Graft Protection Against Cold Ischemia Preservation: An Institute George Lopez 1 and Histidine-tryptophan-ketoglutarate Solution Appraisal

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ABSTRACT

Cold storage of organs in preservation solutions, such as Institute George Lopez 1 (IGL-1) or histidine-tryptophan-ketoglutarate (HTK), is a mandatory step for organ transplantation. This preservation leads to an ischemic injury that affects the outcome of the organ. This article studies the liver graft eluate after organ recovery using IGL-1 or HTK solutions. We explore the influence of the volume used for washing out the liver and the consequences in the graft preservation when both solutions are used. Livers were washed out with different volumes of HTK and IGL-1 according to manufacturers' instructions and then preserved in both solutions for 24 hours at 4°C. Tissue and eluates were collected for subsequent analyses. We measured transaminases (aspartate aminotransferase and alanine aminotransferase), histology by hematoxylin/eosin staining, and red blood cell and hemoglobin counts, respectively. After washing out and cold storage, the IGL-1 processed livers showed better preservation than those with HTK solution; however, in this latter case, an important accumulation of erythrocytes was found when compared to IGL-1. These data were consistent with the higher hemoglobin and red blood cell counts observed for IGL-1 eluates after 24 hours. The volume used for washing out the organ depends on the composition and properties of the organ preservation solutions (ie, IGL-1 and HTK); this is an important factor for the graft cold preservation. The total volume used for washing out the graft should be considered because it has a direct impact on the total cost for clinical transplantations.

COLD ischemia reperfusion injury is a limiting step for the success of liver transplantation. This is injury can be modulated during graft cold storage by the use of different organ preservation solutions, including Institute George Lopez 1 (IGl-1) and histidine-tryptophan-ketoglutarate (HTK) which are currently used for abdominal transplantation purposes (Table 1). Recently it has been reported by the European Liver Transplant Registry (ELTR) that HTK shows some limitations in front of other solutions, including IGL-1 solution [1]. ELTR results were in accordance with those previously published by the United Network for Organ Sharing (UNOS) [2]. Taking this into account, we have explored the relevance of pathophysiological mechanisms involved in graft preservation when IGL-1 and HTK solutions were used [3]. We studied an aspect that has been poorly investigated; that is, the influence of the volumes used for washing out and preserving organs with different solutions such as IGL-1 and HTK (used according to the manufacturer instructions) [4–6] on the quality of graft preservation. Here we present a detailed

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Table 1. Composition of HTK and IGL-1 Solutions

	IGL-1	HTK
Colloids (g/L)		
Polyethylene glycol-35	1	
Antioxydants (mmol/L)		
Glutathione	3	
Allopurinol	1	
Precursors (nmol/L)		
Adenosine	5	
Ketoglutarate		1
pH	7.4	7.2
Osmolarity (mosmol/L)	290	310
Buffers (mmol/L)		
Diphosphate	25	
Histidine		180
Histidine-HCl		18
Tryptophan		2
Electrolytes (mmol/L)		
K^+	25	9
Na^+	125	15
Mg_2^+	5	4
Cl^-		50
SO_4^{2-}	5	
Impermeants (mmol/L)		
Raffinose	30	
Lactobionic acid	100	
Mannitol		30

washing out appraisal for comparing IGL-1 and HTK solutions in a controlled experimental preservation rat model when a longer time of cold preservation time (24 hours at 4°C) was used. Results show that the volumes of the organ preservation solutions should be considered during graft preservation.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats weighing 250 g from Charles River (France) were used. All animals had free access to water and dry food. All animals were used in accordance with protocols approved on July 14, 2016 (NO-483116). The study was performed in accordance with the European Union Directive 2010/63/EU for animal experiments and approved by the Ethics Committees for Animal Experimentation of the University of Barcelona (Directive 396/12). Animals were randomly distributed into groups as described below.

Experimental Design

In a sterile room, we anesthetized the rats (Sprague-Dawley, male) with isoflurane with a silicone mask at 4% during 1 to 2 minutes for induction, and then 2%, 2 l/min air flow for liver procurement. After cleaning and shaving the abdomen, we performed a medium xifopubian incision. Retractors of the abdominal wall were placed and then the small intestine was moved and covered with wet gauze. Sections of the falciform, left triangular, and gastrohepatic ligaments were done before the division of the left diaphragmatic vein from the suprahepatic IVC. Next, the hepatoesophageal ligament was cut and the artery coagulated. To isolate the infrahepatic IVC down to the left right renal vein, we sutured using 4-0 silk that had been placed around it. We proceeded to separate the proper hepatic artery and dissect the portal vein and the bile duct to fix the hepatic hilum. Then, 2 IU of heparin was injected through the dorsal vein of the penis and we used a bulldog clamp for the abdominal aorta and the IVC just before its ileac bifurcation and diaphragm transection. A bulldog clamp was placed at the suprahepatic IVC level just after the heart. A cannula was placed in the aorta and a 14 G catheter was placed in the infrahepatic IVC and secured with 4-0 silk sutures. We flushed livers with the 40 mL of IGL-1 or 100 mL of HTK through the aorta and we collected effluents drained by the infrahepatic IVC through the catheter (t0 at the beginning of the flush, t1 after 40 mL of both solutions, and t2 just in the HTK group after 100 mL). We also flushed with 10 mL of IGL-1 or 25 mL of HTK through a 22 G catheter by the portal vein and perfusate was collected (t = Portal). Finally, we cut the suprahepatic and infrahepatic IVCs, portal vein, and bile duct just below the catheter, and liver specimens were placed into a basin filled with the preservation solutions IGL1 or HTK, respectively, for 24 hours at 4°C (Fig 1). After 24 hours, all livers were rinsed with 20 mL Ringer lactate solution and samples were obtained from the flush (t = 24 hours). Liver samples were then stored at −80 °C for the subsequent biochemical determinations.

Experimental Groups

- Group 1 (HTK; n = 6): After organ wash and recovery, the livers were stored in HTK preservation solution for 24 hours at 4°C as previously described.
- Group 2 (IGL-1; n = 6): After organ wash and recovery, the livers were stored in IGL-1 preservation solution for 24 hours at 4 °C as previously described.

Determinations

Liver injury. Hepatic injury was evaluated by measuring alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels using commercial kits from RAL (Barcelona, Spain), as previously reported [3]. Briefly, 100 µL of effluent washout liquid or perfusate was added to 1 mL of the substrate provided by the commercial kit, and then transaminase activity was measured at 340 nm with an ultraviolet spectrometer and calculated following the supplier's instructions.

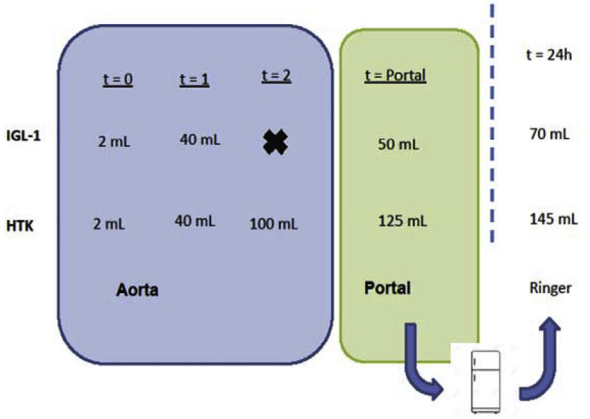

Fig 1. Washing procedures for histidine-tryptophan-ketoglutarate (HTK) and Institute George Lopez 1 (IGL-1) solutions.