Pentoxyphylline inhibits M1 polarization and favors M2 of murine macrophages treated with TLR4 agonist.

María Cecilia Montero¹ MVD MSc, Julia Guerrero¹,² MD PhD

¹ Laboratorio de Inmunomodulación Neuro- Endocrina del Programa de Fisiología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina Universidad de Chile.
² Unidad de Cuidados Intensivos, Clínica Alemana de Santiago

Presentado en Congreso Europeo de Medicina Intensiva (ESICM), 20 al 24 de octubre, París, Francia.

Introduction
Pentoxyphylline (PTX) is a phosphodiesterase inhibitor that increases intracellular cAMP. Recently, PTX has been recognized as a pharmacological modulator of inflammation that may improve outcomes in septic patients (1). In neonatal sepsis, the use of PTX as an adjunct to antibiotics therapy decreased all-cause mortality and the length of hospital stays, without significant adverse effects (2). Authors had proposed its effect may be mediated by adenosine-dependent pathways for polymorphonuclear leukocytes and T cells (3). Results of studies in whole new born umbilical blood showed that PTX inhibited the inflammatory cytokine response induced by Toll-like receptors (TLR) agonists, TLR4, TLR7 and TLR8 (4). Considering that peripheral blood macrophages can reprogram their phenotype and orchestrate the inflammatory response, we tested if PTX modifies inflammatory cytokines profile in response to a TLR-4 agonist in a macrophage cell line.

Objective: To assess if PTX modulates macrophage TLR4-dependent polarization in vitro.

Methods: Murine macrophages (Raw 264.7 cells) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) (control), or in the presence of lipopolysaccharide (LPS, Sigma - Aldrich Chemie®, Germany) 25ng/mL, LPS + PTX (dose-response curve; Sigma-Aldrich Chemie®, Germany) or PTX alone. We analyzed cell viability by trypan blue exclusion assay and the time-course of changes in TNF-alpha and IL-10 mRNA content (as surrogate markers of M1 or M2 polarization, respectively) in the presence or absence of LPS, PTX or LPS plus PTX by real-time qPCR.
**Results:** PTX (100-250-500 and 1000μg/ml by 1-1.5 or 2h) had no effect on cell viability or TNF-alpha mRNA abundance. LPS induced TNF-alpha mRNA (3 times of control level; n=4, p<0.05) and PTX inhibited TNF-alpha mRNA induced by LPS (p<0.05). The inhibitory effect of PTX on LPS-dependent TNF-alpha mRNA induction was greater with 250 μg/ml and over 1h of exposition. Interestingly, after 1.5 h of exposure, PTX+ LPS significantly increased the cellular content of IL-10 mRNA.

**Conclusions:** PTX modulates macrophage inflammatory cytokines response induced by a TLR-4 agonist. We postulate that PTX modifies the polarization profile of macrophages (M1 to M2). In the context of TLR4 activation, the increase of cAMP induced by PTX may activates PKA, stabilize the IκB inhibitor and suppress NF-κB nuclear translocation. Besides, cAMP-PKA-dependent CREB phosphorylation may explain the induction of IL-10 mRNA at a transcriptional level.

**References**


**GRANT ACKNOWLEDGMENT:** this study was financed with own resources.