SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

EXAMINATION OF BETA-CYCLODEXTRINS AND THEIR PACLITAXEL COMPLEXES ON CACO-2 CELL LINE

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, 1st December 2016, 1 pm

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1.1. Introduction

Today's drug development faces new challenges. Newly synthetized active substances mostly belong to Class IV of the Biopharmaceutics Classification System (BCS), as they are (almost) insoluble in water and they just poorly absorb from the GI tract. On the other hand generic and supergeneric preparations get more and more importance beside the original ones. To solve the above mentioned problems modern excipients and technologies are necessary. Among others this challenge helps the cyclodextrin research to advance.

The number of cyclodextrin applications and publications is rising continuously (in the last years approximately 3000 new printed scientific articles were published per year). More and more new results and applications are submitted, and there are almost 600 new patents yearly (Jicsinszky & Fenyvesi 2014).

Cyclodextrins are used in many industries, such as pharmaceutical-, food-, pesticide-, diagnostic-, cosmetical-, explosive-, plastic-industry. Last, but not least cyclodextrins have important role in biotechnology as well. According to their special properties, these molecules are very good stabilizers, solubilizers, they improve bioavailability, and they also can be used to reduce smells, tastes and irritative effects (Szejtli 1990).

β-cyclodextrins are also used in cell biology research for the removal of cholesterol from cell membrane (Kilsdonk et al. 1995) and to study the role of cholesterol on cellular functions (Mahammad et al. 2014).

Some cyclodextrins have been included in various pharmacopoeias as solubilizers and absorption enhancers for long, but recently hydroxypropyl-β-cyclodextrin (HPBCD) itself has also been recognized as orphan drug for the treatment of Niemann-Pick type C1 disease (Matsuo et al. 2013; Ottinger et al. 2014). Based on this result, there are further researches to treat other diseases int he central nervous system with cyclodextrins (Vecsernyés et al. 2014). In addition - the new result of year 2015 - researchers found that HPBCD can be used as a novel anticancer agent, because it can selectively disturb the cholesterol homeostasis of leukemic cells (Yokoo et al. 2015).

We need complete knowledge in case of such widely used excipients (and active substances), especially about their interaction with cells and organisms and about their safety. It is important to know what is the mechanism through the applied excipient (in our case the cyclodextrin) acts (for example improves drug absorption) and if it has any unwanted side effect. Many researches and many aspects are needed to have a holistic knowledge about an excipient. With our research we answered still opened questions, especially around the safety and behaviour of cyclodextrins in biological systems.

1.2. Cyclodextrins

Cyclodextrins can be produced from partially prehydrolyzed starch (acyclic dextrin mixture) by cyclodextrin-glycosyltransferase enzyme. These cyclodextrin molecules are non reducing cyclic oligosaccharides (Szejtli 1990). According to the ring size, cyclodextrins have three basic types: α-, β- and γ-cyclodextrins containing 6, 7 or 8 glucopyranose units, respectively (Szejtli 2004). All of these glucopyranose units have C-1 conformation, that is why the inner cavity of these molecules are lipophilic, while the outer surface is rather hydrophilic.

Various derivatives of cyclodextrins can be formed by substitution of these rings. The number of published derivatives is more than 1500. Production should be simple and cheap, the ring should keep its complexation capacity and the new product should have no toxic effects. In the industry mainly methylated- (DIMEB, RAMEB), hydroxyalkylated- (HPBCD),

sulfobutylated- (SBE-CD), acetylated- (acetyl-γCD) and branched (glucosyl-, maltosyl-βCD) cyclodextrins are produced (Otta 2014).

The complexation properties and also the safety of a cyclodextrin molecule are very important when it is applied as an excipient. Cyclodextrins can have toxic effects, as they are able to complex physiologically important lipophilic molecules such as lipids (in case of α-cyclodextrins) or cholesterol (in case of β-cyclodextrins). Therefore we need to know their complexation affinity to endogen molecules for each derivative, hence also the toxicity.

Because of many reasons (for example price, availability, ring size, etc.) β-derivatives are the most prevalent cyclodextrins. There are 21 free hydroxyl groups on these molecules where many different substituents can be attached, hereby creating many different derivatives. Best-known ones are methyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin (HPBCD), both of them are heterogenous, amorphous and water-soluble. They are preferred, because they can not form crystalline complex with cholesterol in contrast with β-cyclodextrin (which forms insoluble cholesterol-complex after administered parenterally). This complex has toxic effects on kidneys. Methyl-β-cyclodextrin is much more lipophilic and so more stable than β-cyclodextrin, but it forms water-soluble complex with cholesterol in aqueous media. Extracting cholesterol from erythrocyte-membrane is a serious disadvantage, therefore methyl-β-cyclodextrin causes haemolysis in a concentration dependent manner. Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin is a crystalline substance and the best solubilizer agent at present. However its production is expensive and not environmentally sound, and it can easily cause haemolysis as well. That is why, in most cases randomly methylated β-cyclodextrin (RAMEB) is used instead of DIMEB. RAMEB is not as good solubilizer as DIMEB, but its production is much more cheaper (Szejtli 1997). The preferred cyclodextrin derivative must have as low toxicity as possible. From this view, 2-hydroxypropyl-β-cyclodextrin (HPBCD) and sulfobutylated-β-cyclodextrin (SBE-CD) are the most favorable. The latter is a newer derivative, it is used not so long ago, but it is already used in many medicines (also distributed in Hungary), due to its good solubilizing and toxicity properties. (Stella & He 2008; Sebestyén et al. 2013; Fenyvesi 2015). Safety studies revealed, that HPBCD and SBE-CD are well tolerated in humans and have no adverse effects on the kidneys or other organs (Stella & He 2008).

In the case of β-cyclodextrins a relationship could be identified among the substituents of the cyclodextrin ring, cholesterol solubilization, hemolytic activity and cytotoxicity. Cholesterol-solubilizing properties and cytotoxicity depend from the structure of the cyclodextrin molecule. For example, more methyl groups attached to the ring improve the cholesterol-solubilizing ability, in contrast with presence of ionic groups, and in case of β-cyclodextrin, attaching hydroxypropyl group results very low toxicity (Kiss et al. 2010).

Cyclodextrins can function as molecular capsules due to their special structures (they are able to bind other lipophilic molecules into their inner cavity). These filled cyclodextrin rings are called inclusion complexes, in which van der Waals and apolar interactions can be found between respective function groups (Rekharsky et al. 1997; Anjana et al. 2013), so the complex can easily dissociate in certain circumstances. In the host-guest complex formed such way, the guest molecule can be located partially or totally in the cyclodextrin ring, but this process cannot change the structure and the conformation of the cyclodextrin molecule.

Cyclodextrin complexes with proper stability constants are able to improve the absorption and bioavailability of the complexed drug. There are numerous mechanisms published, which can explain this behavior.

The first mechanism is based on the solubility-increasing effect of cyclodextrins. The complexed lipophilic molecule can get hydrophilic properties by the cyclodextrin ring.

The second action is based on the effect of cyclodextrins on intestinal epithelium. According to *in vivo* experimental results, just insignificant amount of hydrophilic

cyclodextrin can penetrate into lipophilic biological membranes, such as skin or GI mucosa. Only the free drug molecule can absorb through lipophilic membranes, so excess amount of hydrophilic cyclodextrin can decrease the penetration of drugs (Loftsson et al. 2007). Lipophilic cyclodextrins (e.g., randomly methylated β-cyclodextrin) are able to decrease the barrier function, and by this the drug transport is increased through biological membranes (e.g., nasal mucus membrane) (Loftsson et al. 2007). The disruption of cholesterol rich membrane rafts alters the integrity of tight junctions and barrier functions of cell layers (Lambert et al. 2005; Deli 2009).

The third mechanism can be explained with the water layer which covers absorption epithelium and so takes part in barrier function. Viscous mucus membranes have an adsorbed, unstirred water layer (UWL) on their surface which can be 100 μm thick (Lennernäs 1998; Loftsson et al. 2007). The guest molecule can be transported through the unstirred water layer directly to the biological membrane with the help of cyclodextrin, where the complex dissociates and only the free drug molecule penetrates through the membrane (Loftsson et al. 2005). Hydrophilic cyclodextrins can increase the drug transport only if the resistance of UWL on donor side is equal or higher than the resistance of membrane barrier (Loftsson et al. 2007; Másson et al. 1999). On the other hand if the binding force is too strong between the cyclodextrin and the guest molecule, only a small portion of the drug is released from the complex, resulting in lower bioavailability (Brewster et al. 2007).

Beside the above mentioned effects, active transporter inhibition can be important as well. Many active transporter proteins (e.g., P-glycoprotein) can decrease the absorption of their substrates by pumping them back from the membrane to the lumen of the small intestine. Methylated-β-cyclodextrins are able to prevent the operation of the transporter proteins by reducing the cholesterol content of the membrane (Fenyvesi et al. 2008; Garrigues et al. 2002; Arima et al. 2004; Bacso et al. 2004). These effects can also increase the permeability and absorption of drug molecules from the intestine. On the other hand membrane cholesterol depletion with high cyclodextrin concentration inhibits endocytotic processes (Zuhorn et al. 2002; O' Neill et al. 2011) and increases exocytosis (Chen et al. 2010).

The last action can be based on the endocytosis of cyclodextrins. The chemical structure, number of hydrogen donors and acceptors, relatively high molecular weight (>1000 Da) and the hydrophilicity of cyclodextrins predict that these molecules are not able to permeate biological membranes and have poor absorption (Lipinski et al. 2001); only lipophilic cyclodextrins are considered to be absorbed from the gastrointestinal tract to some extent (Loftsson et al. 2005). In general, only the free form of drug, which dissociates from the cyclodextrin complex, is thought to be absorbed. According to this mechanism cyclodextrin delivers the drug to the surface of cell membrane, the drug molecule penetrates into the lipophilic membrane, but after delivery the cyclodextrin remains extracellular (Loftsson et al. 2005).

Although cyclodextrins most likely cannot permeate the cell membrane by diffusion, recent findings revealed that they are able to enter cells. Methyl-β-cyclodextrin-dextran conjugates and hydroxypropyl-β-cyclodextrin were found to enter cells by endocytosis, as they reduced intracellular cholesterol accumulation in Niemann-Pick type C mutant cells acting at the level of endocytotic organelles inside the cells (Rosenbaum et al. 2010). Intracellular accumulation of the fluorescent mono-4-(N-6-deoxy-6-amino-β-cyclodextrin)-7-nitrobenzofuran (NBD-β-CD) was also detected in HepG2 and SK-MEL-24 cells, and endocytosis as a possible mechanism for the transmembrane passage of NBD-β-CD was suggested (Wei et al. 2011). Macropinocytosis of amphiphilic cationic cyclodextrin transfection complexes were also observed in Caco-2 intestinal epithelial cells (O' Neill et al. 2011), and clathrin-dependent endocytosis of a fluorescent methyl-β-cyclodextrin by HeLa cells was demonstrated (Plazzo et al. 2012).

1.3. Aims of the work

These results raised the possibility that cyclodextrin molecules not only increase the solubility of poorly soluble drugs and act as permeation enhancers in the intestine, but are able to enter intestinal cells by the endocytotic pathway.

This mechanism, the intracellular route and fate of cyclodextrins have not been investigated on intestinal epithelial cells yet, although transcytosis is known in the case of intestinal epithelial Caco-2 cells (Artursson et al. 2001). There is also limited information about the permeability of cyclodextrins on Caco-2 monolayers.

In the present study our first aim was to examine the interaction of the fluorescently labeled randomly methylated β-cyclodextrin (FITC-RAMEB) with Caco-2 colon cell layer and examine the cellular uptake of cyclodextrins on intestinal epithelial cells. Because of RAMEB is the most applied cyclodextrin in biological researches, we also tested our hypothesis with this derivative. According to our first results FITC-RAMEB is able to enter inside the cells, thus we aimed to study the way of this uptake.

Further aims were to test the permeability and endocytosis of fluorescently labeled (2-hydroxypropyl)-β-cyclodextrin, random methyl-β-cyclodextrin and soluble β-cyclodextrin polymer and their inclusion complexes with fluorescently labeled paclitaxel (Flutax-1) on Caco-2 cells. We also investigated the possibility of the cellular uptake of Flutax- rhodamine-labeled random methyl-β-cyclodextrin complex via the endocytotic pathway.

2. New scientific results in the thesis

In the present study at first we investigated the permeability and cellular uptake of the fluorescent methyl-β-cyclodextrin (FITC-RAMEB) in intestinal Caco-2 cells. The available data regarding the absorption and oral bioavailability of cyclodextrins is very limited and there are no Caco-2 permeability values in the literature. In early studies of intestinal absorption of ^{14}C-labelled β-cyclodextrin in rats only 5% of the administered activity could be detected in the blood. It was concluded that β-cyclodextrin was not absorbed from the stomach and the small intestine, and the low absorption was explained with the amylase action: only the open-chain dextrins and the glucose formed from cyclodextrins were absorbed (Szejtli et al. 1980). According to recent publications the oral bioavailability of HPBCD is less, than 0.03% and it is approximately 0.3% for β-cyclodextrin (Kurkov & Loftsson 2013), while RAMEB has an oral bioavailability of about 12% in rats (Loftsson & Brewster 2011).

Our permeability results with FITC-RAMEB are in accordance with the low intestinal absorption of cyclodextrins, as the apparent permeability coefficient (P_{app}) values were $3.35\pm1.29\times10^{-8}$ and $4.23\pm1.46\times10^{-8}$ cm/s for FR (0.05 mM FITC-RAMEB) and FRR (0.05 mM FITC-RAMEB + 5 mM RAMEB) treatments, respectively. These data are also in agreement with the permeability results of natural cyclodextrins (α-, β- and γ-cyclodextrin) on pulmonary Calu-3 cell layers, which were in the same order of magnitude (Matilainen et al. 2008).

Methylated cyclodextrins are also used for membrane cholesterol depletion in 5–10 mM concentration (Kilsdonk et al. 1995; Fenyvesi et al. 2008) and can enhance the penetration of drugs (Deli 2009). 0.05 mM cyclodextrin presumably does not affect membrane cholesterol significantly, since it is 1/100 of the usually applied concentration. **At 5 mM RAMEB concentration we did not observe cytotoxicity on Caco-2 cells previously** (Kiss et al. 2010)**, therefore we investigated the effect of 5 mM RAMEB on FITC-RAMEB permeability and the TEER of the monolayers. No significant difference could be observed on permeability and resistance values of FR and FRR treatments ($p<0.05$), and 5 mM RAMEB had no effect on the permeability of the monolayer.**

Examining the fluorescence of the cells at the end of the permeability experiments we found that a significant amount of FITC-RAMEB accumulated in the cell layers. These cyclodextrins could not be removed by extensive washing. We investigated the time-dependence of FITC-RAMEB accumulation in the monolayers and **Caco-2 cells successively accumulated both FR and FRR up to 120 min of the experiment.** To reveal the fate of the accumulated FITC-RAMEB we loaded the cells with 0.5 mM FITC-RAMEB solution, using 10 time higher concentration than in permeability studies. This resulted in 10 time higher accumulation, but the permeability of FITC-RAMEB did not increase ($2.28\pm0.346*10^{-8}$ cm/s). **After 120 minutes, the release of the accumulated cyclodextrins was followed both in apical and basolateral directions. Interestingly FITC-RAMEB appeared in both the apical and basal chambers, but the majority of the accumulated cyclodextrin was released to the apical direction. Only 7.4% of the accumulated FITC-RAMEB reached the basal chamber.**

These results indicated that although the intestinal Caco-2 monolayer is an almost impermeable barrier for the cyclodextrin molecules, the cells are able to take up cyclodextrins from solutions with a mechanism different from simple diffusion. Studies on Calu-3 monolayers suggested that cyclodextrins traverse these monolayers by paracellular route, although transcytosis could not be excluded (Matilainen et al. 2008). Recent publications revealed that certain cell types are able to internalize cyclodextrins by endocytosis (Rosenbaum et al. 2010; Wei et al. 2011; Plazzo et al. 2012); therefore we investigated the intracellular localization of FITC-RAMEB by confocal microscopy. **We found, that FITC-**

RAMEB is able to enter into the cytoplasm of both undifferentiated and differentiated Caco-2 cells. Since the labeled cyclodextrin was localized in vesicles in the cytoplasm, the possibility of endocytosis was investigated hereafter. **In the cell membrane RFP-Rab5a fusion protein and FITC-RAMEB showed colocalization.** Rab5 is a key organizer of early endosomes, but it cannot be detected in late endosomes (Rink et al. 2005). **In our confocal microscopy images Rab5a and FITC-RAMEB did not exhibit colocalization in vesicles in deeper layers. The colocalization of RFP-Rab5a and FITC-RAMEB suggests that endosome formation is involved in the initiation of cyclodextrin internalization.** According to further examinations of my colleagues the highest degree of colocalization between FITC-RAMEB and Rab5a protein can be measured after 2 minutes incubation, but after 30 minutes colocalization is still significant. These results clearly show the continuous working of endocytotic processes (Fenyvesi et al. 2014).

Endocytosis has two major routes, phagocytosis and pinocytosis or fluid-phase uptake. Fluid-phase endocytosis, which requires the cargo molecules to be dissolved, can be subdivided into macropinocytosis, clathrin-mediated, caveolin-mediated and clathrin- and caveolin-independent endocytosis (Conner & Schmid 2003).

The widely used marker of macropinocytosis is Lucifer Yellow (Sarkar et al. 2005; Swanson et al. 1985; Sallusto et al. 1995). Flow cytometry analyses revealed that in Caco-2 cells Lucifer Yellow was internalized in a concentration dependent manner and its uptake could be inhibited at 0 °C (Swanson et al. 1985; Sallusto et al. 1995). **FITC-RAMEB showed similar cellular uptake: at 37 °C accumulated in the cells as a function of concentration, while at 0 °C FITC-RAMEB uptake was diminished.** On the other hand lipophilic calcein AM showed the same cellular accumulation at 0 °C and 37 °C, as it rapidly permeated the lipid membrane (Homolya et al. 1993), and the intracellular accumulation was not inhibited by cooling. **The macropinocytosis inhibitor rottlerin** (Sarkar et al. 2005) **had similar inhibitory effect on FITC-RAMEB and Lucifer Yellow accumulation.** These results indicate, that **in Caco-2 cells macropinocytosis is involved in the entry of FITC-RAMEB.** It also explains why the majority of the accumulated FITC-RAMEB was released to the apical direction. It was demonstrated in human epidermoid A431 cells, that macropinosomes recycle their content to the cell surface (Hewlett et al. 1994). It seems that the same mechanism could be observed in Caco-2 cells, as the mechanism of internalization was macropinocytosis, the majority of accumulated cyclodextrin was guided to the apical cell surface. In accordance with our results, researchers found, that Niemann-Pick C1 type cells can internalize fluorescently labeled β-cyclodextrin by endocytosis, and can release it by exocytosis (Dai et al. 2015).

Nevertheless, the total recycling of the internalized cyclodextrin molecules took at least one hour, which means that this process prolongs the contact between cylodextrins or cyclodextrin-drug complexes and the membrane of macropinosomes. It is important to note, that other endocytotic mechanisms should be also taken into consideration. Previous studies implicated fluid-phase endocytosis and clathrin-dependent endocytosis (Rosenbaum et al. 2010; Wei et al. 2011; Plazzo et al. 2012) for the mechanism of cyclodextrin internalization. Nevertheless, phagocytosis could be also a possibility for cyclodextrin internalization in concentrated cyclodextrin solutions. It is reported that at high concentrations, natural β-cyclodextrin (González-Gaitano et al. 2002) and the fluorescent tetraamino rhodaminyl hydroxypropyl-β-cyclodextrin (Puskás et al. 2012) form large, nano-sized aggregates in water. However, the substitution of OH groups with methyl groups on the cyclodextrin ring inhibits the aggregation of RAMEB and at 12 mM no aggregation was observed (González-Gaitano et al. 2002).

In this study 0.05 mM FITC-RAMEB was applied alone or in combination with 5 mM RAMEB, which is 40–240 times lower cyclodextrin concentration than what was found to

form aggregates above, thus phagocytosis can be excluded from the possible mechanisms of cyclodextrin uptake.

In summary, our results on Caco-2 cells are in accordance with earlier findings, the cellular internalization of water soluble FITC-RAMEB is governed by fluid phase endocytosis in intestinal Caco-2 cells. It is hard to predict the significance of this mechanism quantitatively. Even if permeability data are suitable to value the extent of absorption of cyclodextrins, it is difficult to quantify the amount of continuously internalized and released cyclodextrins with this setup of the model. The intestinal absorptive surface area relative to the volume of the gut is much bigger than the surface area of Caco-2 monolayers and on the other hand the peristaltic movement should be also considered. Thus the extent of internalization can be much higher *in vivo*, even if cyclodextrins are released back to the lumen of the gut and as the process can be continuous along the small intestine its efficiency can be much higher.

In the second part of our work we involved other cyclodextrin derivatives and the behavior of fluorescently labeled HPBCD and BCDpolymer was investigated on Caco-2 cells compared to fluorescently labeled RAMEB. Furthermore, we aimed to investigate the uptake of these cyclodextrin derivatives complexed with fluorescently labeled paclitaxel (Flutax-1) on Caco-2 cells.

At first the permeability test of the cyclodextrins was performed on Caco-2 monolayers, and in accordance with our previous results, **very low and not significantly different apparent permeability values were measured for all the studied derivatives (FITC-HPBCD, FITC-RAMEB and FITC-BCDpolymer). Surprisingly, all three types of labeled cyclodextrins could be detected both in the basal chamber and in the cytoplasm.**

It was confirmed by fluorescent microscopy that after 30 min of incubation all the labeled derivatives could be detected in attached cells. **FITC-HPBCD, FITC-BCDpolymer and Rho-RAMEB were found in the cytoplasm in vesicles of different size.**

Nevertheless, flow cytometry results showed that in the case of Caco-2 cell suspension only the monomer derivatives (FITC-HPBCD and FITC-RAMEB) were detectable in the cells after 30 min of incubation. This uptake could be inhibited by keeping samples on ice and with rottlerin pretreatment. However Rho-RAMEB internalization could not be inhibited by rottlerin. In our previous publication (Fenyvesi et al. 2014) we demonstrated that rottlerin decreased the endocytosis of FITC-RAMEB. These results raise the possibility that FITC and Rhodamine derivatives of methylated β-cyclodextrins are internalized by different processes. More types of endocytosis need to be examined to conform this hypothesis.

Cyclodextrins are hydrophilic molecules with low octanol–water partition coefficients (log P) (Kurkov & Loftsson 2013; Loftsson 2015). The fluorescein and rhodamine labeling increased their molecular weight and altered the properties of the parental cyclodextrins, but they kept their good water solubility. We predicted log P values (c log P) for FITC-HPBCD, Rho-RAMEB and their parent cyclodextrins and found that fluorescent derivatives retained their hydrophilicity. These data confirm that fluorescent cyclodextrins are not able to cross cell membrane by passive diffusion, similarly to the unlabeled cyclodextrins.

Finally, paclitaxel-cyclodextrin complexes were investigated on Caco-2 cell layers. **It was clearly shown that the complexes of RAMEB and HPBCD were able to increase Flutax uptake in Caco-2 monolayers, while BCDpolymer had no effect on it.** It is in accordance with our previous finding, where we have shown that RAMEB and its derivatives were able to enhance paclitaxel permeability on Caco-2 monolayers (Fenyvesi et al. 2011).

We also made Flutax-Rho-RAMEB complex, in which both molecules are fluorescently labeled (with different dyes), **and we investigated the uptake of fluorescent complexes at cellular level. The intracellular colocalization of the fluorescent paclitaxel derivative, Flutax-1 and Rho-RAMEB could be identified. We demonstrated for the first**

time that fluorescent cyclodextrins entered the cells through endocytotic pathways with a highly lipophilic substrate and host-guest molecules could be detected together in an intracellular endosome.

3. Summary

Cyclodextrins are used to increase solubility, bioavailability and stability for drugs with low water solubility. Our results demonstrate for the first time that randomly methylated-β-cyclodextrin, hydroxypropyl-β-cyclodextrin and β-cyclodextrin-polymer can enter into intestinal epithelial cells by endocytosis. This process can contribute to the enhancement of the intestinal delivery and bioavailability of drugs by cyclodextrins in several ways. It can help to overcome the intestinal membrane barrier, the endosome formation increases the contact surface area between the cyclodextrin-drug complexes and the cell membrane and prolongs the retention time of cyclodextrins in the epithelial cells.

Then we demonstrated for the first time that β-cyclodextrins can improve the bioavailability of drugs with poor solubility and absorption not only by solubility improvement but by transporting the complexed drug into the cytoplasm of enterocytes via endocytosis. The permeability and absorption enhancing effect of cyclodextrins might involve several mechanisms, which may act simultaneously and thus, it is difficult to examine separately in cellular systems. However, we emphasize that in some special cases also endocytotic processes should be considered.

Since this study has demonstrated the role of macropinocytosis in the uptake of methylated-β-cyclodextrin in intestinal cells, this mechanism merits further investigations in connection with drug absorption mediated by cyclodextrins.

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5. Related publications by the candidate

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Registry number: DEENK/138/2016.PL
Subject: Ph.D. List of Publications

Candidate: Katalin Réti-Nagy
Neptun ID: EUDID0
Doctoral School: Doctoral School of Pharmaceutical Sciences
MTMT ID: 10036779

List of publications related to the dissertation

1. **Réti-Nagy, K.**, Malanga, M., Fenyvesi, É., Szente, L., Vámosi, G., Váradi, J., Bácskay, I., Fehér, P., Ujhelyi, Z., Róka, E., Vecsernyés, M., Balogh, G., Vasvári, G., Fenyvesi, F.: Endocytosis of fluorescent cyclodextrins by intestinal Caco-2 cells and its role in paclitaxel drug delivery. *Int. J. Pharm. 496*, 509-517, 2015.
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2. Fenyvesi, F., **Réti-Nagy, K.**, Bacsó, Z., Gutay-Tóth, Z., Malanga, M., Fenyvesi, É., Szente, L., Váradi, J., Ujhelyi, Z., Fehér, P., Szabó, G., Vecsernyés, M., Bácskay, I.: Fluorescently Labeled Methyl-Beta-Cyclodextrin Enters Intestinal Epithelial Caco-2 Cells by Fluid-Phase Endocytosis.
PLoS One. 9 (1), e84856, 2014.
DOI: http://dx.doi.org/10.1371/journal.pone.0084856
IF:3.234

List of other publications

3. **Szászné Réti-Nagy Katalin**, Fenyvesi F.: Ciklodextrinek alkalmazása 2..
Gyógyszerészet. 58, 568-570, 2014.

4. **Szászné Réti-Nagy Katalin**, Fenyvesi F.: Ciklodextrinek alkalmazása 3.
Gyógyszerészet. 58, 663-666, 2014.

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Pharmazie. 66 (7), 549-550, 2011.
IF:1.006

Total IF of journals (all publications): 8,893
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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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