

Delivery of Immunosuppressive DNA Drug for Treatment of Autoimmune Diseases

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Abstract:

The focus of this project was drug delivery methods for delivering various deoxyribonucleic acid (DNA) fragments to treat autoimmune disease. A cationic liposome was successfully used to transfect the DNA drugs into the mouse fibroblast cells, significantly reducing the immune response to large B-DNA molecules. The cationic liposomes have some limitations when utilized in other cell types, so other nanoparticle drug delivery methods involving silica nanoparticles were explored. Our experiments have shown that silica nanoparticles are ineffective carriers for these types of DNA drugs.

Introduction:

In autoimmune diseases, large DNA fragments such as the body's own "self" DNA can stimulate an immune response, attacking your own cells. Small DNA fragments, however, do not elicit such a response, so these small fragments can be administered to a patient to occupy the receptors, stopping cells from responding to self-DNA fragments. When the naked DNA fragments were added to cells, there was a small immune suppression effect. In order to increase this effect, a more efficient drug delivery method had to be developed. Three different drug delivery methods were tested: cationic liposomes, silica nanoparticles coated in positively charged PEI, and streptavidin-coated silica nanoparticles bound to biotin-labeled DNA fragments. Experiments involving the two types of silica nanoparticles showed that both failed to suppress the immune response. This demonstrated that silica nanoparticles are an ineffective method of delivering the DNA drugs to cytosolic receptors.

Experimental Procedure:

We first transfected different DNA fragments into cells using a cationic liposome. Single-strand and double-strand versions of three types of DNA fragment were combined with lipofectamine, a cationic liposome, and transfected into cells in a 24-well plate. In each well, 10 μg of the DNA drug were added. After waiting one hour, 10 μg of B-DNA/lipofectamine were added into all of the wells. After 24 hours, the supernatant was collected from

each well and tested to see the concentration of two cytokines (IL-6 and IFN- β). A greater amount of these cytokines indicates a strong immune response, while a lesser amount means that the DNA drug suppressed the immune response. To measure the cytokine concentrations, a process called enzyme-linked immunosorbent assay (ELISA) was used. This process uses antibodies to bind to the cytokines, creating a color assay that can then be read by a plate reader. The absorbance levels were compared to a standard curve, revealing the concentrations of the two types of cytokines.

After that experiment was finished, we employed a similar technique to test the silica nanoparticle coated with PEI. First, we made the DNA drug/nanoparticle complex. A silica/PEI solution was prepared with 0.05% PEI and 10 mg of silica nanoparticles. This was mixed for one hour and centrifuged at 150,000G for 30 minutes. The pellet was re-suspended in 5 mL of water. Three tubes were then prepared: one with double-stranded CpG, one with single-stranded CpG, and one control with only silica and PEI. In each, 1 mg of silica nanoparticles and 80 μg of DNA were mixed with water for a total volume of 1 mL. The three tubes were then placed in a rotating machine for one hour, centrifuged at 150,000G for 30 minutes, and re-suspended in 1 mL of water. Once the silica/PEI/CpG complex was finished, they were transfected into cells and the cytokine levels were measured in the same manner described above.

The next experiment involved streptavidin-coated silica nanoparticles bound to biotin-labeled CpG. The silica/streptavidin nanoparticles were first washed in a wash buffer, mixing 400 μl of the silica/streptavidin nanoparticle with 1 mL of wash buffer and centrifuging at 5,000 rpm for five minutes, discarding the supernatant, and re-suspending the pellet in 400 μl of wash buffer. Three tubes were then prepared: one with double-stranded CpG, one with single-stranded CpG, and one control with only silica/streptavidin nanoparticles. In the two tubes containing DNA, 80 μg of either single or double-stranded CpG were mixed with 2.5 mg of silica/streptavidin nanoparticles for a final volume of 200 μl . Once the silica/streptavidin/CpG-biotin complexes were finished, they were transfected into cells and the cytokine levels were measured using ELISA, as described previously.

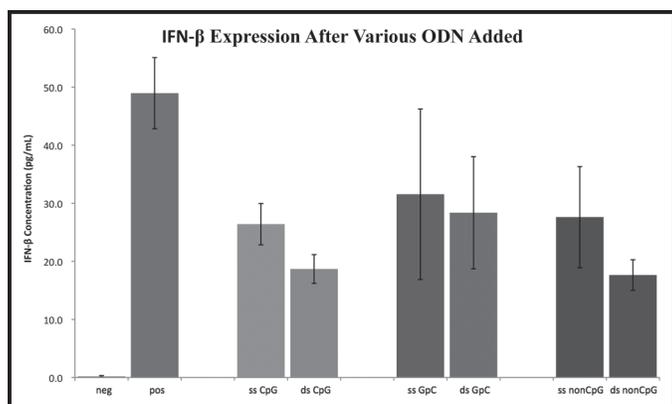


Figure 1: Cytokine suppression effect of DNA drugs with cationic liposome delivery method.

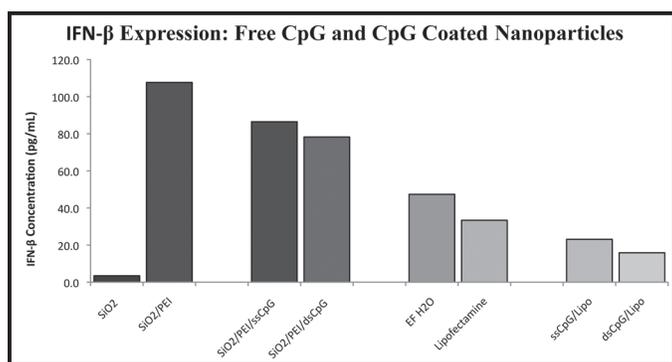


Figure 2: Cytokine suppression effect of DNA drugs with silica/PEI nanoparticle delivery method.

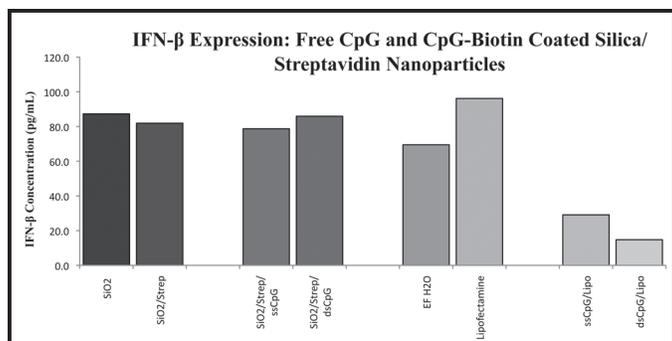


Figure 3: Cytokine suppression effect of DNA drugs with silica/streptavidin/CpG-Biotin delivery method.

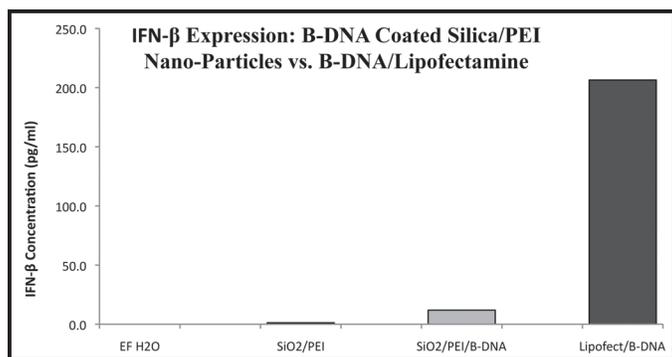


Figure 4: Cytokine expression after silica/PEI/B-DNA nanoparticle delivery method vs. cationic liposome/B-DNA delivery method.

When these silica-based delivery methods failed to suppress the immune response, one last experiment was conducted to confirm a hypothesis on why they were failing. Instead of binding the DNA drug to the silica/PEI nanoparticle, transfecting that into the cells, and then adding the B-DNA (to aggravate the immune system), the B-DNA was bound to the silica/PEI nanoparticle and then transfected into the cells. To make the silica/PEI/B-DNA complex, the same silica/PEI procedure described above was used, only the B-DNA was substituted for CpG.

Results and Conclusions:

The only drug delivery method that effectively suppressed the immune response was the cationic liposome, as can be seen in Figure 1. Double-stranded CpG showed 62% suppression in IFN-β and 74% suppression in IL-6. The two silica nanoparticle delivery methods showed no suppression. Figure 2 shows that PEI actually created an even greater cytokine output than the controls. In Figure 3, it can be seen that cells treated with the silica/streptavidin/CpG nanoparticles had the same cytokine production as the controls.

From this data, it was hypothesized that the silica nanoparticles were getting stuck in the endosomes of cells when taken up by endocytosis, so the DNA drugs weren't able to access the receptors in the cytosol. This was tested by transfecting silica/PEI/B-DNA nanoparticles into cells, and the cytokine outputs seen in Figure 4 support this hypothesis, as even the B-DNA wasn't able to get to the cytosolic receptors and elicit an immune response. From this data, it can be concluded that silica nanoparticles are ineffective carriers of DNA drugs when targeting receptors in the cytosol.

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