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学位論文名	PPARα and GR Differentially Down-Regulate the Expression of Nuclear Factor-κB-Responsive Genes in Vascular Endothelial Cells （血管内皮細胞においてPPARαとGRはNF-κB誘導性遺伝子の発現に対して異なる抑制作用を示す）
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論文内容の要旨

〔目　的〕

Up-regulation of cytokines and cell adhesion molecules by proinflammatory cytokines in vascular endothelial cells (ECs) represents part of the inflammatory process affecting vascular walls. To inhibit the expression of these inflammatory molecules is therefore one of the targets of anti-inflammatory drugs. The anti-inflammatory action of glucocorticoids is mediated partly by the inhibition of the expression of several cytokines and adhesion molecules via glucocorticoid receptor (GR). Some other nuclear receptor activators have also been sbown to inhibit the expression of these inflammatrory molecules, although their molecular mechanisms remain unidentified. We therefore compared the effects of the pharmacological activators of PPARα and GR on TNFα-induced expression of IL-6 and VCAM-1 both of which promoters contain binding sites for NF-κB transcription factors that have key roles in inflammation.

〔方法ならびに成績〕

Human umbilical vein endothelial cells (HUVECs) and bovine aortic endothelial cells (BAECs) were used. ECs were stimulated for IL-6 and VCAM-1 production with TNFα after pretreated with or without ligands for GR, PPARα and PPARγ respectively.

Immunfluorescence studies were performed to reveal the cellular localization of GR and PPARα. In the absence of the ligand, PPARα was localized predominantly to the cytoplasm in HUVECs. When the cells were treated with the PPARα activator fenofibrate, PPARα staining was observed in the nuclei. In contrast, GR was localized in the nuclei of the cells regardless of whether the cells were treated with GR activator dexamethasone.

Effects of fenofibrate and dexamethasone on IL-6 secretion and cell surface VCAM-1 expression induced by TNFα in the HUVECs were determined by ELISA. When HUVECs were treated with TNFα, the IL-6

concentration in the culture supernatants was increased to about 3-fold. TNFα-induced IL-6 production was inhibited by fenofibrate in a concentration-dependent manner, whereas it was not apparently inhibited by the PPARγ activator pioglitazone. Dexamethasone also showed significant inhibition of TNFα-induced IL-6 production in a concentration-dependent manner, and this effect could be blocked by the GR antagonist RU486. Fenofibrate inhibited TNFα-induced cell surface VCAM-1 expression in a concentration-dependent manner in HUVECs. Whereas, dexamethasone had no effect on the TNFα-induced VCAM-1 expression.

Transient transfection assays showed that fenofibrate significantly inhibited TNFα-induced IL-6 and VCAM-1 promoter activities. Meanwhile, in GR expression vector transfected BAECs, dexamethasone significantly inhibited the TNFα-induced IL-6 promoter activity, but failed to inhibit the TNFα-induced VCAM-1 promoter activity.

To investigate whether fenofibrate and dexamethasone affect NF-κB binding to its recognition sites in IL-6 as well as VCAM-1 promoters, EMSAs were performed. The TNFα-induced NF-κB complexes consisted mainly of p65 and p50. Both fenofibrate and dexamethasone suppressed TNFα-activated NF-κB binding to the oligonucleotide probe derived from human IL-6 gene promoter. By contrast, only fenofibrate inhibited TNFα-activated NF-κB binding to the oligonucleotide probe from human VCAM-1 gene promoter. Pioglitazone elicited no effect on the NF-κB binding to the both oligonucleotide probes.

Immunostaining studies indicated that neither fenofibrate nor dexamethasone could prevent TNFα-induced nuclear translocation of NF-κB.

〔総　括〕

Down-regulation of nuclear factor-κB activity by PPARα occurs in both the IL-6 and VCAM-1 genes, whereas that by GR occurs only in the IL-6 gene in vascular endothelial cells. This strongly suggests the existence of a target gene-specific mechanism for the nuclear receptor-mediated down-regulation of NF-κB activity. And, PPARα activator may have the potential to relieve vascular inflammation.

論文審査の結果の要旨

炎症によって惹起される遺伝子の多くは、核内転写因子 NF-κB によってその発現が促進される。グルココルチコイドが抗炎症作用を発揮するメカニズムの分子基盤として、グルココルチコイドレセプター（GR）が NF-κB の活性を抑制し、炎症性遺伝子の発現を転写レベルで抑制することが報告されている。しかしグルココルチコイドが NF-κB によって促進される全ての炎症性遺伝子の発現を一様に抑制するわけではなく、その分子機構は不明である。本研究は、血管内皮細胞における IL-6 と VCAM-1 の発現に対する、GR と Peroxisome proliferator-activated receptor α（PPARα）の作用について解析したものである。本研究により、IL-6・VCAM-1 の発現に対して GR と PPARα が特異的な制御を示すこと、そしてその制御の特異性は NF-κB の転写活性および遺伝子のプロモーター活性のレベルで認められることが示された。このことは、同じ NF-κB により誘導される遺伝子であっても、NF-κB の活性に対する２種の核内受容体の抑制作用が異なることを示した新しい知見である。本知見は、抗炎症薬がなぜ特定の炎症性遺伝子の発現のみを抑制し得るか、という疑問に対する一つの回答を示したものであり、特定の遺伝子をターゲットとした抗炎症薬の開発にも繋がるものであり、学位の授与に値するものと考えられる。