Parasitol Res (2008) 103:713–715
DOI 10.1007/s00436-008-1017-1

SHORT COMMUNICATION

Skin prick test of *Kudoa* sp. antigens in patients with gastrointestinal and/or allergic symptoms related to fish ingestion

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Received: 14 September 2007 / Accepted: 23 April 2008 / Published online: 20 May 2008

Abstract A majority of *Kudoa* spp. infects the somatic muscle of fish establishing cysts. Previously, elevated humoral responses were detected in BALB/c mice immunised with *Kudoa* sp. pseudocyst extracts and in BALB/c mice orally inoculated with *Kudoa* sp. pseudocysts, as well as the presence of anti-*Kudoa* sp. antibodies in human sera by enzyme-linked immunosorbent assay. The objective of this work was to test *Kudoa* sp. pseudocyst extracts by the skin prick test. Fifteen patients with gastroallergic and/or allergic symptoms related to fish ingestion were examined. *Kudoa* sp. pseudocyst extracts were administered (1 mg/ml) on the volar forearm skin. Four of the 15 selected patients were positive to *Kudoa* sp. extracts. The saline solution negative control did not induce any reaction.

Introduction

Kudoa species infect the somatic muscle of marine and estuarine fish, establishing cysts which contain many spores which provoke the tissue degradation. Whilst the parasite is within a muscle fibre, it remains undetectable by the immune system of the host and the infected fibres appear white. However, as the parasite grows, it breaks the sarcolemma and is duly recognised by the host (Moran et al. 1999). This recognition results in the rapid development of a fibroblast layer around the parasite (Stehr and Whitaker 1986; Morado and Sparks 1986), and the cyst, more properly pseudocyst, quickly acquires a black appearance.

In Spain, *Kudoa*-infected fish have lately been detected in both fresh and frozen imported Chilean hake (*Merluccius gayi gayi*; Guichenot 1848) destined for human consumption. Since the consumption of imported Chilean hake in Spain is higher than the hake proceeding from the Cantabric Sea and there are not effective methods to detect parasitised fish, these reach the consumer.

We have previously shown (Martínez de Velasco et al. 2002; Martínez de Velasco and Cuéllar 2003) that BALB/c mice immunised with "white" and "black" *Kudoa* sp. pseudocyst soluble extracts or orally inoculated with "white" *Kudoa* sp. pseudocysts developed high levels of immunoglobulin (Ig)G1 and IgE antibodies, showed the possible allergenic nature of some of the components of the Kudoa parasitic extracts. Likewise (Martínez de Velasco et al. 2007), we investigated the seroprevalence of anti-*Kudoa* sp. antibodies in a healthy Spanish population and the possible association between the manifestation of allergic reactions after fish consumption and the humoral response to *Kudoa* sp. antigens. Specific anti-*Kudoa* sp. antibody levels in sera of patients diagnosed with several digestive pathologies were also determined. In the present study, *Kudoa* sp. antigens were assayed by means of the skin prick test in human patients.

Materials and methods

Fifteen patients were studied at the Immunology and Allergy Service of the "Hospital del Aire" (Madrid, Spain).

This work was supported in part by the Santander Central Hispano bank under Contract PR27/05-13950-BSCH

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These patients presented gastroallergic and/or allergic symptoms (anaphylaxis, acute and chronic urticaria, antral oedema, abdominal pain, appendicitis, intestinal adherences or intestinal obstruction) related to fish ingestion.

"White" and "black" *Kudoa* sp. pseudocysts were manually obtained from the skeletal musculature of Chilean hake (*M. gayi gayi*) from local fish markets and destined for human consumption. Pseudocysts were carefully separated from any associated fish tissue and then homogenised in a hand-operated glass tissue grinder in phosphate-buffered saline (PBS) at 4°C (Martínez de Velasco and Cuéllar 2003).

The homogenates were frozen at −80°C and later sonicated by 20 pulses of 10 s with a Virsonic 5 (Virtis, NY, USA) set at 70% output power, in an ice bath. Then, they were extracted in PBS at 4°C overnight at 8,500 g for 30 min at 4°C. The supernatants were dialysed overnight at 4°C in PBS, the protein contents were estimated and the extracts were frozen at −20°C until used.

"White" and "black" *Kudoa* sp. pseudocyst extracts (1 mg/ml) were used to investigate the sensitisation of each patient to these parasites. Besides, a commercial extract of *Anisakis simplex* (1 mg/ml; International Pharmaceutical Immunology, ASAC Pharmaceutical International, Alicante, Spain) was used for the study of cross-reactivity. Commercial skin tests (ALK-ABELLÓ, Madrid, Spain) of sensitisation to blue fish [sea bream (*Pagellus centrodontus*), anchovy (*Engraulis encrasicoulus*) and red mullet (*Sardina pilchardus*)] and white fish [cod (*Gadus morrhua*), common sole (*Solea solea*), common bass (*Roccus labras*) and hake (*Merluccius merluccius*)] were also performed. All the antigens were tested using prick tests on the skin of the volar forearm and, 15 min later, the formation of a weal measuring at least 3 mm in diameter was considered as a positive result. Histamine (10 mg/ml) and saline solution were used as positive and negative controls, respectively. Following scanning of the weals, the areas were vectorised and, subsequently, these areas were calculated using the SuperCAD 2000 software expressed in mm^2.

Results

The areas (mm^2) of the papules obtained in the 15 selected patients are presented in the Table 1. The 26.66% of the patients assayed were positive to "white" and/or "black" *Kudoa* extracts (patients FPA, MIVM, MHB and FPS).

Two of the 15 selected patients were positive to both *Kudoa* sp. extracts (patients MIMV and FPS), while only one patient was positive to the "white" pseudocyst extract (patient MHB) and other one from "black" pseudocyst extract (patient FPA). The saline solution did not induce any reaction in these four cases and, although in one of the two patients who were positive to both *Kudoa* sp. extracts, the histamine positive control produced an exacerbated reaction (patient MIMV), it did not occur, with the other patient who was also positive against both extracts (patient FPS).

None of the five patients tested for the possible sensitisation against white and blue fish showed positive results. One of these late patients was positive to both *Kudoa* sp. extracts (patient MIMV), who was *A. simplex* negative.

Table 1 Areas (mm^2) of the papules obtained by prick test in the 15 selected patients

Patient	Saline solution	Histamine	White/blue fish	WK Ag	BK Ag	*A. simplex*
VRM	0	43.9	na	0	0	9.4
ECO	0	84.3	na	0	0	0
AIM	0	59.5	na	0	0	28.3
FPA	0	70.7	na	0	13.7	9.0
FVL	0	57.6	na	0	0	61.9
FPM	0	21.7	na	0	0	5.3
MIVM	0	173.3	0/0	22.2	12.8	0
DCN	0	72.4	0/0	0	0	0
PLM	0	83.3	0/0	0	0	0
ACLG	0	67.885	0/0	0	0	54.5
MHB	0	75.2	0/0	15.9	0	40.1
PGP	0	60.7	na	0	0	32.1
FPS	0	25.1	na	8.1	8.4	15.1
FVC	12.1	102.4	na	0	0	78.3
MJVG	0	84.2	na	0	0	23.7

Commercial skin tests to white and blue fish and to *A. simplex* (1 mg/ml) and with the "white" (WK Ag) and "black" (BK Ag) *Kudoa* sp. pseudocyst extracts (1 mg/ml). Areas obtained with histamine (10 mg/ml) and saline solution (positive and negative controls, respectively) are also presented.
na Not available

Discussion

Many species of the myxosporean order Multivalvulida, included *Kudoa* species, are parasites of the musculature of marine fishes and there is not any effective method to detect parasitised fish. For this reason, it is inevitable that these parasitised fish reach the consumer.

In the Spanish daily diet, fish represents a considerable percentage of the proteic and energic support, according to the data from the Spanish Nutrition and Feeding Study of 1991, published in 1995. Thus, from 63 g person/day consumed in 1964, 72 g was reached in 1991.

In this study, we have investigated the possible allergenic nature of some of the components of the *Kudoa* parasitic extracts, using the skin prick test method in 15 patients with gastroallergic and/or allergic symptoms related to fish ingestion. Skin prick test is nowadays a routine method largely employed by allergologists to determine patient sensitisation to the most common allergens. So, parasite extracts as the *A. simplex* ones have been incorporated in the latest years among the antigens used in these tests, since the frequent sensitisation with this nematode by eating infected fish was known (Fernández de Corres et al. 1996). For these reasons, to determine if the *Kudoa* sp. extracts could be used to detect, by this easy test, patients sensitised to the antigens of this parasite means an important application in a practical point of view.

The "white" and "black" *Kudoa* sp. pseudocyst extracts were administered on the volar forearm skin, at a concentration of 1 mg/ml. The 26.66% of the patients assayed were positive to "white" and/or "black" *Kudoa* extracts. Previously, we had observed the allergenic nature of some components of the *Kudoa* parasitic extract when BALB/c mice were immunised by the oral administration of *Kudoa* pseudocysts provoking a high levels of IgG1 and IgE immunoglobulins. The elevated IgE responses obtained in all the immunised mice confirms the possible allergenic nature of some of the components of these parasites. These components could be responsible for the type I hypersensitivity reactions after their ingestion (Martínez de Velasco et al. 2002; Martínez de Velasco and Cuéllar 2003).

None of the five patients tested for the possible sensitisation against white and blue fish showed positive results, in spite of two of them were positive against *Kudoa* extract. On the other hand, the experiments carried out on the possible existence of cross-reactions between the *Kudoa* sp. antigens and the skeletal musculature of the Chilean hake revealed that the parasite did not share any antigens with *M. gayi gayi* (Martínez de Velasco et al. 2002). The inexistence of common antigenic community between these parasites and their hosts capable of provoke allergenic reactions was confirmed in these patients because none of them was positive against white and blue fish extracts.

Two of these 15 patients resulted positive to both extracts, while only one patient was positive against the "white" pseudocyst extract and the other to the "black" pseudocyst extract. Previously, we had observed the presence of cross-reactions between sera of animals immunised with "white" *Kudoa* extract and tested against "black" *Kudoa* extract and vice versa (Martínez de Velasco et al. 2002). The recognition of both "black" and "white" *Kudoa* extracts by MIVM and FPS patients demonstrated the presence of common allergenic proteins between both antigens.

It was not possible to obtain sera from these four patients to confirm these results by enzyme-linked immunosorbent assay or Western blot and, although in one of the patients that were positive against both extracts, the histamine positive control produced an exacerbated reaction, while the histamine reaction was moderated with the other patient. In conclusion, it seems that the precise study of the antigenic characterisation of Myxosporidia not only could permit a deeper knowledge of the parasite–host interaction and the support to the fish industry of a weapon against a serious problem but, moreover, it could be justified since the point of view of the possible implication of these parasites in the nowadays very frequent immunopathologic processes related to fish consumption.

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