PREPARATION OF *GNATHOSTOMA* PROTEIN BY ULTRAFILTRATION METHOD USING NANOSEP MEMBRANE

Suphan Sugaroon[1], Wilai Saksirisampant[2], Kanyarattana Kraivichian[2], Jamsai Suwansaksri[3], and Viroj Wiwanitkit[4]

[1]Department of Clinical Microscopy, Faculty of Allied Health Sciences, Chulalongkorn University; [2]Department of Parasitology, Faculty of Medicine, Chulalongkorn University; [3]Department of Clinical Chemistry, Faculty of Allied Health Science, Chulalongkorn University; [4]Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Abstract. We report our experience with *Gnathostoma* protein preparation by the ultrafiltration method. Crude antigen was sonicated and ultrafiltrated using the Nanosep 100 K membrane. SDS-PAGE electrophoresis showed protein bands at 43, 41, 24, 22, 21, 19.5 kDa. Use of the ultrafiltration method can provide specific protein (24 kDa), similar to the non-ultrafiltration method, with the other 5 non-specific proteins. Using the non-ultrafiltration method, there were more (20) non-specific protein. The ultrafiltration method can be an alternative method for the preparation of protein, which can provide better results than non-ultrafiltration.

INTRODUCTION

Crude somatic aqueous extract of advanced third stage larvae (AL3) was used as antigen in enzyme-linked immunosorbent assay (ELISA) for the detection of antibody to *Gnathostoma* in patients with intermittent cutaneous migratory swelling (Dharmkrong-at *et al*, 1986). The composition of the AL3 larvae antigens was composed of 40 protein bands with MW ranging from 13 kDa to 150 kDa (Nopparatana *et al*, 1988). Approximately, 20 bands of AL3 were found to be the predominant bands, at 150, 135, 120, 94, 84, 82, 72, 55, 54, 49, 43, 38, 35, 32, and 28 kDa, respectively. Sera were taken from people who were clinically normal, without a history of cutaneous migratory swelling and whose stool and sputum examinations were also negative for parasite ova. The majority of these sera were positive for band 38, while some of them were, in addition, reactive to 49 and 43 kDa (Nopparatana *et al*, 1988).

The above-mentioned results indicate that considerable cross-reactivity inadvertently occurred with patients with other helminthic infections. Thus, the use of crude somatic aqueous extract in routine ELISA is not recommended. In 1991, Chaicumpa *et al* found that 24 kDa is a specific protein of *G. spinigerum* and monoclonal antibody was produced. In the same year,Tapchaisri *et al* (1991) reported that 24 kDa was found to consistently react with sera from all of the proven gnathostomiasis cases by SDS-PAGE and Western blot analysis.

Correspondence: Suphan Sugaroon, Department of Clinical Microscopy, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand.

Crude aqueous extract antigen can be used only in Western blot analysis, which needs sophisticated equipment and experienced personnel. We tried to separate 24 kDa by using ultrafiltration in order to reduce the non-specific protein. This antigen can be used in routine ELISA with comparable results to Western blot analysis.

MATERIALS AND METHODS

Antigen was prepared by using advanced third stage larvae of *G. spinigerum* (AL3G) collected from eel's livers bought from local markets. Liver samples were pooled and digested at 37˚C with 1.5% pepsin for 3 hours. Living larvae were identified under a dissecting microscope using morphological criteria, as described by Daengsvang (1980). The larvae were individually picked from digested materials. They were washed twice in 0.85% normal saline and kept at -20˚C before sonication. Crude somatic aqueous extract was prepared by homogenization of the collected larvae in a small amount of distilled water with a ground-glass tissue grinder, followed by intermittent sonication for 1 hour. The protein concentration of the AL3G antigen was determined by the method of Lowry *et al* (1951) using bovine serum albumin as the standard.

Ultrafiltration was done using Nanosep 100 K (Gelman Sciences), which has a capacity of 0.5 ml. The resulting filtrate was centrifuged at 5,000*g* for 15 minutes. Twenty to fifty microliters of PBS pH 7.2 was added to the filtrate and the mixture was centrifuged again; repeat this step once. The filtered solution was transferred into a second Nanosep, 3 K and centrituged. The filtrate was discarded and the protein of interest was concentrated above the

membrane. This was then used for the SDS-PAGE which we followed the method described by Nopparatana *et al* (1988). The molecular weights (MW) of unknown proteins were estimated by comparing the relative migratory ratios against those of the MW standard (Bio-Rad Laboratory, CA, USA).

RESULTS

As shown in Fig 1, the crude aqueous extract antigen revealed a complex pattern comprised of more than 40 protein bands. After the process of ultrafiltration we found 6 bands: 19.5, 21, 22, 24, 41 and 43 kDa.

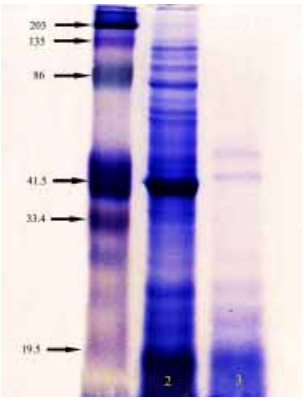

Fig 1- SDS-PAGE. Lane 1 standard marker protein, Lane 2 crude *Gnathostoma spinigerum* antigen, Lane 3 protein after ultrafiltration.

DISCUSSION

Ultrafiltration is a membrane separation technique used to separate extremely small particles and dissolved molecules in fluids. The primary basis is molecular size. Molecules larger than the membrane pores will be retained at the surface of the membrane and concentrated during the ultrafiltration process.The 100 K Nanosep retained 17.8 kDa by 0-4%, 24.5kDa by 0-4%, 160 kDa by 11-79%, and 669kDa or more can be retained by 90-100% (Nanosep®, Gelman Sciences). It has also been used for minimizing artifacts in reagents, samples and primers prior to performing polymerase chain reaction (PCR). Otherwise, the high sensitivity of PCR increases the probability that false priming contamination will lead to the amplification of the wrong molecule. The 24 kDa band is one of the 40 bands in the crude aqueous extract. Using Nanosep, it can be identified in the filtrate because the membrane will allow molecular weights below 67 kDa to pass into the filtrate. We found that 38kDa, 49 kDa did not exist in the filtrate, which had been proven reactive at low to relatively strong reactivities with healthy adult sera. However, the band 43 kDa can be identified in the filtrate and can cause cross-reactivity because the 43 kDa reacts against some healthy normal sera (Nopparatana *et al*, 1988). However, Kulkaew and Chamniyanta (2000) found that the band 43 kDa exhibited IgE positivity of up to 90.9% with those cutaneous gnathostomiasis cases. They also found the positivity of IgE-specific proteins of 54 kDa by 45.45%, 37 kDa (9%), 36 kDa (9%), 26 kDa (9%), 24 kDa (27.3%), 22.5 kDa (18%), and 19.5 kDa (36.6%), respectively. The 19.5, 21, 22, 24, 41, and 43 kDa, found in the filtrate after ultrafiltration, may be used as antigen in the identification of specific IgE in gnathostomiasis. Ultrafiltration may be proposed as the method of choice for the purification of antigens for the ELISA test, which would have fewer cross-reactions. Thus, it can be used comparably with Western blot analysis.

REFERENCES

Chaicumpa W, Ruangkunaporn Y, Nopparatana C, Chongsa-Nguan M, Tapchaisri P, Setasubun P. Monoclonal antibody to a diagnostic Mr 24,000 antigen of *Gnathostoma spinigerum*. *Int J Parasitol* 1991;21:735-8.

Dharmkrong-at A, Migasena S, Suntharasamai P, Bunnag D, Priwan R, Sirisinha S. Enzyme-linked immunosorbent assay for detection of antibody to *Gnathostoma* antigen in patients with intermittent cutaneous migratory swelling. *J Clin Microbiol* 1986;23:847-51.

Daengsvang S. A monograph on the genus *Gnathostoma* and gnathostomiasis in Thailand. Tokyo: SEAMIC/IMFJ, 1980.

Kulkaew K, Chamniyanta R. An analysis of IgE-specific protein(s) by Western blot. Health Sciences Research, Faculty of Allied Health Sciences, Chulalongkorn University, 2000.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-phenol reagents. *J Biol Chem* 1951;193:265-75.

Nopparatana C, Tapchaisri P, Setasubun P, Chaicumpa W, Dekumyoy P. Antibody response in human gnathostomiasis. *Southeast Asian J Trop Med Public Health* 1988;19:219-24.

Tapchaisri P, Nopparatana C, Chaicumpa W, Setasuban P. Specific antigen of *Gnathostoma spinigerum* for immunodiagnosis of human gnathostomiasis. *Int J Parasitol* 1991;21:315-9.