

Carry and Cramp – Cyclodextrins with dual function

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Introduction

Cyclodextrins are well known about their potential in complexation of certain poorly water soluble molecules, like cholesterol or hydrophobic drugs. Moreover some earlier studies showed that randomly methylated or dimethyl-β-cyclodextrin treatment of cells can modulate activity of membrane proteins like P-glycoprotein (Pgp, MDR1). Among other factors elevated expression level of various multidrug resistance proteins results in undesired chemotherapy resistance of cancer cells. Methyl cyclodextrin treatment can change membrane lipid surrounding of transmembrane type efflux transporter proteins by extracting cholesterol (and other components) from the lipid bilayer. This way improved bioavailability of drugs could be reached due not only to the drug solubilizing ability but also to the inhibitory effect on the transporter mediated efflux.

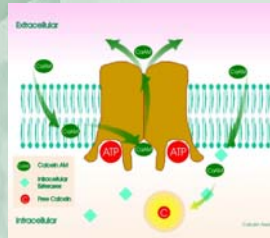


Figure 1: Schematic view of an ABC transporter and basics of Calcein assay

Cyclodextrin	Abbreviation	Degree of Substitution (Substituent/CD)
Randomly methylated-βCD	RAMEB	~12
Heptakis(2,3,6-tri-O-methyl)-βCD	TRIMEB	~21
Heptakis(2,6-di-O-methyl)-βCD	DIMEB	~14
Carboxymethylated-βCD	CMBCD	~3
Quaternary amino-βCD	QABCD	~4
Soluble carboxymethylated-βCD-polymer	CMBCDP	~3
Quaternary amino-βCD-polymer	QABCDP	~2

Table 1: short name and degree of substitution of investigated cyclodextrines

Results

Results of ³H-cholesterol extraction experiments showed that efficiency of CDs in cholesterol depletion from living cells depends strongly on (i) chemical nature of CD, (ii) original lipid composition of the cell membrane (insect and mammalian cells) and (iii) transporter expression profile. Methylated derivatives proved to be the most effective in cholesterol depletion (Figure 2) from Sf9 insect cells. RAMEB and DIMEB showed the highest efficacy also in case of mammalian cell lines. Cholesterol loss was higher in MDR1 overexpressing K562 cells (Figure 2B and 2C). This later phenomenon is presumably linked to recently described cholesterol export modulating ability of the MDR1 overexpression.

Cytotoxicity on different cells did not fully correlate. DIMEB was the most cytotoxic in all systems, however TRIMEB was much less cytotoxic on erythrocytes than on cell lines (Figure 5). Pgp inhibition (Figure 4) did not correlate completely with cholesterol extraction efficacy in K562MDR cells. Although the effective cholesterol extractor DIMEB shows the most characteristic inhibitor profile, RAMEB seems to be lot more potent than TRIMEB at removing cholesterol from K562 MDR membranes but equally active in blocking Pgp activity. More strikingly, CMBCD and QABCD that are more effective than TRIMEB in cholesterol extraction are totally inactive in the Calcein Assay.

Experimental

Cholesterol depletion effect of variously substituted β-cyclodextrin (CD) derivatives (Randomly methylated-βCD, Heptakis(2,3,6-tri-O-methyl)-βCD, Heptakis(2,6-di-O-methyl)-βCD, Soluble carboxymethylated βCD polymer, Carboxymethylated βCD, Quaternary amino-βCD-polymer, (2-Hydroxy-3-N,N,N-trimethylammonium)propyl-βCD) was investigated on different cell lines. Sf9 and recombinant baculovirus infected MXR-Sf9 (insect) cells, K562 and K562MDR (human leukemia) cell lines were cultured for 1 day in a medium containing 0.5 mCi/ml ³H-cholesterol to determine residual cholesterol content (activity) after cyclodextrin treatment (Figure 2). Cytotoxicity of CD derivatives and concentration dependence was measured by the CellTiter 96 (MTS) method (Figure 3). Inhibitory effect of the CD treatment on the transporter mediated efflux was investigated in Calcein assay (Figure 4), a fluorescence dye transport assay using MDR1 overexpressing K562MDR human cell line.

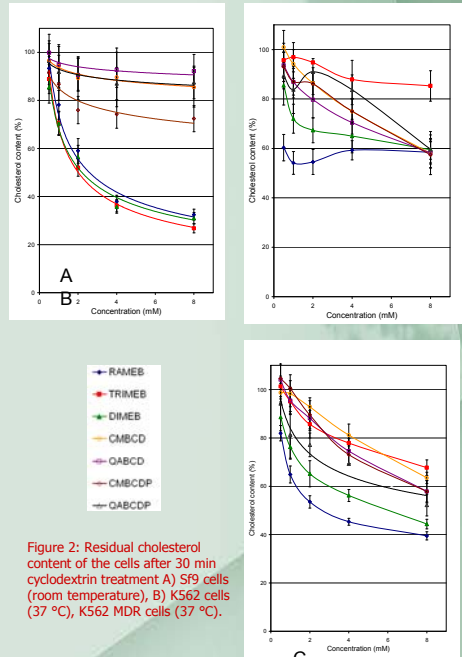


Figure 2: Residual cholesterol content of the cells after 30 min cyclodextrin treatment A) Sf9 cells (room temperature), B) K562 cells (37 °C), K562 MDR cells (37 °C).

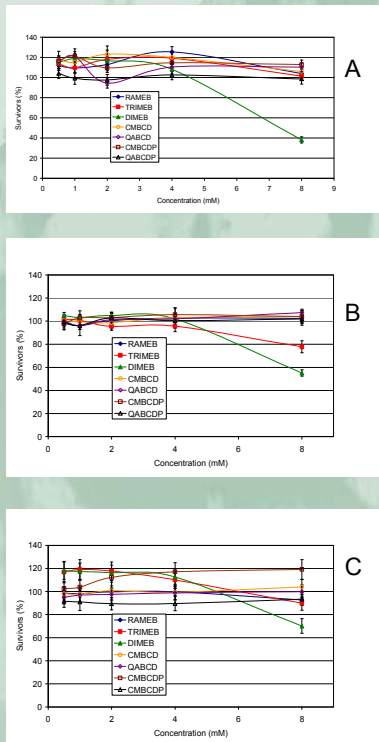


Figure 3: Cytotoxicity (MTS) test after 30 min CD treatment A) Sf9 cells (room temperature), B) K562 cells (37°C), C) K562 MDR cells (37°C)

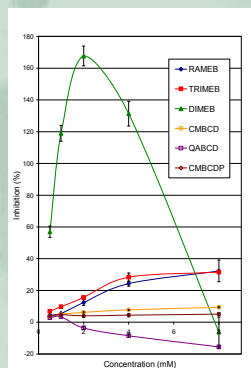


Figure 4: Inhibition of MDR1 transporter in K562 MDR cells after 30 min cyclodextrin treatment at 37 °C measured in Calcein Assay

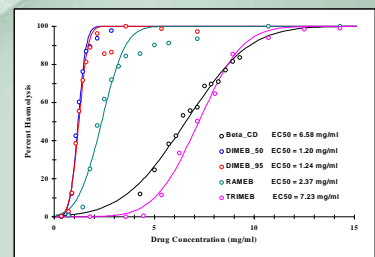


Figure 5: Hemolytic properties of the different methyl-βCD derivatives

Summary

Effect of variously substituted CD derivatives was investigated in cholesterol depletion, cytotoxicity and efflux transporter inhibition experiments using insect and mammalian cell lines. Our data suggest that Pgp inhibition by CDs may have a much more complex mechanism than simple removal of cholesterol. The observation of imperfect concordance between cytotoxic effect and Pgp inhibition inspires further studies to develop CD derivatives with low cytotoxicity but high drug carrier capacity and additional ability to cramp efflux transporter activity.

Acknowledgement

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