Nanodevices For Treatment of Secondary Spinal Cord Injury

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The outcome of spinal cord injury depends on the extent of secondary damage produced by a series of cellular and molecular events initiated by the primary trauma. Secondary injury is a combination of several factors contributing to cell death, including free radical damage and glutamatergic excitotoxicity. Here we study attachment of superoxide dismutase (SOD) and anti NR1 glutamate receptor antibody (NR1) to poly(butylcyanoacrylate) (PBCA) nanoparticles with the ultimate goal to design novel therapy for treatment of secondary spinal cord injury. Ability to penetrate the blood-brain barrier (BBB) is a unique property of PBCA nanoparticles that can be used for drug delivery to the central nervous system (CNS).

Methods: PBCA nanoparticles were prepared using polymerization in acidic medium. We studied the effects of pH (pH 1.0, 2.0, and 3.0) and two different surfactant stabilizers, 1.0% Dextran-70 or 1.0 % Pluronic F68 to determine optimal conditions for the preparation of monodispersed non-aggregated nanoparticles. Particle size was determined using Atomic Force Microscopy (Veeco Dimensions 3100) in ambient conditions. The best results – monodispersed nanoparticles with the diameter of about 150 nm were achieved for polymerization at pH 2.0 using Dextran as the stabilizer. These nanoparticles were used in all future experiments.

Sulfo-HSAB cross-linkers (Pierce, Rockford, IL) containing an N-hydroxysuccinimide moiety reactive towards amine groups of proteins, and an arylazide moiety that can react with a C-H bond upon UV-irradiation were used to covalently attach superoxide dismutase and anti-glutamate receptor antibodies to PBCA nanoparticles. Each of the proteins was first fluorescently labeled using AlexaFluor® Succinimidyl Ester dyes with absorbance maxima at 350 nm (for superoxide dismutase) and 594 nm (for anti-glutamate receptor antibody), and then allowed to react with the sulfo-HSAB crosslinker via their amine groups. The solutions containing mixtures of sulfo-HSAB activated proteins at different ratios were used for attachment to PBCA nanoparticles via arylazide moieties of HSAB under UV-irradiation. Protein-nanoparticle conjugates were separated from unreacted proteins by centrifugation. Yield of covalent binding for each of the proteins was estimated by comparing fluorescence intensities for labeled proteins in the initial mixtures and in resuspended nanoparticles. SOD demonstrated constant binding yield of ~25±2%, while binding yield for the antibody increased with the increase of its initial concentration.

Results: To determine activity of covalently attached enzyme, SOD Assay Kit - WST (Dojindo Molecular Technologies, MD) was used. Generally, about 70% of SOD activity was retained by the enzyme attached to nanoparticles (Figure 1). It was found that presence of Tween family of surfactants (adsorption of Tween 80 on PBCA nanoparticles is believed to be necessary for their effective delivery to CNS [1]) does not adversely affect enzymatic activity of SOD. Also notable is that enzymatic activity does not sufficiently decrease during the storage for 21 days in refrigerator (4°C). The observed high activity and storage stability are very important for future therapeutic applications.

Targeting of nanoparticles to neurons was studied using

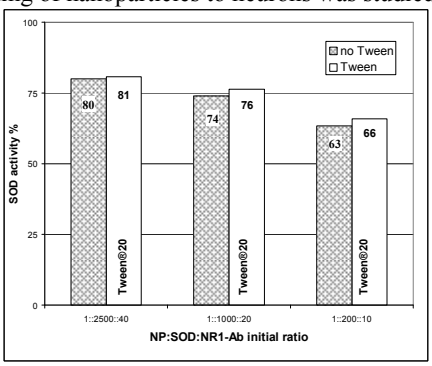

Figure 1. Results of SOD activity assay.

fixed dorsal root ganglion neurons and spinal cord neurons harvested from chicken and rat embryos, respectively. Strong binding of the anti-NR1-PBCA nanoparticles to the neurons was observed using fluorescent microscopy (Figure 2).

In conclusion, we found that SOD and anti- NR1 glutamate receptor antibody can be covalently attached to 150 nm PBCA nanoparticles without considerable changes in enzymatic activity or receptor-binding ability. Aggregation of protein-nanoparticle conjugates was not observed if Dextran was used as a stabilizer. E*x vivo* studies of therapeutic effect of these conjugates on neurons challenged by generated superoxide and/or glutamate are currently in progress.

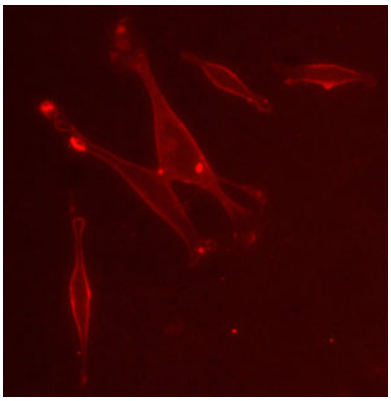

Figure 2. Targeting of AlexaFluor®594 labeled SOD-anti-NR1-PBCA nanoparticles to fixed rat neurons.

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References: 1. J. Kreuter, Nanoparticulate Systems for Brain Delivery of Drugs, Advanced Drug Delivery Reviews, 47, 65 (2001)