SUPPLEMENTARY MATERIAL

Supplementary data: Materials and methods

Patients

The study was conducted in patients from the allergy department of the Lucus Augusti hospital, Lugo, Spain, who experienced a systemic reaction after being stung by a bee. All patients had been diagnosed according to the recommendations of the European Academy of Allergy and Clinical Immunology (EAACI) (9) with positive results in skin tests, tryptase levels, total IgE and sIgE to *Apis mellifera* venom. The clinical and demographic characteristics of the patients were collected. The study was approved by the local ethics committee, and patients gave written informed consent to participate.

Serological analysis

Total IgE, tryptase levels, specific IgE (sIgE) against *Apis mellifera* venom and the molecular allergens, rApi m 1, rApi m 2, rApi m 3, sApi m 4, rApi m 5 and rApi m 10 were measured with the ImmunoCAP assay system (Thermo Fisher Scientific) following the manufacturer's instructions. Values of sIgE ≥0.35 kU/L were considered positive.

SDS PAGE and IgE Western blot

Honeybee venom (HBV) from Inmunotek S. L. was analysed and used for SDS-PAGE and Western blot. The protein concentration of the extract was determined by Bradford, using the Protein Assay kit (Bio-Rad). The protein profile of the HBV samples (Inmunotek S. L.) was obtained by SDS-PAGE, using Mini-Protean TGX Stain-Free Precast Gels, Any kD (Bio-Rad) (10), and silver stained with the Silver Stain Kit (Thermo Fisher Scientific), according to the manufacturer's protocol. An HBV standard (Latoxan) was used as control.

Allergenic profiles were obtained by Western Blot. HBV proteins were separated by electrophoresis and transferred onto a nitrocellulose membrane. The membranes were incubated with the patient's serum, diluted in PBS-Tween 0.25%, overnight at 4°C. After incubation, the membrane was blocked with PBS-Tween 0.5% for 1 hour at room temperature, and then incubated with mouse anti-human IgE conjugated to peroxidase (Southern Biotech), for 1h at room temperature. The reaction was developed by chemiluminescence. An HBV standard (Latoxan) was used as control.

The HBV extract (Inmunotek S. L.) with and without stabiliser (Human Serum Albumin, HSA) was analysed at different times to study its stability: T0 (inmediately after reconstitution of the lyophilised extract), T1 (1 week), T2 (2 weeks), T1 (1 month), T2 (2 months), and T3 (3 months). Western Blot assays were developed as described before, using a pool serum from the allergic patients.

Accepted Article

© 2024 Esmon Publicidad
doi: 10.18176/jiaci.1050

LC-MS/MS

The HBV extract (Inmunotek S. L.) was analysed by LC-MS/MS to identify the allergens contained in the extract. Twenty micrograms of protein were loaded onto an S-Trap™ column, after a reduction and alkylation step with TCEP and chloroacetamide. The sample was digested using trypsin and cleaned with a StageTip C18 prior to LC-ESI-MS/MS analysis. The sample was then quantified at the peptide level by fluorimetry, and 500 ng of the digest was injected. The method used for data acquisition was a combination of 60-minute liquid chromatography separating peptides by polarity (using a C-18 reverse phase column) and then the eluted peptides were fragmented on the Orbitrap Exploris 240 mass spectrometer (LC-MS/MS). The raw data were exported, and a search was launched using the MASCOT search engine against the *Apis mellifera* database.

Accepted Article

 © 2024 Esmon Publicidad
doi: 10.18176/jiaci.1050