Proteolytic Activity of Corneal Amoeba and Effects of Protease Inhibitors

Carvalho, F.R.S.[1], Carrijo-Carvalho, L.C.[2], Chudzinski-Tavassi, A.M.[2], Foronda, A.S.[1], Freitas, D.[1]

[1]Lab. of Ocular Protozoology,UNIFESP, São Paulo, SP, Brazil; [2]Lab. Biochemistry and Biophysics, Butantan Institute, São Paulo, SP, Brazil; Email: frscarvalho@unifesp.br

Introduction: Corneal infections, designed as keratitis, due to free-living amoebae have potentially devastating consequences. If not successfully treated, it can progress into the eye, causing corneal ulcer, loss of visual acuity and eventually blindness. In general, *Acanthamoeba* spp is the main etiological agent of amoebic keratitis. Extracellular proteases are the major virulence factors, which are commonly involved in adhesion and invasion of corneal tissue by trophozoites. **Objectives:** To evaluate the proteolytic activity of proteins secreted by a free-living amoeba isolated from a patient with severe keratitis, which clinical symptoms followed to corneal transplantation. Proteases in the culture supernatant were characterized with regard to sensitivity to specific protease inhibitors, and their molecular weight distribution in substrate electrophoresis gels. **Results:** A sample of 0.5µg of soluble proteins, from approximately 0.1 mg/ml secreted by the clinical amoeba strain in the culture medium, was analyzed by SDS-PAGE-gelatin, after dialysis in 0.85% NaCl. After 4-hour incubation, the zymographic profile observed consisted of three major bands of high intensity. The estimated molecular mass of each protein band was 141kDa, 100kDa and 69kDa, respectively. The proteolytic activity was totally inhibited by phenylmethylsulfonylfluoride (PMSF), which is inhibitor of serine proteases. Other protease inhibitors tested, such as E-64; EDTA; 1, 10-phenanthroline; pepstatin and soybean trypsin inhibitor (SBTI) showed no inhibitory effect. **Conclusions:** Serine proteases secreted by this atypical amoeba strain showed intense activity at a lower concentration, which could explain the severity of clinical symptoms observed in the patient. The study of secreted proteases by free-living amoebae could provide new perspectives in the therapeutic procedures.

Key words: proteases, keratitis, free-living amoeba.
Financial Support: FAPESP, CAPES and FADA/UNIFESP.