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Advantages of replacing hydroxyethyl starch in University of Wisconsin solution with hyperbranched polyglycerol for cold kidney perfusion

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ABSTRACT

Background: Efficient and effective perfusion during organ procurement is required for the best prevention of donor organ injury preceding transplantation. However, current organ preservation solutions, including hydroxyethyl starch (HES)–based University of Wisconsin (UW) solution, do not always yield the best outcomes. Our previous study demonstrated that replacing HES with hyperbranched polyglycerol (HPG) reduced donor heart injury during cold storage. The current research was designed to examine the advantages of HPG-based solution for cold kidney perfusion.

Methods: Perfusion efficiency of HPG *versus* UW solution was tested using mouse kidneys at 4°C. The blood washout was evaluated by using a semiquantitative scoring system and tissue damage by histologic analysis. The interaction of HPG or UW solution with human red blood cells (RBCs) was examined by measuring RBC sedimentation and aggregation.

Results: The lower viscosity of HPG solution was correlated with faster and more efficient perfusion through donor kidneys as compared with UW. HPG solution was also more effective than UW in removing RBCs from the kidney and was associated with less tissue damage to donor kidneys. *In vitro* UW solution caused significant RBC sedimentation and hyperaggregation, whereas HPG showed minimal impact on RBC sedimentation and prevented RBC aggregation.

Conclusions: This experimental study demonstrated that compared with UW, HPG solution was more efficient and effective in the removal of the blood from donor kidneys and offered better protection from donor tissue damage, suggesting that the HPG solution is a promising candidate to supplant standard UW solution for donor kidney perfusion in transplantation.

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Introduction

During procurement of donor organs, surgeons need to use an organ preservation solution, such as University of Wisconsin (UW), Celsior (CS) or histidine–tryptophan–ketoglutarate (HTK), to wash out the blood and to limit donor organ ischemic injury. However, with a push to expand the donor pool to include more marginal quality organs, a high percentage (11.25%-56.1%) of delayed graft function (DGF) is observed despite the use of any of these perfusion solutions.[1-6] Increased DGF rates could limit the ongoing exploration of even more marginal kidneys, potential donor organs currently deemed "unusable" for the transplantation. Thus, to maximize the use of available donor organs, particularly from marginal donors, and to reduce DGF, more effective organ preservation technologies are needed.

UW solution is the most common preservation solution for the preservation of intra-abdominal organs[7] and has been shown equivalency or better performance than other solutions (i.e., CS and HTK) in the cold preservation of various donor organs.[5,7-12] Hydroxyethyl starch (HES) is included in this solution as a colloid to prevent cell swelling or tissue edema.[13] It has also been noticed that HES is a component in other newly developed organ preservation solutions, such as ET-Kyoto[14] and KPS-1.[15] However, numerous studies have documented that HES is associated not only with coagulopathy, pruritus, and acute kidney injury[16-20] but also with impairment of immediate donor kidney function after transplantation.[21] In addition to these adverse effects, there are other two limitations of HES for organ preservation. First, HES increases the viscosity of the solution, resulting in an increase in vascular resistance of donor organs during initial flushing and/or perfusion and shortening of graft survival.[22,23] Second, HES causes the hyperaggregation of human red blood cells (RBCs), probably due to the linear nature of HES that bridges RBCs together.[24] RBC agglutination may result in stasis of blood and/or incomplete removal of donor blood from organs before transplantation. Therefore, for better initial blood washout and better protection from graft dysfunction after transplantation, an alternative colloid for donor organ preservation is needed.

In contrast to HES that has large hydrodynamic radii and predominantly linear structures, hyperbranched polyglycerol (HPG) is a branched compact polyether polymer.[25] The supporting evidence of using HPG as a viable alternative colloid for flushing and/or storage of donor organs has been well documented in literature. (1) HPG is a highly water-soluble (>400 mg/mL) synthetic polymer, and is blood compatible, nonimmunogenic, and nontoxic with intravenous administration up to 1 g/kg in mice and 15 g/kg in rats, and its potential is currently be evaluated in many biomedical applications.[26-31] In fact, HPG has equal or better biocompatibility profile compared with polyethylene glycol (PEG; an Food and Drug Administration–approved polymer).[27,32,33] (2) HPG has low intrinsic viscosity similar to that of proteins and is approximately 10 times lower than those of linear polymers with similar molecular weight (MW; i.e., PEG, HES, dextran).[27,33] And (3) unlike linear polymers, HPG neither precipitates proteins nor aggregates cells (e.g., RBCs) even at very high concentrations.[33-36] Recently, we investigated the potential of replacing HES with HPG for organ preservation and demonstrated that the HPG-based solution was superior to HES-based UW solution in the protection of mouse hearts from cold ischemia injury during static cold storage.[37] To further expand our understanding of this novel colloid for an organ preservation solution, here, we investigated the efficacy of HPG-based solution compared with UW in cold flushing and/or perfusion of donor kidneys and its interaction with human RBCs.

Materials and methods

Animals and reagents

Male C57BL/6 (B6) mice, weighing 25-30 g, were provided by a breeding colony at the Jack Bell Research Centre at the University of British Columbia (UBC). The animal experiments were performed following the protocol (A11-0409) approved by the Committee on the Ethics of Animal Experiments of UBC in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Canadian Council on Animal Care. Glycidol was purified by distillation under reduced pressure before use and stored over molecular sieves at 4°C. UW solution was purchased from DuPont Canada (Mississauga, Ontario, Canada).

Polymer synthesis and characterization

As described previously,[31,37] HPG (1 kDa) was synthesized by anionic ring opening multibranching polymerization of glycidol, and its molecular characteristics were verified using gel permeation chromatography and proton nuclear magnetic resonance spectroscopy.

Preparation of HPG solution

HPG-based solution (denoted as HPG solution here) was prepared by dissolving HPG (1 kDa. 3% w/v) as described previously.[37] Similar to UW solution, the osmolality of HPG solution was 335 ± 5 mOsm/kg, determined by using The Advanced Osmometer Model 3D3 (Advanced Instruments, Inc, MA). The relative viscosity of the HPG was 1.244 at 25°C or 1.375 at 4°C, whereas of UW solution was 3.175 at 25°C or 3.514 at 4°C, which were measured by using an Ubbelohde Viscometer with a sample volume approximately 1.0 mL (Cannon-Manning 50, A 412 Semi-Micro Viscometer; CANNON Instrument Company, PA).

Kidney perfusion model

A mouse kidney perfusion model was established as shown in Figure 1. Briefly, mice were anesthetized by an intraperitoneal injection of a mixture of ketamine and xylazine, and anesthesia was maintained using isoflurane when required. After the abdominal cavity was exposed via a ventral midline incision, both the aorta and the inferior vena cava