BINDING OF A VERSATILE TARGETING SYSTEM TO L-132 CELLS

G. Borchard[1], A. Kalaghi-Nafchi[1,2], T. Schiestel[2], C. Lesniak[2], H. Schmidt[2], C.-M. Lehr[1]

[1]Dept. of Biopharmacy and Pharm. Technology, University of the Saarland, Germany

Introduction

Nanoparticles have extensively been investigated as carriers in modern drug delivery. A multitude of synthesis methods and materials used resulted in a variety of nanoparticle formulations that differ in size, surface charge, hydrophilicity, etc. [1]. In order to achieve site-specific deposition, the particles have to "communicate" with their biological environment (e.g., immune system, target tissue).

This interaction is conducted via the particle surface, and may be influenced by altering the surface parameters through functionalization with e.g., surfactants [2], antibodies, ligands, or lectins. Functionalization in most cases is achieved through either mere physical adsorption, or covalent binding. Both techniques may impair the activity of the molecules bound to the particle surface. In order to screen the rate of cell binding of lectin-functionalized nanoparticles, we developed a simple method to synthesize nanoparticles of varying size and surface charge. For particle functionalization with concanavalin A- (Con A) lectin, the avidin-biotin technique was employed, which allowed for the preservation of biological activity. Binding to an embryonic lung cell line (L-132), and short-term cytotoxicity were studied.

Experimental methods

As model drug carriers, silica nanoparticles were synthesized by a controlled growth reaction with defined size and negative surface charge. The nanoparticle surfaces were then fluorescently labeled with fluorescein-isothiocyanate (FITC), and coated with avidin through electrostatic interaction.

Successively, biotinylated Con A was bound to the particle surfaces through avidin/biotin complex formation. Size and zetapotential of uncoated, avidin coated and lectin functionalized nanoparticles were determined. The amount of avidin and lectin bound to the particles was assessed by gel chromatography, lectin activity by hemagglutination assay. For determination of cell binding, nanoparticle suspensions (uncoated, avidin-coated, functionalized) were incubated with L-132 cells for 1h at 37°C and 4°C. The amount of binding was determined by fluorescence measurement after washing. Particle uptake was calculated after cytolysis. Cytotoxicity was assessed using Cell Proliferation Test (WST-1).

Results and discussion

By variation of reaction conditions particles with hydrodynamic diameters in the range of 50 to 500 nm were obtained.

Particles of 260 nm size were used for further experiments. Under physiological conditions (pH 7.4, PBS-buffer) such particles have zetapotentials of -45 to -50 mV.

After coating with avidin and following lectin functionalization, the particle size increased to 600 nm, the zetapotential to -8,1 mV. The amount of avidin bound was 1.85 μg, the amount of Con A 11.4 μg per mg particles. Hemagglutination test gave positive results for lectin-labeled, but not for un-coated or avidin-coated particles. At 1% particle concentration, no toxic effects were determined during 48h.

After incubation for 1h at 4°C (Fig. 1), about 17% of the functionalized particles were bound, and no uptake occurred. At 37°C, 22% of the functionalized particles were bound to L-132 cells, an increase by a factor of 4.4 compared to uncoated particles. 2.5% were taken up by the cells (increase factor 5). Binding at both temperatures was blocked by incubating with a lectin inhibitor (methyl-α-D-pyranoside).

Conclusions

By synthesizing negatively charged nanoparticles, that were coated with avidin, we were able to create a versatile model system to evaluate cell binding potentials of functionalized nanoparticles. As was shown for a lectin, biotinylated compounds can be non-covalently bound to the particle surface, retaining their biological functions of binding to cell membranes. This opens up the possibility to screen a wide variety of biomolecules with regard to their capacity to target nanoparticles to specific cells.

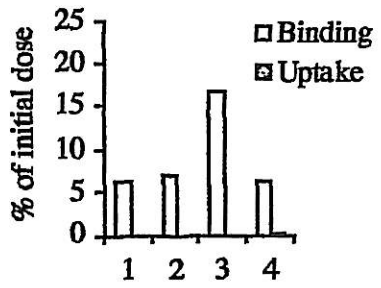

Fig. 1: Incubation at 4°C.

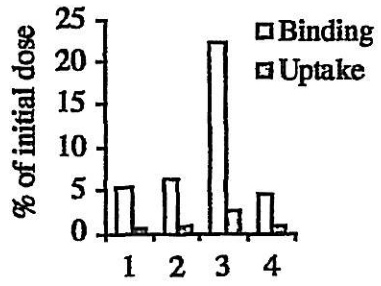

Fig. 2: Incubation at 37°C.
1: uncoated, 2: avidin-coated, 3: lectin-functionalized nanoparticles, 4: inhibitor

References

[1] Kreuter, J., Colloidal Drug Delivery Systems, Marcel Dekker, 1994.

[2] Borchard, G. and Kreuter J., J. Drug Target. 1 (1993) 15-19.

[2]Institute for New Materials, Saarbrücken, Germany.