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Polyethylene glycols: An effective strategy for limiting liver ischemia reperfusion injury

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Abstract

Liver ischemia-reperfusion injury (IRI) is an inherent feature of liver surgery and liver transplantation in which damage to a hypoxic organ (ischemia) is exacerbated following the return of oxygen delivery (reperfusion). IRI is a major cause of primary non-function after transplantation and may lead to graft rejection, regardless of immunological considerations. The immediate response involves the disruption of cellular mitochondrial oxidative phosphorylation and the accumulation of metabolic intermediates during the ischemic period, and oxidative stress during blood flow restoration. Moreover, a complex cascade of inflammatory mediators is generated during reperfusion, contributing to the extension of the damage and finally to organ failure. A variety of pharmacological interventions (antioxidants, anti-cytokines, *etc.*) have been proposed to alleviate graft injury but their usefulness is limited by the local and specific action of the drugs and by their potential undesirable toxic effects. Polyethylene glycols (PEGs), which are non-toxic water-soluble compounds approved by the FDA, have been widely used as a vehicle or a base in food, cosmetics and pharmaceuticals, and also

as adjuvants for ameliorating drug pharmacokinetics. Some PEGs are also currently used as additives in organ preservation solutions prior to transplantation in order to limit the damage associated with cold ischemia reperfusion. More recently, the administration of PEGs of different molecular weights by intravenous injection has emerged as a new therapeutic tool to protect liver grafts from IRI. In this review, we summarize the current knowledge concerning the use of PEGs as a useful target for limiting liver IRI.

Key words: Ischemia reperfusion injury; Polyethylene glycol; Liver preconditioning; Liver transplantation; UW solution; IGL-1 solution; SCOT solution; PEG rinse solution; Machine perfusion

Core tip: Pharmacological treatments for preventing liver ischemia reperfusion injury are limited, due to the complex pathophysiology of this condition. The drugs currently used for preventing ischemia-reperfusion injury (IRI) all have local and specific activity with potentially damaging side effects. This review focuses on the current understanding of polyethylene glycols, which are non-toxic polymers, as new emerging agents for limiting liver IRI, and proposes directions for future investigations.

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INTRODUCTION

It is well known that the interruption of blood to an organ (ischemia) and its subsequent restoration (reperfusion) leads to irreversible damage which is termed ischemia reperfusion injury (IRI). IRI is inherent to liver surgical procedures such as hepatic resection and liver transplantation[1-3]. During liver resections, the damage is commonly a consequence of the vascular occlusion of the liver hilum (Pringle' s maneuver) under normothermic conditions[1]. In the case of transplantation, the damage is sustained during cold storage of the liver graft (at 4 ℃) in preservation solution following explantation from the donor, and during subsequent warm reperfusion and implantation into the recipient[4].

There are several steps between organ recovery and transplantation that can exacerbate the damage to the graft. The most important are organ procurement (pre-preservation), conservation in preservation solution (cold storage), and rewarming (graft washout) before transplant (reperfusion). The cumulative injuries due to each step are determinant for the successful graft outcome after transplantation but the most significant lesions occur during cold ischemia, graft rewarming and normothemic reperfusion after transplantation.

At the cellular level, prolonged ischemia leads to ATP breakdown and provokes the accumulation of hypoxanthine, mitochondrial de-energization and ionic alterations which finally lead to liver cell necrosis. Upon oxygenation during reperfusion, reactive oxygen species (ROS) generation by uncoupled mitochondria promotes oxidative stress and a complex cascade of inflammatory mediators (nitric oxide, cytokines, adhesion molecules, chemokines, *etc.*) which all contribute to the spread of the damage and finally to cell death[4].

Because of the range of mechanisms involved in hepatic IRI, the choice of preventive or therapeutic strategies is very difficult[5]. Pharmacological strategies for preventing IRI focus on the use of specific agents, but the benefits of these drugs are limited because of their local actions, side effects and potential toxicity. In this situation, there is a clear need to test the use of non-toxic, water-soluble and protective agents for tissues such as PEGs as "preconditioning agents" for preventing IRI and also as potential targets for therapeutic interventions in organ transplantation.

This review is an update of the most significant advances in the use of polyethylene glycols (PEGs) as therapeutic tools for protecting the liver against IRI, placing specific emphasis on future perspectives in liver graft preservation and transplantation.

PEGS: CHEMICAL STRUCTURE AND MEDICAL USE

PEGs are non-immunogenic, non-toxic and water-soluble polymers which show no electric charge and no affinity for any specific organ. They are composed of repeating units of ethylene glycol which form polymers with a linear shape of different molecular weight[6-9]. PEGs with different shapes can be obtained by using different initiator molecules during the polymerization reaction (*e.g.,* hexa-glycerin instead of methanol to form a tri PEG) or by joining different linear PEGs to create different structures, as shown in Figure 1.

PEGs are negligibly metabolized *in vivo* and are mainly unaltered when eliminated from the body either by the kidneys (for PEGs < 30 kDa, slowly for 30 kDa < PEGs < 40 kDa) or in the faeces (for PEGs > 20 kDa)[10]. PEGs are generally considered to have low toxicity *via* all routes of administration, as demonstrated by tests in many animals[11]. Due to their high flexibility, hydrophilicity, and the large number of water molecules coordinated by their chains, PEGs present a greater hydrodynamic volume than would be expected from their molecular weight, and they show

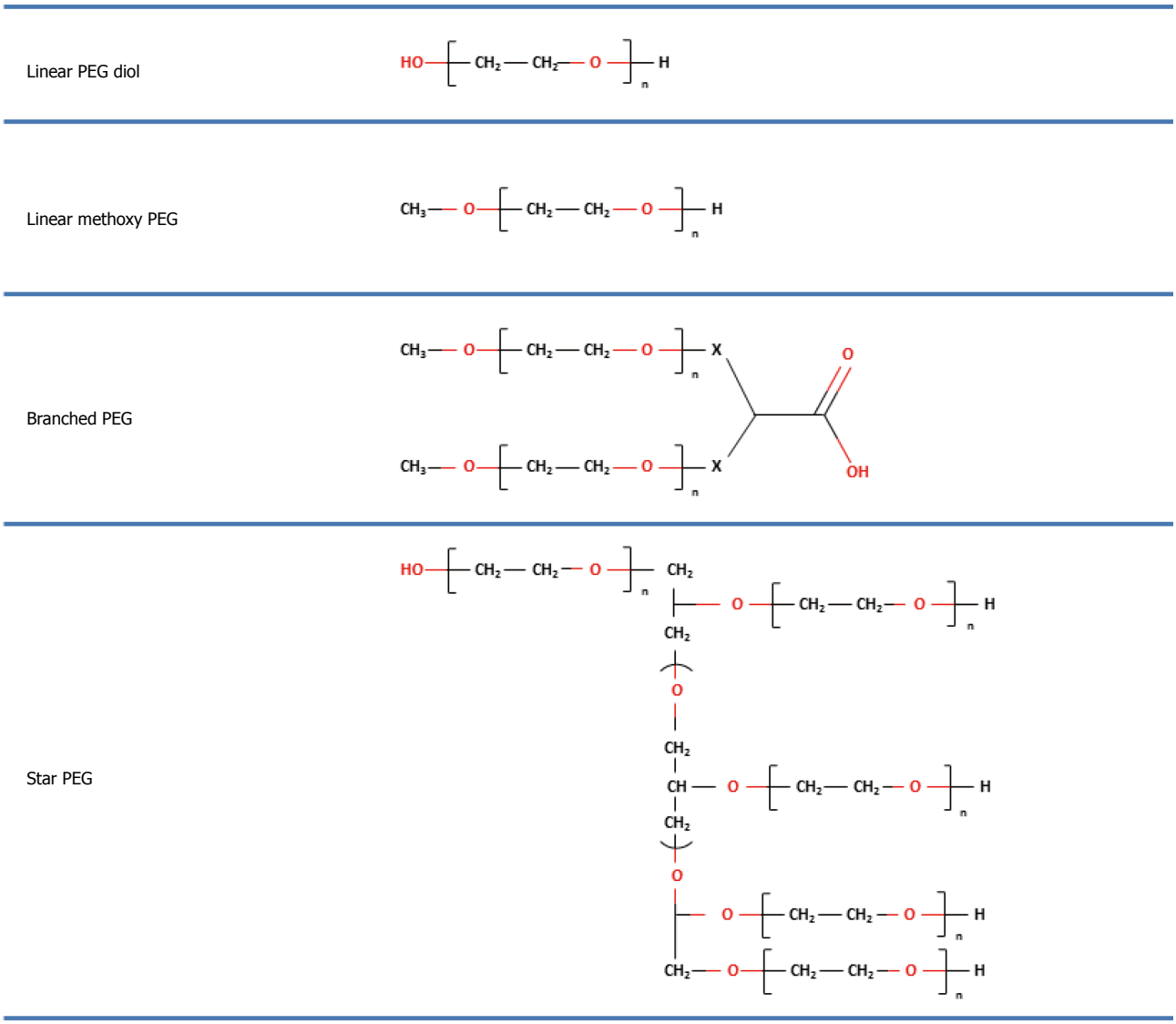

Figure 1 Schematic composition of linear, branched and star polyethylene glycols. Polyethylene glycols (PEGs) are synthesized by a process of linking repeating units of ethylene glycol. The reaction gives products with one or two end chain hydroxyl groups termed methoxy-PEG or diol-PEG, respectively. Then, the linear PEG with branches at irregular intervals along the polymer chain forms branched PEGs. Star-shaped PEGs are the simplest class of branched PEGs with a general structure consisting of several (more than three) linear chains connected to a central core.

high protein-rejecting properties[12].

PEGs have an apparent molecular weight which, depending on the molecular weight of the polymer, can be 5-10 times higher than a corresponding soluble protein of similar molecular mass, as shown by gel permeation chromatography[13]. The Food and Drug Administration (FDA) has approved the use of PEGs as a vehicle or a base in food, cosmetics and pharmaceuticals, including injectable and bowel solutions[14,15]. For example, low molecular weight PEGs are widely used as the basis of a number of laxatives and soft capsules, and high molecular weight PEGs have been used as components in organ preservation solutions to attenuate injury from cold perfusion in animal organs such as pancreas[16], small bowel[17], kidney[18] and liver[19]. In addition, the attachment of PEG (PEGylation) to drugs, peptides, proteins, nanoparticles, micelles, and liposomes is spreading as a technology for enhancing the bioavailability, stability, safety, and efficacy of a wide range of therapeutic agents[20,21]. Examples include PEGylated interferon alpha, which is used to treat hepatitis C[22], or PEGylated antihuman TNF-alpha for rheumatoid arthritis[23].

PEG STRATEGIES TO PREVENT ISCHEMIA-REPERFUSION INJURY: AN APPROACH TO THE LIVER

The use of PEGs to minimize the deleterious effects of IRI has not been studied in depth. However, PEGs have been shown to be effective in cell protection against hypoxia/oxygenation, as additives in preservation and perfusion solutions for organ transplantation and, more

recently, as "preconditioning agents" for preventing IRI in heart and liver. As a result, PEGs may offer new therapeutic strategies for applications in clinical liver surgery and transplantation, as indicated below.

PEG and cryopreservation ("supercooling")

Current technologies can preserve livers outside the body for about 12 h using a combination of cold temperatures and a preservation solution. This has helped increase the number of successful liver transplants, but extending the time a liver can survive outside the body even further would provide many extra benefits.

The presence of PEGs has been shown to be determinant for hepatocyte preservation in hypothermic conditions[24-26]. The addition of PEG8 to the preservation solution suppressed cell swelling in cultured hepatocytes, keeping them relatively well-preserved and restoring membrane integrity[24-26]. This is consistent with the further development of a slow-cooling method that first chills rat livers at 4 ℃ and then drops the temperature to below freezing (named "supercooling"), allowing them to be stored in a "supercooled" but non-frozen state[27]. In this connection, a recent study by Berendsen *et al*[28] presented a method for extended liver storage combining supercooling and machine perfusion. An essential step in this method was the addition of PEG35 to the preservation solution. Similar results were found by the same researchers with "supercooled" hepatocytes: this addition of 5% PEG35 to the storage solution prevented cold-induced lipid peroxidation and maintained hepatocyte viability and functionality during supercooling[25-27].

PEGs in organ preservation solutions

The cold static preservation of solid organs using preservation solutions is the gold standard in clinical organ transplantation today. PEG35 and PEG20 have been used as oncotic agents in IGL-1 and SCOT 20 preservation solutions respectively to prevent cell swelling[29-32]. The presence of PEG35 in IGL-1 makes this solution a good alternative to UW solution (the standard goal for liver transplantation), especially in the presence of moderate to severe hepatic steatosis[33,34]. PEG20 is the basic component of the SCOT solution, which furthermore contains low K+/high Na+ concentrations. PEG20 at 15 g/L has been found to reduce alloantigen recognition after liver reperfusion in comparison to UW solution[35]. However, the use of this PEG20 in preservation solutions has not shown a greater benefit than PEG35[35].

PEG35 (at 1 g/L) plays a key role in reducing the higher vulnerability of fatty livers to IRI[33]. This is mainly due to the production of nitric oxide (NO), whose vasodilatory properties contribute to counteracting the exacerbated alterations of microcirculation in steatotic livers due to the accumulation of fat in the sinusoids, which makes it more difficult to obtain an adequate hepatic revascularization after transplantation. Moreover, the NO generated may act as a suitable scavenger for preventing the impairment of lipid peroxidation in fatty livers against reperfusion[36].

It has also been demonstrated that PEG interferes with the coagulation system and reduces platelet adhesion *in vitro* and *in vivo*[37,38] by forming a molecular barrier on the glycocalyx. This PEG barrier prevents acute platelet deposition on damaged arteries. When a relatively low molecular weight PEG (< 10 kDa) was conjugated to pericardium, it reduced the deposits of calcium and decreased platelet and leukocyte surface attachment[39,40]. Therefore, the conjugation of PEG to the surface of endothelial cells seemed to reduce inflammation and control water content. Longer PEG chains, such as that of PEG35, might be expected to interact with the surface of endothelial cells of blood vessels and/or remain in the interstitial fluid of transplanted liver, thus promoting the above mentioned beneficial effects even after the washout of the organ graft.

The presence of PEG35 in IGL-1 solution also promotes the activation of several protective cell signaling pathways during liver cold storage, as a self-response of the organ to oxygen deprivation. This leads to the induction of cytoprotective factors such as adenosine monophosphate protein kinase (AMPK), an enzyme which is involved in the glucose metabolism breakdown and modulates the energy balance towards an energy preserving state. PEG35 also activates other protective factors associated with the deprivation of oxygen supply to the organ such as the hypoxia inducible factor HIF alpha[41].

PEG in perfusion solutions (graft washout and machine perfusion)

Rinse solutions help to wash the liver graft preserved in organ preservation solutions by avoiding air emboli and the secondary effects of the remnants of preservation solution, such as the excessively high concentration of intravascular potassium and metabolic waste during cold storage[42,43]. Although there is no consensus among physicians on how the graft flushes should be carried out, the most widely used solutions are Ringer Lactate and 5% human albumin[42].

PEG35 is a suitable additive in rinse solution for an efficient liver graft washout, and also ensures additional protection against reperfusion injury[44]. Protective mechanisms induced by PEG35 against liver reperfusion injury mainly involve the preservation of liver mitochondrial status[44], as shown in Figure 2.

Rhodamine 123 cell viability marker (in green) shows the preserved membrane potential of liver mitochondria in liver grafts preserved in UW and then rinsed with PEG35 solution when compared to livers rinsed with Ringer lactate. In this case, Evans Blue labeling (in red) shows the albumin content and the disrupted mitochondrial membranes. Thus,

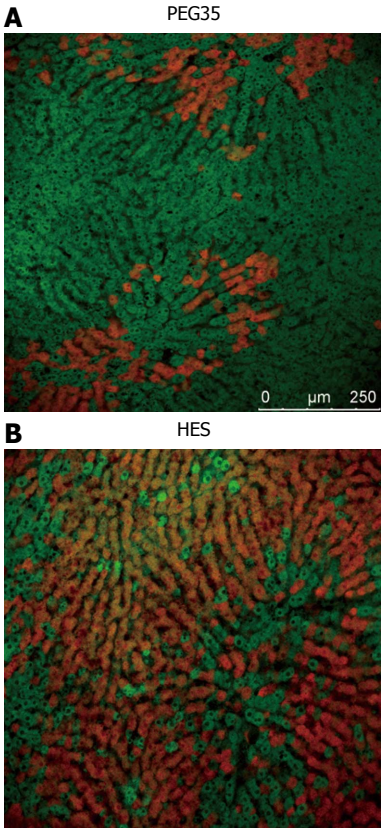

Figure 2 PEG35 preserves liver graft function. Confocal microscopy images showing green fluorescence of rhodamine 123 cell viability marker. Liver grafts were better preserved when they were rinsed with a solution containing PEG35 (A) rather than Ringer lactate solution (B).

PEG35 preserves the cytoskeleton structure and cell morphology against the effects of IRI[44,45].

Static cold preservation remains the gold standard, but the growing needs of liver transplantation oblige physicians to use machine perfusion (MP) techniques in different temperature conditions: hypothermia (HMP), normothermia (NMP) and subnormothermia (SNMP) for graft preservation purposes[46-48]. The use of PEG in MP solutions is very limited in contrast to UW-gluconate and KPS solutions[46-48]. Bessems *et al*[49,50] have shown that substitution of hydroxyethyl starch by PEG in Polysol perfusion solution achieved equal or better function and less damage in rat liver after 24 h of HMP, and that this Polysol-PEG solution was more efficient than UW-Gluconate perfusion solution. In rat steatotic livers, cold storage using Polysol resulted in significantly better integrity and functions of the liver[51] and thus improved the preservation quality of partial liver transplantation[52]. More recently, it was shown that addition of PEG35 to SNMP at 5 g/L using the "supercooling" technique was necessary to achieve successful liver transplantation after six days' preservation[28,53]. Thus, PEG contributes to the rapid extension of cooling and the lower temperatures attained also contributed to preserving the membrane and cytoskeletal structure of hepatocytes during HMP[25].

PEGs as "preconditioning" agents for IRI prevention

Recent investigations in the heart have found that high molecular weight PEG (15-20 kDa) protected cardiac myocytes from hypoxia reoxygenation[54]. More recently, these cardioprotective benefits for PEG 15-20 were observed when it was administered just before reperfusion[55].

With this in mind, our group explored the benefits of using PEG35 to limit IRI in different experimental models of cold ischemia and warm ischemia reperfusion in rats[56,57]. Intravenous administration of PEG35 to rats before the induction of cold ischemia-reperfusion insult (a single 10 mg/kg dose) protected fatty livers from the lesions associated with IRI[56]. The prevention of liver damage was accompanied by a high protection of liver cytoskeleton and mitochondria, which was concomitant with increased phosphorylation of pro-survival protein kinase b (AKT) and the activation of cyto-protective factors such as e-NOS and AMPK respectively[56].

These investigations reveal that *in vivo* PEGs improve the initial conditions of organs against the cold ischemia reperfusion insult. This PEG strategy can be considered as a useful tool for multi-organ preconditioning before organ recovery and then static cold storage/machine perfusion preservation.

These protective mechanisms of PEG were also corroborated in a warm ischemia-reperfusion model in the rat[57]. Intravenous administration of PEG35 at 10 mg/kg was protective against IRI. In this case, PEG35 not only prevented mitochondria damage, but also promoted the activation of prosurvival pathways (AKT, AMPK), and reinforced the cytoskeleton structure and preservation of the hepatocytes' morphological features[44,56]. However, the precise mechanisms by which PEGs interact with the cytoskeleton remains to be elucidated.

FUTURE PERSPECTIVES

Many studies have been designed to prevent mitochondrial dysfunction and to increase endothelial NO generation as a tool for favoring a rapid recovery of liver graft viability after reperfusion. The use of new NO-releasing molecules covalently linked to PEG, as oncotic agents for fatty liver preservation, could help to prevent exacerbated microcirculation in steatotic liver grafts. This practice has been extended to the pharmacological preconditioning of fatty livers, with very promising results (unpublished data).

Moreover, the use of another alternative molecule similar to butanediol mononitrate, conjugated to the carboxylic groups of PEG derivatives by an ester linkage, may provide a new kind of PEG derivative obtained by preparation of PEG-dendron polymers. This may be useful for defining new PEG molecules for supercooling purposes either in combination with MP or not.

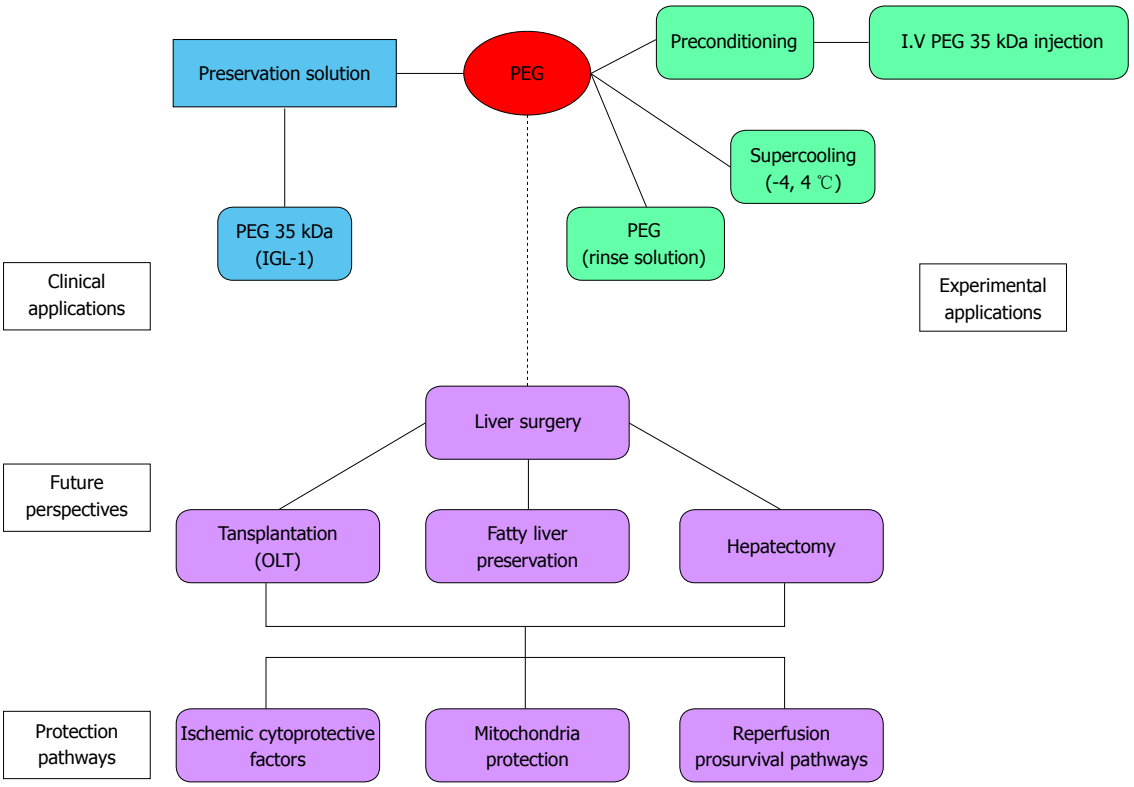

Figure 3 State of the art and future perspectives of polyethylene glycols as effective tool for limiting hepatic ischemia-reperfusion injury. Currently, the clinical applications of polyethylene glycols (PEGs) focus on their use as oncotic agents in IGL-1 solution for liver transplantation (in blue). Recent experimental investigations confirm the value of PEG35 for "supercooling" strategies combined with machine perfusion, as well as its use as a component in rinse solution for liver graft washout. Intravenous PEG35 treatment has also been shown to induce liver protection against cold and warm ischemia-reperfusion injury (IRI) (in green). Future clinical applications should be investigated (in violet). PEG protection mechanisms are characterized by a prevention of mitochondrial damage and by the promotion of several cyto-protective factors during IRI (in violet).

On the other hand, given the potential of PEGs as therapeutic targets for liver protection against IRI, it will be important to identify new PEG derivatives for use in liver transplantation as preconditioning agents. PEG treatment in donors and during reperfusion could minimize the deleterious effects of IRI, such as oxidative stress, cytoskeleton disruption and apoptosis. Moreover, in reduced orthotopic liver transplantation, the prophylactic administration of PEG to donor or/and recipient could contribute to a better liver regeneration of the implanted reduced graft and thus contribute to preventing the small-for-size syndrome present in living-living donor transplantation. In this particular case, the use of new derivatives based on growth factors releasing molecules covalently linked to PEG could offer potential advantages for rapid liver regeneration. Hepatic Growth Factor may be a suitable candidate. The benefits and perspectives of PEGs for limiting liver IRI are summarized in Figure 3.

CONCLUSION

The use of PEG may improve the initial conditions of organs available for transplantation, especially in the case of the most vulnerable ones such as steatotic livers. PEG is a very promising tool for limiting IRI in liver surgery (hepatectomy and transplantation) but further investigation in clinical trials is needed.

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