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Cyclodextrin (CD) complexes of cholesterol – their potential use in reducing dietary cholesterol intake

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The main objective of this investigation was to test and see if cyclodextrins, a type of molecule which form inclusion complexes, could effectively prevent the absorption of food derived cholesterol. Cyclodextrins are nontoxic and easily tolerated, having only a slight sweet taste. α,β,γ-Cyclodextrins, 2-Hydroxypropyl-beta-cyclodextrin (2-HPCD), and heptakis-*O,O*-dimethyl-beta-cyclodextrin (DMCD) were tested in solutions of different concentrations. These solutions were then saturated with cholesterol and the excess cholesterol was removed. The clear solutions left were then analyzed by HPLC to assess the amount of cholesterol in the solutions, and compared to a standard. DMCD gave the best results in successfully dissolving (complexing) most of the cholesterol followed by HPCD and alpha-cyclodextrin. Beta- and gamma-CD showed nearly insignificant complex formation. After administration of 10 mg of cholesterol to mice through a gastric tube, the cholesterol level increased about 125–130%, and only 15–20%, if the cholesterol was administered together with 20 mg of DMCD. That means, the DMCD formed complexes with approximately 80–85% of the cholesterol administered in the mice gastrointestinal tract.

1. Introduction

High cholesterol blood levels significantly contribute to a variely of cardiorascular diseases (Small et al. 1974; Gale 1985).

If it is desired to reduce the blood cholesterol level, the first major possibility is to prevent its absorption (Frijlink et al. 1991; Riottot et al. 1993). The main hypothesis of the present work is that food derived cholesterol can be prevented to be absorbed to the blood if it is bound to specific, unabsorbable molecules. Cyclodextrins (CD) are molecules, which form water soluble complexes with lipophilic water insoluble molecules, such are various steroids, including cholesterol. This process is called inclusion phenomenon. The complexed cholesterol cannot be absorbed to the blood and thus it will be excreted in urine (Frijlink et al. 1990). Inclusion phenomena are characterized by the physical interaction between two molecules, a host molecule and a guest molecule. This non-chemical bond is helpful in stabilizing and solubilizing chemicals in water which would otherwise be unstable or insoluble (Szejtli 1982).

Cyclodextrins are cyclic carbohydrates consisting of six, seven, or eight glucose units and are called alpha, beta, and gamma cyclodextrins, respectively (Pitha et al. 1987). The cyclodextrins are produced by an enzymatic conversion of starch, followed by an elaborate purification process (Szejtli 1982). Cyclodextrins can be simplistically represented as cylinders (Fig. 1), with a hydrophobic inside and hydrophilic outside.

The hydrophilic inside forms a cavity in which poorly water-soluble molecules can cover their most hydrophobic parts. The cavity of cyclodextrins is of a fixed shape and size so that if there is a molecule with poor water solubility which contains a part of the correct size, it will be attracted to the cavity (Yoshida et al. 1988).

The effect of 2-hydroxypropyl-β-cyclodextrin (2-HPCD) on the aqueous solution of 18 drugs was investigated. The solubility of selected drugs was tested only in 25% (w/w) aqueous 2-HPCD solution, but the solubility of cholesterol was not studied (Loftsson 1989). CD complexation influences the stability of drugs (Brewster et al. 1992).

In order to optimize the complexation of cholesterol, five different cyclodextrins, in different concentrations were tested: alpha-, beta-, gamma-cyclodextrins, 2-hydroxypro-

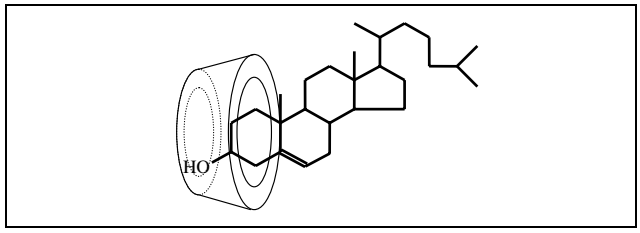
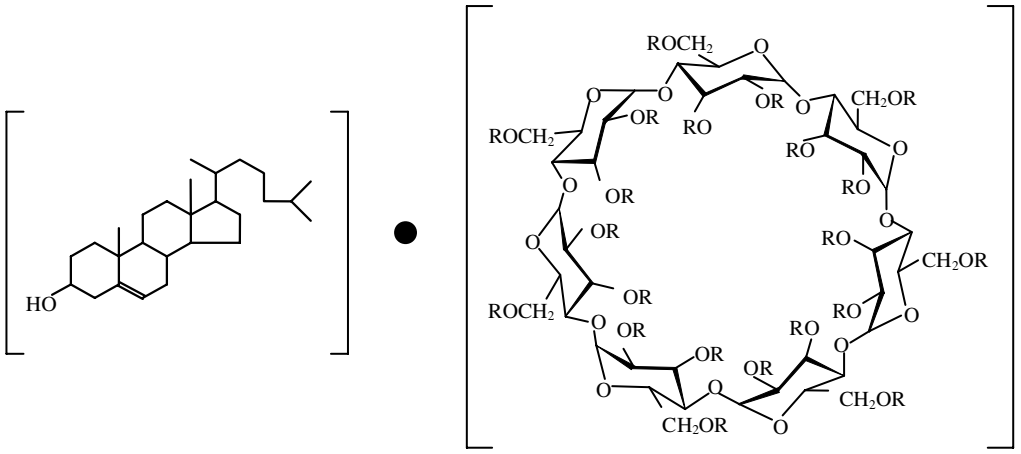


Fig. 1: Cholesterol-cyclodextrin complex. Illustration of the fit of a part of the cholesterol molecule in the hydrophobic cavity of a cyclodextrin



Cholesterol Beta-Cyclodextrin

R=[(CH$_2$CH(CH$_3$)O)]$_n$H: HPCD
R=(CH$_3$): DMCD
R=H: α-Cyclodextrin (GM: 6, MW: 972, ICD: 5.2 Å)
β-Cyclodextrin (GM: 7, MW: 1135, ICD: 6.4 Å)
γ-Cyclodextrin (GM: 8, MW: 1297, ICD: 8.3 Å)
(HPCD: 2-Hydroxypropyl-beta-cyclodextrin, DMCD: Heptakis-O,O-dimethyl-beta-cyclodextrin, GM: glucose monomer, MW: molecula weight, ICD: internal cavity diameter)

Fig. 2: Representation of a 1 : 1 complex between cholesterol and a beta-cyclodextrin derivative

pyl-beta-cyclodextrin (2-HPCD), and heptakis-*O*,*O*-dimethyl-β-cyclodextrin (DMCD). Alpha-, beta-, and gamma-CD are the three natural cyclodextrins with six, seven and eight glucose units respectively. On all natural cyclodextrins, there are three free hydroxyl groups on each glucose unit. The hydrogen on these hydroxyl groups can be replaced by a substituent (Szejtli 1982). In the case of 2-HPCD an average of one hydrogen per glucose unit is replaced by a hydroxypropyl function (Fig. 2), while in DMCD an average of two hydroxy hydrogens per glucose unit is replaced by methyl groups (Szejtli 1982). The addition of these substituents allows the cyclodextrins to be better water soluble, by reducing the attracting forces between the cyclodextrin molecules (Irie et al. 1988). The more water soluble cyclodextrins should be able to dissolve (complex) more cholesterol. Ultimately, a good complexing cyclodextrin should produce a dramatic reduction in the absorption of cholesterol (Garcia-Mediavilla et al. 2003; Nishijo et al. 2004). Cholesterol is lipophilic, and thus it is attracted the cyclodextrins cavity.

2. Investigations and results

2.1. Solubility of cholesterol in Cyclodextrin solutions of various concentration

In order to optimize the complexation of cholesterol, five different cyclodextrins (CDs), in different concentrations were tested: alpha-, beta- and gamma-CD, and two chemically modified CDs: 2-hydroxypropyl-beta-CD (2-HPCD), and heptakis-*O*,*O*-dimethyl-beta CD (DMCD).

Two concentrations of alpha-CD were tested: 6.25%, and the saturated solution of 10.0%. Beta-CD is only soluble in water to the extent of 1.3%. A 1.0% solution was tested. Gamma-CD solutions of 6.25%, 12.5%, and 25.0% were investigated. The more soluble DMCD and 2-HPCD were used in 10, 20, 30, 40 and 50% concentrations, prepared by serial dilution. All the sample solutions were then saturated with cholesterol.

The various concentrations of the cyclodextrins, and the solubility of cholesterol in the different CD solutions are shown in Table 1.

It was found that the natural cyclodextrins do not solubilize cholesterol too well. Thus, alpha-cyclodextrin solubilized maximum 0.09 mg/ml cholesterol, the 12.5% and 25.0% gamma-cyclodextrin solubilized 0.01 and 0.012 mg/ml cholesterol, respectively, while the 6.25% gamma-cyclodextrin and beta-cyclodextrin solutions did practically not dissolve any cholesterol.

However, the chemically modified cyclodextrins, and particularly DMCD showed dramatic complexing power, as shown in Table 1.

The complexing difference among the various cyclodextrins is clearly illustrated in Fig. 3. The maximum cholesterol amount complexed by 1 ml of 50% DMCD is almost 50 mg. As 1 ml of 50% DMCD contains 500 mg of DMCD, 10 g of DMCD (about one full tablespoon) could complex 1000 mg cholesterol.

Table 1: Solubility of cholesterol (mg/mL) in various concentrations of the cyclodextrins tested

Conc. of CD% (w/v)	Concentrations of cholesterol (mg/mL)				
	2-HPCD	DMCD	α-CD	β-CD	γ-CD
1.00	NA	NA	NA	b	NA
6.25	NA	NA	0.09	NA	b
10.00	0.77	6.94	0.09	NA	NA
12.50	NA	NA	NA	NA	0.01
20.00	4.68	17.29	NA	NA	NA
25.00	NA	NA	NA	NA	0.012
30.00	5.01	24.02	NA	NA	NA
40.00	8.24	35.60	NA	NA	NA
50.00	13.97	49.11	NA	NA	NA

(β-cyclodextrin and γ-cyclodextrin are not shown as they have solubilized negligible amounts of cholesterol)
NA: not available
b: below of detectability (5 μg/mL)
The standard deviation of data were less than 5%

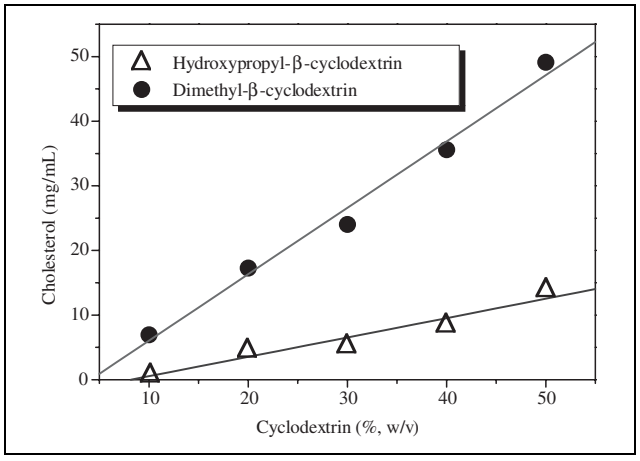


Fig. 3: Saturated solubility of cholesterol in various concentrations of chemically modified beta-cyclodextrin (HPCD and DMCD)

Table 2: Blood cholesterol concentrations (mg/mL) in mice

	Control	1 h after administration of 10 mg cholesterol	1 h after administration of 10 mg cholesterol plus 20 mg of DMCD
1	1.52 ± 0.06	3.59 ± 0.14	1.75 ± 0.08
2	1.47 ± 0.05	3.26 ± 0.11	1.73 ± 0.09
3	1.58 ± 0.06	3.68 ± 0.15	1.88 ± 0.10
4	1.51 ± 0.06	3.41 ± 0.12	1.81 ± 0.07
5	1.53 ± 0.05	3.36 ± 0.13	1.79 ± 0.08
Average	1.52 ± 0.06	3.46 ± 0.13	1.79 ± 0.08

2.2. In vivo study

Five male mice (C57BL/6J) per group, weighing 18–32 g were used. The first group was the control group. In the control group only the solvent was administered. The second group received 10 mg solid cholesterol through a gastric tube, and the third group 10 mg solid cholesterol and an aqueous solution of 20 mg DMCD the same way.
Table 2 *in vivo* cholesterol concentration in control group and the concentration change of cholesterol after administration of 10 mg cholesterol and 10 mg of cholesterol with 20 mg of DMCD solution.
Each value is the mean of five independent determinations.

3. Discussion

The solubility of cholesterol in various concentrations of the cyclodextrins are shown in Table 1 and Fig. 3. It was found that the natural cyclodextrins do not solubilize cholesterol too well. The chemically modified cyclodextrins, and particularly DMCD, however, showed dramatic complexing power, as shown in Table 1.
The results of *in vivo* experiments are shown in Table 2. The experiments demonstrated that DMCD formed complexes with approximately 80–85% of the cholesterol administered, thus the cholesterol level only increased about 15–20%, if the cholesterol and the DMCD were administered concomitantly. As one large egg contains 250 mg of cholesterol, 3 oz. (~85 g) of beef has 77 mg, and 3 oz. of liver has 372 mg, it is entirely possible that DMCD can indeed prevent absorption of substantial amounts of dietary cholesterol, because as already mentioned, 10 g of DMCD could complex 1000 mg of cholesterol.

4. Experimental

4.1. Solubility of cholesterol in Cyclodextrin solutions

Two concentrations of alpha-cyclodextrin were tested: 6,25% and the saturated solution of 12,5%. Beta-cyclodextrin is only soluble in water to the extent of 1.3%. A 1.0% solution was tested. Gamma-cyclodextrin solutions of 25.0%, 12.5%, and 6.25% were investigated. DMCD and HPCD was used in 10, 20, 30, 40, and 50% concentrations. DMCD (5 g) was dissolved in water to obtain a 10 ml total volume. This is the 50% (w/v) solution. This solution (1.6 ml) was diluted to 2 ml resulting in the 40% solution, while 1.2, 0.8, and 0.4 ml were diluted to 2 ml to give the corresponding 30, 20, and 10% solutions. The HPCD solutions were done similarly. All of the sample solutions were then saturated with cholesterol by shaking them in closed vials for a few days. The excess cholesterol was separated by filtration and 40 µl of the clear solution were removed with a syringe and diluted with 450 µl of methanol (a 12.25 times dilution). Ten microliters of these solutions were injected to the HPLC to determine the cholesterol content in the solutions. Apparatus: Hewlett-Packard 1050 model HPLC. NOVAPAK phenyl column (7.6 cm by 3.9 mm I.D.). Mobil phase : acetonitrile : water 70 : 30. Flow rate: 1 ml/min. Detector: UV/VIS operated at 220 nm. Retention time of cholesterol: 4.25 min. External standard was used, as follows: 0.3724 mg/ml cholesterol solution in methanol was prepared. Ten microliters of the solution was injected.

4.2. In vivo study

Five male mice (C57BL/6J) per group, weighing 18–32 g were used. The first group was the control group, where only the solvent was administered. The second group received 10 mg of solid cholesterol through a gastric tube, and the third group 10 mg of solid cholesterol and an aqueous solution of 20 mg of DMCD the same way.
The animals were sacrificed under anesthesia. One hour after the administration the collected blood samples were extracted with chloroform. The clear solution was injected in to the GC-MS system to determine the cholesterol content (HP GC5810, MSD5971, 25 m Ultra-2 column 5% phenylmethyl silicon, 0.52 µm, 0.32 mm I.D. temperature program 80 °C, 16 °C/min, 280 °C, 5 min. carrier gas He).

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