Colloids and Surfaces B: Biointerfaces 164 (2018) 406–413

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Removal of indoxyl sulfate by water-soluble poly-cyclodextrins in dialysis

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ARTICLE INFO

Article history:
Received 6 September 2017
Received in revised form 5 January 2018
Accepted 27 January 2018
Available online 31 January 2018

Keywords:
Cyclodextrin
Adsorbent agent
Indoxyl sulfate
Dialysis
Removal

ABSTRACT

Indoxyl sulfate (IS) is a uremic toxin related to the progression of chronic kidney diseases. Removal of IS from the plasma would reduce the risk of cardiovascular disease. In this study, crosslinked poly-β-cyclodextrins (PCDs) were used as a water-soluble adsorbent agent for IS in dialysis for the first time. The molecular weight of PCDs was found to be proportional to the crosslinking time between β-cyclodextrin monomers and epichlorohydrin, yet the proportion of β-cyclodextrin that reacted with epichlorohydrin decreased. It was observed that PCD after 2 h crosslinking yielded the best IS-binding capability in PBS, while reaching the binding equilibrium within 30 min and yielding a maximum binding capability of 45 mg g^{-1}. Furthermore, the binding mechanism was investigated by two-dimensional nuclear magnetic resonance, Job's plot method, and salt treatments. To simulate the clinical removal of IS we established a macro-dialysis and added PCD obtained from 2 h crosslinking (PCD1) to the dialysate. The removal of plasma IS from the dialysate by PCD1 was about twice as much as that removed from the dialysate without PCD1. Therefore, crosslinked poly-β-cyclodextrins may represent a simple, low-cost, and effective IS removal strategy with great potential for removing other hydrophobic plasma-bound toxins in dialysis. It could also serve as a supplement for the existing non-adsorbent added therapy.

1. Introduction

Indoxyl sulfate (IS), a prototypical protein-bound uremic toxin, is generated from colonic bacterial fermentation of dietary proteins in the colon [1]. It is absorbed into the blood, and finally excreted through the kidney. Therefore, for patients with impaired renal function, IS would continuously accumulate in the blood and reach a concentration of up to 500 μmol L^{-1} (with the normal blood level of 3 μmol L^{-1}) [2]. Usually, uremic toxins are removed by dialysis, but about 90% of the IS in the blood bind to human serum albumin (HSA) and therefore cannot pass through the dialysis membrane. As a result, conventional hemodialysis (HD) cannot effectively clear IS from the blood, which may lead to a high possibility of vascular disease with higher mortality [3].

During dialysis, the effective transfer of IS is crucial for improving uremic therapy efficiency. To enhance the removal of IS, previous studies mainly focused on the modification of lumen or exterior dialyzer surfaces, and the infusion of IS-HSA binding competitors into the blood-side circuit upstream of the dialyzer [4]. For example, Tijink et al. embedded activated carbon particles into the outside of the hollow fiber matrix membranes, which effectively removed protein bound solutes [1,2,4,5]. Tao et al. infused tryptophan or docosahexaenoic acid (DHA) in the blood to competitively bind HSA, improving the removal rates of IS to 19% and 28% as compared to the 10% obtained by adding non-competitors to the blood, respectively [6]. Although these methods have demonstrated significant improvements of IS removal, the modification of the sorbent membranes requires complex techniques, and the addition of drugs into the blood may have potential long-term adverse effects.

We propose here for the first time, a new strategy for IS removal via the addition of a water-soluble sorbent macromolecule, poly-β-cyclodextran (PCD), into the dialysate of the exterior dialyzer. This approach facilitated high mass-transfer efficiency for IS while leaving no negative impact on the dialysis membrane. Moreover, the sorbent did not come into contact with the blood, making it a safe approach for clinical applications. β-cyclodextran (β-CD) is a cup-shaped cyclic oligosaccharide consisting of seven glucose units,

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https://doi.org/10.1016/j.colsurfb.2018.01.056
0927-7765/

with an inner hydrophobic cavity and an external hydrophilic shell [7,8]. β-CD was selected in our study for the removal of IS due to its sensitive recognition of diverse hydrophobic molecules, including hydrophobic drugs and guest molecules, by forming inclusion complexes with these molecules [9–15].

To ensure the safety of the additive, the molecular weight of the IS-bound adsorbent should be larger than the molecular weight cut-off of the dialysis membrane, so as to prevent the transfer of β-CD across the dialysis membrane and subsequent leaking into the blood. Thus different reaction times were used to crosslink β-CD monomers to epichlorohydrin (ECH) to produce polymeric β-CDs (PCDs) with different sizes. The characteristics and chemical compositions of these PCDs were determined and their binding with IS was established before their ability to remove IS was finally tested. Data obtained from IS removal experiments suggested that PCDs of a certain size might have a potential to be applied in the clinics for the removal of IS from the blood of patients because of its good solubility, high binding capability, ease to prepare and low cost.

2. Materials and methods

2.1. Materials

β-cyclodextrin (β-CD) was purchased from Liquan Chemical (Shaanxi, China). Indoxyl sulfate potassium salt was purchased from Sigma-Aldrich (St Louis, MO, USA). Urea was bought from Solarbio (Beijing, China). Acetonitrile (HPLC grade) was obtained from J&K Chemical. Epichlorohydrin (Damao Chemical, Tianjian, China) and other chemicals were of analytical grade. Water used in all experiments was purified by a Milli-Q water system (Millipore, Billerica, USA).

2.2. Preparations of poly-cyclodextrins

Poly-β-cyclodextrins (PCDs) were synthesized according to the method described by Olteanu et al. [16]. Briefly, 5 g of β-CD was first dissolved in 8 mL of 8.4 mol L^{-1} NaOH. After the addition of 3.45 mL of epichlorohydrin (ECH), the mixture was vigorously stirred at 30 °C for different times (2 h, 2 h 20 min and 2 h 40 min). The reaction was stopped by the addition of 30 mL acetone, and the resulting precipitate was dissolved in water and neutralized with 10 mol L^{-1} HCl. All the PCDs were filtered through a FX80 dialyzer (Fresenius Helixone, Tokyo, Japan) to remove any unreacted ECH, monomers and PCDs with molecular weight under 10 kDa. After purification, ECH concentration in PCDs solutions was below the detection limit (10 μmol L^{-1}) under a $Na_2S_2O_3$ measurement (for details, see Supporting Information 1.1). The final products were stored at 4 °C until use.

2.3. Characterization of poly-cyclodextrins

The hydrodynamic diameter of PCDs was detected by a Malvern Zetasizer Nano ZS90 (Malvern, UK) at 25 °C. 2D NOESY NMR spectra of the inclusion complexes of IS and PCD in D_2O were obtained with a Varian INOVA 400 MHz spectrometer (Varian, US). The molecular weight of PCDs was determined by gel permeation chromatography (GPC) using an Agilent 1200 Series with a G1362A RID (Agilent, US). A series of near-monodisperse pullulan standards was used for the calibration [17].

The effective β-CD content in PCDs was measured by a colorimetric method using phenolphthalein [18]. Briefly, phenolphthalein and PCD (or β-CD) were dissolved in 1 mL of 0.1 mol L^{-1} $NaHCO_3$ buffer (pH = 10.5). The final concentrations of phenolphthalein and PCD in the sample were 0.005 mg mL^{-1} and 0.1 mg mL^{-1}, respectively. After a 15 min incubation, the intensity of the pink phenolphthalein solution was reduced and the level of decolorization was measured with a UV–vis spectrophotometer (Thermo Scientific, US) at 550 nm. The effective cyclodextrin content in the PCD was calculated using the standard curve for the β-CD monomer.

2.4. Binding of IS to PCD in PBS

Ten millers of 100 mg L^{-1} IS solution was prepared by dissolving IS in phosphate buffer solution (PBS). PCD was then added to the IS solution to a final concentration of 2% (w/w) and the mixture was incubated for 2 h. There are two kinds of IS in this solution. One kind of IS would bind to the PCD (PCD-bound IS), while the other would exist freely in this solution (free IS). An ultrafiltration method was used to separate the free IS from the PCD-bound IS. Ultrafiltration was achieved with a membrane having a molecular weight cut-off (MWCO) of 3000 Da (Amicon® Ultra-15, Merck Millipore, Billerica, MA). As the molecular weight of PCD was more than 2.5×10^5 Da, the IS that bound to PCD could not pass through the ultrafiltration membrane. Thus, after 7 min of filtration conducted at 4000 × *g*, free IS was effectively collected in the filtrate. The concentration of free IS was determined by UV absorbance at 280 nm using a UV–vis spectrophotometer (Lambda 35, PerkinElmer, US). The IS-binding capability of PCD (Q_e, mg g^{-1}) was calculated via dividing the mass difference of IS before and after PCD binding by the PCD mass.

To analyze the equilibrium isotherm of IS, 2% (w/w) of PCD in PBS was incubated with different initial concentrations of IS (ranging from 20 to 1200 mg L^{-1}) for 2 h.

NaCl or urea was added to a mixture of IS and PCD to different concentrations while keeping the final concentrations of IS and PCD at 1 mg mL^{-1} and 10 mg mL^{-1}, respectively. After 2 h of incubation, the free IS was separated by ultrafiltration, and the concentration of free IS was measured by UV absorbance at 280 nm to evaluate the interaction between IS and the PCD.

2.5. Binding of IS to PCD in a dialysis system

A micro-dialyser system for 2 mL of priming plasma volume in the hollow fiber membranes, was assembled in our laboratory using the hollow fiber membranes of FX80 dialyser (Fresenius Helixone, Tokyo, Japan) (Fig. 1). The IS removal capability of PCD in the micro-dialyzer system was assessed in a two-compartment recirculation dialysis model (Fig. 1). In a normal experiment, 30 mL of plasma containing 100 mg L^{-1} IS was circulated through the blood compartment at a flow rate of 42 cm min^{-1} (equals to 200 mL min^{-1} in clinical), and 30 mL of a 2% (w/w) PCD-spiked dialysate was pumped through the extraluminal compartment of the dialyzer at a flow rate of 30 cm min^{-1} (equals to 400 mL min^{-1} in clinical), which is close to the clinical flow rate. Unless otherwise stated, dialysis was carried out for 4 h at room temperature. The measurement of IS-PCD

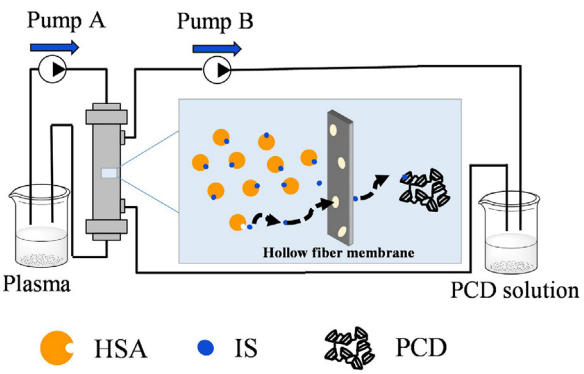

Fig. 1. Schematic representation of the two-compartment recirculation dialysis system with PCD as the adsorbent.