“Liposomal carrier of genetic drug for targeted therapy of blood cancers”

Abstract

Leukemias and lymphomas include a heterologous group of hematological neoplastic diseases. Both lymphoid and myeloid stem cell line can overproliferate at each stage of the differentiation process giving a various form of acute or chronic leukemia. The standard treatment of blood cancers is chemotherapy, however, approximately 20% of patients, show resistance to standard cytostatic drugs. The cells of chronic lymphocytic leukemia and B-cell lymphoma are characterized by overexpression of *BCL2* gene, and there is a clear correlation between high levels of expression of *BCL2* gene and the low efficacy of chemotherapy. In the case of genetic diseases, such as CLL, the most promising method for treatment is gene therapy. Gene therapy is a treatment of genetic diseases based on transfer of a genetic material to modify temporary or permanently genotype of the cell, tissue, organs or even organism. Most suitable methods involve asODN, siRNA, shRNA, miRNA, or DNAzymes, which act by reducing the expression of the gene responsible for the disease. Thus, a potential treatment for blood cancer with overexpression of *BCL2* gene is to use the antisense oligonucleotides. Antisense oligonucleotides bind complementary target mRNA and induce its degradation. However, the main problem in gene therapy is efficient delivery of the genetic drug to the target cell. Genetic drug is DNA or RNA molecule, which generally is not able to pass across the cell membrane. Another important problem is the sensitivity of the "naked" nucleic acid molecules to extracellular nucleases in blood. Due to its structure, the nucleic acid molecule has a high negative net charge, which promotes opsonization and elimination from the circulation by macrophages of the reticuloendothelial system. It is necessary to use a suitable carrier which delivers the drug into the target cell and overcomes mentioned above barriers. The most effective carriers used for short nucleotide sequences or genes are viral vectors, due to their natural ability to infect cells. However, this type of carriers has some major limitations such as induction of strong immune response, cytotoxicity, ability to introduce the transgene only to dividing cells, high probability of insertional mutations and difficulties in quality control of those carriers. Liposomal carriers of genetic drugs have some advantages and are a very good alternative. The presented project aims to solve two very important problems in the treatment of leukemias: to overcome the resistance to chemotherapy using gene/antisense

therapy and to enhance the effectiveness of the therapy by using the ligand specific for target cells. Antibodies, thiolated first, were covalently attached to liposomal bilayer through DSPE maleimide-derived polyethylene glycol (DSPE-PEG-Mal). As an example therapeutic antibodies against-CD20, specifically recognizing human lymphocyte B, were used. Presented liposome formulation was stable during long-term storage in suspensions or as lyophilized powder. They were also stable in the presence of human serum or plasma. Liposomes were characterized by low non-specific toxicity towards CD20- cells. For *in vitro* studies of therapeutic activity, cell lines expressing the CD20 surface marker and overexpressing *BCL2* gene were used. The formulation reduced *BCL2* gene expression *in vitro* in Daudi and Raji cell lines and mononuclear cells isolated from blood of CLL patients. Higher therapeutic efficiency was observed in combination with low doses (IC10) of mitoxantrone. Very promising results obtained in *in vitro* experiments led to study the *in vivo* efficiency. Liposomal formulation was characterized by prolonged circulation in blood when compared to PE/PC liposomes which were neither PEG-modified nor targeted with antibodies. In biodistribution analysis high accumulation of targeted liposomes tL-D in tumor (CD20+) was observed. Therapeutic efficacy of antibody-targeted liposomes carrying a genetic drug was tested as a monotherapy or in combination with low dose of cytostatic drug *in vivo* in a mouse model. NOD/SCID mice were inoculated subcutaneously with Daudi human Burkitt lymphoma cells (CD20+). When a tumor started to grow, the drug was applied to mice. As a control separated groups of mice were treated separately with immunoliposomes tL-D scODN containing a scrambled sequence, mitoxantrone (0.3 mg/kg), antibody against CD20, or PBS. The therapeutic effect of immunoliposomes containing asODN anti-*BCL2* (tL-D asODN), alternatively in combination with low doses of mitoxantrone (0.3 mg/kg.b.w.) was also tested. Liposomes tL-D asODN revealed high therapeutic efficiency, which was reflected by total tumor remission. Due to this fact no synergistic effect could be observed in combined therapy. In conclusion, the formulation fullfils requirements of an efficient targeted genetic drug carriee. At this stage of development it is now ready for further preclinical trials, including additional *in vivo* test in another animal model, and up-scaling it production in GMP standard.