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The size-dependent efficacy and biocompatibility of hyperbranched polyglycerol in peritoneal dialysis

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ABSTRACT

Glucose is a common osmotic agent for peritoneal dialysis (PD), but has many adverse side effects for patients with end-stage renal disease. Recently, hyperbranched polyglycerol (HPG) has been tested as an alternative osmotic agent for PD. This study was designed to further examine the efficacy and biocompatibility of HPG over a range of different molecular weights. HPGs of varying molecular weights (0.5 kDa, 1 kDa, 3 kDa) were evaluated in a preclinical rodent model of PD. HPG PD solutions were standardized for osmolality and compared directly to conventional glucose-based Physioneal™ PD solution (PYS). The efficacy of HPG solutions was measured by their ultrafiltration (UF) capacity, solute removal, and free water transport; biocompatibility was determined *in vivo* by the histological analysis of the peritoneal membrane and the cell count of detached peritoneal mesothelial cells (PMCs) and neutrophils, and *in vitro* cytotoxicity to cultured human PMCs. All the different sized HPGs induced higher UF and sodium removal over a sustained period of time (up to 8 h) compared to PYS. Urea removal was significantly higher for 1–3 kDa than PYS, and was similar for 0.5 kDa. Our analyses indicated that the peritoneal membrane exhibited more tolerance to the HPG solutions compared to PYS, evidenced by less submesothelial injury and neutrophil infiltration *in vivo*, and less cell death in cultured human peritoneal mesothelial cells. Free water transport analysis of HPG indicated that these molecules function as colloids and induce osmosis mainly through capillary small pores. We attribute the differences in the biocompatibility and osmotic activity of different sized HPGs to the differences in the polymer bound water measured by differential scanning calorimetry. These preclinical data indicate that compared to PYS, low MW HPGs (0.5–3 kDa) produces superior fluid and waste removal with better biocompatibility profile, suggesting that they are promising osmotic agents for PD.

1. Introduction

End-stage renal disease (ESRD) is the most severe form of kidney disease and is of growing concern worldwide. Although the best option for the treatment of ESRD is kidney transplantation, the availability of donor organs and the presence of significant comorbidities may preclude transplantation as a viable option for all ESRD patients. Therefore, as a method of renal replacement therapy, most people will require hemodialysis (HD) or peritoneal dialysis (PD), and the outcomes of these two treatment modalities are similar [1,2].

PD utilizes the patient's own abdominal lining – peritoneal membrane – as a filter for fluid removal (ultrafiltration, UF) and solute transport (e.g. urea, sodium, potassium). In PD, glucose has been used as the most common osmotic agent for several decades to achieve adequate fluid removal. Although alternative osmotic agents, such as amino acids and polyglucose (icodextrin, ICO) solutions are now available, glucose still remains the most widely used osmotic agent in PD. However, clinical studies have documented that the use of glucose-based PD solutions is linked to many adverse metabolic complications, such as hyperglycemia, hyperinsulinemia, hyperlipidemia and obesity [3–5]. In addition to these systemic side effects, glucose has been shown

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to directly damage the peritoneal membrane. Evidence from both *in vitro* and *in vivo* studies has shown that the non-physiologic levels of glucose and glucose degradation products in PD solutions activate many molecular cascades that lead to peritoneal tissue inflammation, injury, and remodeling [6–10]. These pathological changes are characterized histologically by peritoneal membrane fibrosis, reduplication of the peritoneal basement membrane, diabetiform vasculopathy, and maladaptive neo-angiogenesis [11]. Clinically, this damage is manifested by increased permeability of the peritoneal membrane to small solutes and the loss of ability to remove fluid via the peritoneal membrane – termed Ultrafiltration Failure. This phenomenon occurs reliably and progressively with increasing duration of peritoneal dialysis and correlates in part with the burden of glucose exposure over this time period [12–15]. Ultimately, this phenomenon is associated with increased mortality in PD patients [16]. Therefore, a new osmotic agent with superior biocompatibility, as well as chemical and physical stability is needed for PD solutions in the treatment of patients with ESRD.

Hyperbranched polyglycerol (HPG) is a branched polyether polymer, synthesized by a multi-branching ring opening polymerization of glycidol [17–19]. HPG is a highly hydrophilic, blood compatible, non-immunogenic, non-toxic, compact and water-soluble polymer [20–23]. Its intrinsic viscosity in water is in the order of 4–7 cc g^{-1} depending on the molecular weight (MW), and is similar to proteins, and approximately 25-fold lower compared to linear polyethylene glycol (PEG) of similar MW [22,23]. Unlike linear polymers, HPG does not precipitate proteins, does not bind to cells or aggregate the cells even at high concentrations [20,23,24]. Recent biodistribution studies in mice have shown that low MW HPGs are rapidly eliminated from the circulation without organ accumulation [23,25]. Our group has reported for the first time the potential of HPG (3 kDa) as an osmotic agent for use in PD [26]. Since the size of the osmotic agent can influence the ability of the PD solution to remove water and solute [27,28], in the present study, we examined the efficacy, kinetics and biocompatibility of HPG-based PD solutions containing different MW (0.5 kDa, 1 kDa, 3 kDa) HPG, and compared with glucose-based PD solution in a rodent model of PD. In addition, the dependence of molecular size of HPG on their osmotic activity and bound water was determined.

2. Experimental methods

2.1. Reagents

All the laboratory general reagents and chemicals were purchased from Sigma–Aldrich Canada (Oakville, ON, Canada) and used without further purification unless mentioned. Glycidol was purified by distillation under reduced pressure before use and stored over molecular sieves at 4 °C.

2.2. Synthesis and characterization of HPGs

HPGs (0.5 kDa, 1 kDa, and 3 kDa) were synthesized by anionic ring opening multi-branching polymerization [29]. The following conditions were used for the synthesis of 1.0 kDa HPG as a typical protocol. Trimethylolpropane (10 g) initiator was stirred with potassium methylate (25% solution in methanol, 6.52 mL) for 5–6 h, followed by the evaporation of methanol at 80 °C for 24 h. Glycidol (36 mL) was added at a rate of 1.9 mL per hour at 95 °C. After the addition of glycidol, the mixture was stirred for 6 h. The newly synthesized polymer was precipitated in acetone and dried, and then dissolved in water and dialyzed with 500 MWCO dialysis tubing against deionized water for 6 h with water change in every hour. The final solution was lyophilized to recover the polymer.

The MW of each polymer was determined by using Gel Permeation Chromatography (GPC) on a Waters 2695 separation module fitted with a DAWN HELEOS II multiangle laser light scattering (MALLS) detector coupled with Optilab T-rEX refractive index detector (Wyatt Technology, Santa Barbara, CA, USA). GPC analysis was performed using Waters ultrahydrogel columns [Guard, 250 (7.7 × 300 mm), and 120 (7.7 × 300 mm)] and 0.1 N $NaNO_3$ as an eluent. The value of differential index of refraction (dn/dc) of HPG in the solvent was 0.12 mL/g and was used for the MW determination. The MW of the HPG was also calculated based on the proton NMR integration data. NMR spectra were collected on a Bruker Avance 300 MHz NMR spectrometer using D_2O as a solvent. The average hydrodynamic radius of the molecule was determined by pulsed-field gradient NMR experiments (see below).

2.3. Hydration determination by using differential scanning calorimetry

Since hydration differences of polymers could influence their cell and tissue interaction, and osmotic activity [30,31]. The amount of free water in different sized HPGs in solution was determined using Q 2100 differential scanning calorimetry (DSC) (TA instruments, New Castle, DE, USA). Briefly, approximately 20 μL of HPG solution in water was heated at a rate of 0.2 °C/min after cooling rapidly to −30 °C. The enthalpy of fusion of pure water ($\Delta H_{f(H_2O)}$) vs. HPG solution ($\Delta H_{f(ps)}$) was calculated using the TA instrument analysis software. The number of water moles bound per mole of HPG (y) was calculated using the following equation, where x is the weight percent of HPG in the solution [32,33]:

$$y = \frac{(1-x)\left[\frac{\Delta H_{f(H_2O)} - \Delta H_{f(ps)}}{\Delta H_{f(H_2O)}}\right]}{xM_{n(H_2O)}} \quad (1)$$

2.4. Measurement of the hydrodynamic radius using the pulsed-field gradient NMR (PFG-NMR)

PFG-NMR experiments were performed using a Bruker Avance 400 MHz spectrometer at 298 K, using D_2O as a solvent. T_1 spin/lattice relaxation, P_1 (90° pulse), δ (the width of a bipolar pair), and Δ (the diffusion time) were optimized, and the experiment was acquired using Ledbpgp2s, a pulse program developed by Bruker. The gradient strength (G) was varied from 1 to 32 G/cm in 16 steps. Each of the free induction decays was averaged over 8 scans, using 4 dummy scans. The diffusion coefficient was extracted using topspin NMR software, by performing a non-linear square curve fitting to the relationship between echo attenuation and pulse field gradient, given by the following equation:

$$\ln\left(\frac{A}{A_0}\right) = -D\left[\gamma^2 G^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right)\right] \quad (2)$$

The hydrodynamic radius was calculated using the Stock's equation [34]. For HPG experiments, attenuation of ether backbone signals, located between 3.3 and 3.95 ppm was used to evaluate the diffusion coefficient [33]. An excellent fit between experimental data and the model indicated to an accurate optimization of different parameters. An example of a used parameters for HPG 0.5 kDa were ($T1 = 0.5497$ s), ($P_1 = 8.13$ μs), ($\delta = 2400$ μs), and ($\Delta = 0.2$ s).

2.5. Calculation of osmotic reflection coefficient

In PD, osmotic agents are characterized by their osmotic reflection coefficient (σ), which defines the ability of the peritoneal membrane to reject the particular solute of interest; values close to 0 represent total permeability across the membrane whereas values close to 1 represent total impermeability. Larger solutes have a higher reflection coefficient and will remain inside the peritoneum for longer; these molecules usually remove more fluid *per unit solute* compared to small molecules. Reflection coefficients for HPG were calculated using equation (4) from Rippe and Levin [35].

$$\sigma_{pore,solute} = 1 - \frac{(1-\lambda)^2\left[2-(1-\lambda)^2\right](1-\lambda/3)}{1-\lambda/3+2/3\lambda^2} \quad (3)$$

In this equation λ represents the ratio between solute radius and pore radius. HPG radius is determined by NMR diffusion measurements as given in the previous section. For the three pore model, the final reflection coefficient represents the weighted sum of reflection coefficients of each pore. The reflection coefficient of glucose is 0.045.

2.6. Preparation of HPG PD solutions

Two sets of HPG PD solutions were prepared by dissolving HPG polymer of various sizes (0.5 kDa, 1 kDa, or 3 kDa) in buffered solution. The pH of the solutions was measured using Fischer Scientific Accumet pH meter with probe-13-620-290 (Fisher scientific, Ottawa, ON, Canada) that was calibrated using buffer solutions of pH 4.0, 7.0 and 10.0 from Fisher scientific. Osmolality (mOsm/kg) was measured using Advanced® Model 3320 Micro-Osmometer (Advanced Instruments, Inc., Norwood, MA, USA) in the Vancouver Coastal Health Regional Laboratory Medicine (Vancouver, BC, Canada).

For experiments which studied the influence of MW and concentration of HPG on the osmolality of solutions (see Section 3.1.3 below), we used buffer composition similar to Dianeal™ solution (Baxter Healthcare Co., Deerfield, IL, USA). Buffer composition (mg/100 mL): sodium chloride (NaCl)-538; sodium Lactate ($C_3H_5NaO_3$)-448; calcium chloride ($CaCl\ 2H_2O$)-18.3; magnesium chloride ($MgCl_2\ 6H_2O$)-5.08.