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Reduction of the haemolytic effect in a biologically recognisable β-cyclodextrin

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β-cyclodextrin derivatives having azido, amino and bioactive galactosylamido spacer functions were tested for haemolytic effect and compared with that of hydroxypropyl-β-cyclodextrin. The cyclodextrin coupled to the bioactive saccharide galactose via a spacer and which has bio-recognition properties for cell-wall lectin shows an extremely reduced haemolytic effect.

INTRODUCTION

The cyclodextrins are widely used in the pharmaceutical, cosmetic and food industries for their aptitude to include a large number of organic molecules.[1] Particularly, in the pharmaceutical field, they are used for the solubilisation, stabilisation and transport of biologically active molecules.[2]

Toxicity studies have shown that for α-, β- and γ-cyclodextrin oral doses up to 10g.kg^{-1} may be administered without adverse results.[3] However, β-cyclodextrin, the most readily available and least expressive cyclodextrin (20 USD kg^{-1}), presents haemolytic activity[4,5]. A diminution in the haemolytic activity is observed for the branched cyclodextrins (substituted at the primary face by glucosyl or maltosyl groups).[6] At present the hydroxypropyl derivatives of β-cyclodextrin show the best combination of high solubility and reduced haemolytic activity.[7] Their use for humans is still limited to oral administration although parenteral administration is possible.[8]

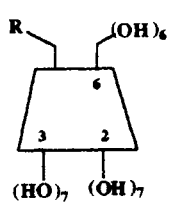
In spite of this promise for the transport of drugs, the lack of functionalities for biological recognition renders this transport non-specific. We have recently developed the synthesis of modified cyclodextrins carrying bioactive saccharides coupled to the macrocycle via a flexible spacer and capable of being recognised by biological receptors.[9] For such systems to be effective for the vectorisation of drugs, it would be helpful if they showed zero or extremely low haemolytic activity.

In this communication we report the haemolytic activity of the β-cyclodextrin derivatives **2**, **3** and **4**, as shown in Figure 1, and compare them with that of β-cyclodextrin, **1**, and hydroxypropyl β-cyclodextrin, **5**.

EXPERIMENTAL

Materials and methods

Chemicals. β-cyclodextrin (β-CD) was a gift from Wacker GmbH and hydroxypropyl β-cyclodextrin (HP β-CD) (MS 0.6) was purchased from Acros. Compounds **2**, **3** and **4** were prepared as previously published.[9] The mono-6-azido derivative **2** was obtained from treatment of the mono-6-tosyl[10] with NaN_3, reduction with PPh_3 and NH_4OH to give the amine **3**. The β-CD galactosyl derivative **4** was obtained after peptide coupling of the spacer-Gal to **3**. Cyclodextrin solutions were prepared by dissolution of the appropriate compound in phosphate buffered saline 10x and were then diluted tenfold. Subsequently the pH was adjusted to 7.4 by addition of $NaHCO_3$.



1 R = OH
2 R = N_3
3 R = NH_2
4 R = β-D-Gal-NHCO-$(CH_2)_7$-CONH

Figure 1 Structure of the new compounds tested

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Haemolysis Tests. Haemolysis tests were carried out on freshly-drawn human whole blood. Erythrocytes were diluted using PBS (1/10) before assays. 50 µL of dilute sample was added to 1mL of the CD-derivative solution for increasing values of the CD concentration, depending on the CD solubility. Five concentrations were measured for each derivative. The mixtures were stirred and incubated for 30 min at 37°C, then centrifuged at 1000g for 10 min. The haemoglobin concentration in the supernatant was assayed spectrophotometrically at 543 nm. The percentage haemolysis was expressed as the ratio of the absorbance of the samples with derivatives to the absorbance of a control (complete haemolysis of erythrocytes in water).

RESULTS AND DISCUSSION

In Figure 2 is shown the haemolytic activity of the series of β-CD derivatives for whole blood cells. The percentage of haemolysis is plotted against concentration of the derivatives.

In Table 1 are reported two characteristic values obtained from every haemolysis curves: TD_0 = CD concentration at the beginning of the haemolytic effect, and TD_{50} = CD concentration for 50% haemolytic effect. TD_{100} = CD concentration for maximal haemolytic effect cannot be obtained under current experimental conditions. The tests were carried out at the same cell density as in reference 7, but the pH is here 7.4, which may explain a shift of TD_0 and TD_{50} to higher concentrations for β-CD and HP β-CD. From Figure 2 and Table 1, the order of decreasing haemolytic activity is:

β-CD > NH_2 β-CD > HP β-CD ≫ N_3 β-CD > β-Gal-sp β-CD

From these results, NH_2 β-CD is almost as haemolytic as β-CD itself and thus would be of little interest for parenteral use as compared to HP β-CD. This could be expected, since the simple variation from OH to NH_2 at one of the glucopyrannose units should have little effect on the hydrogen bonding pattern and inclusion properties. Effectively, the haemolytic activity of the CD derivatives seems conditioned partially by the ability of the derivative to include and remove from the cell wall lipids such as cholesterol and certain phospholipids.[7,11]

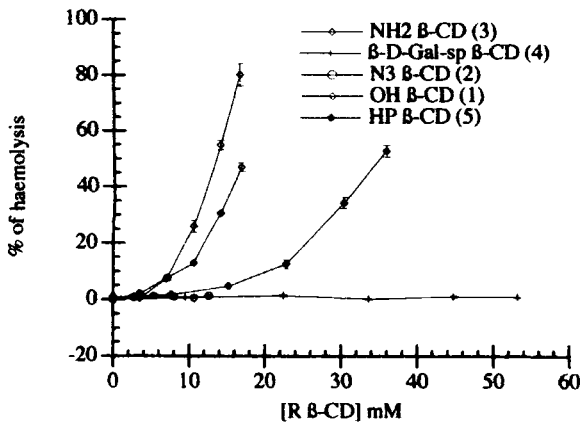

Figure 2 Haemolysis curves for the β-CD derivatives (n = 3)

Table 1 Characteristic values of the haemolytic curves of R β-CD.

CD	*β-CD*	*N_3 β-CD*	*NH_2 β-CD*	*β-D-Gal-Spacer β-CD*	*HP β-CD*
	1	**2**	**3**	**4**	**5**
DT_0 (mM)	4	*	4	>55	8
DT_{50}(mM)	13	*	≥17	>55	35

(*: no toxicity even at saturation, very low solubility)

In contrast, the mono-azido derivative **4** shows little or no haemolytic activity until its saturation concentration, 12.6 mM. Here the inclusion properties may be modified by auto-inclusion of the azido-group as observed for the X-ray structure of the heptakis-β-CD derivative[12]. However, the low solubility of the compound reduces its interest.

Compound **4**, the cyclodextrin derivative coupled to bio-active saccharide galactose via a spacer arm and which shows useful bio-recognition properties with regard to the cell-wall lectin of *Kluveromyces Bulgaricus*,[14] shows zero haemolytic activity even at concentrations greater than **50mM**. This may arise not from a diminution of the capacity for inclusion which actually retained or even improved[10] but probably from increased residence time on or near the cell wall. In contrast, HP-β-CD shows 50% haemolytic activity at 35 mM, and the β-CD galactose derivative where there is direct saccharide-CD coupling shows haemolytic activities intermediate between those of β-CD and HP-β-CD[13].

In conclusion, **4** is of considerable promise for pharmaceutical applications having a high aqueous solubility, the capacity to be recognised by cell-wall lectins,[14] and an effectively zero haemolytic activity at all concentrations.

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