Synthesis and Controlled Release of Biocompatible Prodrugs of β-Cyclodextrin Linked with PEG Containing Ibuprofen or Indomethacin

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A B S T R A C T

Prodrugs of β-cyclodextrin (β-CD) conjugated with nonsteroidal anti-inflammatory drugs such as ibuprofen and indomethacin were prepared under mild conditions by two different routes. In the first procedure, β-CD was either reacted with ibuprofen-carboxy chloride or treated with ibuprofen in the presence of dicyclohexylcarbodiimide (DCC). Also conjugated β-CD-indomethacin was prepared via reacting of β-CD with indometacin in the presence of DCC. In the second procedure, acid terminated poly (ethylene glycol) (PEG, Mₚ = 600) was connected to the β-CD in the presence of DCC and the resulted polymer reacted with ibuprofen. In the similar reactions, β-CD was linked to PEG then ibuprofen and indomethacin simultaneously reacted with resulted polymer. The hydrolysis of drug-polymers was carried out in the aqueous buffer solutions in pH 1, 7.4, and 8 at 37°C. The quantity of hydrolyzed drugs was determined by using UV spectrophotometer.

Key Words: prodrugs; cyclodextrin; ibuprofen; indomethacin; hydrolysis.

INTRODUCTION

Chemical modification of basic cyclodextrins to provide suitable derivatives with ideal cavities either in size or with chemical properties has been a matter of great interests. The additional capability of cyclodextrins (CDs) makes them ideal molecules in which catalytic functional groups can be attached in order to form enzyme mimic models. As a result, CDs have been the subjects of many detailed investigations in the last decades in order to provide basic understanding of specific
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binding and catalysis of enzyme action. Many research groups have attempted to change the structure and properties of CDs through the chemical modifications of hydroxyl groups. However, due to their symmetry and high functionality and problems associated with their chemo- and regio-selective functionalization, mixtures of compounds are usually obtained. Therefore, subsequent purification of the compounds is still a highly challenging task. In this way, designing methods for regio-selective or per-substitution of CDs with new inclusion and catalytic properties or pharmaceutical applications are important fields of work [1, 2, 3].

The synthetic macromolecules with functional groups of pharmacological potential have received considerable attention in the last two decades. The preparation of macromolecular prodrugs is recognized as an effective way to prolong drug pharmacological activity where polymers are used as drug carriers [4,5,6]. These systems increased potentiality of the drug therapeutic by reducing daily multiusage and side effects [7, 8, 9].

CDs Are potent candidates for such role and also for modification of physical, chemical, and biological properties of drugs through the formation of inclusion complexes or drug-conjugates. The potential use of natural CDs and their synthetic derivatives have been extensively studied to improve certain drug properties such as solubility and/or bioavailability [3]. However, physico- and pharmaceutical properties of CD conjugates in which a drug is covalently bonded to the CD may be significantly different from those of the inclusion complexes [10]. Several CD-drug conjugates and their pharmaceutical properties have been reported [10,11,12].

Here we report the preparation of conjugated ibuprofen-and indomethacin-β-CD as prodrugs and preparation of linked poly(ethylene glycol)-β-CD in conjugation with ibuprofen or indomethacin. The structure of prepared compounds were characterized by FTIR, 1H NMR spectroscopies and elemental analysis. The hydrolysis of CD linked to poly (ethylene glycol) (Mw= 600) containing ibuprofen and indomethacin was studied in aqueous buffer solutions at physiological conditions [13-16].

EXPERIMENTAL

Instruments

FTIR Spectra were measured on a Shimadzu Model FTIR-8101M spectrometer. The 1H NMR spectra were recorded on a FTNMR (400 MHz) Bruker in CDCl3. The amount of released drug was determined by a 2100 Shimadzu UV spectrophotometer. All the solvents were dried according to the literature procedures. Thin layer chromatography was performed on silicagel plates 60F254 in the solvent system A: n-butanol/glacial acetic acid/DMF/water/pyridine (6/3/4/1/2 v/v) and solvent system B: toluene/methanol (2/1 v/v).

Preparation of β-CD prodrugs:

Preparation of Conjugated Ibuprofen-β-CD from Reaction of Ibuprofen-carboxy chloride with β-CD

A solution of β-CD (0.2 g, 0.176 mmol) in 5 mL pyridine was gradually added to the purified ibuprofen-carboxy chloride (1.25 g, 5.57 mmol) dissolved in 5 mL dried pyridine. The resulted solution was cooled in an ice bath and was stirred at 40-50°C for 72 h. The precipitate was filtered and the solvent was evaporated under reduced pressure until about half volume. The concentrated solution was precipitated in methanol/water (50/50, v/v). The precipitate was filtered and washed several times with water and methanol, respectively. The solid product was dissolved in acetone, reprecipitated with addition of 5 mL water, filtered off and then dried in vacuum. The purity of the resulted compound was examined by TLC, Rf = 0.75 (solvent system B); IR (KBr): 2981,1749, 1171, 1047 cm⁻¹; 1H NMR (CDCl3): 0.89 (6H, CH(CH3)2), 1.21 (3H, CH3C), 1.84 ((CH3)2CHCH2), 2.42 (2H, ArCH2-), 3-5.28 (b, -CH(CH3)COCl, and carbohydrate units of β-CD-Hs), 7.11 (4H, Ar-H). Elemental analysis (Calc. for C315H406O56.5H2O; C:73.06; H:8.09. Found C:72.76; H:7.95).

Preparation of Conjugated Ibuprofen-β-CD from Reaction of Ibuprofen with β-CD in the Presence of DCC

A solution of β-CD (0.5 g, 0.44 mmol) in 5 mL pyridine was added to the solution of ibuprofen (2.9 g, 14 mmol) and DCC (2.87 g, 14 mmol) dissolved in 7 mL dried pyridine. The mixture was stirred with adding dimethylamino pyridine (30 mg) and LiCl (30 mg) as a catalyst. The solution was cooled in an ice bath and stirring was continued at room temperature for 24 h. The obtained crude product was worked up and purified as aforementioned procedure.
Preparation of Conjugated Indomethacin-\(\beta\)-CD
A solution of \(\beta\)-CD (0.2 g, 0.176 mmol) in 5 mL pyridine was added to the solution of indomethacin (2 g, 5.63 mmol) and DCC (1.6 g, 7.76 mmol) dissolved in 5 mL dried pyridine and stirred in the presence of dimethylaminopyridine (30 mg) and LiCl (30 mg) as a catalyst. The solution was cooled in an ice bath and stirring was continued at room temperature for 12 h.

During the reaction a lot of white precipitate was formed. The precipitate was filtered, then the solution was precipitated in methanol. The solid product was washed with methanol several times then washed with water. The obtained yellow precipitate was purified by dissolving in acetone and reprecipitated in water. The precipitate was filtered and dried in vacuum. The purity of the obtained compound was examined by TLC, \(R_f = 0.40\) (solvent system B); IR (KBr): 3454, 2930, 1749, 1688, 1115, 1040 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): 2.32 (3H, NCCH\(_3\)), 3.0-5.1 (b, -CH\(_2\)CO, OCH\(_3\), glucose units Hs of \(\beta\)-CD), 6.5-7.5 (7H, Ar-Hs), 8.15 (2 and 3 -OHs).

Preparation of \(\beta\)-CD-PEG
A solution of acid terminated poly (ethylene glycol) (M\(_w\) = 600) (0.275 g, 0.45 mmol) and DCC (0.2 g, 0.97 mmol) in 7 mL dried pyridine was added to the solution of \(\beta\)-CD (0.2 g, 0.176 mmol), dimethylaminopyridine (40 mg) and LiCl (30 mg) dissolved in 4 mL dried pyridine. The solution was stirred at 0\(^\circ\)C for 12 h. The precipitate was filtered and the solution was evaporated under vacuum until about half volume. The obtained solution was precipitated by adding diethyl ether and washed several times with the same nonsolvent. The precipitant was purified by dissolving in pyridine and reprecipitated in diethyl ether. The structure of isolated compound (\(\beta\)-CD-PEG) was confirmed through aforementioned spectroscopy methods.

Preparation of Prodrug \(\beta\)-CD-PEG Conjugated to Ibuprofen
After preparation of \(\beta\)-CD-PEG as similar to the aforementioned procedure, the solution of ibuprofen (0.75 mg, 0.37 mmol) and DCC (100 mg, 0.48 mmol) in 7 mL dried pyridine was added at room temperature and stirred for 24 h. The precipitate was filtered and the solution was evaporated under vacuum until about half volume. The obtained solution was precipitated by adding diethyl ether and washed several times with the same nonsolvent. The precipitant was purified in pyridine and reprecipitated in diethyl ether.

Preparation of \(\beta\)-CD-PEG Conjugated to both of Ibuprofen and indomethacin
The solution of indomethacin (0.171 g, 0.481 mmol), ibuprofen (0.278 g, 1.34 mmol), and DCC (0.412 g, 2 mmol) in 5 mL dried pyridine was added to the solution of prepared \(\beta\)-CD-PEG, and stirred at room temperature for 12 h. The precipitate was filtered and the solution was evaporated under vacuum until about half volume. The obtained solution was precipitated with diethyl ether and the precipitate was filtered and washed with the same nonsolvent several times. The precipitate was purified by dissolving in pyridine and reprecipitated in diethyl ether.

Method of Hydrolysis
The resulted prodrug polymers were dried in vacuum at room temperature. The powdered prodrug (30 mg) was poured in 5 mL of aqueous buffer solution (phosphate buffer pH 7.4-8 and hydrochloric acid buffer pH 1) at 37\(^\circ\)C. The mixture was conducted into a cellophane membrane dialysis bag. The bag was closed and transferred into a flask containing 25 mL of the same buffered solution and was continuously stirred at the same temperature. From the buffer samples (3 mL) was removed at selected intervals and 3 mL of new buffer was replaced. The quantity of hydrolyzed was analyzed by means of a UV spectrophotometer and the amount of the released drug was determined by using the calibration curve obtained previously under the same conditions.

RESULTS AND DISCUSSION
Esterification reactions of \(\beta\)-CD with acylated ibuprofen or ibuprofen in the presence of DCC (Schemes I and II was carried out in dried pyridine. The formation of ester groups was confirmed by means of a variety of techniques including FTIR, \(^1\)H NMR, elemental analysis, and TLC. The insolubility of final products in acetone, CHCl\(_3\) and CH\(_2\)Cl\(_2\) showed that the products are free from \(\beta\)-CD, also the insolubility of products in methanol proved (controlled by chromatography) that the drug have connected to the \(\beta\)-CD backbone.
The \( R_f \) value of conjugated compounds in solvent system A is 1.0 and in solvent system B is 0.75, whereas the \( R_f \) value of \( \beta \)-CD is about 0.50.

In the FTIR spectrum the absorption band at 1749 cm\(^{-1}\) is attributed to the carbonyl groups of ester. The strongly decreasing of the intensity of absorption in the region of 3550 cm\(^{-1}\) due to OH groups indicateds the formation of ester groups between cyclodextrin and acylated ibuprofen.

The calculation and experimental results of elemental analyses showed the presence of water molecules in the sample, which is probably, have been included in the cavity of \( \beta \)-CD. The appearance of small band in the 3550 cm\(^{-1}\) could be related to the water molecules.

The \(^1\)H NMR spectrum showed broad signals of \( \beta \)-CD at the carbohydrates region and 7.1-7.3 ppm is related to the aromatic ring of ibuprofen. The aliphatic part of ibuprofen have signals at 0.89, 1.50, 1.84, 2.42, and 3.86 ppm. As the literature reports the naked and original CD molecules and also their per-substituted derivatives are symmetrical compounds and their \(^1\)H NMR and \(^13\)C NMR spectra are very simple as a single glucopyranose unit. However, one and two, or more substitution of hydroxyl groups dramatically change its simple NMR spectrums to a very complicated one.

Therefore, from elemental analysis and from simplicity and also the integration of \(^1\)H NMR spectrum we concluded that almost near to per-substitution, have been occurred. It means that probably all of primary and secondary hydroxyl groups in the frame of CD have been substituted with ibuprofen molecules. Probably there are 5 water molecules in the cavity of CD.

In the case of indomethacin-\( \beta \)-CD conjugate (Scheme III), the formation of ester group was confirmed using \(^1\)H NMR and FTIR spectroscopy.

The insolubility of final products in solvents such as acetone, CHCl\(_3\), C\(_6\)H\(_5\)CH\(_3\), and CH\(_2\)Cl\(_2\) showed that the products are free from \( \beta \)-CD. Also, the insolubility of products in methanol proved (controlled by TLC) that the drug molecules has connected to the \( \beta \)-CD backbone.
In FTIR spectrum the existence of signals at 1749 and 1688 cm\(^{-1}\) are related to the ester and amide groups, respectively. In \(^1\)H NMR spectrum the signals at carbohydrates region and broad bands at 6.5-7.3 ppm are related to the -CD and aromatic rings of indomethacin. The -CH, -OCH\(_3\), and -CH\(_2\) groups of indomethacin are appeared at 3.32, 3.92, and 3.73 ppm, respectively. The aromatic signals of indomethacin and the free OH groups of cyclodextrin are appeared in the 6.5-7.5 and 8.15 ppm, respectively.

The linkage of carboxylic acid terminated poly(ethylene glycol) to the CD molecule (Scheme IV) was confirmed with the presence of strong absorption at 1757 cm\(^{-1}\) in IR spectra due to the carbonyl group of ester and a very sharp peak at 3.65 ppm related to the -CH\(_2\)CH\(_2\)O- of poly(ethylene glycol). Because resulted product was not soluble in ordinary solvents therefore, it seems that probably a cross-linking have been occurred between CD-polyethylene polymer chains.

The investigation of FTR spectrum of PEG-\(\beta\)-CD-ibuprofen (Scheme V) shows the absorption of the carbonyl group from ester, ether, OH groups, and glycosidic bond at 1757, 1117, 3346, and 1040 cm\(^{-1}\), respectively. The \(^1\)H NMR spectrum showed the signals of aromatic rings of ibuprofen at 6.98 and 7.27 ppm and aliphatic parts including Me\(_2\)CH, CH\(_3\)CH, and Ar-CH\(_2\) at 0.84, 1.41, and 2.33 ppm, respectively. The signal at 3.67 ppm is related to CH\(_2\) groups of poly(ethylene glycol) and the broad signals at carbohydrates region for CD.

In FTIR spectrum of the PEG-\(\beta\)-CD conjugated with ibuprofen and indomethacin (Scheme VI), the absorption of bonds at 1749, 1688, 1101, 1047, and 3416 cm\(^{-1}\) are belonged to the carbonyl group of ester,
and amide, C-O, glycosidic and OH groups, respectively.

The $^1$H NMR spectrum of the PEG-β-CD conjugated with ibuprofen and indomethacin shows the aromatic rings of ibuprofen and indomethacin at 6.98-7.27 ppm. The signals of other segments of drug molecules including Me$_2$CH, CH$_3$CH, CH$_2$Ar, NCCH$_3$, CH$_2$CO, and OCH$_3$ appears at 0.84, 1.41, 2.33, 3.73, and 3.92 ppm, respectively. β-CD Signals appear at carbohydrates region.

We studied the hydrolysis behaviour of drug-polymer adduct in physiological conditions (aqueous phosphate or hydrochloric acid buffers at 37°C). As the polymer compounds were not soluble in water, they were dispersed in buffer solution and the hydrolysis was performed in a heterogeneous systems. The hydrolysis of PEG-β-CD-ibuprofen was studied at 264 nm in pHs 1, 7.4, and 8. In the case of PEG-β-CD-ibuprofen-indomethacin, because of overlapping of the maximum wavelengths of ibuprofen and indomethacin in the region of 264 nm, the $\lambda_{\text{max}}$= 222 nm relating to the ibuprofen and $\lambda_{\text{max}}$= 320 nm relating to the indomethacin were chosen for the detection of released ibuprofen and indomethacin, respectively. As seen in Figures 1 and 2 the hydrolysis of ibuprofen in pH 1 is almost constant at different times. In pHs 7.4 and 8 the rate of hydrolysis of ibuprofen in both prodrugs β-CD-PEG-ibuprofen and β-CD-PEG-ibuprofen-indomethacin are increased as a function of time. In Figure 3 the rate of hydrolysis of indomethacin in pro-

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Figure 1. Release of ibuprofen from prodrug PEG-β-CD-ibuprofen as a function of time at 37°C.
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