TITLE: LAPACHOL AND β-LAPACHONE DO NOT ACT AS EFFLUX PUMP INHIBITORS IN *Mycobacterium tuberculosis*

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ABSTRACT: The control of tuberculosis (TB) represents a public health challenge, mainly due to the current treatment conditions, which consists in the administration of drugs over a long period, causing serious side effects. In this context, adherence to treatment is difficult, with default rates of almost 30%, generating consequences such as the emergence of resistant isolates to existing drugs, treatment failure and increased transmission. The study of new compounds with anti-TB action is important to change this scenario. In previous study, lapachol and β-lapachone have shown great action against *Mycobacterium tuberculosis* and search that help elucidate the mechanism of action of these compounds, as the action in efflux pump (EP), are important. The aim of this study was to evaluate if lapachol and its natural derivative, β-lapachone, act as efflux pump inhibitors (EPIs). Ethidium bromide efflux assay (EtBrE) and Ethidium bromide accumulation assay (EtBrA) were performed. First, a *M. tuberculosis* H_{37}Rv culture in 7H9 medium with oleic acid, albumin, dextrose and catalase (OADC) was growing at 35 ºC for 15-21 days. The culture was centrifuged at 2880 g for 10 min. The pellet was washed, resuspended in phosphate-buffered saline (PBS) and adjust the OD_{600} to 0.4 with PBS 0.05% of tween 80. Especially for EtBrE, the bacterial suspension was in contact to EtBr for 60 min at 37 ºC to achieve steady state and then it was washed and adjust the OD_{600} to 0.4. For both assays, the bacterial suspension was transferred to microplate wells containing verapamil (VP) or lapachol or β-lapachone in concentration equivalent to ½ and ¼ of minimum inhibitory concentration (MIC). The reading was performed immediately and after 15 minutes of incubation at 25 °C for EtBrE and EtBrA, respectively. VP is a known inhibitory efflux pump that was used as standard parameter. Relative fluorescence to EtBr-loaded cells was acquired every 3 min for 60 min at 35 ºC in a VICTOR2 D fluorometer (530/25 nm and 590/20 nm as the excitation and detection wavelengths, respectively). Fluorescence was determined in the absence of VP as a reference assay. Lapachol and β-lapachone have not shown the ability to prevent the accumulation and efflux of EtBr. Therefore, this study may infer that the activity of these compounds in *Mycobacterium tuberculosis* H_{37}Rv has no involvement with bacterial efflux pumps. Additional studies to delineate the mechanism of these potencial compounds for TB treatment should be conducted.

Keywords: tuberculosis, efflux pump inhibitor, ethidium bromide, lapachol, β-lapachone