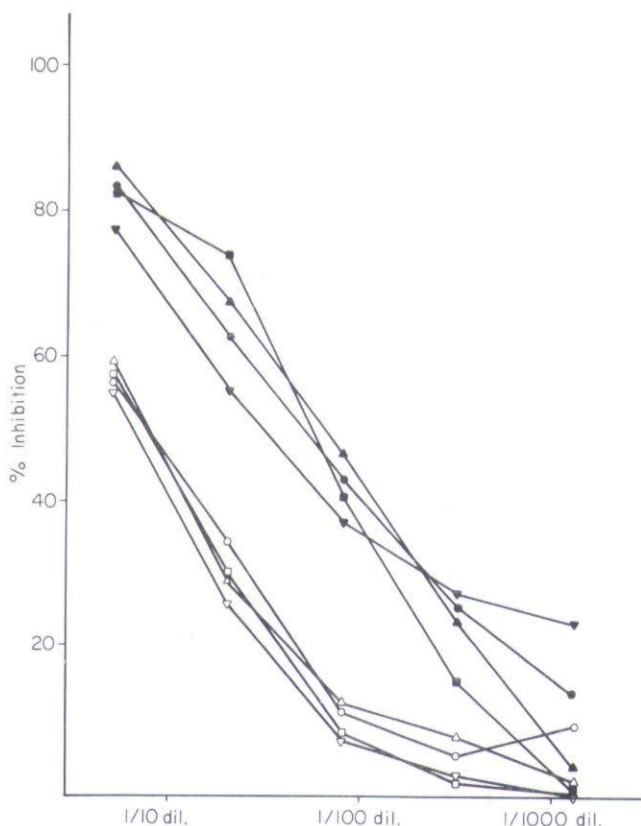


Fig. 1. African maple inhibition assay: Per cent inhibition induced on the four patients by decreasing amounts of African maple (black symbols) or Ramin (white symbols).

etc. It is imported to Europe in large quantities. Obeche and Samba are other common names used to describe this wood in other countries. Two cases of African maple IgE-mediated occupational asthma have recently been reported by our group [3].

Ramin (*Gonystylus bancanus*) is a South East Asian tree belonging to the Thymelaceae family. Sarawak and Borneo are the main exporter countries. It is commonly used for furniture, toys, handles mouldings and interior joinery. Imports to Europe especially to England, West Germany and Spain are increasing steadily. Allergic contact dermatitis due to Ramin has been described [12,13]. A syndrome resembling extrinsic allergic alveolitis has been reported in one patient after occupational exposure to Ramin dust [1]. However, to the best of our knowledge no previous cases of Ramin induced occupational asthma have been reported.

The respiratory syndrome caused by occupational exposure to these wood dusts was fairly characteristic. Upper respiratory symptoms of rhinorrhea, sneezing and nasal obstruction were the initial symptoms present in all patients. Cough, wheezes and shortness of breath were the subsequent symptoms. With continuous exposure, symptoms tended to occur more intensively. Later on, some patients had severe asthma attacks and constant breathlessness with effort while working. After cessation of exposure all patients recovered completely after a period of a few days. The absence of a previous family history of atopy was a prominent feature. However, one patient

Table 4. Results of bronchial provocation tests with African maple and Ramin extracts*

Patient no.	Extract concentration (w/v)	FEV ₁ before provocation (% predicted)	% FEV ₁ change from baseline after provocation							
			5 min	10 min	15 min	30 min	1 hr	3 hr	4-8 hr	
1	1%/(1%)	80/(89)	-37/(+2)	-41/(-16)	-46/(-22)	-46/(-11)	-43/(-7)	-8/(-7)	-4/(0)	
2	1%/(10%)	86/(84)	-23/(-7)	-21/(-12)	-24/(-12)	-30/(-13)	-27/(-13)	-8/(0)	+1/(-1)	
3	0.1%/(10%)	94/(102)	-46/(-8)	-54/(-13)	-54/(-25)	-50/(-14)	-25/(-2)	-11/(+3)	0/(+2)	
4	0.1%/(10%)	103/(94)	-13/(-13)	-18/(-18)	-21/(-21)	-12/(-12)	-1/(-1)	+4/(0)	+2/(-1)	

* Results concerning ramin are expressed in brackets.

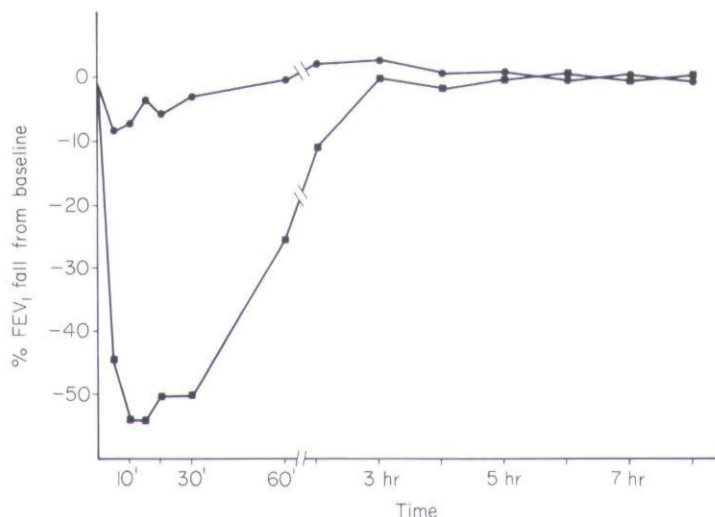


Fig. 2. ■ Bronchial provocation test with 0.1% w/v African maple extract (patient 3); ● the same test performed after 30 min of 40 mg cromolyn sodium inhalation.

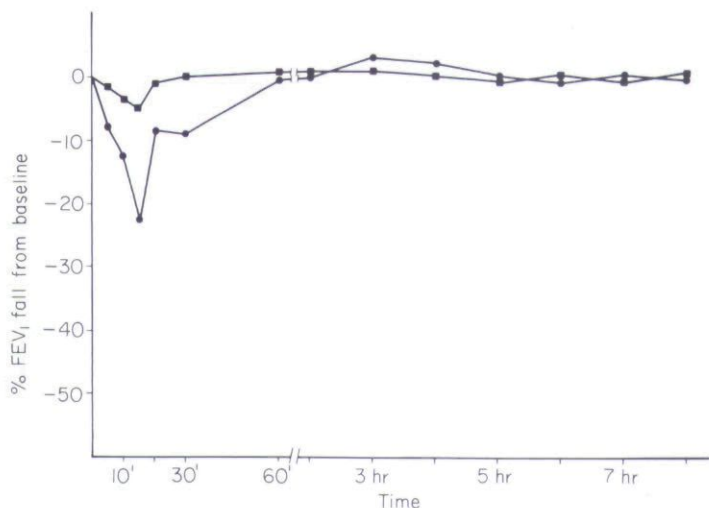


Fig. 3. ● Bronchial provocation test with 10% w/v Ramin extract (patient 3); ■ the same test performed after 30 min of 40 mg cromolyn sodium inhalation.

suffers from hay fever and showed skin sensitivity for several grass pollens.

An IgE-mediated immunologic mechanism appeared to be operational in the patients' responses to both African maple and Ramin dusts. Skin tests and bronchial provocation tests performed with both wood extracts, evinced in all patients an immediate positive response without late reaction. This immediate reaction was markedly inhibited by the pretreatment with disodium cromoglycate. Furthermore, the presence of specific IgE antibodies in the patients' sera was demonstrated by the REIA study. In contrast, the control group composed of exposed asymptomatic individuals, unexposed asthmatics and normal subjects did not respond to skin, REIA

nor BPT tests with both wood extracts. These findings demonstrate the specificity of the patients' response.

Cross reactivity between African maple and Ramin was demonstrated in all patients. Patients 3 and 4 had not previously been exposed to Ramin dust; however, they had positive immediate skin and BPT test reactions to Ramin wood extracts. The REIA inhibition study confirms the existence of cross sensitivity between African maple and Ramin. Amounts 100 times greater of Ramin than African maple are necessary to obtain a 50% inhibition in the African maple REIA inhibition assay. This fact suggests that the antigens responsible for this hypersensitivity are present in higher concentrations in the African maple dust. Another possible explanation is that IgE antibodies present in the patients' sera cross react with a lower affinity to antigens present in Ramin. In fact, more concentrated extracts of Ramin dust were necessary to obtain a positive response to skin and provocation tests. On the contrary, other wood extracts tested in the REIA inhibition assay (Elondo, *Mansonia*, a non-dialysed Western red cedar extract and colophony) were ineffective as inhibitors (data not shown).

It is noteworthy that two woods proceeding from different botanical families and from different continents show cross reactivity. These facts could lead one to believe that identical or similar substances with allergenic properties are present in different timbers coming from different sources. Further studies directed to investigate the possible existence of cross reactivity among woods able to induce allergy would be necessary.

References

- 1 Howie AD, Boyd G, Moran F. Pulmonary hypersensitivity to Ramin (*Gonystylus bancanus*). *Thorax* 1976; 31:385-7.
- 2 Chan-Yeung M. Immunologic and nonimmunologic mechanism in asthma due to Western red cedar (*Thuja plicata*). *J Allergy Clin Immunol* 1982; 70:32-7.
- 3 Hinojosa M, Moneo I, Dominguez J, Delgado E, Losada E, Alcover R. Asthma caused by African maple (*Triplochiton scleroxylon*) wood dust. *J Allergy Clin Immunol* 1984; 74:782-6.
- 4 Outcherlony O. Antigen antibody reactions in gels: Types of reactions in coordinated systems of diffusion. *Acta Pathol Microbiol Scand* 1953; 32:231-40.
- 5 Moneo I, Cuevas M, Ureña V, Alcover R, Bootello A. Reverse enzyme immunoassay for the determination of *Dermatophagoides pteronyssinus* IgE antibodies. *Int Archs Allergy Appl Immunol* 1983; 71:285-7.
- 6 Cuevas M, Moneo I, Ureña V, Dominguez J, Bootello A. Reverse enzyme immunoassay for the determination of *Lolium perenne* IgE antibodies. *Int Archs Allergy Appl Immunol* 1983; 72:184-7.
- 7 Dominguez J, Cuevas M, Moneo I, Ureña V, Ferreira A, Bootello A. Egg hypersensitivity as measured by RAST and a reverse enzyme-immunoassay. *Allergy* 1984; 39:529-33.
- 8 Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: A method and clinical survey. *Clin Allergy* 1977; 7:235-7.
- 9 Juniper EF, Frith PA, Dinnet C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and methacoline. *Thorax* 1978; 705-10.
- 10 Bradford M. A rapid and sensitive method for the quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72:248-54.
- 11 Bois et Forêts des tropiques. Nogent sur Marne. France: Centre Technique Forestier Tropical, 1976; 176:39-53.
- 12 Woods B, Calnan MM. Toxics woods. *Br J Dermatol* 1976; 94, suppl 13:68.
- 13 Beck MH, Roberts MM. A case of Ramin wood sensitivity. *Contact Dermatitis*. 1982; 8:74-6.

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