学位論文内容の要旨
Abstract of the dissertation

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学位論文題名
The title of the doctoral dissertation

Modulatory Potential of Fuzapladib and Pentosan Polysulfate Sodium on Adhesion Molecules and Chemokine Receptors in canine endothelial cells

（犬血管内皮細胞におけるケモカイン受容体機能と接着因子発現に対するフザプラジブおよびポリ硫酸ペントサンの干渉能）

Neutrophil recruitment is a critical step in the inflammation response, regulated by a multistep cascade involving endothelial selectins, integrin activation, and chemokine signaling. In veterinary medicine, fuzapladib sodium hydrate (FZP) and pentosan polysulfate sodium (PPS) are clinically utilized agents proposed to possess anti-inflammatory properties. However, their specific effects on adhesion molecules and the chemokine receptor in dogs remain not fully elucidated. This thesis examined the modulatory effects of FZP and PPS on key adhesion and chemokine pathways using an *in vitro* canine model consisting of canine aortic endothelial cells (CnAOEC) and freshly isolated canine neutrophils.

For the FZP study, CnAOEC were stimulated with recombinant canine tumor necrosis factor alpha (rh-cTNFα) or lipopolysaccharide (LPS) in the presence of FZP, while neutrophils were pre-incubated with FZP prior to rh-cTNFα stimulation. For the PPS study, both CnAOEC and neutrophils were pre-treated with PPS before rh-cTNFα challenge. The mRNA expression of endothelial E-selectin, P-selectin, and Intercellular adhesion molecule-1 (ICAM-1), as well as neutrophil C-X-C motif chemokine receptor 2 (CXCR2), P-selectin glycoprotein ligand-1 (PSGL-1), and L-selectin, was quantified by qPCR. Additionally, C-X-C motif chemokine ligand 1 (CXCL1) secretion by CnAOEC was measured via ELISA, and neutrophil lymphocyte function-associated antigen-1 (LFA-1; CD11a subunit) surface expression was assessed by flow cytometry. The functional relevance of these molecular changes was evaluated using a static neutrophil–endothelial adhesion assay.

The results demonstrated that FZP exhibited broad, dose-dependent modulation across both endothelial and neutrophil targets. In cytokine-stimulated CnAOEC, FZP altered adhesion molecule expression, specifically suppressing endothelial P-selectin, whereas its

effect on CXCL1 secretion was limited. In neutrophils, high-dose FZP reduced the expression of rolling-associated molecules (PSGL-1 and L-selectin) and induced a shift in CD11a expression distribution. Despite these molecular alterations, static adhesion was not significantly reduced, suggesting that under static conditions, integrin-dependent firm adhesion remained sufficient to maintain cell attachment despite the reduction in rolling molecules.

In contrast, PPS exhibited a more selective, endothelium-focused profile. PPS was non-cytotoxic to CnAOEC and significantly decreased endothelial P-selectin expression at low doses, while E-selectin and ICAM-1 expression remained unchanged. Furthermore, PPS did not significantly affect CXCL1 secretion, neutrophil adhesion molecule gene expression, CD11a surface expression, or static adhesion.

In summary, both agents predominantly influenced selectin-associated processes – specifically tethering and rolling – rather than integrin-dependent firm adhesion under the static conditions employed. Overall, FZP demonstrated broader modulation across both endothelial and neutrophil molecules, whereas PPS exerted a more selective, endothelium-biased effect, primarily targeting P-selectin.