IGL-2 as a Unique Solution for Cold Static Preservation and Machine Perfusion in Liver and Mitochondrial Protection

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ABSTRACT

Hypothermic static cold storage and machine perfusion strategies remain the clinical standard of care for liver graft preservation. Recently, the protection of the mitochondrial function and the energetic levels derived from it has emerged as one of the key points for organ preservation. However, the complex interactions between liver mitochondrial protection and its relation with the use of solutions/perfusates has been poorly investigated. The use of an alternative IGL-2 solution to Belzer MPS one for hypothermic oxygenated perfusion (HOPE), as well as in static cold storage, introduce a new kind of perfusate to be used for liver grafts subjected to HOPE strategies, either alone or in combination with hypothermic static preservation strategies. IGL-2 not only protected mitochondrial integrity, but also avoided the mixture of different solutions/perfusates reducing. Thus, the operational logistics and times prior to transplantation, a critical factor when suboptimal organs such as donation after circulatory death or steatotic ones, are used for transplantation. The future challenges in graft preservation will go through (1) the improvement of the mitochondrial status and its energetic status during the ischemia and (2) the development of strategies to reduce ischemic times at low temperatures, which should translate in a better transplantation outcome.

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INTRODUCTION

TRADITIONALLY, liver graft preservation strategies have been based in static cold storage (SCS) [1,2], but more recently, a variety of dynamic perfusion techniques using machine perfusion (MP) strategies in normothermic and hypothermic conditions using the oxygenated perfusion techniques (hypothermic oxygenated perfusion [HOPE]) [2−9] resulted in a promising tool to rescue marginal livers, such as the ones presenting with steatosis for transplantation purposes [4,10,11].

Since the first investigations on hepatic perfusion carried out by Guarrera et al [3] in 2010, the interest has grown in the field of MP, both for normothermic hepatic perfusion [7,8] and for HOPE (with less operative complexity than normothermic perfusion) [3]. Recent advances have made it a very promising strategy to increase the donor pool in the face of the pressing shortage of organs for transplantation [4,10,11].

It is well known that the HOPE benefits are tied to the oxygenation of the perfusate, which is responsible of maintaining the integrity and function of mitochondrial machinery [12], and this applies either by using HOPE itself or in combination with SCS using a commercial preservation [13]. Recent investigations have shown that the emerging interest of mitochondrial protection during hypothermic graft preservation and its energetic status is growing [14]. In this sense, the induced HOPE protection mechanisms, defined recently by Schlegel et al [12], are associated with the sustaining of the mitochondrial state that contributes to: (1) the prevention of energy breakdown with the subsequent sustaining of intracellular ATP levels; (2) the prevention of damage-associated molecular pattern formation; and (3) the induction of underlying mechanisms related to mitochondrial repair and endothelial protection. However, the relevance of the interactions between mitochondrial graft protection and preservation solutions/effluents would need to be considered further.

In this context, the relevance of mitochondrial consequences of organ preservation techniques in organ transplantation should be specially considered in future organ hypothermic preservation strategies, especially when new solute/effluents could be a useful tool to increase mitochondrial protection for the liver graft [14].

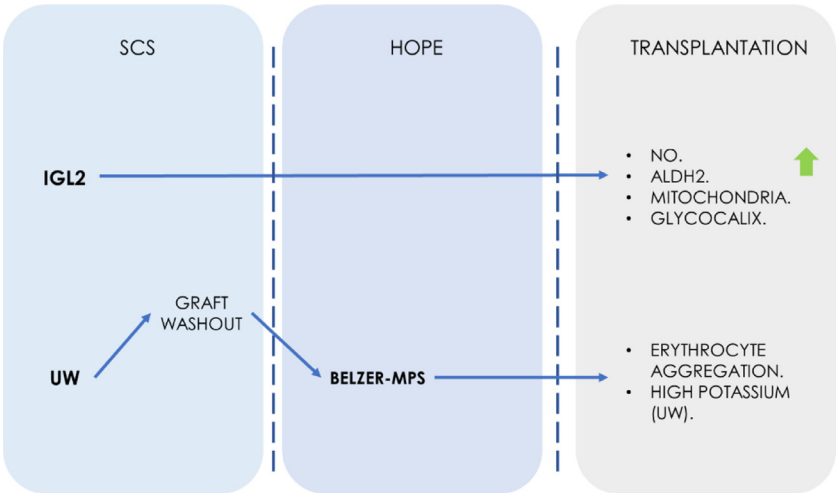
The perfusion solutions normally used for HOPE are a modification of Belzer's solution (Table 1) used for the static preservation of the graft [2,3], which on one hand contains hydroxyethyl starch (HES) as an oncotic agent, and on the other hand, shows a higher decreased K+ concentration than its analogue University of Wisconsin, among other components (Table 1). It is well reported that HES presence may lead to hyperaggregability of the red blood cells during static hypothermic preservation [15]. HES could also interfere with further HOPE strategies using Belzer MPS, where the presence of HES is responsible for increasing the viscosity of the perfusate during hypothermic perfusion vs IGL-2 (Table 1). This is especially relevant for steatotic liver grafts in which the fluid disturbances due to dynamic of fluids in HOPE [4] may destroy the luminal sugar thin layer covering liver endothelia, also known as glycocalyx [16,17]. However, the lower concentration of K+ in Belzer perfusate seems to be not relevant to affect to vascular resistance in hypothermic conditions given that it is well known that perfusates with physiologically low content prevented the vascular resistance increases when livers are subjected to cold perfusion [3,18].

Table 1. Composition of IGL-2 and Belzer MPS solutions

	Belzer-MPS	IGL-2
Electrolytes (mmol/L)		
K+	25	25
Na+	120	125
Mg2+	5	5
SO4 2-	5	5
Ca+		0.5
Zn2+		0.091
Buffers (mmol/L)		
Phosphate	25	25
HEPES		10
Histidine		30
Impermeants (mmol/L)		
Mannitol	30	60
Lactobionic acid		100
Dextrose	10	
Ribose	5	
Gluconate	85	
Colloids (g/L)		
Hydroxyethyl starch		50
Polyethylene glycol−35	5	
Antioxidants (mmol/L)		
Glutathione	3	9
Metabolic precursors (mmol/L)		
Adenosine		5
Adenine	5	
NaNO2 (nmol/L)		50
pH	7.4	7.4
Osmolarity (mosmol/L)	320	360
Viscosity (cP)	2.6	1.7

HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Recently, we have proposed the use of IGL-2 solution as a good alternative to Belzer MPS for HOPE strategies alone or combined with cold static preservation [19,20] (Table 1). The substitution of HES by polyethylene glycol 35 (PEG35); as well as the presence of glutathione (among other components) constitute the main difference between IGL-2 and Belzer MPS; glutathione content in IGL-2 (9 mM) is responsible for a higher antioxidant capacity compared with Belzer MPS (3 mM glutathione), which is translated as an enhanced protection against radical oxygen species formation and their potential damage against mitochondria in hypothermic static preservation followed by HOPE strategies [19−20], where the transient oxygenation sustains the liver mitochondrial machinery at basal levels. We compared Sprague Dawley rats' liver grafts subjected 1h HOPE after 7 hours SCS in both solutions (Belzer MPS and IGL-2)[20]. No significant differences in transaminases (alanine transaminase/aspartate transaminase) were found. However, significant lower levels of glutamate dehydrogenase (as a mitochondrial damage marker), were found in the IGL-2 rats' group vs Belzer MP, which were concomitant with



Scheme 1. IGL 2 Mechanisms of liver graft protection suggested for HOPE and hypothermic SCS preservation. The use of IGL-2 facilitates the logistics of using different solutions/perfusates besides favouring mitochondrial function, NO generation (vasodilation agent) and diminishing the disturbances associated with low viscosity that affect to endothelial glycocalyx.

higher levels of the mitochondrial enzyme aldehyde dehydrogenase 2 (ALDH2). These results are in agreement with the results of Schlegel et al [12], which confirm that the quality and protection of the mitochondria will greatly determine the capability of the graft to recover from ischemia-reperfusion injury insult, although further post-transplant studies are needed.

In addition, it is well known that in glutathione-based solutions, such as Belzer MPS and IGL-2, the presumed vs actual oxidation of glutathione over time is a key point that needs to be carefully overseen [21]. This is especially relevant when the hypothermic storage conditions of the preservation solution are not maintained properly and according to manufacturer instructions. To avoid the oxidation of the glutathione, additional factors such as high-quality package or the maintenance of the cold chain during transport are of utmost importance. However, these points are only valid if the initial quantity of glutathione is the optimal one, which is a differential point between original solutions and white brands. [22].

The use of IGL-2 in hypothermic preservation strategies also prevents the generation of aldehydes such as 4-hydroxynonenal through the activation of ALDH2 and its related protective mechanisms [13,19,20], contributing to HOPE benefits when PEG35 is used (Scheme 1). Scheme 1 summarizes the potential protective mechanisms of PEG35 solutions/perfusate in hypothermic static preservation [13] and HOPE [19,20] for liver transplantation purposes.

Scheme 1. IGL-2 mechanisms of liver graft protection suggested for HOPE and hypothermic SCS preservation. The use of IGL-2 facilitates the logistics of using different solutions/perfusates besides favoring mitochondrial function, nitric oxide generation (vasodilation agent), and diminishing the disturbances associated with low viscosity that affect to endothelial glycocalyx.

In accordance with the relevant investigations of Schlegel et al [12] and Horváth et al [14], we reported for the first time the benefits of using a novel IGL-2 solution for a combined use of SCS and HOPE strategies to rescue marginal livers, facilitating the logistics and avoiding the mixture of preservation solutions/perfusates for transplantation purposes. With this in mind, the use of a unique solution, such as IGL-2, for static and HOPE preservation strategies, could also be a useful tool in combination with "ex vivo" liver splitting and HOPE strategies, as recently reported by Mabrut et al [23].

In conclusion, the actual strategies used in liver graft hypothermic preservation suggest that the use of and unique preservation solution for the protection of mitochondrial functions should be considered as a priority in the actual studies of liver preservation solutions [24]. Future investigations on the mitochondrial protection induced by polyethylene glycols need to be explored in depth.

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