Tips for optimizing organ preservation solutions

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Organ injury during ischemia is one of the clinical problems of today’s transplantation. It occurs during warm ischemia time (WIT) when the blood flow is cut off and during cold ischemia when a graft is chilled in situ until the circulation is restored to the recipient organism. Fast cooling of the organ slows down metabolism and activates intracellular enzymes, which minimizes the effects of warm ischemia. Unfortunately, hypothermia also results in inhibition of ATP synthesis, cell swelling and intracellular acidity. That is why research is continually being conducted to develop new fluids for rinsing and storing organs, as well as to optimize the composition of those that are already in use, which will allow for longer and more effective graft storage and restoration of their optimal functions after transplantation. This article provides current information on rinsing and storage fluids available on the global market. It also discusses tips for the fluid modifications with hormones and micronutrients.

Key words: organ transplantation, preservation solution, hormones, micronutrients

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INTRODUCTION

In recent years, organ transplantation has become a life-saving routine. In 2016, Poland was reported to have a total of 1469 transplants from deceased donors (978 kidneys, 38 kidneys and pancreases, 317 livers, 101 hearts, 35 lungs) and 78 transplants from living donors (50 kidneys, 28 liver fragments). However, the number of people waiting for transplants is constantly increasing. In order to increase the effectiveness of transplants, intensive research is undertaken to ensure a sufficient number of grafts for transplantation, improve organ procurement and storage techniques, and reduce toxicity of the immunosuppressive treatment.

Fluids for organ rinsing and storage are an important factor affecting the success of transplantation. They are a transient environment in which the organ is stored between its removal from the donor organism and implantation into the recipient one. There are many fluids registered in the world (including one developed in Poland) dedicated to the preservation of specific organs. They differ in terms of composition, but all are intended to minimize the risk of cell damage, so as to restore proper functions of the organ after reperfusion. Ischemic damage occurs during warm ischemic time (WIT) when the blood flow is cut off and during cold ischemia when a graft is chilled in situ until the circulation is restored to the recipient organism. Fast cooling of the organ slows down metabolism and activates intracellular enzymes, which minimizes the effects of warm ischemia. Unfortunately, hypothermia also leads to inhibition of ATP synthesis, cell swelling and intracellular acidity. That is why research is continually being conducted to develop new organ preservation fluids, as well as to optimize the composition of those that are already in use, which will allow for longer and more effective graft storage and restoration of its optimal functions after transplantation (Birks et al. 2001; Rao et al., 2001). The optimal time of storing kidneys in preservative fluids is about 24 hours, liver – up to 15 hours, heart and lung – less than 6 hours, pancreas – up to 24 hours, and intestines – up to 8 hours. Longer storage results in disturbances of cellular respiration, oxidative shock and induction of inflammation (Forsythe 2001; Rowiński et al. 2004).

FLUIDS FOR RINSING AND STORING ORGANS

Viaspan® (UW solution, University of Wisconsin solution) is a fluid used for perfusion and preservation of organs within the abdominal cavity. Its composition was developed in the 1980s by the Belzer and Southard’s team at the University of Wisconsin, hence its name. The complex formula of the solution was repeatedly analysed in terms of the efficacy of individual components. The significance of some of them was not confirmed. Viaspan is an intracellular solution with high potassium (125 mmol/l) and low sodium (29 mmol/l) levels. The significant concentration of potassium is expected to counteract the escape of K+ ions from the inside of the cell, but there is a risk of vasocstriction. Hydroxymethylated starch (HES) retains the fluid in the endovascular space and prevents swelling of the extracellular space. It has been found that the addition of HES at 5% triples the viscosity of the fluid at 4°C relative to crystalloids, which in turn reduces the perfusion efficiency (Tullius et al., 2002), and causes aggregation of erythrocytes (Mo-
that after 7, 30 and 60 days, the values were 4.64 mg/dl, 1.75 mg/dl, 1.7 mg/dl (Biosol group) and 3.2 mg/dl, 1.53 mg/dl, 1.62 mg/dl (Viaspan group). The results obtained after using the Biosol and Viaspan solutions were comparable (Jóźwik et al., 2016).

IGL-1® liquid is based on Viaspan, where the concentration of K⁺ (25 mmol/l) and Na⁺ (120 mmol/l) ions was reversed, thereby minimizing the risk of cardiovascular complications. Hydroxyethyl starch (HES) is replaced in some countries with 35 kDa polyethylene glycol (PEG-35), which stabilizes the lipid layer of the cell membrane (Duthieil et al., 2009; Badet et al., 2005; Codas et al., 2009; Dondero et al., 2010). In the USA, in accordance with the decision of FDA (Food and Drug Administration), HES is still present in the composition of IGL-1. The osmolality of the solution is 290 mOsm/l, pH 7.4. The fluid is intended for the preservation of kidneys, livers and pancreases under hypothermic conditions. Experimental and clinical studies confirmed the superior efficacy of IGL-1 in liver and kidney transplants as compared to Viaspan. Renal studies demonstrated decreased levels of apoptotic markers and lower creatinine parameters 15 days after transplantation, whereas based on the liver transplant model, improvement of biochemical and histological parameters of the organ and improvement of microcirculation after perfusion were observed (Franco-Gou et al., 2007; Maathuis et al., 2008; Mosbah et al., 2012; Zoaouli et al., 2011).

HTK® is a cardioplegic fluid initially developed for open heart surgery. Later, its effectiveness in the storage of livers, kidneys, pancreases and lungs was confirmed. The name of the solution is derived from its three main components: histidine, tryptophan and α-ketoglutaric acid. It is an extracellular fluid (potassium and sodium concentrations are 9 mmol/l and 15 mmol/l respectively) and has a high buffer capacity (acidic – 97 mmol/l at T=5°C). Tryptophan stabilizes cell membranes and removes oxygen free radicals, whereas α-ketoglutarate is the substrate for anaerobic metabolism during organ storage. The fluid is characterized by low viscosity so it easily penetrates the microcirculation and can be used for in situ perfusion. It shows comparable efficacy in liver storage to IGL-1 (Meine et al., 2015). It was found that porcine pancreatic islets stored in HTK showed higher survival rates under in vitro conditions than those preserved in Viaspan (Steffen et al., 2017). For liver rinsing, IGL-1 is more cost-efficient than HTK. Although the prices of both fluids are comparable, IGL-1 consumption (4 litres) is lower than that of HTK (6–10 litres) (Meine et al., 2015).

Celsior® is an extracellular (potassium and sodium concentrations are 15 mmol/l and 100 mmol/l, respectively), hypertonic (320–360 mOsm/l) solution intended for the storage of thoracic organs – hearts, lungs, and organs of the abdominal cavity – kidneys, livers and pancreases, under hypothermic conditions. The acidic buffer capacity of the fluid is about 11 mmol, alkaline – approx. 7 mmol, viscosity: 1.15 mm²/s. The fluid contains antiedematous substances (lactobionate and mannitol), oxygen free radical scavengers (histidine and glutathione), and a high energy substrate (glutamate). Glutamate also prevents the increase of calcium concentration in the cell, and histidine prevents acidosis thanks to its buffering properties. It was found that heart storage in HTK and Celsior under hypothermic conditions using the static method had a comparable effect (Li et al., 2016). Randomized, multicentre studies demonstrated high efficacy of this fluid in the recovery of bile duct functions af-
ter liver transplantation (Pedotti et al., 2004) and in lung preservation in the animal model (Wittwer et al., 1999).

Plegisol® is a crystalline cardioplegic solution for cardiac perfusion after stopping coronary circulation and preservation of this organ prior to transplantation. It is an extracellular fluid (K+ – 16 mmol/l, Na+ – 110 mmol/l) with osmolarity of 328 mOsm/l and pH=7.8. It also contains Ca2+ (2.4 mmol/l), Mg2+ (32 mmol/l), Cl- (160 mmol/l) and HCO3- (10 mmol/l) ions (Cannata et al., 2012). Calcium ions stabilize cell membranes, counteract sarcolemma cracking and loss of Ca2+ transport capacity. Phosphate buffer counteracts the effects of metabolic acidosis. Magnesium is involved in myocardial stabilization by inhibition of myosin chain phosphorylation. Plegisol has a protective effect on the heart, comparable to HTK and Celsior in the early post-transplant period. The incidence of graft injury during storage and the occurrence of post-transplant failure were not significantly different in the analysed groups (Aldemir et al., 2014; Latchana et al., 2014).

Perfadex® is an extracellular fluid (osmolarity of 292 mOsm/l, pH=7.4) mainly intended for lung transplants under hypothermic conditions. It is often called “low-potassium dextran” (LPD) due to the K+ concentration (6 mmol/l) and the presence of dextran 40 (50 g/l), which allows the fluid to be maintained in the endovascular space. The phosphate buffer in the fluid prevents metabolic acidosis, whereas glucose is a source of energy for ATP resynthesis (Fermg et al., 2017; Ohsumi et al., 2017). Comparative studies of the efficacy of Celsior and Perfadex in lung preservation were conducted. Menezes and coworkers reported similar effectiveness of both fluids using an animal model. Gas exchange parameters and results of histopathological analyses were comparable in both groups. There were no statistically significant differences in pulmonary arterial pressure after 6 and 12 hours of storage. The extent of pulmonary oedema was comparable for both ischemic times. No differences were observed in type II pneumocytes. Few cells have been observed to have chromatin condensation, which may suggest an early stage of apoptosis. The basement membrane thickness was comparable in both groups (Menezes et al., 2012). However, clinical studies by Gohrbandt et al. demonstrated that Celsior protected lungs during transplantation better than Perfadex. Three-year survival of the graft after transplantation was significantly higher in the group of patients where Celsior was used (Gohrbandt et al., 2015).

Polysol (PS) is a solution intended for organ storage and perfusion under hypothermic conditions. It is an extracellular fluid (total concentration of sodium is 120 mmol/l, potassium – 15 mmol/l) with pH=7.4 and osmolality of 320 mOsm/l. It contains about 60 substances, including: electrolytes, colloid (PEG, 35kDa), buffering systems (phosphate, histidine, HEPES (buffer of N-(2-hydroxyethyl)piperazine-N-2-ethanesulfonic acid), a precursor for ATP production (adenosine), antioxidants (glutathione, α-tocopherol, ascorbic acid), raffinose, trehalose, vitamins B1, B2, B3, B4, B5, B6, B7, B9, B12, C, A, D2, E, K3, and 21 amino acids (including alanine, arginine, asparagine, cysteine, glutamine, glycine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine) (Schreinemachers et al., 2009a). The effectiveness of individual fluid components and the potential for interaction between them have not been investigated. Due to the multi-component nature of the Polysol solution, it is important to carry out research on the proper application of all ingredients. In addition, the presence of various organic and inorganic compounds entails the risk of interaction (Schreinemachers, 2010). The solution is characterized by low viscosity (1.8 cP), high buffer capacity and high oncotic pressure. This is mainly due to the presence of polyethylene glycol in the fluid, which stabilizes the lipid membrane structure and reduces the permeability of the vascular membranes (Hauet et al., 2001; Schreinemachers et al., 2009a). Preclinical studies demonstrated high effectiveness of Polysol compared to other fluids typically used for rinsing organs. Bessem et al. found that the application of continuous perfusion under hypothermic conditions for storing rat liver using Polysol was more beneficial compared to ViaSpan and HTK. The number of released ALT, AST, LDH and alpha-GST was significantly lower (Bessems et al., 2005a; Bessems et al., 2005b). Rinsing of the rat liver in Polysol using the cold hypothermia method also caused smaller mitochondrial changes in this organ compared to HTK (Hata et al., 2007). The use of Polysol solution for storage of porcine kidneys improved their functions and structural integrity compared to HTK (Schreinemachers et al., 2009b). Polysol also proved to be superior to ViaSpan fluid in storing the rat large intestine (Wei et al., 2007). Schreinemachers et al. conducted a clinical pilot study in nine human recipients of kidneys that were stored in ViaSpan and Polysol. A higher percentage of rejection was observed in patients whose kidneys were rinsed with Polysol (Schreinemachers et al., 2013).

ET-Kyoto is an extracellular fluid with sodium concentration of 107 mmol/l and potassium concentration of 42 mmol/l. The solution osmolarity is 366 mOsm/l. Trehalose, which has a cytoprotective effect, is not included in any other fluid. Gluconate and HES counteract cell swelling. Phosphate buffer prevents metabolic acidosis. Db-cAMP (cyclic adenosine monophosphate dibutylin) and nitroglycerine protect the vascular endothelium. NAC (N-acetylcysteine/antioxidant) acts as a free radical scavenger (Bando et al., 1998; Chen et al., 2004; Wada et al., 1996). This fluid was originally developed for lung storage, but it also proved to be effective in preserving kidneys, livers, pancreases and tracheae (Chen et al., 2004). It is suggested that ET-Kyoto has comparable efficacy in storing kidneys and livers to ViaSpan (Yoshida et al., 2002; Zhao et al., 2008).

HORMONES AFFECTING THE EFFICIENCY OF RINSING FLUIDS

An increasing attention is paid to hormones as substances that can significantly reduce the effects of organ ischemia during their storage. Prolactin (PRL) has a broad spectrum of therapeutic effects and is involved in more than 300 processes occurring in living organisms. It affects the regulation of calcium homeostasis by stimulating active transport in the duodenum (Charoenphandhu & Krishnamtra 2007; Charoenphandhu 2001; Dolinska et al., 2012a). It participates in angiogenesis processes, stimulates immune cell proliferation and differentiation, inhibits apoptotic and inflammatory processes. It induces T and B cell multiplication and secretion of immunoglobulins and cytokines (Parada-Turska et al., 2006; Vera-Lastra et al., 2002). Prolactin acts on specific membrane receptors located at different sites, including liver and kidney cells, and hence it can affect their tissues. It stimulates the process of anaerobic glycolysis under hypoxic conditions, increasing the survival of organs (Parada-Turska et al., 2006; Ryszka et al., 2011). PRL has a positive effect on the regeneration of rat liv-
er after partial hepatectomy, increasing cell proliferation and differentiation (Olazabal et al., 2009). When added to preservative solutions, it slows down the release of transaminases, which might suggest hepatoprotective properties of this hormone. A study on an isolated rabbit liver showed that the addition of PRL to Ringer’s fluid significantly reduced the amount of released ALT and AST. ALT and AST release into the intercellular space proves that structural integrity of the cell membrane is affected. Their level of activity correlates with the degree of cell damage (Ryszka et al., 2002; Szulc-Musiol et al., 2004). Inclusion of PRL in HTK positively influenced 24-hour storage of an isolated porcine liver (Ryszka et al., 2011). Addition of PRL to ViaSpan showed its significant effect on the speed and dynamics of the changes in calcium and magnesium ion concentration in the preservative solution. This hormone probably blocks access of Ca^{2+} to hepatocytes (Ryszka et al., 2006). Addition of recombinant human prolactin (rhPRL) to Biosolad reduced the transaminase activity during reperfusion of porcine kidneys (Ryszka et al., 2016). It has been also found that rhPRL can protect the islets of Langerhans during pancreas storage prior to transplantation (Yamamoto et al., 2010).

**Melatonin** is a hormone secreted by the pineal gland, and, to a lesser extent, by the retina, enterochromatophoric gastrointestinal cells and blood cells (Carrillo-Vico et al., 2005). It exhibits strong antioxidant properties and is one of the most effective free radical scavengers. It has been demonstrated that it is five times more effective in eliminating hydroxyl radicals than glutathione (present in the composition of Celsior, ViaSpan, IGL-1) and vitamins C and E (Beyer et al., 1998; Popławski & Derlacz 2003; Zań et al., 2011). In addition, it increases the activity of antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Reit et al., 2000). It stimulates the production of thermal shock proteins, reduces neutrophil infilrows, modulates cytokine production (IL-10 growth and TNF-α decrease), and reduces apoptosis and necrosis of damaged tissues. Strong antioxidant and immunostimulatory properties indicate its cytoprotective effect during ischemia and reperfusion of organs (Jaworek et al., 2007; López-Burillo et al., 2003). Aslaner et al. showed that 30 mg/l of melatonin added to ViaSpan effectively protected kidneys during cold ischemia within up to 48 hours. Histological examination showed less damage when compared to kidneys stored in melatonin-free fluid, and significantly lower LDH release (Aslaner et al., 2013). Li and coworkers found that melatonin reduced damage to kidneys stored in HTK. It induces superoxide dismutase (SOD) activity and reduces the activity of lipoydroperoxides (LPO). Its overactivation may contribute to the reduction in oxidative stress indicators: expression of NF-kBp65, inducible nitric oxide synthase (iNOS), and caspase-3 (Li et al., 2009). Gunal et al. studied the effect of ViaSpan modified with 30 mg/l of melatonin on ischemic hepatic injury in Wistar rats. They found that, compared to the control group, melatonin had a protective effect on the Kupffer cells. The amount of released LDH, AST and ACP enzymes was significantly lower, and there was increased expression of HSP 70 thermal shock proteins and decreased lipid peroxidation (Gunal et al., 2010).

The effects of thyroid hormones: **thyroxine** (T4) and **triiodothyronine** (T3), on graft damage during storage have also been studied. Imberti et al. suggest that administration of thyroxine to rats increases ischemia-reperfusion injury of a liver stored in the Euro-Collins fluid (Imberti et al., 1998). Yang and coworkers have shown that administration of triiodothyronine to mice has protective effects on the liver under conditions of ischemia and reperfusion. T3 activated autophagy mediated by MEK (MAP-ERK kinases)/ERK (extracellular signal regulated kinase)/mTORC1 (mTORC1 kinase) (Yang et al., 2015). Triiodothyronine induces hepatocyte proliferation in rat livers (Malik et al., 2003), stimulates liver regeneration after partial hepatectomy (Cervinková et al., 1998), and reduces apoptosis (Sukoecheva & Carpenter 2006).

**Insulin** is a component of ViaSpan. It transports glucose, which is the substrate for anaerobic glycolysis and the main source of energy in ischemia, into cells. **Dexamethasone** (synthetic hormone of the adrenal cortex), also present in ViaSpan, has long-lasting, potent anti-inflammatory and immunosuppressive effects (Sarč et al., 2009).

**Insulin-like growth factor-1** (IGF-1) belongs to insulin-like polypeptide hormones. It influences the growth processes of the body and normal cell homeostasis (Filius & Zdrojevicz 2014). Zaouali and coworkers (2010b) added 10 μg/L IGF-1 to IGL-1 and found improvement in liver function parameters after 24-hour storage. They observed an increase in the activity of AKT (serine-threonine kinase) and eNOS (endothelial nitric oxide synthase) and inhibition of TNF-α pro-inflammatory cytokine release. IGL-1 was also enriched with the **epidermal growth factor** (EGF - 10 μg/l). They found that it slowed down the release of transaminases, increased the content of adenosine triphosphate (ATP) and minimized mitochondrial damage (Zaouali et al., 2010a).

**Erythropoietin** (EPO) is a glycoprotein peptide hormone that is involved in the development of erythroblasts and promotes the release of reticulocytes from the erythroblast islets. It has been found to have anti-inflammatory, antioxidant, angiogenic and cytoprotective properties. Progressive renal and cardiac failure leads to a decrease in EPO (Jackevicius, 2014; Kopčić-Szlęzak, 2009; Saganowska, 2008). Schmeding et al. administered 8.4 μg of rhEPO to rats and observed smaller ischemia-reperfusion liver injury after orthotopic transplantation when compared to the control group (Schmeding et al., 2009). Eipel et al. demonstrated that HTK supplemented with rhEPO at a dose of 0.084 was beneficial for rinsing isolated, fatty mouse livers and storing them under hypothermic conditions. The modified fluid improves the function of endothelial cells, blood vessel lining, reduces expression of UCP2 (uncoupling protein 2) and phosphorylation of ERK (extracellular regulated kinases) (Eipel et al., 2012). Addition of erythropoietin (0.042 mg/l) to Celsior has a cardioprotective effect on isolated rat hearts (Kumarasinghe et al., 2016; Watson et al., 2013).

**MODIFICATION OF FLUID COMPOSITION WITH MICRONUTRIENTS**

The addition of micronutrients in order to modify the composition of preservative solutions is an innovation. It has been found that the inclusion of selenium in HTK significantly affects normal functioning of kidneys after transplantation. It lowers the concentration of malondialdehyde, which increases under conditions of increased generation of free oxygen radicals (Treska et al., 2003). As a component of Euro-Collins fluid, selenium protects lungs during ischemia and reperfusion (Soneul et al., 1994). Zinc added to St. Thomas’ Hospital No.2 fluid (Plegisol) has a beneficial effect on maintaining...
normal cardiac functions and can be used in cardiopulmonary bypass fluids (Bessem et al., 2005b). Micronutrients can also significantly affect the durability of organ rinse solutions. It has been shown that the addition of zinc to Biolasol decreases its durability by 30.5%, while the addition of selenium increases its durability by 8.2%. This indicates the synergism of antioxidant effect of vitamin C and Se in the fluid (Ostróžka-Cieślik et al., 2015).

**SUMMARY**

The development of transplantation is conditioned by the achievements of many areas of science. Genetic discovery and improvement of genetic engineering techniques may help to develop methods for obtaining fragments or whole organs using the genetic material of the recipient. Advances in biotechnology and pharmaceutical science will enable the development of an optimal fluid for organ storage that will effectively and safely prevent the consequences of ischemia-reperfusion injury and the effects of hypothermia. The next step in fluid development will be their modification with the addition of proper hormones and bio-nutrients.

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