BIOCHEMICAL CHARACTERIZATION OF TRYPANOSOMATID METALLOPROTEINASES

(LEISHMANIA SPP., T. CRUZI E T. B. BRUCEI)

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Leishmania spp., Trypanosoma cruzi and Trypanosoma brucei are etiologic agents of leishmaniasis, Chagas disease and sleeping sickness, respectively. These diseases are among the 17 neglected tropical diseases (NTD) recognized by the WHO. Despite the efforts to control or even eradicate these diseases, currently NTD are endemic in 149 countries. Taken into account the above considerations, we aimed to biochemically characterize metalloproteinases, a set of enzymes common to Leishmania and Trypanosome that are involved in several aspects of parasite virulence. The enzymatic profile of extracts of L. infantum, L. amazonensis, L. guyanensis and L. shawi cultured promastigotes, of T. cruzi (strains Y, Bolívia, QMM5) epimastigotes and trypomastigotes and, of T. b. brucei trypomastigotes were evaluated in collagen, gelatin and casein gels. Minimum inhibitory concentrations (MIC) were estimated in the presence of 1,10-phenanthroline, a specific inhibitor of metalloproteinase. To evaluate the metalloproteolytic activity of each extract, an enzymatic fluorescent assay was optimized to quantify gelatinolytic activity. All extracts were enzymatically active in different substrates and each parasite evidenced a specific zymographic profile. An enzyme with a molecular mass of 50-80 kDa, possibly the 63 kDa glycoprotein, was detected in Leishmania species. Bands of approximately 40 and 20 kDa with enzymatic activity were observed in extracts of T. cruzi and T. b. brucei, respectively. The declining of total enzymatic activity in the presence of increasing concentrations of 1,10-phenanthroline confirmed the metalloproteolytic nature of Leishmania and T. cruzi enzymes. Furthermore, metalloproteolytic profile of L. shawi was different from other Leishmania species and each strain of T. cruzi exhibited a characteristic metalloproteolytic pattern. From all the studied parasites, T. b. brucei presented the lowest enzymatic reaction rate. Given the biological importance of metalloproteases, clarifying their role in the establishment of infection and in the survival of trypanosomatids may constitute an opportunity to identify new targets and, consequently, for the design and development of new therapeutic and prophylactic strategies.

Key words: Trypanosomatids, metalloproteinases, neglected tropical diseases