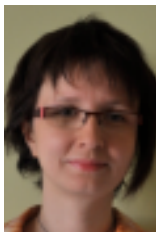
P-063 **Targeting of encapsulated clozapine towards heteromers of dopamine D_2 - serotonin $5HT_{2A}$ receptors.**



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INTRODUCTION AND OBJECTIVES

Various evidences have indicated the formation of clinically relevant heteromers of G protein-coupled receptors (GPCRs) in plasma membrane. Association of GPCRs have therapeutic implications in human diseases (Kroeger 2003). The interaction between the dopamine D_2 and the serotonin 5-HT_{2A} receptors plays an essential role in schizophrenia (affects about 1% of human population. Both of these receptors have been implicated as important site of action of antipsychotics (clozapine) due to well documented serotonin-dopamine interaction and its relevance to schizophrenia. In the light of recent data (Łukasiewicz 2011) selective action of clozapine only on heteromers D_2-5-HT_{2A} may have better therapeutic properties then when the drug interacts via mono- or homomers which may have a key significance in novel therapy of schizophrenia. One of the "hot" topics of nanomedicine is the incorporation of the therapeutic agents inside biocompatibile nanocarriers. The application of nanoparticulate pharmaceutical carriers increases bioavailability and efficiency of many drugs. Surface modification of nanocarriers is used to control their biological properties in a desirable fashion and to enable them to perform therapeutically or diagnostically important functions in proper place and at right time. The nanostrategy also leads to the lowering of drug dose – reducing unfavorable side effects. The present study aims at development of new form of clozapine specific for D_2-5-HT_{2A} heteromer. Clozapine nanocapsules will be functionalized by using synthesized human scFv antibody which recognize only D_2-5-HT_{2A} heteromer. In this way we can obtain efficient delivery of the encapsulated clozapine preferentially to heteromer site in selective tissues.

MATERIALS & METHODS

All used materials were from Sigma, Gibco and BD. The oil phase for capsules formation was prepared by dissolution of 340 g/dm^3 AOT in chloroform. Biocompatible polyelectrolytes poly-L-lysine hydrobromide PLL and Poly-L-glutamic acid sodium salt PGA were dissolved in NaCl solutions of ionic strength (0.015 M) at 1 g/dm^3. Nanocapsules were formed by addition of AOT/chloroform to polycation (PLL) solution during mixing with a magnetic stirrer at 300 rpm. Clozapine was dissolved in chloroform (0,1mg/ml) prior to emulsification with AOT. The multilayer shells were constructed by Layer-by-Layer technique using saturation method. To create pegylated shell, PLL-terminated nanocapsules with seven polyelectrolytes layers were coated with layer of PGA-*g*-PEG. Pegylated nanocapsules with $–NH_2$ groups available for immobilization of antibody were synthesized by coating PLL-terminated capsules with PGA-g-PEG($-NH_2$). The nanocapsules will be functionalized by immobilization of synthesized scFv human antibody selective towards the heteromers D_2-5-HT_{2A}. Human antibody scFv phagemid library (single chain variable fragments) Tomlinson I+J (Geneservice) was used. To separate phages specifically binding D_2-5-HT_{2A} heteromer three rounds of selection on HEK 293+ cells (HEK 293 cells with overexpression of both investigated receptors) as well as HEK 293- cells (HEK 293 cells with overexpression of each investigated receptors separately) were conducted. ELISA tests were used to confirm that obtained phages are specific for HEK 293 + cells. Cells were incubated with diluted suspensions of (obtained after each round of selection) phages (polyclonal and monoclonal, respectively). To detect phages which bind to the cells, tagged HRP monoclonal antibody anti-phage KM13 were used. The cytotoxicity of the nanocapsules was tested in a proliferation assay (MTT test). Experiments were done as described by Siegel (2002).

RESULTS & DISCUSION

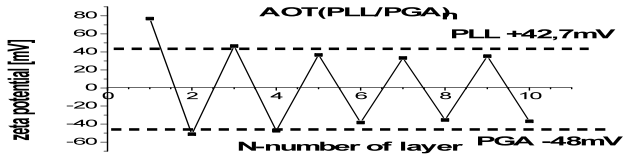

Figure 1 : Clozapine nanocapsules –dependence of zeta potential of capsules on the adsorption of layers

For preparation of the suspension of nanocapsules containing clozapine, 0.05 ml AOT and clozapine in chloroform solution (340 g/dm^3) was added to 100 ml of aqueous PLL solution (c=0,1 g/dm^3) during continuous mixing. The average drop size measured by DLS was around 100 nm and the zeta potential of the emulsion drop was 77 ± 8 mV. The formation of the multilayer polyelectrolyte shells on cores prepared as such were performed by subsequential adsorption of polyelectrolytes from their solutions without the intermediate rinsing step. Figure 1 illustrates a typical zigzag dependence of the zeta potential of capsules on the adsorption of layers. Pegylated nanocapsules were synthesized by adsorption of PGA-*g*-PEG at the surface of PLL-terminated nanocapsules with seven polyelectrolytes layers. The measured zeta poten-

tial of pegylated nanocapsules was close to zero (-7± 4 mV). PEG corona at the capsule surface provides sufficient steric stabilization, capsules were stable up to 30 days. Additionally, the cytotoxicity of the nanocapsules AOT(PLL/PGA)$_{3,5}$PGA-g-PEG on HEK 293 cells was tested (MTT assay). The results of this bioassay have shown that the capsules can be used in high concentrations without harming the cells.

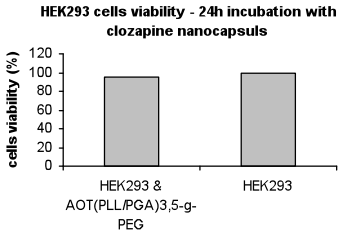

Figure 3 : MTT assay

Pegylated nanocapsules with –NH_2 surface groups were synthesized using PGA-g-PEG(-NH_2). These groups will be used for immobilization of specific antibody. Monoclonal scFv antibody which specifically binds D_2-5-HT_{2A} receptor heteromer will be used for surface functionalization of the clozapine nanocapsuls. The antibody specifically recognizes the heteromer formed by both receptors, and - simultaneously - it doesn't recognize the serotonin 5-HT_{2A} nor the dopamine D_2 receptors alone. For preparation of this kind of antibody we used antibody phage display technique. To separate phages specifically binding the D_2-5-HT_{2A} heteromer the immunoselection of HEK 293+ cells was conducted 3 times. Phages which bound to receptors monomers or other HEK 293 cell surface proteins were eliminated as a result of negative selection (HEK 293 -). After each round of selection the titre of polyclonal phages eluted from antigen were estimated.

a b

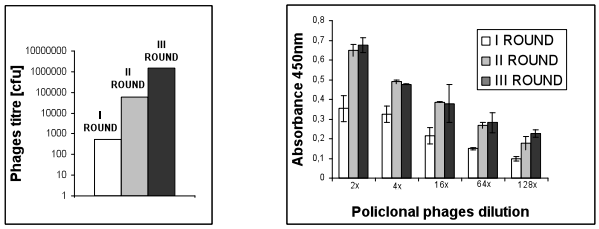

Figure 2 : Selection of HEK293+ specific phages a) titre of phages after each round of selection b) polyclonal phages affinity for HEK+ cells

Increasing titre of phages indicates the correctness of selection process and corresponds with enrichment of phages in those which specifically recognize the D_2-5-HT_{2A} heteromer, after each round of selection. By using ELISA tests it was shown that polyclonal phages obtained after 3rd round of selection contain phages specific for HEK 293 + cells. Phages obtained after 3rd round of selection revealed the highest degree of affinity for D_2-5-HT_{2A} antigen. Moreover, specific binding of monoclonal phages to cells HEK 293 + was tested.

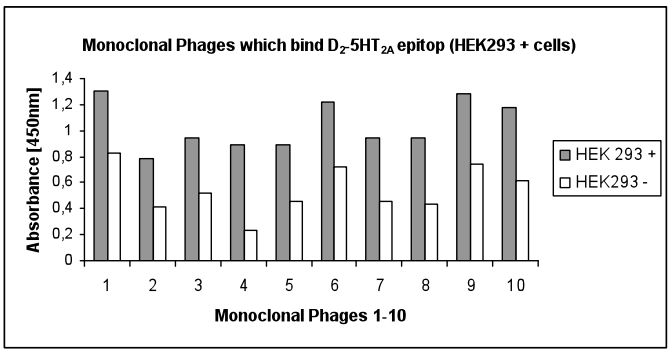

Figure 4 : Monoclonal ELISA assay

In the next part of the studies it is planned to select phages with the highest affinity to D_2-5-HT_{2A} heteromer and to produce the soluble human monoclonal scFv antibodies. Eventually the obtained antibody would be used to modulate the surface properties of clozapine nanocapsules. In this way we can obtain efficient delivery of the encapsulated clozapine preferentially to hetero-dimer D_2-5-HT_{2A} site in selective tissues.

CONCLUSIONS

Development of nanocarriers that are selective for heteromers is important, especially if one takes into account that the physical interactions between two receptors can take place only if they are concomitantly expressed in the same cell, therefore tissue specific action can be obtained. The new way of controlled clozapine delivery may lead to more specific action with lesser side effects connected with drug action on undesirable sites, and may have a key significance in novel therapy of schizophrenia.

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ACKNOWLEDGMENTS

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