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ORIGINAL ARTICLE

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Physiologic and metabolic results of pancreatic cold storage with Histidine-Tryptophan-Ketoglutarate-HTK solution (Custodiol)® in the porcine autotransplantation model

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Abstract Delayed graft function (DEGF) remains an obscure phenomenon in organ transplantation. For the optimal washing of the compounds of the different organ flush solutions, adequate temperature and equilibrium of electrolytes have to be provided. A total of 29 landrace pigs weighing 37.3–5.4 kg were included in this study. According to the model, the left hemipancreas was perfused with Histidine-Tryptophan-Ketoglutarate (HTK)-solution and autotransplanted after 24 h (G1, $n = 13$) and 48 h (G2, $n = 4$) of cold storage (CS). Results were compared with grafts perfused with UW-solution and autotransplanted after 24 h (G3, $n = 8$) and 48 h (G4, $n = 4$) CS respectively. Daily measurements of glycemia, glucosuria, amylase and lipase were carried out. HTK perfusion resulted in an increase in wet weight of the grafts after 24 h and 48 h CS ($P = 0.031$ vs UW). Postoperative glycemia levels in pancreases flushed with HTK-solution were higher after 48 h than after 24 h CS until the 6th postoperative day, when the glycemia returned to normal range ($P = 0.02$), suggesting a delayed endocrine graft function. The mean IVGTT values attained after full function were comparable in G1 and in G3 (-1.22 ± 0.23 vs. -1.5 ± 0.65). The rises in serum amylase and lipase levels were more pronounced after 48 h CS in both HTK and UW groups, (P = n.s.). Appearance of interstitial and intracellular edema after CS and reperfusion did not influence the function.
Conclusion: HTK-solution is suitable for 24 h pancreatic preservation in vivo; the perfusion requires at least 4 min for electrolyte equilibration. Long preservation time (48 h) resulted in a transitory DEGF.

Key words Pancreas preservation · HTK-solution · Segmental porcine pancreatic autotransplantation · Delayed endocrine graft function

Introduction

University of Wisconsin (UW) solution is the preservation solution most commonly used for the transplantation of abdominal organs. This solution was first investigated for experimental pancreas transplantation in dogs [29] and successively employed for renal [19] and liver [23] transplantation. In recent years, Bretschneider's histidine-tryptophan-ketoglutarate (HTK) solution, originally developed for cardioplegia [3], has been used for cardiac [20], renal, [10] and hepatic transplantation [7]. However, despite the widespread use of HTK-solution as an alternative to organ preservation with UW solution, no results have been published for clinical pancreas transplantation. This might be explained by the lack of experimental data on metabolic results in a large animal model. The protective effect of Brettschneider's Histidine-Tryptophan-Ketoglutarate (HTK) solution is based on a lowering of the energy requirements by reduction of the sodium, and a slight elevation of potassi-

um and magnesium levels, as compared with the serum. The addition of histidine provides a strong buffering capacity. UW-solution however, contains impermeants suppressing cell swelling (lactobionate, raffinose and hydroxyethyl starch), electrolytes and phosphate as buffer. To facilitate a rapid restoration of normal metabolism and to stabilize cellular membranes, other substances are added like adenosine, gluthatione, allopurinol and steroids. Compared to UW solution, HTK has a low viscosity facilitating organ flushout. However, the buffering capacity depends on an equilibration of the electrolyte composition in the graft's extracellular space to the electrolyte content of the protective solution. This equilibration has previously been studied for canine and human kidneys, emphasising the importance of adequate organ perfusion with HTK-solution [2]. The influence of this equilibration process and functional results in vivo are unknown since pancreatic physiology and metabolism were studied only in vitro using a perfusion chamber [14, 15]. As these conditions are unlike the clinical setting, we investigated these issues in a porcine autotransplantation model focusing on electrolyte equilibration, functional (in particular the correlation of extended ischemia time and delayed graft function) and histological results in vivo, following autotransplantation of pancreatic grafts preserved with HTK-solution.

Animals and methods

Twenty-nine landrace pigs weighing 37.3 ± 5.4 kg were considered in this study. Pancreatic autotransplantation was carried out by engraftment of the left hemipancreas after 24 h (G1, $n = 13$) and 48 h (G2, $n = 4$), cold storage (CS) using HTK-solution (Custodiol Dr. Franz Köhler Chemie, Alsbach-Hähnlein, Germany). These results were compared with those obtained from pancreata perfused with UW-solution (Viaspan, Dupont Pharma, the Netherlands) and transplanted after 24 h (G3, $n = 8$) and 48 h (G4, $n = 4$) CS respectively. Anaesthesia was effected by injection of 10–15 mg/kg Thiopental (Abbott, Ottigny-Belgium) intravenously with continuous infusion of 0.4 mg/kg Pavulon (Organon Technika, Veedijk, Turnhout, Belgium) and 15 g/kg per h Fentanyl (Janssen-Cilag, Berchem, Belgium). Lungs were ventilated with Halotane-Oxygen mixture following intratracheal intubation. Fluids administration consisted on 500 ml Glucose 5% given via a central venous catether and one shot antibiotic prophylaxis with 800 mg Bactrim (Roche, Brussels, Belgium) given in 100 ml NaCl 0.9% before the start of the operation. After retrieval of the left hemipancreas, a back table perfusion was carefully performed until the venous effluent was clear, for 10 min at least, at a perfusion pressure of 60-cm H_2O (40–50 mm/Hg). At that time, and for pancreases stored with HTK-solution, ten samples were taken from the effluent at intervals of 1 min during the first 10 min and analyzed for sodium, potassium, chlor and magnesium content, using the ion selective electrode method (Hitachi). Thereafter the grafts were stored for 24 h and 48 h at 4 °C. Pancreatic autotransplantation was carried out by a technique previously described [24, 25]. Briefly, the pancreatic vascular pedicle consisted of the celiac trunk including the proximal part of the hepatic and splenic artery. Hepatic blood flow was restored by interposition of an iliac artery autograft between celiac trunk and common hepatic artery, after removal of the left pancreas. Pancreatic autotransplantation was performed using a standard surgical technique: end-to-side anastomosis of the splenic vein to the right common iliac vein, end-to-end anastomosis of the arterial pedicle to the right common iliac artery. On the day of transplantation, the pancreatic head and the uncinate process were successively isolated from the duodenum and portal vein preserving the duodenal arcade. Only the grafted pancreas sustained endocrine function. Exocrine secretions were drained freely into the peritoneal cavity. The weight of the pancreatic graft was measured prior to flushing and prior to reperfusion. Graft function was assumed when daily fasting blood-glucose levels were lower than 150 mg/dl and glucosuria was absent, as measured by Gluketur test (Böringer-Mannheim, Mannheim, Germany). Untreated, unoperated pigs ($n = 3$) served as normal controls, and totally pancreatectomized ($n = 8$) animals that had not undergone transplantation served as diabetic controls. The pigs were closely observed for the first 10 postoperative days undergoing daily measurement of glycemia, glycosuria, serum amylase and lipase, using common reagents for enzymatic method (Böringer-Mannheim). An intravenous glucose tolerance test (Fig. 4) (IVGTT, 0.5 g. Glucose/Kg body weight) was performed within 10 days post-transplant, and K-values were calculated as the decrease of glucose per minute. Pigs were than sacrificed for autopsy and histo-pathological studies. Specimens for histological studies using H and E staining were taken as follows: a) native pancreas; b) after CS; c) immediately after the reperfusion; d) 30 min following the reperfusion; e) at the time of autopsy. For statistical analysis, the student t-test was used. P was significant when < 0.05.

Results

Neither sepsis nor graft thrombosis arose during the observing period, and all the animals that underwent transplantation were suitable for the evaluation of graft viability.

Perioperative data

Total warm ischemia time was 63.8 ± 8.8 s. for all the groups and corresponded to the time occurring for a selective cross clamping of the vascular structures and the graft retrieval. The flushing time was 11.2 ± 2.1 min, and anastomosing time for subsequent engraftment was 18.4 ± 12.9 min.

Physiological data

The time necessary for almost complete electrolyte – equilibration of the porcine pancreases was 10 ± 2 min flushing with 50 ml HTK-perfusate. In particular, the difference in the sodium content between the perfusion solution and the splenic venous effluent was 9 mmol/l after 1 min, with a marked reduction after 4 min (Fig. 1), strongly demonstrating this equilibration process. The difference in the chloride content was

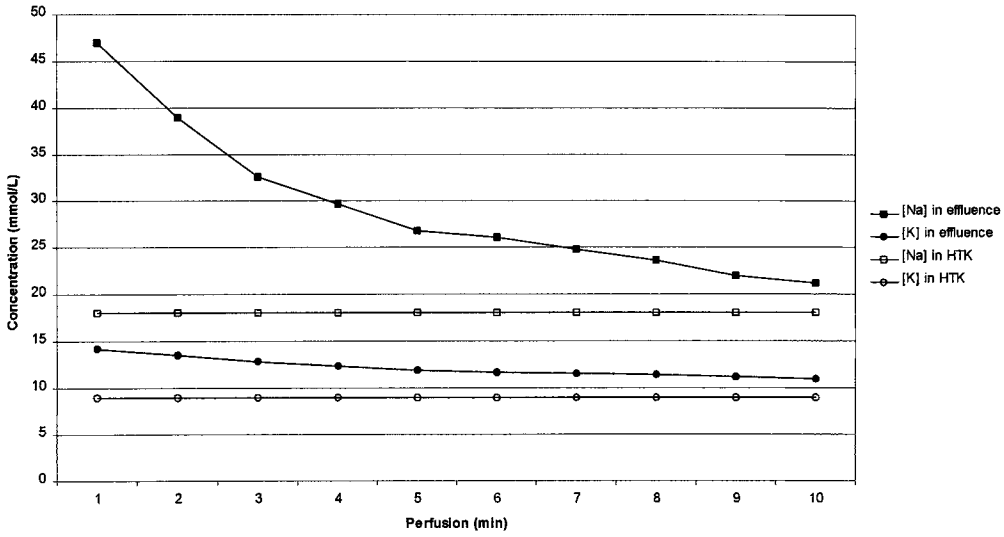


Fig. 1 Differences in the sodium and potassium concentration between the perfusion solution and the venous effluence during perfusion with HTK solution

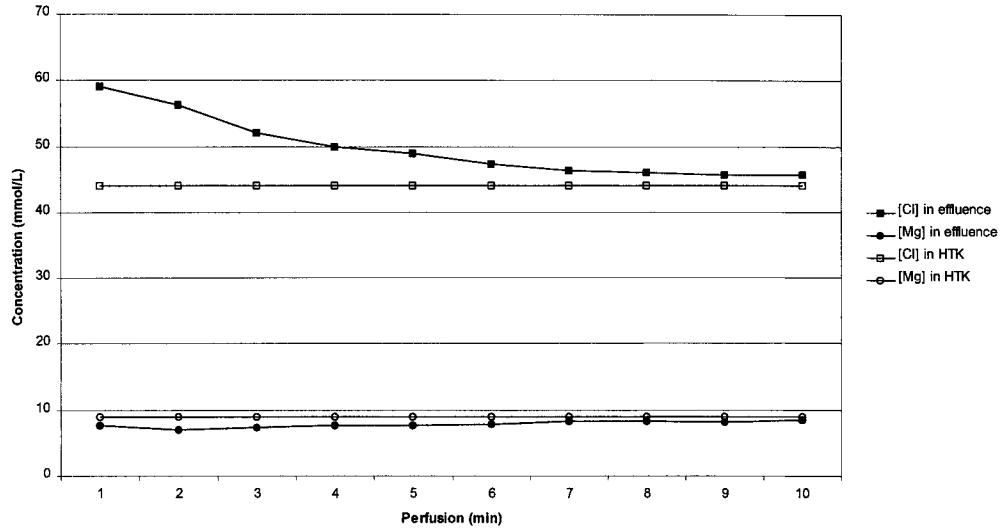


Fig. 2 Differences in the chloride and magnesium concentration between the perfusion solution and the venous effluence during perfusion with HTK-solution

6 mmol/l after 3 min, slowly reaching the level of 48 mmol/l after 10 min (Fig. 2). The potassium content progressively reached the equilibration levels during the entire perfusion time, and no changes were noticed for the magnesium. HTK perfusion resulted also in an increase in wet weight of the grafts after 24 h and 48 h CS, with a mean ± SD of 2.32 ± 2.6 and 1.5 ± 2.5 g respectively ($P = 0.6$) for 24 h versus 48 h and for weight of all the grafts before and after CS. In the UW groups (G3 and G4), the total wet weight decreased at a mean of –3.1 ± 0.2 g ($P = 0.031$ vs G1 and G2).

Metabolical studies

All the animals with 24 h CS (G1 and G3) were normoglycemic after autotransplantation. Glycemia levels taken immediately postoperatively and one day thereafter were higher after 48 h CS in G2 vs G1 [HTK] ($P = 0.02$), suggesting a delayed endocrine graft function (DEGF) for that group (Fig. 3). However, abnormal glycemia levels were noticed until the 6th postoperative day, when the glycemia returned to normal range. In G4 (UW-48 h CS), only 1 pancreas graft displayed normal function without abnormal postoperative glycemia levels, whereas 3 showed severe necrotizing pancreatitis resulting in graft loss. The mean ± SD K-values were –1.18 ± 0.23 for G1, –1.22 ± 0.23 for G2 and –1.5 ± 0.6 for G3 (P = n.s. for G2 vs G1 and G3 vs G1

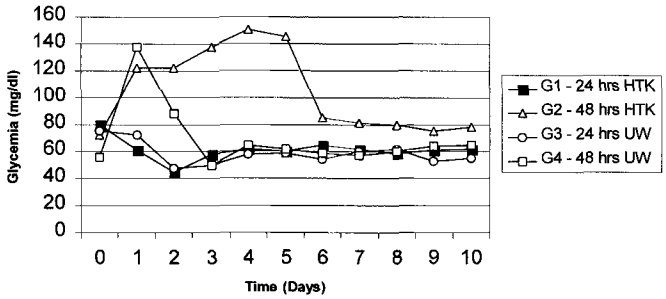


Fig. 3 Evolution of glycemia

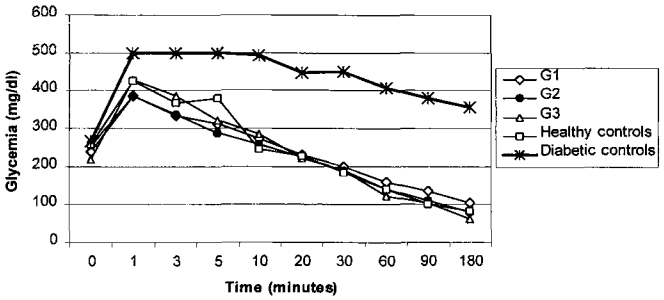


Fig. 4 Intravenous glucose tolerance test

and G2). The mean ± SD K-value of normal controls was −1.53 ± 0.81 and −0.52 ± 0.19 for diabetic controls ($P = 0.001$ vs all functioning grafts). Glucosuria was maximal (i.e. > 1 g/dl) in this latter group and absent in controls. The rise in serum amylase and lipase levels measured postoperatively during the observation time were all in all more pronounced after 48 h vs 24 h CS in the HTK groups. These differences, however, were statistically not significant (mean ± SD serum amylase of 2890 ± 1063 U/l and lipase of 827 ± 1308 U/l in G1; 3339 ± 2382 U/l and 1587 ± 1492 U/l respectively for serum amylase and lipase in G2; 4960 ± 3995 U/l and 2109 ± 2238 U/l respectively for serum amylase and lipase in G3).

Histological features

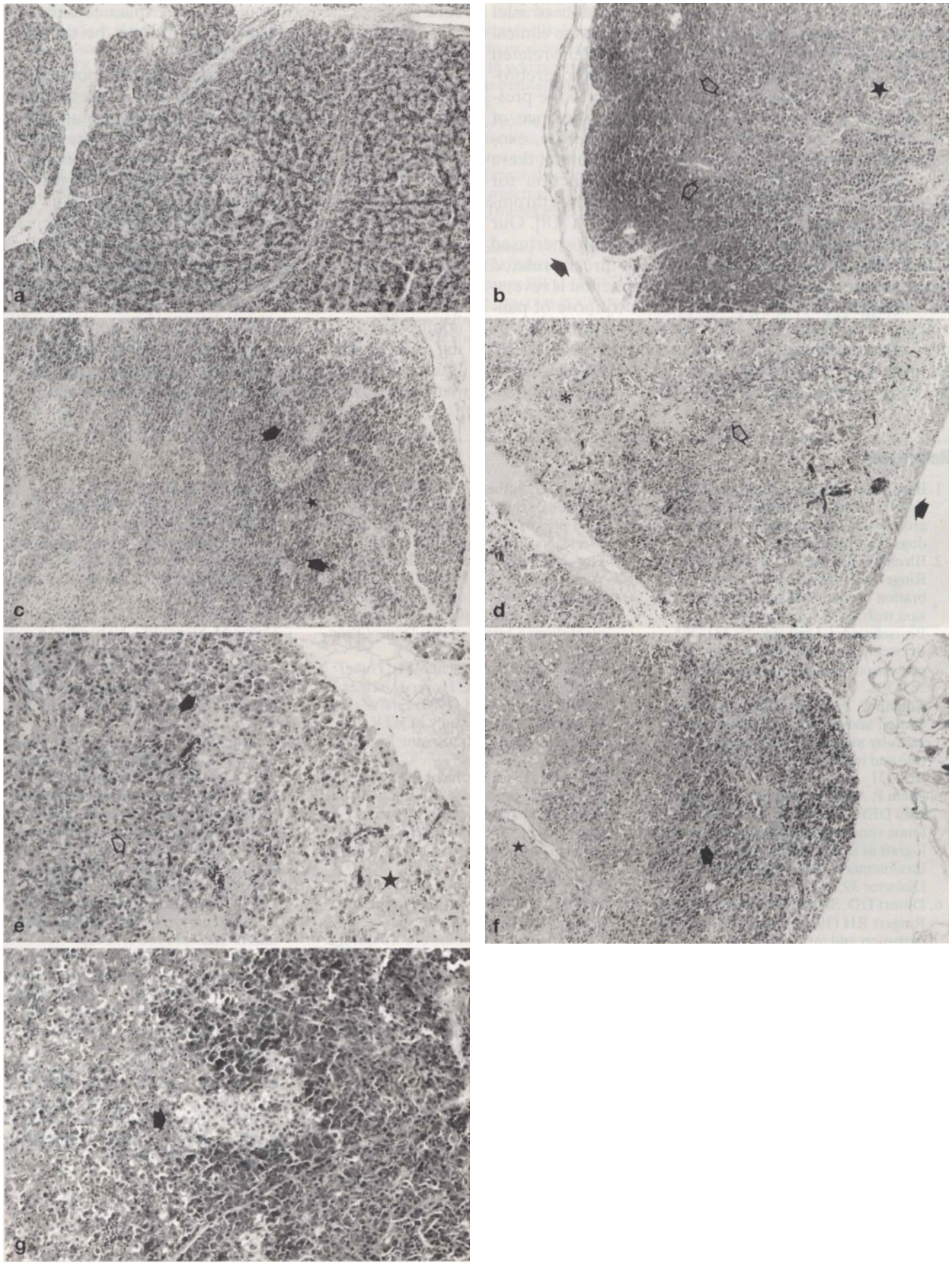
Histological studies of the native pancreas showed preserved lobular architecture with normal islets and no signs of inflammation or necrosis (Fig. 5a). Interstitial and intracellular edema was noticed after CS, (Fig. 5b, c) and this increase after the reperfusion was a time dependent effect (Fig. 5d, e). At the time of reperfusion there were some foci of necrosis within areas which were well preserved. The necrosis was more pronounced 30 min after the reperfusion, and necrotic areas were most prominent at the periphery (Fig. 5d). Inflammation appeared also after the reperfusion but was not correlated with the degree of edema. Grafts stored for 48 h showed more necrosis and less islet-cells but no differences in edema. No abscesses or local infections were seen at the moment of autopsy, and no vascular thrombosis was encountered. However, signs of local chemical peritonitis with fibrosis and adhesions were encountered in the pelvis. The pancreatic lobar architecture was irregular, and a periductular fibrosis was noticed around preserved islet-cells. An interstitial infiltration of chronic inflammatory cells was also seen in the exocrine tissue (Fig. 5f, g). However, the degree of organ damage varied slightly depending on the tissue area investigated, but features were similar in all the grafts. Neither differences nor specific histology features were seen in both HTK and UW-flushed pancreases.

Discussion

UW-solution as well as HTK-solution are routinely applied in clinical transplantation with similar intention, but their components differ greatly. UW-solution contains basically osmotic effective substances (lactobionate, raffinose and hydroxyethylstarch) combined with a phosphate buffer and high potassium. In comparison, HTK-solution contains less potassium and a strong histidine buffer that increases the osmotic effect of mannitol, which is also included in this solution. The tryptophan is a membrane stabiliser and the ketoglutarate serves as a substrate for the metabolism during ischemia. It appears that the electrolyte equilibration process is very important for the optimal function of this buffer-system of protecting membranes, [21, 6] whereas electrolyte equilibration is not required with UW-solution because of different action of the components. First results comparing different preservation solutions including HTK date from 1989–90 and focus on experimental pancreatic ischemia in vitro using reperfusion chambers [14, 15] for organ flushing. Results showed some advantages for HTK perfusion, these being a reduced arterial vasospasm due to the lower K^+ content and a high arterio-venous volume flow-rate decreasing organ temperature of the gland with a fast washout due to its low viscosity. Less anaerobic glycolysis with subsequently low levels of lactate was also noticed, due to the strong buffer-capacity of the HTK-solution. The release of insulin, amylase, and lactate during reperfusion after 24-h cold ischemia in the same model resulted in similar results for the integrity of the vascular system (of arterio-venous volume flow), oxygen consumption (marker of mitochondrial function) and amylase/lipase levels (indicating ischemic damage to the exocrine tissue) for HTK and UW-solutions [16]. However, even if in vitro studies allowed a reproducible standardised assessment of pancreas damage and remaining organ quality after cold ischemia, it becomes clear that only the transplantation of the organ in large animal species can repro-

duce the clinical setting, eventually correlating with the results obtained with the isolated perfusion system. The perfusion of porcine pancreata with HTK was very easy because the graft was retrieved with the celiac trunk, resulting in a wide arterial pedicle permitting a homogeneous perfusion of all the branches encompassed in the pancreatic segment [24]. This resulted in a slow electrolyte equilibration process, most noticable after 4 min for the sodium rather than for potassium, with appearance of interstitial graft edema. This process is already known from experimental HTK perfusion models with human kidneys [2]. In human kidneys, the difference in the sodium concentration between the perfusion solution and the venous effluence is more prominent during the first 2 min of perfusion. In a corresponding comparison of the potassium concentration between the perfusion solution and the venous effluence, an almost complete equilibration is attained after 1 min. Electrolytes equilibration is protracted because of increased perfusion resistance, as compared with canine kidneys, indicating the necessity of a perfusion during at least 10 min in clinical applications of HTK-solution, i.e. longer than in animal experiments. The porcine pancreas, however, because of its property as a low-flow organ, requires also a perfusion time of 10 min with HTK for electrolyte equilibration. This different equilibration time could be probably explained by organ specific differences regarding the blood flow of the pancreas as compared to the kidney (1% of the cardiac minute volume for the pancreas, versus 20% of the cardiac minute volumes for the kidney) [13]. In vitro studies [16] and our results showed that pancreas protection with HTK-solution result in increased post-perfusion weight, due to its lowest oncotic pressure. This phenomenon is due to a post-flushing edema with fluids without colloids, and it is well-described in vitro, using a perfusion pressure of 90–100 mm/Hg [15, 27]. In vivo, interstitial and intracellular edema were noticed immediately after flushing with a perfusion pressure of 40–50 mm/Hg, resulting in a uniform gain in wet weight in all the grafts after 24 h and 48 h CS. These results could be confirmed on histology. Increasing of interstitial edema and reperfusion pancreatitis appeared after reperfusion in all organs but, despite some areas of cellular swelling, no further parenchymal destruction was noticed, suggesting that this initial edema did not influence the function. It is well described that pancreatic function is lost only when cellular endocrine and/or exocrine swelling (vacuolization) appear [8], and we previously reported a high degree of intracellular edema and extended necrosis after 48 h and 72 h CS [11], resulting in graft failure. The importance of colloids in pancreas preservation has previously been discussed, remaining controversial [27, 28] since not only interstitial edema but also other factors such as cold storage and ischemia/reperfusion injury may be responsible for pancreatic graft damage [27, 12]. Tissue edema were seen in all functioning grafts and in both preservation solutions. Differences in water content were difficult to evaluate on histological examination so that the gain in wet weight for pancreases flushed with HTK was the only parameter which was clearly different for HTK and UW. Post-reperfusion pancreatitis, such as noticed by measuring serum amylase and lipase, was observed in both solutions as decreasing during the observation period. All the grafts transplanted after 48 h CS showed a positive trend of biochemical pancreatitis and abnormal glycemia values, suggesting increased preservation damage of the graft with delayed endocrine graft function (DEGF). The onset of postoperative biochemical pancreatitis was also recorded after 24 h CS and in the UW groups. However, serum amylase and lipase values were higher than those measured in vitro [15, 16]. In the clinical setting, the intraperitoneal absorption of secretions (according to the surgical model) but also the preservation time is responsible for this phenomenon. Abnormalities of the insuline secretion and the glucose homeostasis are known in this model (but also in humans) using systemic venous drainage as compared with the portal venous drainage [22, 9, 5, 17]. This has been attributed to several factors such as the suboptimal mass of transplanted β-cells [9], the effect of denervation [1], and the effect of the systemic diversion of the hormon bypassing the hepatic clearance [4]. However, this phenomenon may occur late and after

Fig. 5 **a** Histological section of a native pancreas (original magnification, × 10, ematoxylin and eosin staining). Figure shows normal lobular architecture and islet-cells without signs of inflammation. **b** Histological section of a graft preserved for 24 h with HTK-solution before implantation and reperfusion (original magnification, × 10, ematoxylin and eosin staining). Figure shows peripheral fat necrosis (*solid arrow*) and well-preserved lobular structure (*open arrow*). Presence of a central interstitial and intracellular edema (*black star*). **c** Histological section of a graft preserved for 24 h with HTK-solution before implantation and reperfusion (original magnification, × 10, ematoxylin and eosin staining). Figure shows well-preserved islet-cells (*solid arrow*) in the context of a normal lobular architecture (*black star*). **d** Histological section of a graft preserved for 24 h with HTK-solution after reperfusion (original magnification, × 10, ematoxylin and eosin staining). Figure shows peripheral necrosis (*black arrow*) with vital pancreatic tissue (*open arrow*) and edema (*black star*). **e** Histological section of a graft preserved for 24 h with HTK-solution after reperfusion (original magnification, × 20, ematoxylin and eosin staining). Figure shows vital islet-cells (*black arrow*), normal exocrine tissue (*open arrow*) and peripheral necrosis with intracellular edema (*black star*). **f** Histological section of a graft preserved for 24 h with HTK-solution 2 weeks after transplantation (original magnification, × 10, ematoxylin and eosin staining). Figure shows well-preserved pancreatic tissue (*black arrow*) with enhanced periductular and perilobular fibrosis (*star*). **g** Histological section of a graft preserved for 24 h with HTK-solution 2 weeks after transplantation (original magnification, × 20, ematoxylin and eosin staining). Figure shows well-preserved and irregularly shaped islets (*black arrow*)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several months, resulting in early exhaustion of islet cells and progressive graft fibrosis [22, 29]. In the clinical setting, DEGF has been correlated to recipient-related factors such as obesity, donor age, and cardiocerebrovascular causes of donor death, rather than to the preservation time [26]. Others reported no difference of preservation time (up 30 h CS) on survival rate, exocrine and endocrine graft function [30]. Therefore, there is a suggestion of decreased endocrine function for 24–30 h CS and an increased risk of arterial graft thrombosis for a preservation time longer than 30 h [18]. Our results, however, suggest that DEGF, in grafts perfused with HTK-solution and stored for 48 h is directly related to the preservation time, but this phenomenon is reversible, resulting in IVGTT's comparable to those of pancreata stored for 24 h at the 10th postoperative day. A decreased number of islet-cells seen on histological examination in G2 could not explain DEGF which was followed by normal graft function. The occurrence of severe graft pancreatitis could not be explained either by these findings. Long-term follow-up may be useful to characterise the sequelae of DEGF.

In conclusion, our results show that the porcine autotransplantation model is suitable for preservation studies of the pancreas. Successful transplantation can be safely achieved with up to 24 h cold storage with HTK-solution, and the results are absolutely comparable to those obtained using UW-solution. A longer cold ischemia time is possible but may led to endocrine dysfunction or severe pancreatitis with graft loss. Pancreatic edema that occurs after flushing with both solutions appear more pronounced with HTK-solution but do not influence the function itself. HTK-solution requires a flushing time of at least 10 min for the electrolyte equilibration to be acquired and for the optimal working of the buffer system. Further evaluation of HTK-solution in the clinical setting for the preservation of the pancreas is needed to evaluate its clinical potential.

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