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The effect of prolactin concentration on the efficacy of storing isolated porcine kidneys in a modified Biolasol solution

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ABSTRACT
Introduction: Biolasol is a solution developed in Poland for flushing the kidneys, liver, heart and pancreas by simple hypothermia method prior to transplantation. The solution supports the cellular integrity of grafts during ischemia-reperfusion injury (IRI). The aim of the study was to evaluate the impact of the concentration of prolactin added to Biolasol on selected biochemical parameters of kidney injury.
Materials and methods: Biolasol was modified by the addition of prolactin at 1 µg/L, 10 µg/L and 100 µg/L and by ascorbic acid at 0.5 mmol/L. After 2 h and 48 h of storage, the levels of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase enzymes, sodium and potassium concentrations, pH and osmolarity parameters were assessed in the perfusates.
Results: The addition of prolactin to Biolasol significantly improves the biochemical parameters of grafts in the models of rinsing, perfusion and reperfusion of isolated porcine kidneys.
Conclusions: The study indicates the nephroprotective role of Biolasol with the addition of vitamin C and prolactin at a 100 µg/L.
Keywords: Biolasol; kidney; perfusion; preservation; prolactin.

INTRODUCTION

Biolasol is intended for flushing the kidneys, liver, heart and pancreas by simple hypothermia method prior to transplantation [1, 2, 3]. Biolasol supports the structural and functional integrity of grafts and minimizes ischemia-reperfusion injury (IRI) [4, 5]. Histopathological examinations confirm the absence of damage in the structure of the renal cortex [5]. Clinical trials confirm that the efficacy of Biolasol is not smaller than that of HTK (Histidine-Tryptophan-Ketoglutarate, Custodiol) and Viaspan solutions [4, 5, 6].

Prolactin (PRL) is a hormone with a molecular weight of 23 kDa, made up of 198 amino acids. In mammals, it stimulates over 300 biological processes. Prolactin acts as a growth factor, regulator of the reproductive cycle, metabolism and homeostasis (osmoregulation), neurotransmitter and immune regulator. It stimulates receptors located on the cell membrane of the breast gland, as well as the prostate, uterine, gonadal, adrenal, liver and kidney cells [7, 8, 9]. In humans, 3 isoforms of the PRL receptor exist, comprising the extracellular part (responsible for PRL binding), transmembrane part (binding the receptor in the cell membrane) and cytoplasmic part (responsible for signal transduction). Signals are transmitted through Janus Kinase 2 (JAK2) tyrosine kinases and signal transducer and activator of transcription 5 (STAT5) proteins [7]. Prolactin, due to the presence of membrane receptors (PRL-R) located at various sites of the immune system, is involved in the regulation of the immune response [7, 10, 11]. Prolactin receptors are present in the proximal tubular epithelial cells and in the parietal epithelial cells lining the Bowman's capsule in the renal tubules [12, 13, 14]. Prolactin affects the homeostasis of salt and water in the kidneys and regulates the secretory activity of renal tubules [15]. The synergistic activity of PRL with exogenous antioxidants (e.g. ascorbic acid) has been confirmed [16].

The aim of the study was to evaluate the impact of the concentration of PRL added to Biolasol on selected biochemical parameters of kidney injury.

MATERIALS AND METHODS

Chemicals

Biolasol comprising: K^+ (10 mmol/L), Na^+ (105 mmol/L), Ca^{2+} (0.5 mmol/L), Mg^{2+} (5 mmol/L), Cl^- (10.5 mmol/L), dextran 70 kDa (0.7 g/L), NaHCO3 (5 mmol/L), citrate (30 mmol/L), glucose (167 mmol/L), EDTA (5 mmol/L) and fumarate (5 mmol/L). The osmotic pressure of the fluid is 330 mOsm/L, pH = 7.4. Biolasol was supplied by FZNP Biochefa (Sosnowiec, Poland), ascorbic acid was sourced from PLIVA (Kraków, Poland), PRL was supplied by FZNP Biochefa (Sosnowiec, Poland). All substances used in the study were of analytical grade.

Experimental groups and sample collection

Forty kidneys from Polish "Large White" breed adult pigs aged 175–180 days and weighing 90–110 kg were used for the study. The kidneys were collected in the Meat Plant H.A.M in Radzionków (permission from the II Local Ethics Commission for Animal Experiments in Kraków; No. 1046/2013). Kidneys were immersed for 2 h in 1 of 4 solutions: 500 mL of fluid: Biolasol (10 kidneys), Biolasol + 1 µg/L PRL + vitamin C (10 kidneys),

Biolasol + 10 µg/L PRL + vitamin C (10 kidneys), Biolasol + 100 µg/L PRL + vitamin C (10 kidneys), in isothermal containers at 4°C. Organs were then transported to the FZNP "Biochefa" laboratory. The period of warm ischemia was 30 min. The renal artery was cannulated using a catheter, Nelaton CH08, 40 cm (Conva Tec, Deeside, United Kingdom). The study was carried out according to the plan presented in Figure 1.

Biochemical analyses

The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), as well as sodium and potassium concentrations were determined by colorimetric methods with a diagnostic kit (BioMérieux, Lyon, France). The pH value was determined by potentiometric method using a pH meter (model CP-215 Elmetron, Poland) using a combined electrode, to a measurement accuracy of ±0.01 pH. Osmolarity was determined by measuring the freezing point using an osmometer 800 cL (Trident Med, Poland), to an accuracy of ±1 mOsm/kg H2O (±0.4%).

We used ascorbic acid at 0.5 mmol/L because we found that it increased the stability of Biolasol by 25% and affectd the maintenance of the normal cytoskeleton of the stored isolated kidneys in the animal model [16, 17].

The normality of distribution of the variables of the tested parameters was verified by Shapiro–Wilk test. We used one-way ANOVA and *post hoc* Tukey tests for multiple pairwise comparisons to test the impact of preservation solutions. Statistica v13.1 software (StatSoft, Poland) was used. Differences were considered significant at $p < 0.05$.

RESULTS

Based on the analyses performed, we found that the activity of ALT and AST enzymes (Tab. 1) at each stage of the study were within the physiological norms for adult pigs (ALT < 84 U/L, AST < 113 U/L) [18, 19]. During rinsing as well as perfusion and reperfusion of the kidneys with Biolasol, Biolasol + 1 µg/L PRL + vitamin C, Biolasol + 10 µg/L PRL + vitamin C, Biolasol + 100 µg/L PRL + vitamin C, there was no increased membrane permeability indicating the cells degradation. The integrity of mitochondrial and cytoplasmic membranes was preserved [20].

After 2 h of storage in the perfusates of Biolasol + ascorbic acid + PRL at various concentrations, ALT activity remained at the physiological level: 35.0 U/L (100 µg/L PRL) vs. 32.0 U/L (10 µg/L PRL) vs. 43.7 (1 µg/L PRL). After 48 h of storage, there was a decrease in ALT activity in the case of Biolasol + 100 µg/L PRL + vitamin C: 27.2 U/L (22.3%), an increase in ALT in the case of Biolasol + 10 µg/L PRL + vitamin C: 65.1 U/L (50.8%) and no change in the activity of AST in the case of Biolasol

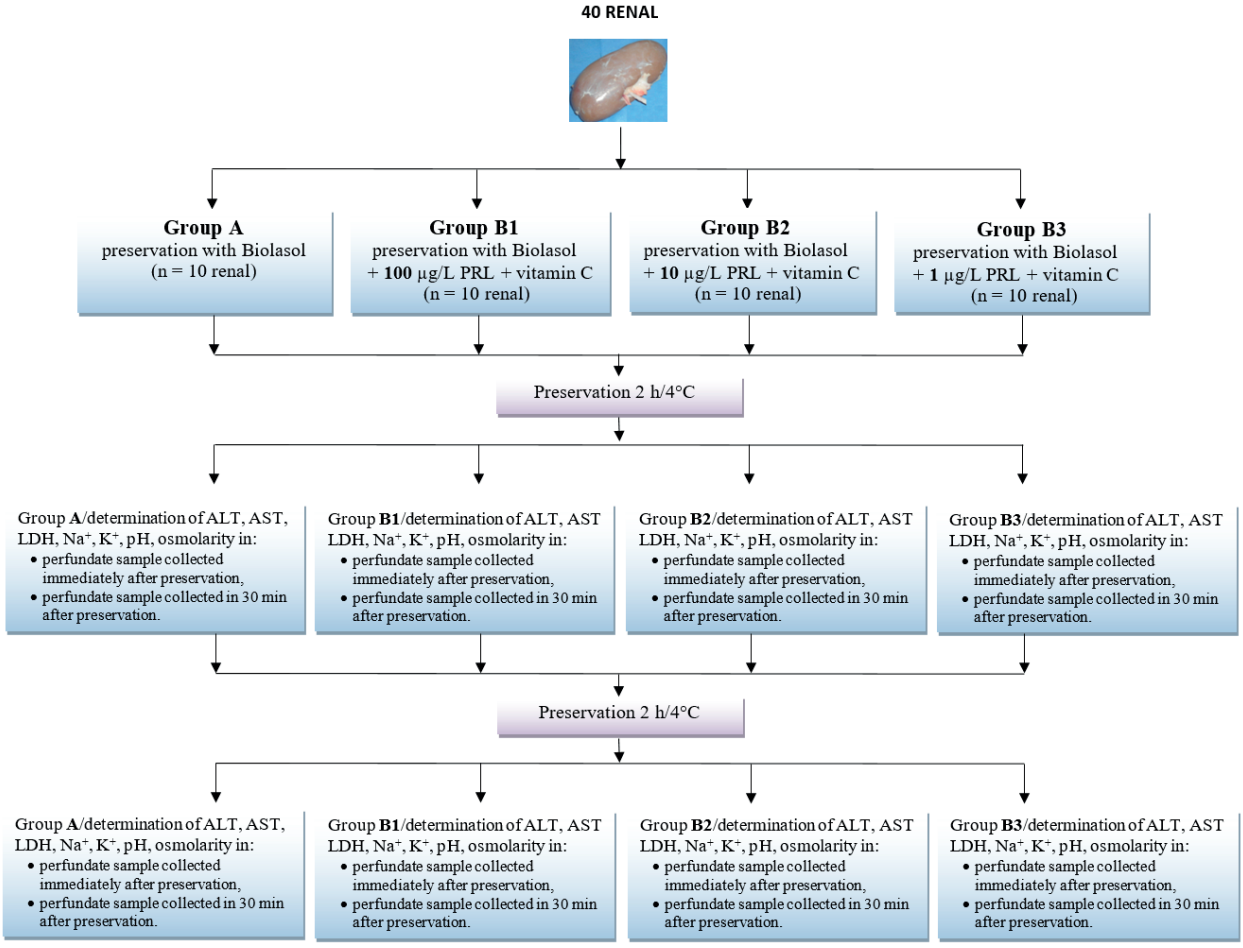

FIGURE 1. Study design

TABLE 1. Mean values ±standard deviation (SD) of the biochemical parameters of the efficacy of kidney storage

Time (min)	Group A Biolasol no PRL Control (renal n = 10)	Group B1 Biolasol + 100 µg/L PRL + vitamin C (renal n = 10)	Group B2 Biolasol + 10 µg/L PRL + vitamin C (renal n = 10)	Group B3 Biolasol + 1 µg/L PRL + vitamin C (renal n = 10)
		ALT (U/L)		
2 h preservation 0'	70.6 ±19.0	35.0 ±8.7[a]	32.0 ±10.1[a]	43.7 ±16.6[a]
2 h preservation 30'	58.8 ±15.5	26.0 ±2.3[a]	61.6 ±16.7[b]	24.3 ±9.1[ac]
48 h preservation 0'	68.6 ±16.9	27.2 ±7.1[a]	65.1 ±16.8[b]	43.2 ±11.2[ac]
48 h preservation 30'	60.1 ±17.9	19.9 ±4.3[a]	34.2 ±15.0[ab]	24.1 ±9.0[ac]
		AST (U/L)		
2 h preservation 0'	60.5 ±16.4	41.8 ±11.9[a]	25.8 ±10.6[ab]	103.4 ±34.6[abc]
2 h preservation 30'	32.6 ±8.9	29.9 ±4.7	36.6 ±10.5	63.9 ±16.8[abc]
48 h preservation 0'	60.3 ±11.1	37.7 ±11[a]	86.4 ±10.8[ab]	63.8 ±17.5[bc]
48 h preservation 30'	35.8 ±9.4	25.2 ±6.12[a]	47.2 ±12.5[ab]	36.0 ±12.3[bc]
		LDH (U/L)		
2 h preservation 0'	720.8 ±164.6	656.7 ±111.5	635.5 ±63.0	602.0 ±171.0[a]
2 h preservation 30'	168.1 ±41.4	208.7 ±122.8	720.5 ±22.8[ab]	305.7 ±161.0[ac]
48 h preservation 0'	416.0 ±59.9	506.9 ±127.0	599.1 ±279.4	473.0 ±95.4
48 h preservation 30'	216.5 ±135.5	380.5 ±57.0[a]	277.2 ±175.9	145.7 ±71.0[bc]
		Na+ (mEq/L)		
2 h preservation 0'	82.2 ±12.4	128.5 ±3.1[a]	77.8 ±23.6[b]	122.4 ±15.3[ac]
2 h preservation 30'	70.3 ±18.1	119.8 ±2.9[a]	65.0 ±20.4[b]	117.5 ±28.2[ac]
48 h preservation 0'	97.1 ±16.2	117.8 ±11.4	75.5 ±19.7[b]	91.3 ±15.1
48 h preservation 30'	99.2 ±13.4	118.8 ±10.1	79.8 ±20.7[b]	95.8 ±11.2
		K+ (mEq/L)		
2 h preservation 0'	14.9 ±2.7	12.9 ±0.6	13.1 ±1.5	22.6 ±7.2[abc]
2 h preservation 30'	13.5 ±3.2	10.5 ±1.1	10.9 ±1.8	17.7 ±3.7[bc]
48 h preservation 0'	16.0 ±1.2	14.2 ±2.8	11.05 ±1.1	17.7 ±4.2[c]
48 h preservation 30'	14.2 ±1.2	9.4 ±1.9[a]	10.2 ±0.7[a]	13.5 ±4.1[bc]
		pH		
2 h preservation 0'	7.1 ±0.0	7.8 ±0.1[a]	7.8 ±0.0[a]	7.0 ±0.0[bc]
2 h preservation 30'	7.3 ±0.0	8.0 ±0.1[a]	8.0 ±0.1[a]	7.3 ±0.1[bc]
48 h preservation 0'	6.8 ±0.1	7.5 ±0.2[a]	7.7 ±0.2[a]	7.0 ±0.2[bc]
48 h preservation 30'	7.1 ±0.1	7.6 ±0.2[a]	7.8 ±0.2[a]	7.2 ±0.0[bc]
		Osmolarity (mOsm/kg H_2O)		
2 h preservation 0'	338.3 ±47.0	341.5 ±5.2	350.8 ±4.3	337.6 ±25.8
2 h preservation 30'	324.3 ±29.6	321.3 ±3.8	314.8 ±13.8	318.0 ±38.1
48 h preservation 0'	300 ±27.7	315.5 ±5.2	321.5 ±4.1[a]	301.9 ±49.2[c]
48 h preservation 30'	293.0 ±37.0	314.3 ±5.1	313.0 ±4.2	264.8 ±40.4[abc]

[a] $p < 0.05$ vs. A; [b] $p < 0.05$ vs. B1; [c] $p < 0.05$ vs. B2; one-way ANOVA and Tukey's *post hoc* test analysis
ALT – alanine transaminase; AST – aspartate aminotransferase; LDH – lactate dehydrogenase; PRL – prolactin

+ 1 µg/L PRL + vitamin C. The results of ALT activity are significantly lower compared to its activity in pure Biolasol solutions (70.6 U/1–2 h, 68.6 U/1–48 h, $p < 0.05$).

The lowest AST activity (after 48 h of graft storage) vs. its activity in the Biolasol perfusates was found for the solution modified with PRL at 100 µg/L (60.3 U/L vs. 37.7 U/L, $p < 0.05$). In turn, the highest decrease in AST activity after 48 h storage was observed in perfusates of the solution modified with the addition of PRL at 1 µg/L: 63.8 U/L (38.3%). The activity of AST in the perfusates of Biolasol + 10 µg/L PRL + vitamin C increased by ~70% (25.8 U/1–2 h, 86.4 U/1–48 h).

The serum activity of LDH is 380–634 U/L [4]. An increase may correlate with the degree of renal impairment. Dysfunction of a small number of nephrons result in a measurable increase in LDH activity [21]. After 48 h of simple cold storage at 4°C, LDH activity in perfusates of all solutions oscillated within normal limits: 416.0 U/l (no PRL) vs. 506.9 U/L (100 µg/L PRL) vs. 599.1 (10 µg/L PRL) vs. 473.0 U/L (1 µg/L PRL) – Table 1.

Sodium is an element mainly associated with the extracellular space (the norm for adult pigs is 139–152 mEq/L) and has the largest impact on osmotic pressure, maintaining the volume of extracellular fluid within safe limits. It was found that Na+ levels were low at every stage of the study, which indicates excess water in relation to sodium ions, and this in turn points to graft swelling. The reduced concentration of Na+ ions, due to water shift from the cells, correlates with the increase in osmolarity of the solution in the perfusates (the norm is 275–295 mOsm/kg) [18, 19]. The concentration of Na+ ions marked in the perfusates decreased in the following order: Biolasol + 100 µg/L PRL + vitamin C (128.5 mEq/1–2 h, 117.8 mEq/1–48 h), Biolasol + 1 µg/L PRL + vitamin C (122.4 mEq/1–2 h, 91.3 mEq/1–48 h), Biolasol (82.2 mEq/1–2 h, 97.1 mEq/l–48 h), Biolasol + 10 µg/L PRL + vitamin C (77.8 mEq/l–2 h, 75.5 mEq/l–48 h). The sudden reduction in perfusate osmolarity associated with the rapid shift of water into the cell results in "acute oedema". The smallest change in perfusate osmolarity after 48 h storage was found for Biolasol + 100 µg/L PRL + vitamin C (decrease by 7.6%).

Potassium is the main intracellular cation. Two percent of K+ ions is found in the extracellular space (the norm for adult pigs is 4.9–7.0 mEq/L) [18, 19]. The shift of potassium into the extracellular fluid may be a consequence of a disturbed acid-base balance. The most optimal in this respect is the composition of Biolasol + 100 µg/L PRL + vitamin C. The concentration of K+ ions was 12.9 mEq/l–2 h vs. 14.2 mEq/l–48 h, whereas the corresponding pH results oscillated around physiological values: 7.8–2 h vs. 7.5–48 h, the norm is 7.4.

DISCUSSION

Ischemia-reperfusion injury of organs occurring between removal from a donor body and implantation into the recipient's body is a serious clinical problem. A lack of optimal protection of the graft at this time may result in a delayed uptake of vital signs, and in extreme cases, an absence. Hemodynamic, metabolic and hormonal disorders occur in the body. The hypothalamic-pituitary pathways are damaged and, as a consequence, the level of hormones decreases. This results in their limited involvement in cellular oxygen metabolism, which intensifies anaerobic transitions [22]. Therefore, modification of the solution with hormones can minimize the consequences of IRI of organs during their storage.

The modification of Biolasol with the addition of PRL improved the biochemical parameters of grafts in the model of rinsing, perfusion and reperfusion of porcine kidneys. This is confirmed by the results obtained by our team in the model of rinsing isolated rabbit and porcine livers. The addition of PRL to the HTK solution significantly reduced the leakage of ALT, AST, LDH and gamma-glutamyl transpeptidase (GGTP) from hepatocytes in the rabbit model [23] and the amount of released transaminases, LDH and Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} ions from hepatocytes in the porcine model [24]. Budziński et al. suggest that HTK-PRL affects the stabilization of the cell membrane, minimizing the number of dead hepatocytes [25]. In turn, the addition of human recombinant PRL (rhPRL) to Biolasol reduced the nephron damage markers (pH, osmolarity, K^{+}, Na^{+}, ALT, AST) determined in perfusates collected during reperfusion of isolated porcine kidneys [26]. Continuing the research on the effectiveness of Biolasol modified with the addition of PRL, it has been found that PRL at a concentration of 1 µg/mL influences the maintenance of a normal actin cytoskeleton of stored kidneys [16].

The modification of Biolasol with the addition of PRL at 1 µg/L, 10 µg/L and 100 µg/L, and ascorbic acid at 0.5 mmol/L contributes to the maintenance of normal activity of ALT, AST and LDH enzymes after 48 h of kidney storage. This confirms our hypothesis that PRL significantly affects the maintenance of the normal structure and kidney function under hypoxic conditions. We assume, similarly to Thébault [27], that PRL presents an antioxidant effect, reducing the reactive oxygen species (ROS) present in the cytosol. Research by Tallet et al. suggests that PRL does not react with ROS, therefore it enhances the action of endogenous [28] and exogenous antioxidants [16]. Moreover, Yamamoto et al. suggest that rhPRL has a cytoprotective effect on the islets of Langerhans, which may improve the survival of pancreatic grafts [29]. It may result from the participation of PRL in the anti-apoptotic mechanism and reduction of pro-apoptotic effectors, and consequently, protect cells against inflammation [30].

Prolactin at a concentration of 100 µg/L had the greatest influence on the sodium–potassium balance. It is believed that PRL regulates the transport of ions across the cell membrane [8]. Prolactin activates sodium transport in renal epithelial cells via epithelial sodium channel (ENaC) [31]. Ibarra et al. suggest that PRL may act as a natriuretic peptide that works by inhibiting the proximal convoluted tubule (PCT), Na+, K+ – ATPase and leads to increased urinary water and sodium excretion [32]. Some authors have stated that PRL induces an antidiuretic response [33]. Mountjoy et al. confirmed the presence of PRL receptors primarily in the proximal tubules, regulating sodium metabolism [13]. Roberts investigated the effect of PRL on renal function in a chicken model. Birds were given PRL in the form of a bolus at doses of 3 IU (150 µg) and 6 IU (300 µg). It was found that the fractional sodium excretion was higher in the group of chickens with the "lower dose" of PRL in comparison to the control group (without PRL). In the case of the "higher dose" of PRL, no changes in renal function were observed in relation to the control group [34]. Caban et al. modified the HTK solution with the addition of PRL at a dose of 0.2 mg/dL, 0.02 mg/dL, 0.01 mg/dL, followed by rinsing and perfusion of isolated porcine kidneys. Based on the biochemical parameters determined in perfusates (ALT, AST, LDH, lactates, total protein, Na^{+}, K^{+}), it was found that the supply of PRL at 0.02 mg/dL showed a cytoprotective effect of nephrons during cold ischaemia [35].

The pleiotropic effect of PRL indicates its great potential in the development of new therapeutic methods [36]. The obtained results suggest that the introduction of PRL to the composition of solutions intended for perfusion, preservation and reperfusion

of kidneys is justified and potentially increases the chance of the uptake of vital signs by grafts after transplantation.

CONCLUSIONS

The addition of PRL to Biolasol significantly improves the biochemical parameters of grafts in the model of rinsing, perfusion and reperfusion of isolated porcine kidneys. Modification of the solution with the addition of vitamin C and PRL at a concentration of 100 μg/L provides the best protection for nephrons.

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