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BIOTECHNOLOGICAL APPLICATION OF METHYL-β-CYCLODEXTRIN FOR CHOLESTEROL CONTENT MANIPULATION IN BIOLOGICAL MEMBRANES

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Investigation is devoted to demonstrate application of methyl-β-cyclodextrin (MβCD) as cargo that attached to magnetic nanoparticles, in particularly magnetite, for the manipulation cholesterol content in synaptic membranes.

Key words: methyl-β-cyclodextrin, cholesterol, magnetite nanoparticles, biological membranes.

Introduction. Membrane cholesterol plays one of the key roles in the regulation of the cellular functions. Membrane microdomains enriched with cholesterol are considered to serve as scaffolding regions where the interface of different signal transduction pathways occurs [1]. Certain level of membrane cholesterol, which is an abundant constituent of eukaryotic membranes, is very important for normal functioning of a number of membrane proteins involved in synaptic transmission. The central nervous system, which is equal to 2 % of body mass, keeps a special place among other systems of the organism, because it contains approximately a quarter of total unesterified cholesterol [2]. Thus, its presence or absence can alter such structures as ion channels, pumps, receptors and transporters, change the fusibility of membranes and the activity of these proteins.

The treatment with cyclodextrins, which are a family of cyclic oligosaccharides composed of a lipophilic cavity and hydrophilic outer surface, is the common methodological approach for cholesterol content manipulation (Fig. 1).

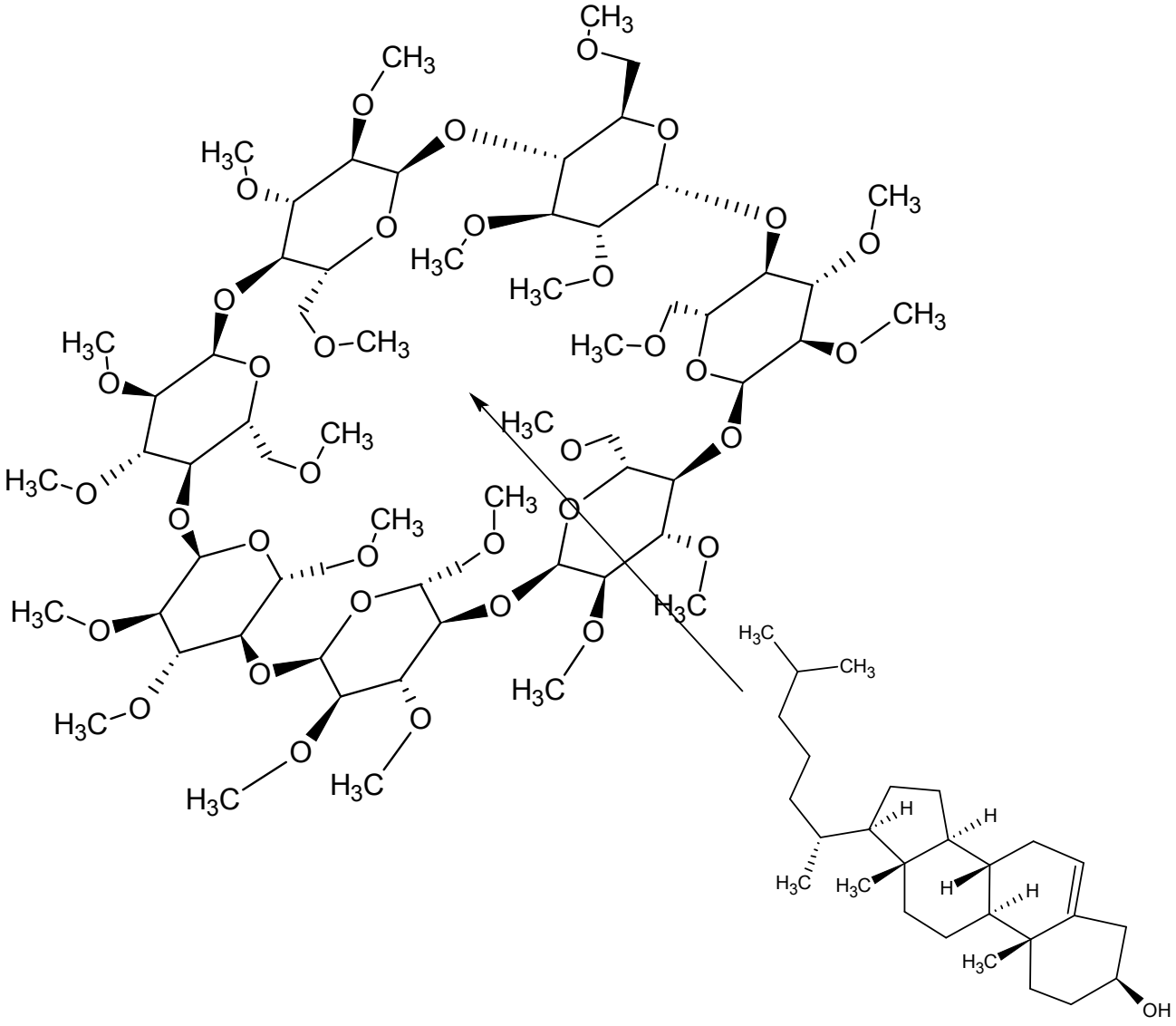

Fig.1. Cholesterol entering into methyl-β-cyclodextrin ring

It is known that methyl-β-cyclodextrin (MβCD), which contains seven α-(1,4)-linked glycosyl units, may be used as an effective cholesterol-depleting agent [3].

Purpose of work is to evaluate the ability of magnetite nanoparticles Fe_3O_4 (NPs) with attached MβCD, manipulate cholesterol content in synaptic membranes with help of external magnetic field. Recently, we demonstrated the ability of MβCD-γ-Fe_2O_3 nanoparticles to modulate cholesterol content of nerve terminals [4].

Materials and methods. MβCD-Fe_3O_4 nanoparticles were obtained as described before [5]. The synaptosomes were obtained by differential centrifugation of rat brain homogenate [6]. Cerebral hemispheres of the animals were homogenized in an ice-cold mixture of 0.32 M saccharose, 5 mM HEPES-NaOH, and 0.2 mM EDTA. Obtained homogenate was centrifuged for 5 min at 2500 rpm. Then, supernatant was centrifuged for 20 min at 15000 rpm to obtain rough fraction of synaptosomes. One animal was used to obtain one synaptosomal preparation. The synaptosome suspensions were used at 4 ºC, 2-4 h after isolation. A standard saline solution was oxygenated and contained (in mM): NaCl – 126.0, KCl – 4.0, $MgCl_2$ – l.4, NaH_2PO_4 – 1.0, HEPES – 20.0, EGTA – 2.0, D-glucose – 10.0 (pH 7.4). Protein concentration was measured as described in common methodic [7].

Quantity of extracted cholesterol by MβCD-Fe_3O_4 nanoparticles measuring was carried out in the following way. The particles (1 mg/ml) were added to isolated nerve terminals (1 ml with concentration 1 mg of protein/ml), then incubated for 10 min, and nanoparticles were magnetically separated. Aliquots from MβCD-Fe_3O_4 and Fe_3O_4 (control) were transferred into Eppendorf tubes, after a magnet (250 mT, gradient 5.5 T/m) was applied, and samples were analyzed for cholesterol [8], both in the supernatant and in the particles. As a control was quantity of cholesterol in entire synaptosomes.

Results and discussions. After magnetic separation of particles from nerve terminals cholesterol content was measured (Fig. 2).

Experimental data shows that unmodified magnetite nanoparticles also significantly decreased cholesterol content in nerve terminals. Both types of the nanoparticles absorb cholesterol, the amount of which ranges from 80 to 90 % of the total cholesterol content of a synaptosome suspension. However, there should be taken into account a significant error that may be caused by several circumstances.

Firstly, buffer solution for synaptosomes hidden may cause aggregation of uncoated NPs [9]. These clusters captured nerve terminals and were withdrawn by magnet. Thus, amount of cholesterol could be changed only in the way of

synaptosomes concentration reduction, not cholesterol extraction from their membranes.

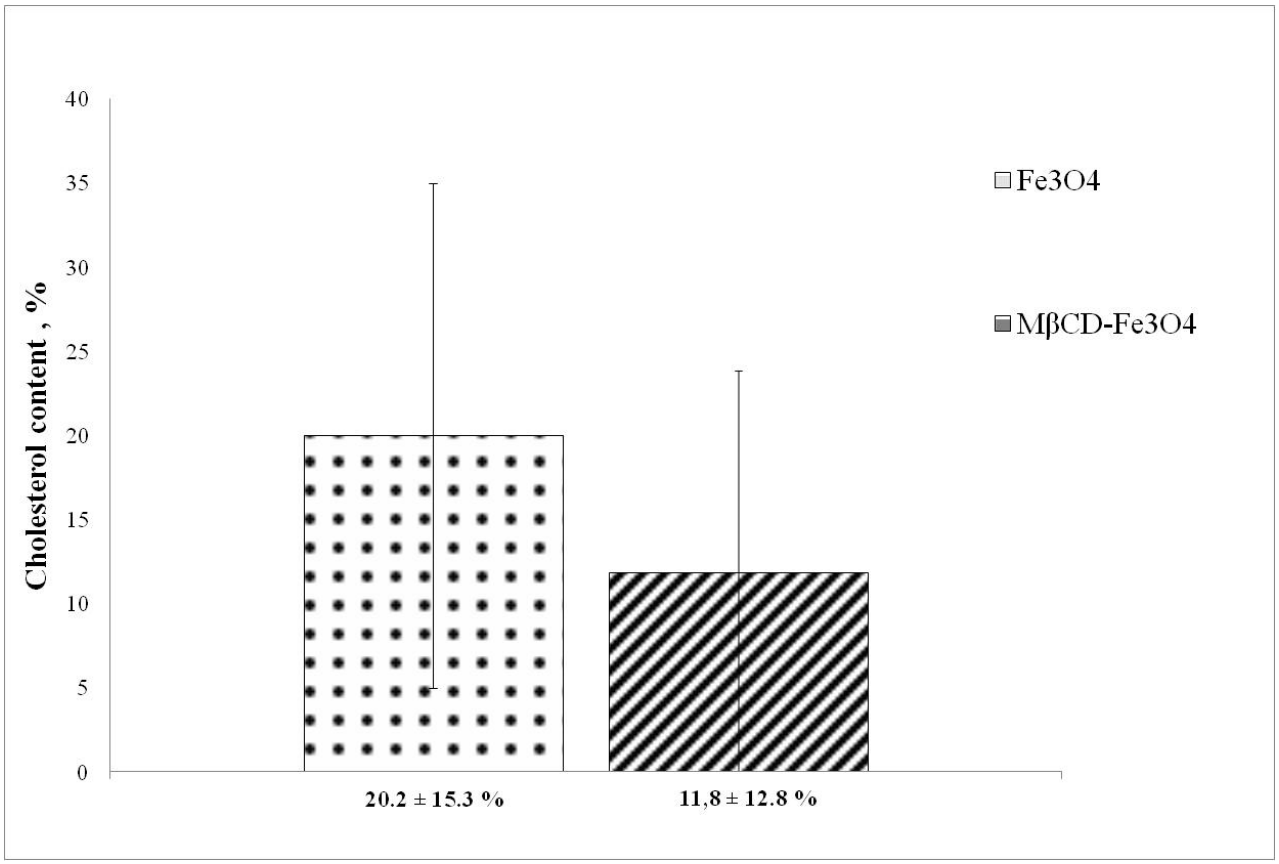

Fig. 2. Cholesterol content in synaptosomal suspension after addition Fe_3O_4 NPs (dotted column) and MβCD-Fe_3O_4 (dashed column)

One more phenomenon could take place. To single synaptosome several MβCD-Fe_3O_4 nanoparticles may attach and after magnetic field applying, this complex removed, as well as in previous case, and gives falls-positive result of cholesterol content depletion.

As a result, the cholesterol content in supernatant most probably directly depends on quantity of absorbed synaptosomes by magnetic nanoparticles, but not to the fetched cholesterol with methyl-β-cyclodextrin in the composition of NPs.

CONCLUSIONS

Methyl-β-cyclodextrin happened to be indeed convenient cargo molecule for the magnetite nanoparticles for the cholesterol binding. However, manipulation by the cholesterol content and its extraction or saturation with it of biological

membranes, particularly membranes of rat brain nerve terminals is significantly hindered. Experiments that are carried out *in vitro*, incorporate mobile phase of cell structures and at conditions, when quantity of these nanoparticles is sufficient, the linkage of membrane cholesterol within MβCD of particles allows withdraw synaptosomes from test solution. It causes erroneous results in membrane cholesterol measuring.

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БІОТЕХНОЛОГІЧНЕ ВИКОРИСТАННЯ МЕТИЛ-β-ЦИКЛОДЕКСТРИНУ ЗА ДЛЯ ЗМІНИ ВМІСТУ ХОЛЕСТЕРЕЛУ У БІЛОГІЧНИХ МЕМБРАНАХ

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Дослідження присвячено демонстрації того, яким чином можна використати метил-β-циклодекстрин (МβЦД) в якості вантажної молекули, що прикріплена до магнітних наночастинок, зокрема магнетиту, за для маніпулювання вмістом холестеролу у синаптичних мембранах.

Ключові слова: метил-β-циклодекстрин, холестерол, маґгемітові наночастинки, біологічні мембрани.

БИОТЕХНОЛОГИЧЕСКОЕ ИСПОЛЬЗОВАНИЕ МЕТИЛ-β-ЦИКЛОДЕКСТРИНА ДЛЯ ИЗМЕНЕНИЯ СОДЕРЖАНИЯ ХОЛЕСТЕРОЛА В БИЛОГИЧЕСКИХ МЕМБРАНАХ

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Исследование посвящено демонстрации того, каким образом можно использовать метил-β-циклодекстрин (МβЦД) в качестве карго молекулы, которая прикреплена к магнитным наночастицам, в частности магнетита, для манипуляции содержания холестерола в синаптических мембранах.

Ключевые слова: метил-β-циклодекстрин, холестерол, магемитовые наночастицы, биологические мембраны.