TITLE: VERAPAMIL DECREASES *Mycobacterium leprae* VIABILITY IN HUMAN MACROPHAGES

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ABSTRACT:

Leprosy is a chronic infectious disease that can manifest itself in different clinical forms. While in multibacillary lepromatous (LL) skin lesion cells there are highly parasitized macrophages, few or rare bacilli are found in paucibacillary tuberculoid (TT/BT) lesion cells. Previously, we demonstrated a positive correlation between the control of bacillary load in tuberculoid macrophages and autophagy and that in multibacillary lepromatous form bacterial persistence is associated with a blockade in beclin 1– mediated autophagy induced by viable *M. leprae*. Although the existence of a multidrugtherapy (MDT) strategy to leprosy control, treatment is longer and the emergence of leprosy reactions and relapses demonstrate the need for inclusion of more effective drugs in MDT. Since our previous data suggested that autophagy might be a target for the development of new therapeutic strategies of disease control, the aim of this study was to investigate if Verapamil, a drug with pro-autophagic properties, could control the bacillary load in human macrophages infected with *M. leprae*. For this purpose, we evaluated the effect of Verapamil in the viability of human macrophages. Peripheral blood mononuclear cells (PBMC) were obtained from healthy volunteers. The recovered monocytes were cultured with 50 ng/mL M-CSF for 6 days at 37^0C/5%CO_2 to obtain macrophages Mφ2. After 72 hours of exposure MTT colorimetric method was performed. The values obtained showed no significant difference in both doses and at different exposure times (24, 48 and 72 hours) when compared to the control, showing that there was no indication of cytotoxic effect. We then evaluated the ability of the drug Verapamil and its analogue Norverapamil to induce the death of *M. leprae*. To perform this assay, the multiplicities of infection (MOI) of 20:1 were used in relation to the number of cells. The intracellular viability of *M. leprae* in the infection experiments was estimated by real-time PCR detection of the 16S RNAr levels of the bacterium. After 48h the intracellular viability of *M. leprae* was reduced in the presence of Verapamil and its analogue Norverapamil in macrophages. Although preliminary, these data suggest that the drug Verapamil may exert antimicrobial activity. Thus, it is believed that the use of drugs with pro-autophagic action, together with MDT, may contribute to reduce bacillary load in leprosy patients.

Keywords: Autophagy, Leprosy, Verapamil

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