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Artesunate inhibits prostaglandin E_2 (PGE_2) production in LPS + IFN-γ activated BV-2 microglia cells.

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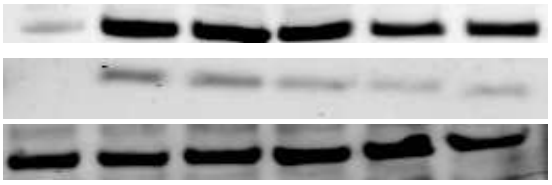
Neurodegenerative disorders, including Alzheimer's disease (AD), associated with the aging process have been shown to be linked with microglia activation and inflammatory processes [1]. Microglial activation releases various mediators including prostaglandin E_2 (PGE_2). The transcription factor, nuclear factor kappa B (NF-κB) has been shown to control inflammatory responses in microglia cells. Artesunate has been reported to have anti-inflammatory properties in experimental colitis [2]. In this study the effects of artesunate were investigated in BV-2 stimulated microglia cells.BV-2 cells were pre-treated with artesunate (0.5-4μM) for 30min and stimulated with LPS (1μg/ml) + IFN-γ (5ng/ml) for 24h.Thereafter, supernatants were analysed for PGE_2 production. COX-2 and m-PGES1 protein expressions were also measured in cell lysates by western blot. The effect of artesunate on the transactivation of NF-κB was assessed in transfected HEK293 cells using luciferase reporter gene assay. Values of all experiments were represented as a mean ± SEM of at least 3 experiments. Values were compared using one-way ANOVA followed by a post-hoc Student Newman-Keuls test. Pre-treatment with artesunate significantly ($p<0.05$) inhibited LPS + IFN-γ-induced PGE_2 production in a dose-dependent manner with an IC50 value of 3.2μM. Artesunate (0.5-4μM) suppressed COX-2 protein expression significantly ($p<0.05$) in LPS + IFN-γ stimulated BV-2 cells. At 4μM, artesunate significantly suppressed COX-2 protein expression by 52% compared to the LPS+IFN-γ control (IC50= 3.8μM) (Fig.1). In addition, artesunate decreased m-PGES1 protein expression significantly ($p<0.05$) with 25% decrease at 4μM compared to LPS+IFN-γ control (IC50= 0.62μM) (Fig.1). However, artesunate significantly ($p<0.05$) inhibited NF-κB transactivation in transfected HEK293 cells with an inhibition of 40% compared to the positive control at all concentrations (0.5-4μM).

These data have demonstrated that artesunate inhibits PGE_2 through interference with COX-2 and m-PGES1 protein expressions in BV-2 activated microglia. Artesunate also inhibited NF-κB transactivation in transfected HEK293. These results suggest that artesunate blocks the NF-κB signalling by inhibiting transactivation of NF-κB and its downstream signals PGE_2, COX-2 and m-PGES1.



LPS (1μg/ml) + IFN-γ (5ng/ml)	-	+	+	+	+	+
Artesunate (μM)	-	-	0.5	1	2	4

Figure 1: Artesunate inhibits COX-2 and m-PGES1 protein expression in LPS-activated BV-2 microglia cells

References

[1] Lee YJ, Choi DY, Choi IS, *et al.* (2011) J Neuroinflammation 8: 132 - 1316.

[2] Yang Z, Ding J, Yang C, et al. (2012) Curr Med Chem 19(26): 4541 – 4551.