Supplementary Appendix

METHODS

1.- Material used for desensitisation: HL was manufactured under Good Manufacturing Practices conditions according to internal procedures(Laboratorios LETI S.L.,Madrid, Spain). In short, frozen hake was washed in bidistilled water and homogenised in PBS 0.01M. After extraction, the material was centrifuged and the supernatant dialysed, sterile-filtered and freeze-dried. At the end of the process a HL is obtained. 1 g of frozen hake produced 3 mg of HL and 3 mg of HL contains 1.5 mg of protein and approximately 0.9 mg of parvalbumin(measured by scanning densitometry). The extract was immunochemically characterised and the protein profile was determined by SDS-PAGE. The material used for desensitization was manufactured in a single batch. All the material used was obtained from the same raw material, manufactured at the same time and conditions and divided in different kits.

2.- Skin prick tests were performed following standardized methodology[1]. End-point SPTs titration technique with dilutions of hake extract (1250, 125, 12.5 and 1.25 mcg protein/ml) were made. The most dilute concentration with a wheal size at least as large as that elicited by the histamine control was considered the positive threshold.

3.- DBPCFC testing to HL was carried out, following the indications of the European Academy of Allergology and Clinical Immunology [2]. The DBPCFC consisted of 6 doses of HL masked in orange juice or placebo orange juice administered orally every 30 minutes containing the following doses: 7 mg, 15 mg, 29 mg, 58 mg, 116 mg and 225 mg (cumulative dose, 450 mg, equivalent to 150 g hake) in a double-blind manner. The challenge procedure was stopped when clinical symptoms appeared or when the highest dose was reached. After completing, the DBPCFC children were kept under observation for at least 2 hours and then discharged.

4.- Classification of severity of the adverse reactions:

The severity of the reactions was classified as as follows: Mild: : oral itching, localized erythema or urticaria, abdominal pain, rhinitis, conjunctivitis. Moderate: Generalized urticaria-angioedema, cough, mild bronchospasm. Severe: Severe bronchospasm, breathing difficulties with inspiratory stridor, hypotension, anaphylactic shock.

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SUPPLEMENTARY TABLES

Table S1. Dose build-up phase protocol of hake oral immunotherapy

	Dose (mg lyophilized extract of hake)	Dose (mg protein)
Day 1 In hospital Doses hourly	0,006	0,003
	0,012	0,006
	0,027	0,0135
	0,054	0,27
	0,111	0,0555
Day 2 In hospital Doses hourly	0,111	0,0555
	0,225	0,1125
	0,45	0,225
	0,9	0,45
	1,8	0,9
Dose maintained at home, with elevation once a week in hospital Total 16 weeks	3	1,5
	4,5	2,25
	6	3
	9	4,5
	12	6
	15	7,5
	21	10,5
	27	13,5
	36	18
	45	22,5
	54	27
	72	36
	96	48
	129	64,5
	174	87
	225	112,5

None of the patients received preventive pharmacological treatment

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Table S2.- Protocol duration and adverse reactions during the desensitization

Subject nº	duration of the protocol (weeks)	adverse reactions during the desensitization (dose: mg of lyophilized extract of hake)	**Treatment**
1	18	AP (21 mg)	Antihistamine
2	18	OP (3, 6 , 129 mg) AP+OP (15 mg)	Antihistamine
3	17	OP (0.006 mg) C (3 mg)	Antihistamine + oral steroid
4	19	FU (4,5 mg) FA (174 mg)	Antihistamine + oral steroid
5	17	NR	No therapy
6	18	R (129 mg)	Antihistamine
7	17	NR	No therapy
8	20	OP (0,006 0,027 0,054 mg) AP +E + FU (0,111 mg) E (4,5 mg) U (12 15 21 mg)	Antihistamine + oral steroid

AP: abdominal pain. C: cough. E: emesis. FA: facial angioedema. FU: facial urticaria. NR: no reaction. OP: oral or pharingeal pruritus.R: rhinitis. U: urticaria

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