P-066 **Microcontainers based on $CaCO_3$ and TiO_2 porous particles for the intranasal drug delivery to the brain**

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INTRODUCTION AND OBJECTIVES

Targeted delivery of functional compounds to the brain – one of the major problems for both basic science and for medical applications. This unsolved problem greatly limits the development of modern approaches for diagnostics and treatment of cerebral pathologies. It is believed now that nasal administration of substances enables to penetrate to the central nervous system bypassing the blood-brain barrier. Furthermore the advantages of this route of administration are clear: it is noninvasive; bioavailable compound is rapidly absorbed and acts within minutes, which is important in the cases of emergency. However, not all compounds can be effectively delivered by this way. In the present work microcontainers based on inorganic porous particles were used as drug carriers for the intranasal drug delivery. It should permit to extend the range of substances administered intranasally. Such containers do not allow particles to penetrate into the brain, allowing the passage of the functional molecules only (Fig. 1).

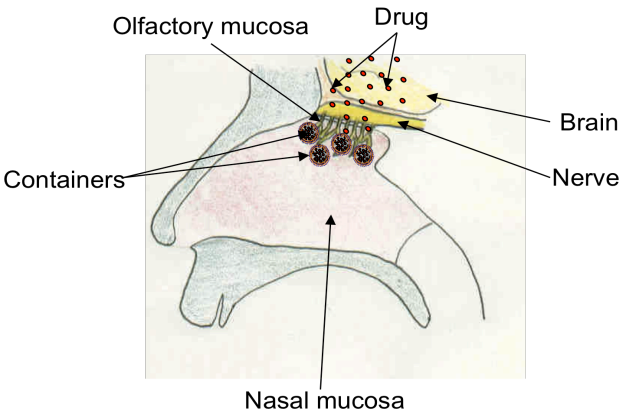

Figure 1: A scheme of intranasal drug delivery to the brain by means of the microcontainers

Central anesthetic loperamide was chosen as a model drug compound because it does not penetrate the blood-brain barrier. Earlier loperamide delivery to the mouse central nervous system (CNS) was successfully implemented using intravenous administration of polybutylcyanoacrylate (PBCA) nanoparticles (Alyautdin 1997). Now nanoparticles of polyalkylcyanoacrylates are one of the most developed and efficient transport systems for the delivery of functional compounds to the brain. In this work the efficiency of the intranasal delivery of loperamide by porous particles of calcium carbonate and titanium dioxide was compared with such data for PBCA particles. In order to prolong the residence time of containers in the nasal cavity the particle surface was modified with hyaluronic acid, which possesses mucoadhesive properties. In addition, the polymer shell can regulate the release of the active substance from the container.

MATERIALS AND METHODS

Calcium chloride dihydrate ($CaCl_2$*$2H_2O$), sodium carbonate (Na_2CO_3), titanium carbide (TiC, fine powder), nitric acid (HNO_3), sodium chloride (NaCl), ethanol, loperamide, hyaluronic acid (Mw 1000 kDa) were purchased from Sigma-Aldrich.

Spherical microparticles of $CaCO_3$ were prepared by mixing 0.33M $CaCl_2$ and 0.33M Na_2CO_3 aqueous solutions according to (Antipov 2003). Mesostructured titanium dioxide particles were synthesized by the interaction of titanium carbide powder with 5M nitric acid (Shieh 2007). PBCA particles with loperamide were synthesized by emulsion polymerization of butylcyanoacrylate (Histoacryl B. Braun) in the presence of loperamide in the reaction mixture (Beck 1994).

Loperamide was adsorbed onto the particles from 1 mg/ml ethanol solution. Loperamide amount included into containers was determined from the difference of the light absorption intensity at a wavelength 259 nm before and after adsorption. Measurements were performed in the interval 210-340 nm in increments of 1 nm using a dual-beam scanning spectrophotometer Lambda-650 (Perkin Elmer).

Particles containing loperamide were modified with hyaluronic acid by exposure to its solution (5 mg/ml in 0.2M NaCl aqueous solution) under stirring on a shaker. The particles were stored in dried form.

The structure of the particles and containers has been investigated by transmission and scanning electron microscopies (TEM, SEM) using FEI Tecnai $G^2$30ST and Jeol 7401F microscopes.

To estimate the delivery of loperamide to the brain a formalin test was used (Matthies 1992). In this test, conclusions about the delivery of anesthetics to the CNS of rodents are based on the change in their pain sensitivity. The containers suspension was administered intranasally to Wistar rats weighing 200-250 g after subcuta-

neous injection of formaldehyde into the rat paw. Animals were placed in a transparent aquarium for observation and after a certain period of time their behavior and pain sensitivity were assessed. All values for each time points were averaged for the group, the data were processed using the program STATISTICA-7. Loperamide in 5% glucose solution without particles, as well as the suspension of the particles without loperamide were used as a control. Results in the control groups of animals were not significantly different, so they were combined and the average control was taken for further calculations.

RESULTS AND DISCUSSION

The synthesized $CaCO_3$ particles reveal a quite narrow size distribution (about 5-6 μm) and mesoporous structure (Fig. 2a). TiO_2 particles have an average size of 1.5 μm and consist of elongated nanocrystallites (Fig. 2b). Amount of adsorbed loperamide was within one mass percent for both kind of particles.

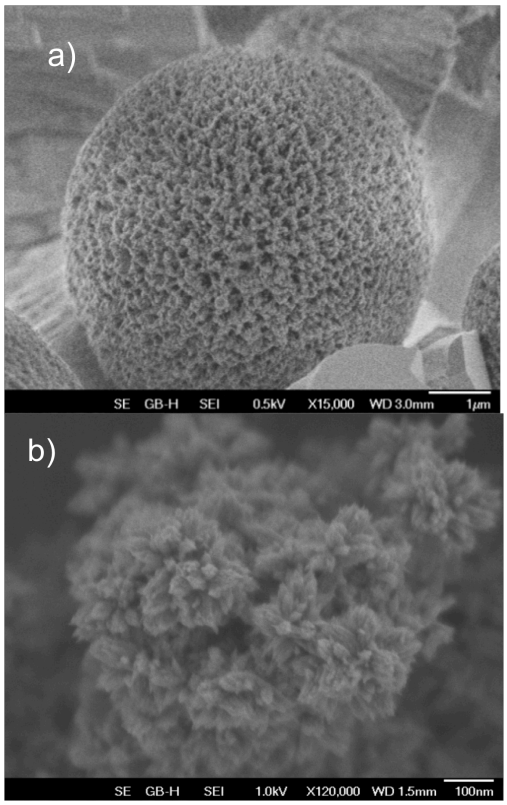

Figure 2: SEM images of the particles of (a) calcium carbonate and (b) titanium dioxide

According to the results of formalin test, all tested containers with loperamide decrease pain sensitivity in rats (Fig. 3). It means that containers based on porous inorganic particles, as well as PBCA particles ensure the delivery of loperamide to the rat CNS. Dynamics of sensitivity reducing is different for the various types of the particles. The particles of titanium dioxide as the PBCA particles are more effective at short times of action (18-24 minutes), but polymeric containers start to act earlier. The particles of calcium carbonate provide a significant reduction in pain sensitivity for long time periods (42-60 minutes). Modification of the containers with hyaluronic acid increases the efficiency of almost twice at the beginning of the test and after prolonged use.

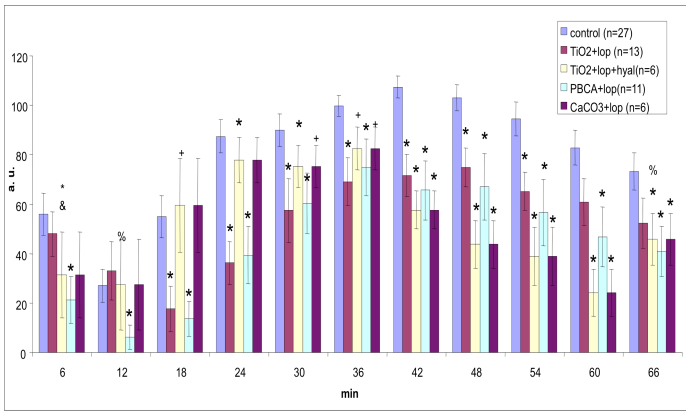

Figure 3: The results of formalin test for the particles loaded with loperamide (* - p<0.05, + - p<0.1, Kraskal-Wallis criterion)

CONCLUSIONS

Containers made of calcium carbonate coated with hyaluronic acid are the most effective of the studied systems for the delivery of loperamide to the CNS of rats. The high efficiency and low toxicity of containers, combined with the simplicity of preparation and the benefits of nasal administration, provide the high promise of the proposed system for medical applications.

ACKNOWLEDGEMENTS

This study was supported by the Program for Basic Research of the Presidium of the Russian Academy of Sciences, no.22.

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