Effects of Immunization with Purified Specific Proteins on the Formation of Secondary Hydatid Cysts in Mice

A. Burgu1, O. Sarımehmetoğlu1, B. Gonenç1*, H. Oge1, G. Sahin1, S. Aypak2

1University of Ankara, Faculty of Veterinary Medicine, Department of Parasitology, Ankara, Türkiye
2University of Adnan Menderes, Faculty of Veterinary Medicine, Department of Parasitology, Aydın, Turkey

*Corresponding author

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Introduction

Hydatid cyst, parasites a variety of mammals in a large series of anatomical sites such as the lungs, liver, heart and brain. The disease is a major public health and has economic importance in several countries and regions (3, 5, 7).

The purifications of proteins can be done by separating according to their molecular weights and isoelectrical points (1). Proteins can be separated according to their isoelectrical points by using Rotofor® device. Before starting purification procedure, proteins being used for developing vaccines and diagnostic kits must be determined whether they are specific or immunoreactive. The most reliable methods for specific proteins are SDS-PAGE and western blotting (1, 9). In this research purified polypeptides of 8, 68, and 116 kDa which are known as specific were given to experimental and control groups of mice to investigate the preventing effect for the development of hydatid cysts.

Materials and Methods

Antigens previously prepared hydatid cysts taken from sheep liver were separated by SDS-PAGE and analyzed. To get previously determined proteins 8, 68, and 116 kDa, a gel (5% stacking+15% separating) was prepared. After separation by SDS-PAGE, a total of 23 protein bands were observed including specific proteins of 8, 68, and 116 kDa. After adding 50 ml of total antigen and 2% ampholyte to Rotofor® cell device, protein purifying procedure was started. Following that, the proteins mentioned above and the verification was done by comparing to the protein standards on the gel. In this point. By using this feature purified proteins can be separated according to their molecular weights and isoelectrical points. Proteins have isoelectrical values (pI) at which net electrical charge is zero and they precipitate at this point. Using this feature purified proteins from viral, bacterial, and parasitic diseases were used for some vaccination and diagnostic kit studies. (1, 2, 4, 6, 8). In our study the proteins were purified with a procedure similar as mentioned above and the verification was done by comparing to the protein standards on the gel. In some studies (1, 2, 8) proteins were purified partially according to their molecular weights by using Gel eluter and Prep-cell devices. In our study, purification was accomplished by using Rotofor® cell device according to isoelectrical points. The main idea and aim in this study was to purify the parasitic proteins which are at different molecular weights to developers vaccines. It is concluded that obtained results can light the way for appropriate further studies for hydatidosis. In next step of this study, the characterization of the studied proteins and determination biochemical and immunologic details. Also the evaluation of immunization will be tried.

Results and Discussion

Cyst Development: The difference in the number of mice that developed cyst between the 116 kDa immunization group and control were statistically significant (p<0.05) The cyst development was 9.3 times higher in control group than that of the immunized group with 116 kDa. The immunized groups of 8 and 68 kDa polypeptides didn’t have significant difference when compared to the control groups (p>0.05).

Reduction in the number of cysts by immunization: The decrease in the number of cysts by immunization when compared to controls were 10.61%, 9.8% and 4.36 for 116, 8, 68 kDa groups respectively.

Statistical analysis: The results from both experimental and control group were compared and statistically analyzed. Chi-Square test was performed for statistical analysis.
References
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Fig. 1 8, 68 and 116 kDa specific proteins were purified in this study.

Fig. 2 After performing Western blotting, it was determined that all the mice were immunized successfully. Paranchimatous hemorrhaging stemmed from taking biopsy material with a “double spoon” biopsy forceps.