World Academy of Science, Engineering and Technology
International Journal of Pharmacological and Pharmaceutical Sciences
Vol:10, No:09, 2016

Phorbol 12-Myristate 13-Acetate (PMA)-Differentiated THP-1 Monocytes as a Validated Microglial-Like Model in Vitro

Authors : Amelia J. McFarland, Andrew K. Davey, Shailendra Anoopkumar-Dukie

Abstract : Microglia are the resident macrophage population of the central nervous system (CNS), contributing to both innate and adaptive immune response, and brain homeostasis. Activation of microglia occurs in response to a multitude of pathogenic stimuli in their microenvironment; this induces morphological and functional changes, resulting in a state of acute neuroinflammation which facilitates injury resolution. Adequate microglial function is essential for the health of the neuroparenchyma, with microglial dysfunction implicated in numerous CNS pathologies. Given the critical role that these macrophage-derived cells play in CNS homeostasis, there is a high demand for microglial models suitable for use in neuroscience research. The isolation of primary human microglia, however, is both difficult and costly, with microglial activation an unwanted but inevitable result of the extraction process. Consequently, there is a need for the development of alternative experimental models which exhibit morphological, biochemical and functional characteristics of human microglia without the difficulties associated with primary cell lines. In this study, our aim was to evaluate whether THP-1 human peripheral blood monocytes would display microglial-like qualities following an induced differentiation, and, therefore, be suitable for use as surrogate microglia. To achieve this aim, THP-1 human peripheral blood monocytes from acute monocytic leukaemia were differentiated with a range of phorbol 12-myristate 13-acetate (PMA) concentrations (50-200 nM) using two different protocols: a 5-day continuous PMA exposure or a 3-day continuous PMA exposure followed by a 5-day rest in normal media. In each protocol and at each PMA concentration, microglial-like cell morphology was assessed through crystal violet staining and the presence of CD-14 microglial / macrophage cell surface marker. Lipopolysaccharide (LPS) from Escherichia coli (055: B5) was then added at a range of concentrations from 0-10 mcg/mL to activate the PMA-differentiated THP-1 cells. Functional microglial-like behavior was evaluated by quantifying the release of prostaglandin (PG)-E2 and pro-inflammatory cytokines interleukin (IL)-1β and tumour necrosis factor (TNF)-α using mediator-specific ELISAs. Furthermore, production of global reactive oxygen species (ROS) and nitric oxide (NO) were determined fluorometrically using dichlorodihydrofluorescein diacetate (DCFH-DA) and diaminofluorescein diacetate (DAF-2-DA) respectively. Following PMA-treatment, it was observed both differentiation protocols resulted in cells displaying distinct microglial morphology from 10 nM PMA. Activation of differentiated cells using LPS significantly augmented IL-1β, TNF-α and PGE2 release at all LPS concentrations under both differentiation protocols. Similarly, a significant increase in DCFH-DA and DAF-2-DA fluorescence was observed, indicative of increases in ROS and NO production. For all endpoints, the 5-day continuous PMA treatment protocol yielded significantly higher mediator levels than the 3-day treatment and 5-day rest protocol. Our data, therefore, suggests that the differentiation of THP-1 human monocyte cells with PMA yields a homogenous microglial-like population which, following stimulation with LPS, undergo activation to release a range of pro-inflammatory mediators associated with microglial activation. Thus, the use of PMA-differentiated THP-1 cells represents a suitable microglial model for in vitro research.

Keywords : differentiation, lipopolysaccharide, microglia, monocyte, neuroscience, THP-1
Conference Title : ICPPS 2016 : International Conference on Pharmacy and Pharmaceutical Sciences
Conference Location : Singapore, Singapore
Conference Dates : September 08-09, 2016