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Artificial Modification of Adhesive Properties of Liver Sinusoidal Endothelial Cells and Its Influence on Interactions with Activated Leukocytes under Controlled Flow

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Background: P-selectin is an adhesion molecule found on activated endothelial cells that facilitates early phases of the leukocyte recruitment cascade. It is known that liver sinusoidal endothelial cells (LSEC) lack P-selectin in general as well as under severe inflammation. The hypothesis predicted that by increasing functional P-selectin density on LSECs, leukocyte recruitment could be optimized at targeted regions of the liver in the presence of varying intravascular shear forces. In this study, an in vitro experimental approach of artificial modification of the adhesive properties of LSEC using Fc-chimera P-selectin was made.

Method: LSECs were isolated from mouse liver using magnetic-activated cell sorting (MACS) and were seeded in microfluidic chambers for flow adhesion experiments. Leukocytes were extracted from mouse spleen and were activated using monoclonal antibodies. Basal expression of P-selectin on LSEC was first examined using immunofluorescence methods. A suitable protocol for P-selectin enrichment was then established by determining the optimal incubation time and prime expression period of chimeric P-selectin. The functionality of the chimeric P-selectin on LSEC was evaluated using a flow adhesion assay. Under different conditions of shear stress (0.5 dyn/cm^2, 1.5 dyn/cm^2, 3.0 dyn/cm^2), the recruitment of activated leukocytes to LSEC was analyzed, particularly rolling and firm adhesion.

Results: As anticipated, untreated native LSECs expressed low levels of P-selectin in immunofluorescence staining. A marked increase in P-selectin presentation on LSEC can be detected after a chimeric protein incubation time of 15 and 30 minutes. The stability of the anchored P-selectin decreased over time. Compared to native LSECs, significant increase of leukocyte rolling and adhesion on P-selectin enriched LSEC monolayers was observed under different conditions of flow shear stress ($P < 0.01$).

Conclusion: This experimental study showed a successful in vitro modification of the adhesive properties of LSEC using P-selectin Fc-chimera protein, leading to a significant increase of leukocyte recruitment. The modification of endothelial adhesion properties could promote better leukocyte infiltration in certain liver regions. This concept has the potential to contribute to the targeted treatment of hepatic pathologies such as hepatocellular carcinoma, in which leukocyte infiltration was associated with favorable clinical outcomes.