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# Effect of inflammation-related compounds on ABCB1 efflux transporter expression and activity in an *in vitro* porcine brain endothelial cell model

The ATP-binding cassette efflux transporter ABCB1 (P-glycoprotein) is highly expressed in endothelial cells of the blood-brain barrier (BBB) and can significantly affect central nervous system drug disposition (1). Evidence suggests that ABCB1 expression and activity could be regulated through activation of nuclear receptors and transcription factors of signalling pathways important in development and progression of chronic inflammatory diseases (2). Recently, it has also been proposed that modulation of ABCB1 activity can occur by modifications of plasma membrane lipid composition by compounds including long chain n-3 and n-6 polyunsaturated fatty acids (PUFAs) obtained from the diet and synthesised endogenously in both physiological and pathological conditions involving inflammation (3). The aim of this study was to determine the actions of pro- and anti-inflammatory compounds on ABCB1 expression and activity.

Primary cultures of porcine brain endothelial cells (PBECs) were treated for 24 h under low-serum conditions with the anti-inflammatory compounds hydrocortisone (HC), dexamethasone (DX) and interleukin-1 receptor antagonist (IL-1RA), the pro-inflammatory cytokine interleukin-1 beta (IL-1β) and the fatty acids arachidonic acid (AA), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Furthermore, PBECs were treated with combinations of the above compounds. ABCB1 activity was assessed by means of the calcein-AM fluorescent uptake assay, whilst ABCB1 expression was determined by western blotting. Data are expressed as mean ± standard deviation and the statistical analysis was performed with the Mann-Whitney test.

Treatment with DX 10 μM and HC 10 μM significantly decreased intracellular calcein accumulation to 75.9 ± 9.2, 80.1 ± 8.7 ($P < 0.0001$ n=3) of the control, suggesting increased ABCB1 activity, and increased ABCB1 protein expression to 187 ± 65 (n=5) and 152 ± 35 (n=6) % of the control ($P < 0.05$), respectively. Treatment with IL-1β 1 ng/ml significantly decreased intracellular calcein accumulation to 74.9 ± 9.1 ($P < 0.001$ n=3) and increased ABCB1 protein expression 174 ± 31 % ($P < 0.01$, n=8) of the control. Use of IL-1RA as co-treatment, significantly counteracted the effects of IL-1β on both ABCB1 activity ($P < 0.0001$, n=3) and expression ($P < 0.05$, n=5). Treatment with the fatty acids AA (3 μM), DHA (5 μM) and EPA (3 μM) significantly increased the intracellular accumulation of calcein to 156.8 ± 33.9, 150.3 ± 37.3 and 147.8 ± 22.1 % ($P < 0.0001$, n=3), respectively, of the control. However, no change in ABCB1 expression was observed (n=4). The HC-, DX- and IL-1β-induced ABCB1 activity was significantly counteracted by PUFAs (n=3), which did not affect the up-regulation of ABCB1 expression mediated by the former compounds (n=3).

In summary, pro-inflammatory and anti-inflammatory compounds can modulate ABCB1 expression and activity in PBECs, with overlapping and antagonising effects. The actions of HC and DX are consistent with previous reports and can be attributed to activation of the glucocorticoid receptor (4). However, up-regulation of ABCB1 expression and activity by IL-1β is through activation of the IL-1 receptor. The lack of effect of PUFAs on ABCB1 expression, despite the increase in intracellular calcein accumulation suggests either reduced activity of the transporter or enhanced permeability of calcein-AM across the plasma membrane.

(1) Miller DS (2010), *Trends PharmacolSci* 31: 246–254

(2) Qosa*et al*. (2015),*Brain Res* 1628: 298-316

(3) Gelsomino*et al*. (2013), *Mol Cancer* 12: 137-157

(4) Iqbal*et al*. (2011), Endocrinology, 152(3): 1067-1079