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Immobilization of alpha lipoic acid onto polysulfone membranes to suppress hemodialysis induced oxidative stress

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ABSTRACT

Alpha-lipoic acid (ALA) immobilized polysulfone (PSF) hemodialysis membranes have been fabricated by the dry–wet phase inversion technique. The antioxidant properties of the membranes were evaluated in terms of inhibition of reactive oxygen species (ROS) in blood plasma, while their blood compatibilities were determined by quantitating plasma protein adsorption, platelet adhesion, activation and cytotoxicity to blood cells. The stability of ALA under typical hemodialysis conditions was improved by immobilization, and the greatest enhancement was achieved when it was sandwiched between two polyethyleneimine (PEI) layers. In vitro antioxidant activity measurements showed that ALA coated membranes are not only capable of reducing ROS levels in blood, protein adsorption and platelet activation on the membranes, but, can also prolong coagulation time. All membranes prepared were noncytotoxic to peripheral blood mononuclear cells. In addition, the high permeation rates of solutes through PSF membrane were not affected by ALA immobilization.

1. Introduction

During hemodialysis significant amount of reactive oxygen species is generated due to leukocyte activation [1]. These free radicals react with the proteins and lipids in blood causing oxidative stress which acts in forming three important problems – cardiovascular disease, cancer, and age-associated neurodegenerative diseases [2]. The potential cause of oxidative stress is the reduction of antioxidant defence in uremic patients. As a result, antioxidants are administrated to hemodialysis patients orally or intravenously [3–4]. Over the last 10 years, researchers focused on developing functional hemodialysis membranes to suppress hemodialysis-induced oxidative stress. The most commonly used approach is to immobilize antioxidants, vitamin E and linoleic acid, onto these membranes [5–11]. Mydlik et al. compared the effectiveness of oral vitamin E intake against the use of a vitamin E coated synthetic hemodialysis membranes on oxidative stress levels in blood [5]. They reported that vitamin E coated dialyzer provided more effective antioxidant defense than peroral administration of vitamin E in hemodialysis patients. Yamamoto et al. observed that vitamin C administration increased antioxidant activity of the vitamin-E coated membrane [7]. Kung and Yang covalently attached conjugated linoleic acid (CLA) onto industrial hemodialysis membranes manufactured from cellulose acetate, polyacrylonitrile and polysulfone. CLA immobilization not only inhibited production of ROS but also reduced platelet adhesion, protein adsorption and prolonged blood coagulation time [8–11]. Recently, Neelakandan et al. modified poly(amide):poly(vinyl pyrrolidone) membranes with a soybean-derived phytochemical, genistein [12]. The modified membranes showed significant suppression of three clinically relevant cytokines and exhibited a dose dependent suppression of ROS levels in blood.

Unlike other antioxidants, alpha-lipoic acid (ALA) and its reduced form dihydrolipoic acid (DHLA) effectively quench a number of free radicals in both lipid and aqueous media, ALA and DHLA together have metal-chelating activity and which may have effects on regulatory proteins and on genes involved in normal growth and metabolism [13]. Therefore, ALA is widely used as a drug for prevention of various chronic diseases associated with oxidative stress, and is administered as a daily supplement for dietary purposes, antiageing, diabetes, and cardiovascular disease. In recent years, some clinical studies showed that administration of ALA therapy could decrease the biomarkers of oxidative stress in end-stage renal disease patients under hemodialysis [14] and prolong clotting time via inhibition of an intrinsic coagulation pathway [15–16]. However, instability of ALA under light or heat and its short biological half-life (30 min), may restrict its effective medical usage [17–18]. To increase its stability and provide controlled delivery, ALA was encapsulated into chitosan [17,19] and chitosan-alginate complexes [18]. In addition, microcrystalline cellulose and poly(vinylidine fluoride)/cellulose ester blend membranes were functionalized with ALA and showed an excellent long-term antioxidant activity [20–21].

In this study, ALA was selected as the antioxidant to modify polysulfone (PSF) based hemodialysis membranes. The source of motivation for the present work is a need for functional hemodialysis

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membranes that are capable of suppressing oxidative stress. In addition, few have utilized the unique antioxidant properties of ALA. The PSF membranes were prepared by dry/wet phase inversion method and modified with ALA through an intermediate polyethyleneimine (PEI) layer. The antioxidant activities of free and immobilized ALA were measured using the free-radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The blood compatibilities of the membranes were evaluated in terms of inhibition of reactive oxygen species in blood plasma, amount of adsorbed plasma proteins, platelet adhesion, activation and cytotoxicity on blood cells. The transport and structural properties of the membranes were also characterized. To the best of our knowledge, this is the first study that suggests immobilization of ALA onto hemodialysis membranes to prevent hemodialysis induced oxidative stress. Furthermore, the immobilization method used in this study is unique in that antioxidants are not attached on the membranes covalently through a complex chemistry. Our method is based on adsorbing positively charged, hydrophilic polyelectrolyte PEI on the negatively charged membrane surface and then site-specifically binding a carboxylic group of ALA to an ammonium group of PEI through electrostatic interactions. Thus, the cyclic disulfide bond that forms the active site of ALA becomes available on the surface of the membrane to scavenge ROS.

2. Materials and methods

2.1. Materials

PSF with a molecular weight of 26,000 g mol^{-1}, 1-2 dichloroethane, chlorosulfonic acid, sodium dodecylsulfate (SDS) were purchased from Aldrich, 1-methyl-2-pyrrolidone (NMP) with a purity of ⩾98% and micro BCA protein assay reagent kit were purchased from Fluka and Thermoscientific, respectively. Bovine serum albumin (MW 65,000), urea, vitamin B_{12}, lysozyme, polyethyleneimine (pKa:8.8), 2,2-diphenyl-1-picrylhydrazyl and alpha-lipoic acid were all supplied by Sigma. Cell viability kits, thiazole orange (TO) and propidium iodide (PI), and the monoclonal antibodies, PAC1, FITC and CD62 PE, used for determining platelet activation were purchased from Becton Dickinson Immunocytometry Systems. H_2NaPO_4 and Na_2HPO_4 used for preparing buffer solutions were obtained from Fluka and Riedel, respectively. Water used in the experiments was distilled ion-exchanged water.

2.2. Preparation of the membranes

The conventional PSF was first modified by sulfonation to induce negatively charged groups (SO_3^-) before preparing the support membrane. For this purpose, PSF was dissolved in dichloroethane (DCE) to obtain a 7.5 wt% solution. Similarly, a solution of chlorosulfonic acid diluted in DCE (10 wt%) was prepared and added drop-wise to the PSF solution. After mixing for 3 h at 24 °C, modified polymer was recovered by precipitation in 50 mL of methanol. Ion exchange capacity (IEC) of sulfonated PSF (SPSF) was determined as 0.9 meq. g^{-1}.

To prepare the PSF and PSF–SPSF blend membranes, a solution of 20 wt% of PSF or 10 wt% of PSF and 10 wt% of SPSF in N-methyl pyrrolidone was cast onto a 10 cm × 24 cm glass substrate, respectively with the aid of an automatic film applicator (Sheen Instrument Ltd., model number: 1133N) at a speed of 100 mm s^{-1}. The initial thickness of the cast film was adjusted by a four-sided applicator with a gap size of 150 μm. Following casting, the support was transferred into an environmental chamber (Angelantoni Industrie, Italy, Challenge Series, model number: CH250) in which the solution was dried for 2 min at 25 °C and 40% relative humidity. Then, it was immediately immersed in a coagulation bath for 18 h, and rinsed with pure water for 1 h. The membranes were allowed to dry further for a period of 72 h in a vacuum oven maintained at 100 °C. They were then kept in a desiccator until their use.

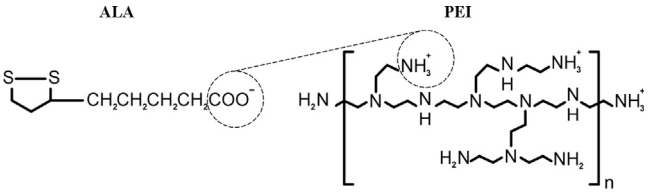

Fig. 1. Structures of PEI and ALA and their possible ionic complexation

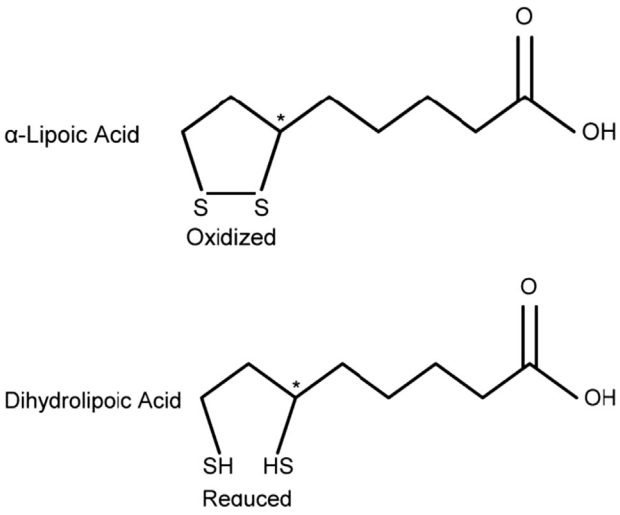

Fig. 2. Chemical structure of alpha-lipoic acid (ALA) and dihydrolipoic acid (DHLA).

The method used for immobilizing ALA is based on initially adsorbing positively charged, hydrophilic polyelectrolyte, PEI, on the negatively charged membrane surface. For this purpose, the PSF–SPSF membrane was dipped in a 1 mg mL^{-1} of PEI solution for 10 min. The pH of the PEI solution was adjusted to a value of 8 by HCl, to obtain a sufficiently protonated form, ensuring a strong bonding on the negatively charged membrane surface through electrostatic attraction. To remove excessive PEI on the surface, the membrane was rinsed with 500 mL water for 10 min and then immersed in a 1 mg mL^{-1} or 5 mg mL^{-1} of ALA solution in 5 vol% ethanol in PBS (pH:7.4) for 30 min at room temperature. The amount of ALA adsorbed on the membrane (mg) was determined from the difference between the initial amount in the solution before contacting with the membrane and that after 30 min of contact. The adsorbed amount was divided to the surface area of the membrane to give the interfacial concentration (mg/cm^{-2}). The concentration of ALA in the solution was measured using a UV spectrophotometer (Perkin Elmer Model No: Lambda 45) at 333 nm. Fig. 1 shows the site-specific binding of the carboxylic group of ALA to the ammonium group of PEI through electrostatic interactions. This method allows the cyclic disulfide bond, which forms the active site of ALA, to become free on the surface of the membrane.

2.3. Measurement of antioxidant activity

Alpha-lipoic acid and its reduced metabolite, dihydrolipoic acid (DHLA) (Fig. 2), form a redox couple and may scavenge a wide range of reactive oxygen species such as hydroxyl radicals, nitric oxide radicals, peroxynitrite, hydrogen peroxide and hypochlorite. The antioxidant activity of ALA was measured using the free-radical scavenging activity of DPPH radicals.