TITLE: Potential Synergistic Effect of ATP with First Line Drugs with applicability to Improve the Treatment of MDR or XDR Tuberculosis

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ABSTRACT:

Tuberculosis treatment is effective with the use of proper antibiotics, although the number of drug resistant cases has increased. In 2016, 10.4 million cases were reported worldwide, from these 600.000 had drug resistance, with strong incidence in the population with the lowest income. In this work we validated, *in vitro*, a hypothesis published by our group (Soares-Bezerra *et al.* 2015) using high content imaging and spectrophotometric techniques. Our protocol was based on primary macrophages activated from human peripheral blood mononuclear cells (PBMC) Infected, or not with BCG expressing GFP, using high content imaging and spectrophotometric techniques as tools for analysis. In this study, we observed the action of extracellular ATP on the infection of primary macrophage activated from PBMC cells with BCG-GFP, in a M.O.I of 5:1. The primary macrophages, when treated with each antibiotic [1µg/mL] for 24 h, together or alone, submitted or not to an extra treatment with ATP [5mM] for twenty more minutes, demonstrate that, the treatments with antibiotics plus ATP lead to an inhibition rate of 70% of the infection regarding those without ATP. However, the treatment with ATP [5mM] alone inhibited 40%. The same results were found in the high content imaging analysis, using infected cells treated with same protocol described above, subsequently fixed with a PFA 4% solution and stained with DAPI [0,4 µg/mL] to access the cell nucleus, and DiR [1 µM/ml] to access the cell membrane. In order to investigate the immune profile from PBMC human primary macrophages after the treatments, we evaluated an ELISA assay for IL-1β, which showed a significant increase between 70% ~ 80% on the release of this cytokine, in the treatments with FLD plus ATP, regarding those without ATP. However, when compared with treatment with ATP [5mM] alone the increase rate was 60%. In conclusion, we accessed cytotoxicity of these applied treatment protocols used in this work, no significant toxicity was found when compared with untreated control. All experimental protocols were approved (protocol nº 535-09) by the Institutional Ethical Committee. Conclusions: Taken together, our results pointed to the potential synergy effect of ATP on improving the action of FLD currently used in TB treatment, which might be useful to treat the multi-drug-resistant cases of TB.

Keywords: Tuberculose, P2X7R, ATP, MDR, XDR.

Development Agency: IOC, CNPq e Faperj.