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Title: Evaluation of stimulatory effect of Naloxone on Th1 and proinflammatory cytokines level in challenge of PBMC with heat killed of Brucella melitensis Rev.1 in vitro

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Abstract: Brucellosis is a major infectious disease afflicting humans and a wide range of domesticated animals worldwide. As intracellular organisms, protection against infection requires cell-mediated immunity, which includes CD4+ and CD8+ T lymphocytes and Th1-type cytokines such as IFN-γ and IL-12. Previous studies have suggested that Naloxone (an opioid antagonist) can shift the immune response toward a Th1 pattern. Thus purpose of this study is evaluation of stimulatory effect of naloxone on Th1 and proinflammatory cytokines level in challenge of PBMC with heat killed of Brucella melitensis Rev.1 in vitro.

Methods: After separation of PBMC from 3 healthy volunteer, 100µl of diluted cell suspensions (1×106 cells per milliliters in RPMI 1640 supplemented with 10% FCS) were dispensed into 96-well culture plates. The dilution of 1, 10 and 100µg/ml of naloxone and also 6×104 heat killed Brucella melitensis Rev.1 was added to each well and the volume was adjusted to 200µl. After incubating for 48h at 37°C in 5% CO2, the cell proliferation was determined by MTT assay. In 2, 6, 24 and 48h after incubation period, supernatants was removed and stored at -70°C. The concentration of secreted IFN-γ, IL-12, IL-6, IL-8 and IL-10 levels in the supernatants was measured with ELISA method.

Results: The mean of the cell proliferation in wells that contains three dose of naloxone and positive and negative control in 2, 6 and 24h was not significant. The result of ELISA showed that the mean of IFN-γ, IL-12, IL-6, IL-8 levels in wells that contain 1, 10 and 100µg/ml of naloxone were increased respectively but in comparison with positive and negative control was not significant.

Conclusion: This study showed that stimulatory effect of naloxone with heat killed Brucella melitensis Rev.1 on PBMC in vitro was low and need to further studies in vivo.

Keywords: Brucella melitensis, Naloxone, PBMC, Stimulatory Effect

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