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Comparative study between trimetazidine and ice slush hypothermia in protection against renal ischemia/reperfusion injury in a porcine model

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ABSTRACT

Purpose: The aim of the study was to compare the effects of renal ice slush hypothermia and the use of trimetazidine in the protection against ischemia/reperfusion (I/R) injury.
Materials and Methods: Fifteen farm pigs were submitted to left kidney ischemia and right nephrectomy during the same procedure. Animals were divided into three groups. Group 1 was submitted to warm ischemia; Group 2 was submitted to cold ischemia with ice slush; and Group 3 received trimetazidine 20 mg one day and 4 hours before surgery. Ischemia time was 120 minutes in all three groups. Serum creatinine (SCr) and plasma iohexol clearance (CLioh) were measured before surgery and on postoperative days (PODs) 1,3,7, and 14. Semi-quantitative analyses of histological alterations were performed by a pathologist. A p value of < 0.05 was considered significant.
Results: All groups showed elevation of serum creatinine in the first week. Serum creatinine was higher in Group 3 in the first and third postoperative days (Mean Cr: 5.5 and 8.1 respectively). Group 2 showed a lower increase in creatinine and a lower decrease in iohexol clearance than the others. Renal function stabilized in the fourteenth POD in all three groups. Analyses of histological alterations did not reach statistical significance between groups.
Conclusion: Trimetazidine did not show protection against renal I/R injury in comparison to warm ischemia or hypothermia in a porcine model submitted to 120 minutes of renal ischemia.

Key words: *kidney; ischemia; trimetazine; hypothermia; reperfusion injury*
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INTRODUCTION

Occlusion of the renal artery and vein may be necessary in many situations during urological procedures and is related to renal ischemia/reperfusion (I/R) injury. Such an injury is often related to acute and chronic renal dysfunction (1,2). Renal hypothermia is frequently used to overcome this problem. However, in some occasions hypothermia is not feasible, and renal protection must be accomplished using other mechanisms.

Several drugs have been studied in the protection of the kidney from I/R injury (3-13). The anti-anginal medication trimetazidine (TMZ) has been shown to protect the myocardium cells through inhibition of fatty acid oxidation and reciprocal activation of pyruvate oxidation, resulting in less production and accumulation of lactate and hydrogen cation, H+, during ischaemia (14). Experimental studies have also shown a protective effect in kidney I/R injury (15,16). Nevertheless, there has been no comparative study between renal hypothermia and trimetazidine in the protection of I/R injury.

MATERIALS AND METHODS

The study was performed with the approval of our Institutional Animal Care and Use Committee (CEEA/UNESP) on fifteen farm pigs weighing 12.2 to 21.4 kg.

Surgical procedures

All animals received preoperative intramuscular administration of 1.0 mg/kg xylazine (Divisão Vetbrands Saúde Animal - SP), 0.1 mg/kg acepromazine (Laboratórios Univet S.A. - SP) and 10 mg/kg ketamine (Divisão Vetbrands Saúde Animal - SP). A 22-gauge polyethylene catheter (Becton Dickinson Ind. Cirúrgicas Ltda - MG) was inserted into an ear vein and induction of anesthesia was achieved with 0.25 mg/kg diazepam (Hipolabor Farmacêutica Ltda - MG) and 5 mg/kg ketamine. After endotracheal intubation, isoflurane (Cristália Produtos Químicos Farmacêuticos Ltda - SP) and 100% oxygen were used for anesthesia maintenence. For additional analgesia during the procedure, intravenous 5 μg/kg fentanyl (Hipolabor Farmacêutica Ltda - MG) was used. The surgical procedures were performed under sterile conditions. Animals were positioned in left lateral decubitus and a right nephrectomy was achieved by lumbodorsal incision. After closing the incision, the animal was placed in right lateral decubitus and a left lumbodorsal incision was made to access the left kidney. One hundred twenty- minute ischemia was accomplished by hilar clamping with a Satinsky clamp. A probe placed 0.5 cm deep in the renal cortex constantly measured the renal temperature in a continuous fashion. The internal jugular vein was dissected for blood sampling and drug administration. In the group submitted to renal hypothermia, this was achieved by surrounding the left kidney with a rubber sheet on which sterile ice slush was placed to completely immerse the kidney.

Experimental groups

Fifteen farm pigs were randomized into three groups. Group 1 was submitted to 120 minutes of warm ischemia (WI) without any kind of renal protection. Group 2 was submitted to 120 min. of cold ischemia with ice slush. Group 3 received 20 mg oral trimetazidine, 24 hours and 4 hours before surgery, and was also submitted to 120 min of ischemia. Five animals in each group were followed for 14 days. Two animals in Group 1 and one in Group 3 died and were replaced to complete the follow-up.

Renal function assessment

Renal function was assessed using serial glomerular filtration rate measurements according to plasma iohexol clearance (CLioh) and serum creatinine (SCr) determination. SCr and CLioh were measured before surgery and on postoperative days (PODs) 1,3,7, and 14. Iohexol clearance was measured by one-compartment single sample clearance (17). High Performance Liquid Chromatography (HPLC) was used to measure iohexol plasma concentration as previously reported (18).

Histology

Animals were sacrificed on postoperative day 14 when renal samples were collected for microscopy. Two conventional stains were applied: hematoxylin - eosin (HE) and periodic acid Schiff (PAS) to evaluate proximal tubule brush border integrity. Seven basic morphological patterns (apical cytoplasm vacuolization, tubular necrosis, tubular dilatation, cell detachment, brush border integrity, intracellular edema, denuded basement membrane) typical of proximal tubular injury were graded in 5-point scales as follows: 1, no abnormality; 2, lesions affecting less than 25% of kidney samples; 3, lesions affecting 25-50% of kidney samples; 4, lesions affecting 50-75% of kidney samples, and 5, lesions affecting more than 75% of kidney samples. A pathologist blinded to the experimental conditions analyzed the histological alterations.

Statistical analysis

Statistical analyses were performed using the SPSS software, Version 15.0. For continuous data ANOVA and Kruskal-Wallis were used. Categorical

data were analyzed using the Fisher's exact test. A 0.05 p value was considered statistically significant.

RESULTS

The outcomes from Groups 1 and 3 differed markedly from the group submitted to cold ischemia. Two animals in Group 1 and one animal in Group 3 died because of acute renal failure confirmed by the increase in serum creatinine before the end of the study and were replaced. One animal in Group 3 presented with a retroperitoneal hematoma by the time of sacrifice, but no histological alterations on renal samples were observed.

Renal temperature was kept around 33-34ºC in Groups 1 and 3, and around 16ºC in Group 2. Body temperature remained constant (around 37ºC) in all three groups (Table-1).

All groups showed elevation of serum creatinine values in the first week after the procedure (Figure 1). Serum creatinine values had the lowest increase in Group 2 in the first and third postoperative days (Mean Cr: 2.2 and 1.2 respectively) compared to group 3 (p = 0.005). Comparisons between Groups 1 and 2 and between Groups 1 and 3 did not reach statistical significance in the first and third postoperative days. There was no significant difference between groups after seven days of surgery (Table-2).

Table 1 *- Comparison of weight, renal and body temperature between groups.*

	Warm Isquemia Group 1	**Hypothermia Group 2**	**Trimetazidine Group 3**	
Weight (Kg)	9.11 ± 6.80	8.1 ± 6.36	7.1 ± 1.43	0.845*
Renal temperature (ºC)	33.4 ± 2.26	16.13 ± 2.38	34.09 ± 1.18	< 0.001*
Body temperature (ºC)	37.03 (36.53 ; 39.00)	37.32 (36.93 ; 38.61)	37.65 (37.36 ; 38.09)	0.852(**)

(*) ANOVA independent samples (α = 0.05) GLRes = 12. Summary in mean & SD.
(**) Kruskal-Wallis (α = 0.05). Summary in median & quartile.

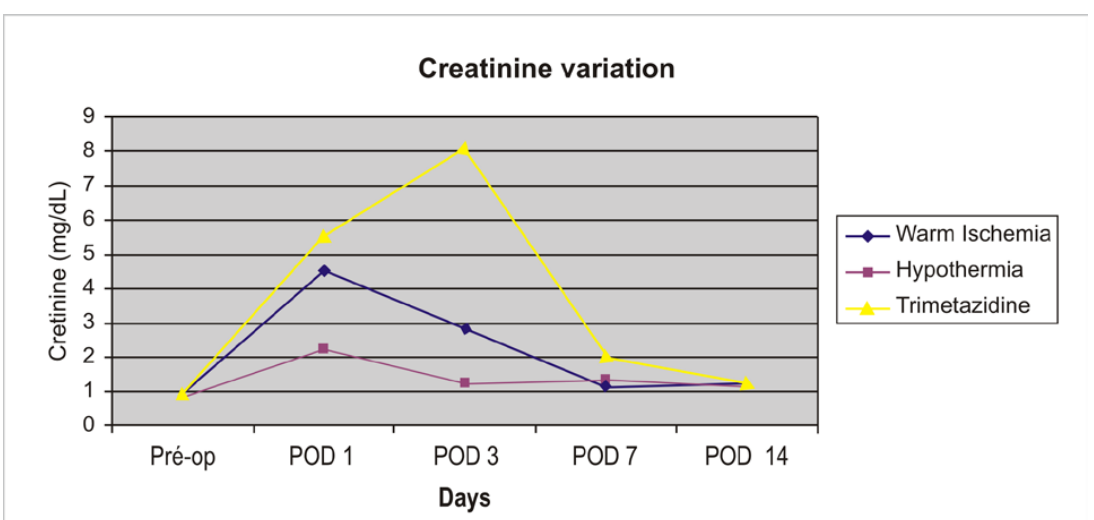

Figure 1. *Increase of creatinine values on POD1, POD3 and return to baseline values on POD14.*

Table 2 *- Creatinine values at baseline and PODs 1, 3, 7 and 14 in each group.*

Moment	Creatinine (mg/dl)			P
	Warm Ischemia Group 1	Hypothermia Group 2	Trimetazidine Group 3	
Pre-op	0.90 (0.75 ; 0.90)	0.80 (0.70 ; 0.85)	0.90 (0.70 ; 0.90)	0.438(**)
POD 1	4.50 (3.45 ; 4.75)	2.20 (2.00 ; 3.20)	5.50 (4.85 ; 6.25)	0.005(**)
POD 3	2.80 (1.75 ; 6.70)	1.20 (1.05 ; 1.35)	8.10 (3.90 ; 9.75)	0.005(**)
POD 7	1.10 (0.70 ; 2.80)	1.30 (1.15 ; 8.35)	2.00 (1.60 ; 9.35)	0.194(**)
POD 14	1.20 (0.95 ; 1.50)	1.10 (1.05 ; 1.40)	1.20 (1.00 ; 2.60)	0.931(**)

(**) Kruskal-Wallis (α = 0.05). Summary in median & quartile.

All groups showed decreased iohexol clearance in all moments assessed after the procedure (Figure 2). Group 2 (hypothermia) showed the lowest decrease in iohexol clearance in the first POD (Table-3) compared to the other two groups. Comparison between Groups 1 and 3 did not reach statistical difference. All groups showed more than 25% decline in iohexol clearance by the end of the follow-up.

Semi-quantitative analyses of histological alterations did not reach statistical difference between groups.

DISCUSSION

Interruption of renal blood flow is often necessary during surgical procedures such as partial nephrectomy, renal transplantation, and vascular surgery. However, vascular clamping is related to increased risk of postoperative complications such as urinary fistula, acute and chronic renal failure, and necessity of temporary dialysis (19). Duration of ischemia is the most important factor related to recovery of renal I/R injury. The historical safe

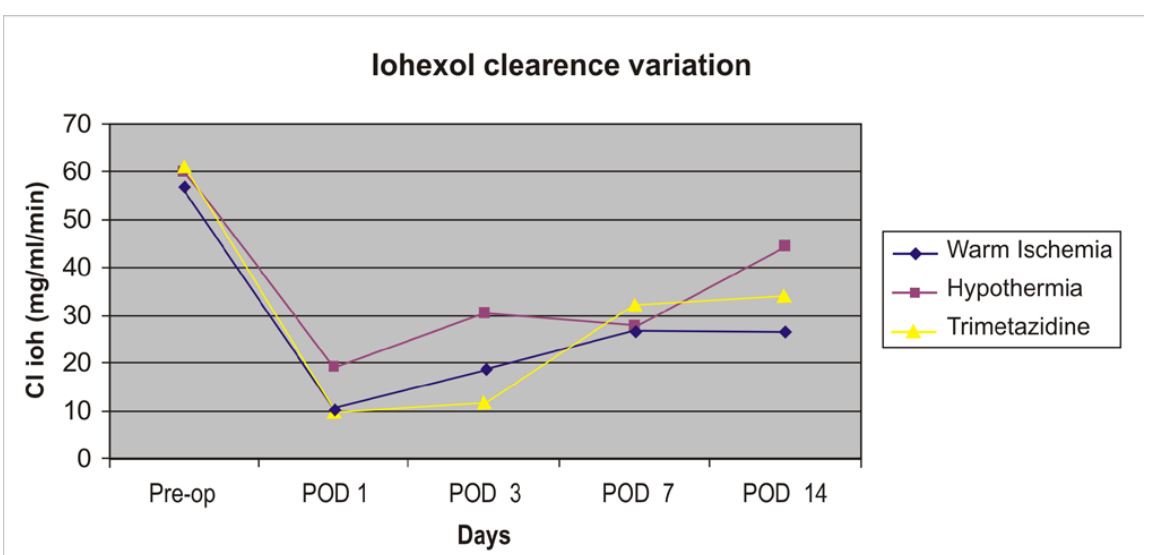

Figure 2. *Decrease of iohexol clearance on POD1 and POD2 with stabilization of the renal function on POD14.*

Table 3 *- Plasma iohexol clearance values at baseline and PODs 1, 3, 7 and 14 in each group.*

Moment	Plasma Iohexol Clearence (mg/ml/min)			p
	Warm Ischemia Group 1	Hypothermia Group 2	Trimetazidine Group 3	
Pre-op	56.6 (46.6 ; 59.1)	59.8 (46.3 ; 63.9)	60.9 (47.6 ; 67.9)	0.595(**)
POD 1	10.1 (7.8 ; 10.9)	19.2 (15.6 ; 29.6)	9.6 (9.3 ; 12.0)	0.009(**)
POD 3	18.8 (10.4 ; 25.4)	30.2 (20.9 ; 31.7)	11.8 (6.9 ; 24.6)	0.125(**)
POD 7	26.9 (12.4 ; 31.1)	27.7 (11.2 ; 36.5)	32.1 (15.1 ; 33.0)	0.827(**)
POD 14	26.6 (17.6 ; 39.0)	44.38 (32.5 ; 46.6)	34.0 (16.0 ; 43.4)	0.357(**)

(**) Kruskal-Wallis ($\alpha = 0.05$). Summary in median & quartile.

duration of warm ischemia time, where full recovery of renal function is expected, is thought to be 30 minutes (20). Based largely on animal models, most studies suggest that warm ischemia longer than 30 minutes results in significant immediate functional loss with either incomplete or absent late recovery (21,22). More recent observations have challenged the maximal safe duration of warm ischemia, suggesting that renal pedicle clamping for 90 minutes is safe in the porcine model (22,23). In our study we have used a 120 minutes ischemia time that is sufficient to cause 25% decline in glomerular filtration rate (24). In those cases where longer ischemia time may be required, adjuvant methods for renal protection are advisable.

One of the most used and effective methods of renal protection is hypothermia. Optimum temperature has been shown to be 15ºC in the canine model (21). Surface cooling of the kidney has been the most popular method of in situ hypothermia and has been accomplished by a variety of techniques such as surrounding the kidney with ice slush, immersing the kidney in a cold material, retrograde ureteral infusion of cold solution, or applying an external cooling device to the kidney (20,25,26). The most popular method has been to surround the mobilized kidney with a rubber sheet on which sterile ice slush is placed to completely immerse the kidney. Surface hypothermia in this manner is technically simple and very effective, and all of the requisite material is readily available in any operating room. Some disadvantages may be related to ice slush hypothermia. The core renal temperature falls slowly, generally taking 15 to 20 minutes to reach the desired level, and renal cooling is often non-homogeneous. Moreover, with the advent of minimally invasive surgery such as laparoscopic partial nephrectomy, hypothermia becomes more difficult to accomplish.

Another approach to in situ renal preservation that does not involve hypothermia is the use of a variety of pharmacologic agents to prevent renal injury. Agents that have been tested fall into four basic categories: diuretic agents, vaso-active drugs, membrane-stabilizing drugs, and agents that act to replenish intracellular levels of ATP. The anti-anginal medication trimetazidine has been shown to increase intracellular ATP levels.

Several papers have shown the protective effect of trimetazidine on kidney grafts from cold preservation and reperfusion (27,28) and against warm ischemia (12,15,16). Hauet et al. (27) evaluated the renal function of isolated perfused pig kidneys after

48 hours of cold storage with Euro-Collins (EC) solution plus trimetazidine (EC+TMZ), standard EC solution, or University of Wisconsin (UW) solution. The author studied the effect of TMZ during cold storage. The addition of TMZ to the EC solution improved the preservation quality and renal tubular function, and gave additional protection from reperfusion injury compared to EC or UW solutions alone. The same investigators studied the effect of TMZ on renal function and lipid peroxidation in an isolated perfused pig kidney (28). Renal function was significantly improved and lipid peroxidation reduced after preservation in Euro-Collins solution plus TMZ. Jayle et al. (12) evaluated the effect of TMZ pretreatment on the injury caused by warm ischemia for 45, 60, and 90 minutes, and reperfusion in a pig kidney model. TMZ pretreatment reduced deleterious effects after 45 minutes and particularly 60 and 90 minutes of WI However, the exact mechanism of action of TMZ on renal tubular cells is not clear.

In our study trimetazidine did not show protective effect on the kidney against I/R injury. When the serum creatinine values were analyzed there was no statistical difference between the warm ischemia (1) and trimetazidine (3) groups. However, creatinine was higher in Group 3 compared to Group 2. When the iohexol clearance was analyzed both the warm ischemia and trimetazidine groups had lower levels of clearance compared to the hypothermia group. However, the differences between the warm ischemia and the trimetazidine group were not so clear.

Some possible limitations of our study may explain these results such as an eventual lower plasmatic concentration achieved by the oral administration of trimetazidine; different metabolic pathways involved in nephron energy production and consumption; or species variability. Although histological alterations were not evident in the kidney of the animal in Group 3 that presented with retroperitoneal hematoma, this surgical complication may have influenced kidney function. However, when this animal was excluded from analyses, the hypothermia group still had a better outcome than the other two groups. Also, none of the previous papers used such a long warm ischemia time (120 minutes) as we did. This may have played a negative impact on our results.

Our results are in contrast to some previous publications that suggested a protective effect of trimetazidine on kidneys submitted to warm ischemia. Larger series with different warm ischemia times are warranted in order to definitely show the exact role of trimetazidine on renal protection to warm ischemia time.

CONCLUSIONS

Trimetazidine did not show protection against renal I/R injury in comparison to warm ischemia or hypothermia in a porcine model submitted to 120 minutes of ischemia.

ABBREVIATIONS

ANOVA - Analysis of variance
ATP - Adenosine triphosphate
CEEA - Comite de ética e experimentação animal
CLioh - Iohexol clearance
EC - Euro-Collins
HPLC - High Performance Liquid Chromatography
I/R - Ischemia/Reperfusion
PODs - Postoperative days
SCr - Serum creatinine
TMZ - trimetazidine
UNESP - Universidade Estadual Paulista
UW - University of Wisconsin solution
WI - warm ischemia

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CONFLICT OF INTEREST

None declared.

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