MALDI-MS method for detection of canine visceral leishmaniasis through differential expression of metabolites in blood plasma

Fábio N. dos Santos[1] (PG), Paulo R. R. Mesquita[2,3] (PG), Jairo T. Magalhães Júnior[2,3] (PQ), Frederico M. Rodrigues[3] (PQ), Manuela S. Solcà[4] (PG), Deborah B. M. Fraga[4] (PQ), Patrícia S. T. Veras[4] (PQ), Stella M. B. Melo[5] (PQ), Marcos N. Eberlin[1,*] (PQ).
***eberlin@iqm.unicamp.br**

[1]ThoMSon Mass Spectrometry Laboratory, University of Campinas, Institute of Chemistry, Campinas, São Paulo, 13083-970, Brazil; [2]Federal University of Bahia, Campus Ondina, Institute of Chemistry, 40170290, Salvador, Bahia, Brazil; [3] Empresa Baiana de Desenvolvimento Agrícola S.A, 40170-110, Salvador, Bahia, Brazil; [4]Oswaldo Cruz Foundation, Salvador, Bahia, Brazil; [5]Federal University of Bahia, Campus Ondina, School of Veterinary Medicine, 40170290, Salvador, Bahia, Brazil.

Keywords: MALDI-MS, canine leishmaniasis, dogs, metabolites, blood plasma.

Abstract

Herein we show that MALDI-MS profiles of proteins and lipids can be used for the differentiation between healthy and leishmania infected dogs.

Introduction

A major limitation of the effective control of canine visceral leishmaniasis (CVL) is the lack of an early diagnostic method that is effective and reliable in detecting infected dogs. Especially animals with a subclinical infection of the disease are difficult to detect. Therefore, an early and accurate diagnostic method to differentiate between healthy and infected dogs is essential to control this disease and thereby preventing the further unnecessary spreading of the parasite. Because of their low sensitivity, traditional CVL detection methods require the presence of a relatively high parasite concentration. This results in a delayed diagnosis after infection. Parasite infection causes a change in the metabolic blood profile of the host. MALDI-MS can detect small alterations at low concentrations of these metabolites in blood plasma.

Results and Discussion

All 28 samples collected were analyzed by ELISA and submitted to a parasitological test. 11 dogs were diagnosed as healthy and 17 as infected. Protein extraction was performed by mixing 200 µL of plasma with 50 µL of 80% TFA for 30 min at 1000 rpm. 100 µL of Milli-Q water and 150 µL of ACN were added, followed by rigorous mixing. The mixture was then centrifuged at 13000×g for 5 min. 1 µL supernatant was spotted onto a MALDI plate and air dried. 1 µL of CHCA matrix solution (50 mg mL^{-1} in ACN:H_2O (1:1, v/v) with 0.1% TFA) was then added. Lipid extraction was performed on 300 µL of plasma, according to the Bligh-Dyer method. 1 µL of the lower phase was added on the MALDI plate followed by addition of 1 µL MALDI matrix (DHB). MALDI analysis was performed on a Bruker Autoflex III MALDI–TOF/TOF. The results show clear differences in protein (Figure 1) and lipid (Figure 2) profiles of healthy (top) and infected (bottom) dogs. The mass spectrum of proteins is most complex in healthy dogs as can be seen in the *m/z* 4–6 kDa region. In contrast, the mass spectrum of lipids is more complex in infected dogs, as can be seen in the *m/z* 700-900 region. These masses are attributed to phospholipids.

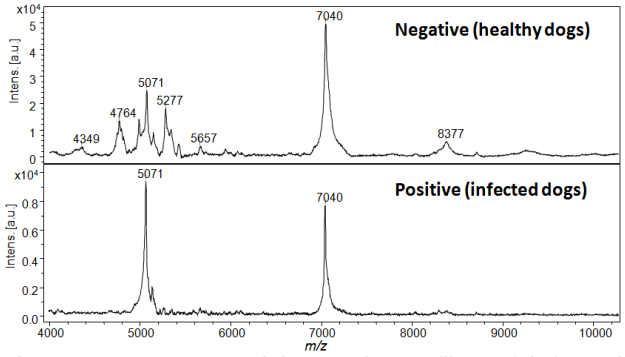

Figure 1. MALDI-MS(+) protein profiles of infected and healthy dogs plasma samples.

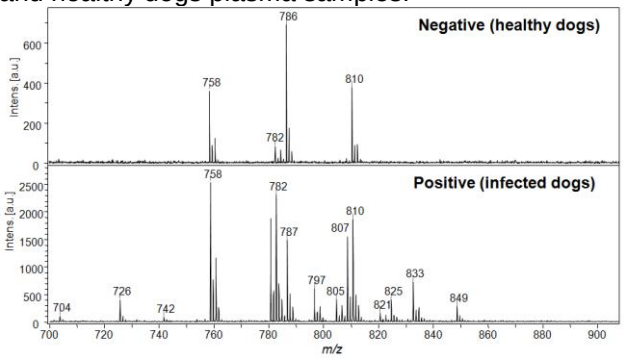

Figure 2. MALDI-MS(+) lipid profiles of infected and healthy dogs plasma samples.

Conclusions

MALDI-MS is a promising method for detecting canine visceral leishmaniasis infection through metabolite alterations of its host.

Acknowledgments

CNPq, FAPESP and CAPES are acknowledged for their financial support.

[1] Carnielli, J.B.T; Andrade, H.M.; Pires, S.F; Chapeaurouge, A.D; Perales, J; Monti-Rocha, R; Lemos, E.M. *J. Proteomics.* **2014**, 108, 198.