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Cytotoxicity of different types of methylated β-cyclodextrins and ionic derivatives

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Cyclodextrins (CDs) are widely used materials and still in the focus of drug development. In spite of the extensive studies, there is limited information about the cytotoxic effect of different derivatives. This study compares the cytotoxic effect of methylated β-CDs and some ionic derivatives. The methylated CDs involved in this study differ in the number and position of the methyl substituents. Heptakis(2,6-di-*O*-methyl)-β-CD (DIMEB) with a degree of substitution (DS) of 14 has two methyl groups in all of the seven glucose subunits mostly at O-2 and O-6 position, each OH group is methylated in heptakis(2,3,6-tri-*O*-methyl)-β-CD (TRIMEB) (DS = 21), and an unsystematic substitution is realized in randomly methylated β-CD (RAMEB). DS is defined as the number of substituents per cyclodextrin ring. Using the above definition, the DS for RAMEB is 12.6. To see the effect of the ionic groups an anionic and a cationic CD derivative were also investigated: (2-hydroxy-3-*N*,*N*,*N*-trimethylamino)propyl β-CD (QABCD) (DS = 2) and carboxymethylated β-CD (CMBCD) (DS = 3,5). The *in vitro* cell toxicity decreases in the order of DIMEB > TRIMEB ≥ RAMEB > QABCD > CMBCD. Ionic β-CDs were less toxic than the methylated derivatives.

CDs are often applied to solubilize biologically active substances. Water-soluble derivatives with low toxicity are required, therefore their biological effects are of great concern (Szente and Szejtli 1999). The *in vitro* toxicity of CDs is usually characterized by their hemolytic activity. The hemolysis-inducing ability of the α-, β-, and γ-CD molecules differs significantly, and this phenomenon is in accordance with their cytotoxic effect on leukaemic cells (Irie et al. 1982; Leroy-Lechat et al. 1994). Different substituents on a certain type of CD ring can confer diverse actions. Relations between the structure and the cytotoxic properties of CDs are essential factors for drug design.
This study demonstrates the effects of various substituents of β-CD on cytotoxicity. DIMEB, TRIMEB, RAMEB, CMBCD and QABCD were tested on HeLa cells using the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] assay and the resulting viability diagrams show that there are significant differences between the cytotoxicity of the derivatives up to 100 mM.

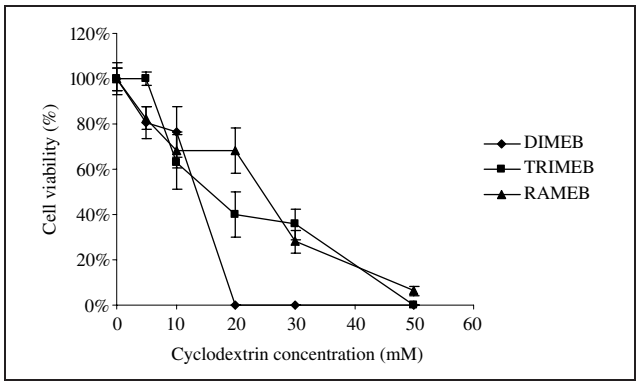

Fig. 1: Concentration-viability diagram of methylated β-CDs (mean ± SD; n = 3)

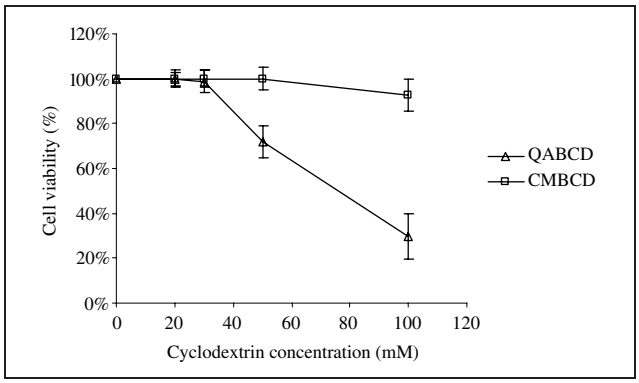

Fig. 2: Concentration-viability diagram of ionic β-CD derivatives (mean ± SD; n = 3)

The cell toxicity of methylated β-CDs decreases in the order of DIMEB > TRIMEB ≥ RAMEB (Fig. 1). An increase in the number of the methyl groups at the molecule decreases the toxic effect in the case of DIMEB and TRIMEB. A similar alteration was observed for the hemolytic effect of these derivatives (Hirayama et al. 1999). The random substitution of the β-CD ring by methyl groups decreased the toxic activity as compared to DIMEB and TRIMEB.
CMBCD and QABCD showed to be less toxic on cells (Fig. 2). Incorporation of the negatively charged carboxymethyl group into the molecule inhibits the development of the cell toxicity in the concentration range examined.
It can be concluded that in the case of β-CD, cell toxicity is dependent on the number and the position of the methyl groups and ionic substituents can decrease the toxic effects as compared to the electroneutral methylated molecules.

Experimental

1. Cells

HeLa cells were obtained from the European Collection of Cell Cultures (ECACC) and maintained in Dulbecco's Modified Eagel's medium

(DMEM), supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine and 100 mg/L gentamycin in 5% CO_2 at 37 °C.

2. Chemicals

Randomly methylated β-CD (RAMEB) is a product of Wacker Chemie, Münich, Germany, all the other CDs were obtained from Cyclolab Ltd., Budapest, Hungary. The CDs were dissolved in phosphate buffered saline (PBS) at a concentration between 1–100 mM. The other reagents were purchased from Sigma-Aldrich (Budapest, Hungary).

3. Cytotoxicity assay

The cytotoxic effect of CDs was evaluated by a colorimetric cytotoxicity method i.e. the MTT test (Mosmann 1983). The test was performed as follows: HeLa cells in complete medium were seeded to 24-well plate at a final density of 8×10^4 cells/well. After 2–3 days the medium was removed, the cells were washed with PBS and the CD test solution was added. The cells were then incubated for 30 min at 37 °C in a 5% CO_2-air incubator. After incubation, the samples were removed, and the cells were washed twice with 1 ml PBS. At the end, 0.9 ml medium and 100 µl MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] solution (5 mg/ml) were added to each well. The plates were further incubated for 4 h, the MTT solution was removed and 2 ml of DMSO were added to dissolve the formed formazan crystals. The absorbance of each sample was recorded at 570 nm by a Shimadzu UV-1601 spectrophotometer. Data were expressed as the percentage of viable control cells calculated from the absorbance at 570 nm, corrected for background absorbance.

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A new arbutin derivative from the herb of *Myrothamnus flabellifolia* Welw

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The ethyl acetate soluble fraction of an acetone/water extract of the air-dried aerial parts from *Myrothamnus flabellifolia* Welw. (Myrothamnaceae) was fractionated by a combination of CC on Sephadex LH-20, MPLC on RP-18 material and LPLC on MCI-gel. This procedure has led to the isolation of 2,3-di-*O*-galloylarbutin, a new representative of the rare 2,3-diacylated glucopyranosides. The structure was elucidated with the help of 2D-NMR and ESI-MS experiments. Conformation of D-glucose was established by CZE (capillary zone electrophoresis).

Myrothamnus flabellifolia Welw. (Myrothamnaceae), a species growing in arid areas of Southern and Eastern Africa has a strong ability to survive regular periods of extreme dryness and high temperatures by dehydrating the vegetative tissue to an air-dry state. When getting in contact with water, the hydrated plant material rearranges colour and shape and is starting to flower within a short time period. Therefore, the plant is recorded to belong to the so-called desiccation-tolerant systems or resurrection plants (for review s. Moore et al. 2007). The plant is widely used in traditional South African medicine. Main diseases treated with *Myrothamnus* extracts are cough, colds, influenza, mastitis, haemorrhoids and a topical use for skin and mucosal irritations (Moore et al. 2007). From the phytochemical point of view the plant contains volatile oil with pinocarvone as main constituent (Viljoen et al. 2002), 3,4,5-tri-*O*-galloylquinic acid (Moore et al. 2005), a range of different flavan-3-ols and proanthocyanidins (Petereit et al. 2006) and arbutin (Suau et al. 1991).

The ethyl acetate soluble fraction of an acetone/water (7 + 3) extract of *Myrothamnus fabellifolia* was fractionated by a combination of column chromatography on Sephadex®LH-20, MPLC on RP-18 material and by a final purification by LPLC on MCI® gel. The purified compound **1** (yield 0.0025%, Fig. 1) was identified by ^1H and ^{13}C NMR as double galloylated arbutin. The key correlations were derived by 2D-NMR experiments (HBMC, Fig. 2) and proved the structure of 2,3-di-*O*-galloylarbutin. D-Glucose was established by CZE (capillary zone electrophoresis) after derivatization with *S*-(−)-1-phenylethylamine and reduction with sodiumcyanoborhydride (Noe and Freissmuth 1995). To the best of our knowledge, **1**