Abstract: P714

2-AG impacts on endothelial cell activation and endothelial cell viability in vitro and impairs endothelial repair in vivo

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Topic(s):
Basic Science - Vascular Biology and Physiology

Citation:
Background: The endocannabinoid (eCB) 2-arachidonoylglycerol (2-AG) is a known modulator of inflammation and few studies have addressed its influence on myeloid cells in the context of atherogenesis. However, the impact of 2-AG on endothelial cell function has not been studied before.

Methods: Endothelial repair was studied in two treatment groups of wildtype mice following electrical denudation of the common carotid artery at a length of 3000 µm. One group received the monoacylglycerol lipase (MAGL)-inhibitor JZL184 [5 mg/kg i.p.], which impairs 2-AG degradation and thus causes elevated 2-AG levels, the other group received vehicle. The residual endothelial gap at five days in either group was visualized by Evan’s blue staining. In vitro, the effect of 2-AG on human coronary artery endothelial cell (HCAEC) viability was assessed by an XTT-based assay. Endothelial activation was studied by an adhesion assay of THP-1 monocytes to 2-AG-preconditioned HCAEC. HCAEC migration, ROS-production, expression of NADPH oxidases, and secretion of inflammatory cytokines were assessed by Boyden chamber, qPCR, and colorimetric assays.

Results: Treatment with JZL184 produced a significant increase in 2-AG levels and impaired reendothelialisation in wildtype mice following electrical injury of the common carotid artery. The residual denudation at 5 days yielded 2291 ± 286 µm in JZL184-treated animals vs. 1505 ± 223 µm in vehicle treated controls (n = 18-19; p < 0.05). In vitro, JZL184 significantly reduced viability of HCAEC at 24 hours (0.31 ± 0.10 vs. 1.00 ± 0.08; n = 3; p < 0.01). Finally, 2-AG promoted HCAEC activation resulting in a significant increase in THP-1 monocyte adhesion to HCAEC following pre-treatment of HCAEC with 2-AG (0.17 ± 0.03 THP-1 cells per HCAEC vs. 0.07 ± 0.01 THP-1 cells per HCAEC; n = 3; p < 0.05). Besides, HCAEC migration, ROS-production, expression of NADPH oxidases and secretion of inflammatory cytokines were unaffected by 2-AG.

Conclusion: Elevated 2-AG levels appear to hamper endothelial repair and to promote HCAEC activation and cell death. Our data suggest that besides its influence on myeloid cells, 2-AG is also adverse to endothelial integrity which might promote early atherosclerotic lesion formation. Thus, decreasing vascular 2-AG levels might represent a promising therapeutic strategy for the prevention of atherosclerosis and coronary heart disease.