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ORIGINAL ARTICLE

Effect of cyclodextrins on the viability of endothelial cells

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Abstract Natural cyclodextrins (CD) and some of their derivatives have been tested in order to evaluate their cytotoxicity on LT2 endothelial cells. After 1 h of treatment with concentrations not higher than 10 mM, the number of cells was quantified by measuring DNA content. No significative decrease in the DNA amount was observed except for the dimethyl-β-CD at the highest tested concentration.

Keywords Cyclodextrins · Cytotoxicity · DNA assay · Endothelial cells · LT2 cell line

Introduction

Cyclodextrins (CD) are widely used in pharmaceutical technology to form inclusion complexes with poorly water-soluble drugs in order to increase their aqueous solubility and to allow intravenous administration. In this context and to compare their safety towards the vascular endothelium, tests on human umbilical vein endothelial cells (HUVEC) were previously performed with a limited number of derivatives (HP-β-CD, Dimeb and SBE-β-CD) [1].

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The present work focuses on testing a larger panel of CD (i.e. the newly commercialized Crysmeb®) on endothelial cells.

The aim of this study was to evaluate the viability of immortalized HUVEC (LT2) in the presence of different CD currently available in pharmaceutical technology. Natural cyclodextrins (α-CD, β-CD and γ-CD) and some of their derivatives (HP-α-CD, HP-β-CD, HP-γ-CD, Crysmeb®, Rameb, Dimeb, Trimeb, SBE-β-CD and sulfated β-CD) have been tested.

Materials and methods

The LT2 cell line originates from human umbilical vein endothelial cells (HUVEC) which have been immortalized by transfection with large T SV40 antigen. Cells were grown at 37°C in a humidified 95% air-5% CO_2 atmosphere, using 2‰ pork skin gelatin-coated culture dish and RPMI medium supplemented with 10% fetal bovine serum (FBS), 90 units/ml penicillin and 90 μg/ml streptomycin. Cells at passages between 12 and 15 were used in the experiments.

The different cyclodextrins studied have been divided into four groups which were tested separately. Natural cyclodextrins (α-CD, β-CD and γ-CD) belong to the first group whereas their hydroxypropylated derivatives (HP-α-CD, HP-β-CD, HP-γ-CD) belong to the second one. Methylated β-CD (Crysmeb®, Rameb, Dimeb, Trimeb) were tested together as part of the third group and the fourth group included the sulfobutylether (SBE-β-CD) and sulfated derivatives (sulfated β-CD).

The cells were harvested by trypsinization, seeded at an initial density of 25,000 cells/well in 24-well coated plate, and allowed to attach overnight. After two washings, they were incubated during 1 h in 1 ml of the culture medium containing concentrations of CD ranging from 0.1 to 10.0 mM or in the medium without CD. After incubation, cells were washed and the remaining attached cells were quantified by measuring DNA content using a fluorimetric method with bisbenzimide as reagent and the SpectraMax Gemini XS apparatus (excitation and emission wavelenghts were 356 and 458 nm respectively).

Results and discussion

The effect of natural CD, hydroxypropylated, methylated and other derivatives are presented in Figs. 1, 2, 3 and 4, respectively.

As it can be seen on Fig. 1, no results are shown for β-CD at concentrations higher than 0.1 mM. As a matter of fact, we observed precipitation for these solutions probably due to the formation of insoluble complexes with serum components.

The number of cells determined as DNA amount is not modified by the 1 h treatement with the different concentrations of CD except for the 10 mM Dimeb

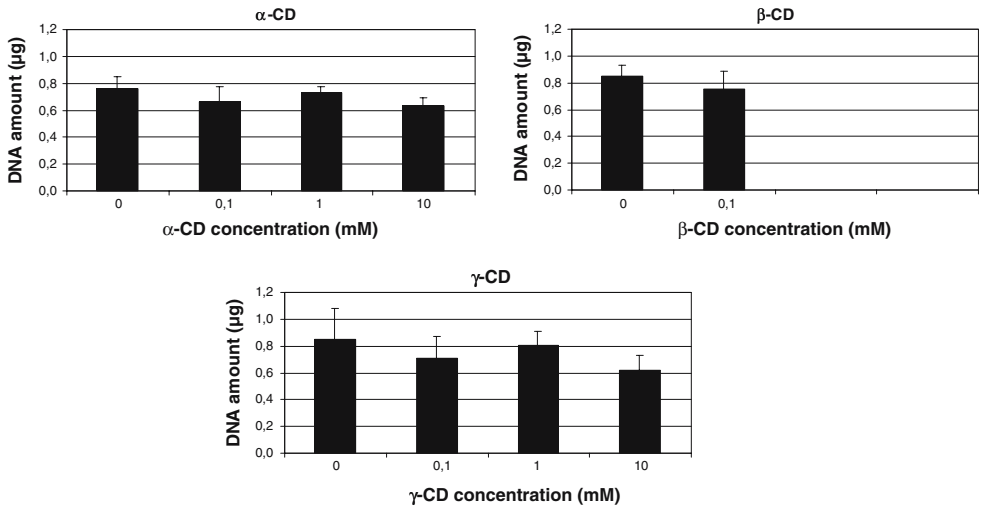

Fig. 1 DNA amount for cells incubated 1 h with solution of α-, β- or γ-CD ranging from 0 to 10.0 mM (except for β-CD) ($n = 3$)

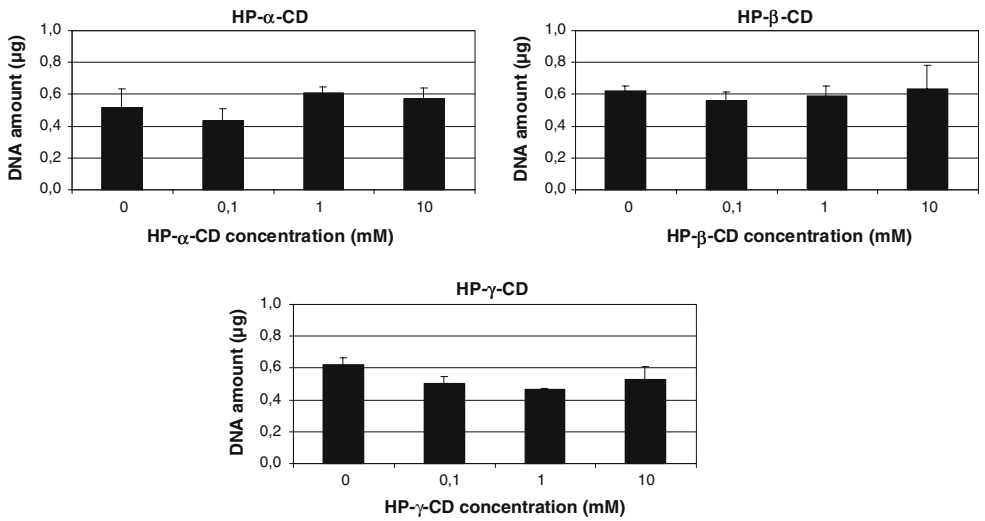

Fig. 2 DNA amount for cells incubated 1 h with solution of HP-α-, HP-β- or HP-γ-CD ranging from 0 to 10.0 mM ($n = 3$)

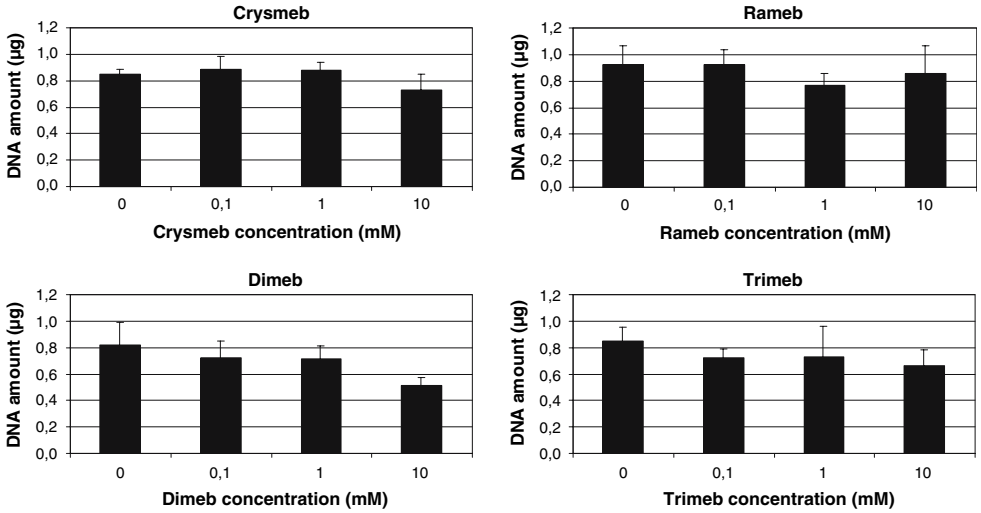

Fig. 3 DNA amount for cells incubated 1 h with solution of Crysmeb®, Rameb, Dimeb or Trimeb ranging from 0 to 10.0 mM (n = 3)

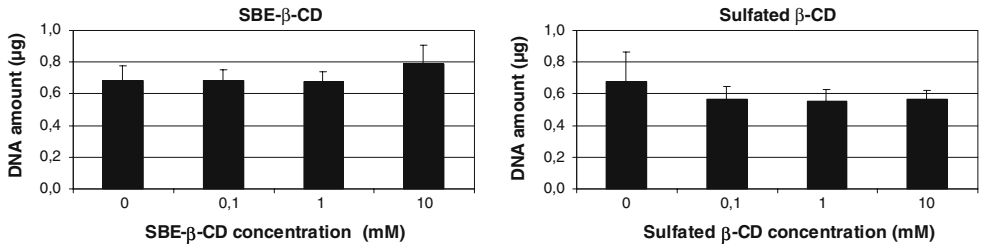

Fig. 4 DNA amount for cells incubated 1 h with solution of SBE-β-CD or sulfated β-CD ranging from 0 to 10.0 mM (n = 3)

solution. As shown on Fig. 3, a significative decrease of DNA amount is observed at this concentration suggesting a most damaging effect on cell viability.

Conclusion

From the experimental study, it can be concluded that Dimeb has the most damaging effect on the integrity of cell membranes. These results can be correlated with those obtained with physiologic HUVEC and with studies comparing the ability of CD to form inclusion complexes with membrane components. It can also be concluded that other tested cyclodextrins are not cytotoxic in the conditions described above allowing to evaluate, in future studies, the effects of CD on cellular functions.

Reference

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