Affinity Microdialysis Sampling of Cytokines

by

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ABSTRACT

Microdialysis sampling is a diffusion-based sampling process used to collect small hydrophilic analytes, e.g. neurotransmitters, from tissue extracellular fluid (ECF) space. Nowadays, there is increasing interest in applying this technique for the collection of proteins. Cytokines are important messenger proteins present in tissue ECF. Microdialysis sampling of cytokines is challenging due to their small aqueous diffusion coefficients. The aim of this dissertation is to develop new affinity agents to increase cytokine recovery during microdialysis sampling, thus allowing easier detection of these proteins.

Antibody-immobilized microspheres were previously used as affinity agents, but the capture antibody binding sites could be saturated by cytokines. Heparin is able to bind multiple cytokines with nM affinity. The method developed in this work is to create and utilize heparin-immobilized microspheres as affinity agents for enhanced microdialysis recovery of cytokines. The affinity agents will also allow for separated recovery event from the analysis process.

In-house prepared heparin-albumin conjugate-immobilized microspheres were included in the microdialysis perfusion fluid to improve the mass transport of the cytokine, tumor necrosis factor-alpha (TNF-α), across the microdialysis probes with 100-kDa molecular weight cut-off membranes. These microspheres served to increase the relative recovery (RR) of TNF-α by three-fold. The binding of other cytokines such as monocyte chemoattractant protein-1 (MCP-1) to the control albumin-immobilized beads suggested that a non-specific adsorption event was possible leading to the need to immobilize only heparin.

Further research was performed to immobilize only heparin onto amine-functionalized microspheres via reductive amination. A bead-based flow cytometric assay was developed to study the binding capacity and specificity of the heparin-immobilized microspheres to four cytokines, MCP-1, acidic fibroblast growth factor (aFGF), vascular endothelial growth factor (VEGF), and RANTES. MCP-1 and RANTES were quantitatively dissociated from the beads before ELISA analysis. Using these heparin-immobilized microspheres, a two- to five-fold increase of microdialysis RR was achieved for the four cytokines from a quiescent solution *in vitro*. *In vivo*

microdialysis sampling of MCP-1 was also performed with the heparin-immobilized beads and a two-fold RR enhancement was observed.

Surface plasmon resonance (SPR) was used to study the binding interaction between heparin and five cytokines (aFGF, VEGF, MCP-1, RANTES, and interleukin-6). The cytokines exhibited different binding kinetics and affinities to heparin and the binding data allowed better understanding of the RR enhancement when the heparin-immobilized beads were used as the affinity agents. This dissertation describes a new way for affinity microdialysis sampling of cytokines and provides the flexibility of sample pretreatment between sampling and analysis.