RESEARCH ARTICLE

The relevance between graft preservation solutions and QTc interval during living donor kidney transplantation and rat cardiomyocytes sampling

Dinc B[1], Aycan IO[1], Ozdemir S[2], Dandin O[3], Hadimioglu N[1], Mercan T[2], Yamasan BE[2], Kisaoglu A[3]

[1]Department of Anesthesiology and Reanimation
[2]Department of Biophysics
[3]Department of General Surgery
Akdeniz University Medical Faculty, Antalya, Turkey

Abstract

Background: The purpose of the retrospective study was to identify the impacts of different solutions on the electrocardiogram and cardiovascular changes. Moreover, the differences between these solutions were analyzed by examining their impacts on rat ventricular cardiomyocytes.

Methods: Eighty renal transplant patients were evaluated retrospectively. The patients were divided into two groups: Group UW (n =40) used the University of Wisconsin solution, and Group HTK (n =40) used the Histidine-Tryptophan-Ketoglutarate solution. Electrocardiograms of the subjects were obtained three times at different periods; during the pre-perfusion, intraoperative kidney reperfusion, and postperfusion phase at the end of the surgery. Any Electrocardiogram or cardiovascular alterations were noted and analyzed.

Adult male Wistar rats were used for *in vitro* experiments. Myocyte contractility, action potentials, and membrane current were recorded in enzymatically isolated ventricular myocytes.

Results: Sinus bradycardia was detected in 19 patients of Group UW, while there was short-term asystole in eight patients. However, no cardiac changes were observed in Group HTK patients. In both Groups, reperfusion and postperfusion corrected QT (QTc) intervals were different from pre-perfusion QTc intervals. Group UW patients' reperfusion and postperfusion QTc's values were higher than those of the Group HTK patients.

In rat myocytes, prominent asystole episodes were observed at specific concentrations of the UW solution compared to the HTK solution. The UW solution depolarized the resting membrane potential significantly and decreased the peak value of action potential, whereas the HTK solution did not elicit a significant change in those parameters. Accordingly, the UW solution elicited a significant inward current at -70 mV, while the HTK solution activated only a modest current, which may not change the membrane potential.

Conclusion: Prolongation of QTc intervals was detected with reperfusion in both groups according to electrocardiography analysis. However, the QTc interval was observed to be longer in cases using the UW solution and required intervention intraoperatively. HIPPOKRATIA 2021, 25 (1):22-30.

Keywords: Kidney transplantation, organ preservation/flushing solutions, QTc interval, electrocardiogram, cardiomyocytes sampling

Corresponding author: Assoc. Prof. Dr. Aycan Ilker Onguc, Akdeniz University Medical Faculty, Department of Anesthesiology and Reanimation, Pınarbaşı street, 07070 Konyaaltı/Antalya, Turkey, tel: +905056702335, fax: +902422278836, e-mail: ilkeraycan@gmail.com

Introduction

The incidence of cardiovascular problems due to prolonged QT interval is higher in patients with chronic renal failure[1]. Several factors, including acid-base and electrolyte variations, several proarrhythmic drugs, and hemodynamic alterations, affect the myocardium during the transplantation process[2,3]. The QT interval has been known to be prolonged, particularly in hemodialyzed patients, for a long time[4]. This QT dispersion is associated with the iron accumulated over time in cardiac muscles due to the intracellular electrolyte shift[5]. Hence, there may be remarkable changes during ventricular recovery periods, and this, in turn, may cause disturbances in ventricular repolarization and malign arrhythmias that may result in sudden cardiac death in some cases[6,7].

During kidney transplantation, particularly early postperfusion, the myocardium may be affected by hemodynamic changes, administered medications, and surgical procedures. In turn, this period may end up with cardiac arrhythmia. Furthermore, the disturbance may progress with the graft preservation solution (GPS), which enters the systemic circulation during the reperfusion phase, although the graft was flushed[8].

These GPSs used during transplantation in kidneys and many other organs such as the liver, pancreas, and small intestines can prevent ischemia and reperfusion injuries, thus maintaining the graft function at the early stages. The most commonly used GPSs are the University of Wisconsin solution (UWS) and the Histidine-Tryptophan-Ketoglutarate solution (HTKS).

Besides their positive effects, they may impact transplantation operations differently depending on their content. However, a limited number of studies demonstrate the effects of GPS on the myocardium, especially after the reperfusion phase. Therefore, this study aimed to identify the impact of different GPSs on the electrocardiogram (ECG) and cardiovascular changes during the re/post-perfusion phase by retrospectively analyzing kidney transplant patients' files. Moreover, to examine the likely cellular mechanism of differences between GPSs used during the transplantation process, an *in vitro* study was also conducted in isolated rat ventricular myocytes. This comparison will provide an opinion about the underlying causes of cardiovascular changes that may develop in the early post-reperfusion phase.

Material and methods

Retrospective Patient Screening

The study included one hundred 18-65 years old Caucasian patients who underwent living-donor kidney transplantations between 2016 and 2017 at the Akdeniz University Organ Transplantation Center. The donor's mean age (± standard deviation) was 41 ± 5 years. The study was approved by Akdeniz University Local Research Ethics Committee (decision No 70904504/90/305, date: 02/05/2018). We excluded patients from the study with a history of severe cardiovascular complaints, cardiac rhythm disorders, medications prolonging QT interval, diabetes, respiratory complaints, oncologic and hematologic diseases, psychoactive medication use, central nervous system disorders, and deceased kidney donor recipients (Figure 1). We recorded the patient's demographic data, preoperative dialysis type and duration, laboratory results, and intraoperative anesthesia observations with arterial blood gases (ABG). The benchtop arterial blood gas analyzer (RapidLab 1265; Siemens Healthcare Diagnostics Inc., Newark, DE, USA) was utilized for obtaining blood sample laboratory results. Routinely, ECGs were recorded during three separate periods, i.e., after induction (pre-perfusion period), intraoperative (right after kidney reperfusion), and at the end of the surgery (post-perfusion phase). We collected the patient characteristics, accompanying diseases, American Society of Anesthesiologists (ASA) classification, primary etiology of kidney failure, intraoperative; pre-anesthetic medications, anesthesia duration, graft warm/cold ischemia duration, type of organ flushing solutions, invasive arterial and central venous pressures, total intravenous volume of replacement therapy, and blood products therapies, administered immunosuppressive medications. Only preoperative and intraoperative data were included in the study.

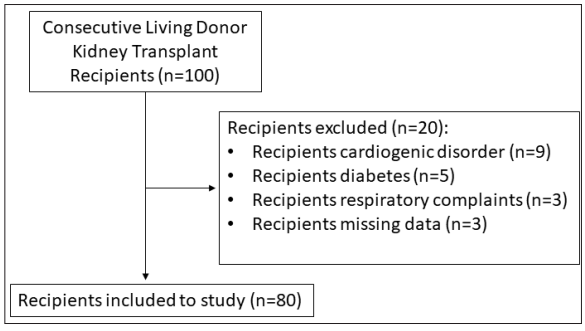

Figure 1: Diagram illustrating the flow of selection of the eighty renal transplant patients retrospectively evaluated in the presented study.

The grafts were perfused with fresh UWS or HTKS before reimplantation. The study consists of two groups according to GPS usage. Group UW consisted of patients on whom the UWS Servator B Salf® (S.A.L.F S.p.A., Cenate Sotto, Italy) was administered, while Group HTK consisted of patients on whom the HTKS (Bretschneider modificata® Galenica Senede, Siena, Italy) was administered (Table 1).

For premedication, weight-dependent intravenous midazolam (0.05-0.1 mg/kg^{-1}), was administered. Anesthesia was induced with fentanyl 1-2 μg/kg, pentothal 3-5 mg/kg, and rocuronium bromide 0.4-0.6 mg/kg. Anesthesia was maintained with desflurane 4-6 % (medical air 60 % in oxygen), intravenous remifentanil (0.2-0.6 μg/kg/min), and rocuronium bromide (0.1 mg/kg). A central venous catheter was inserted through the internal jugular vein in all patients, and blood pressure was monitored from the radial artery. As a standard procedure, ABG samples were recorded right after induction, on the fifth minute after reperfusion, and at the end of the operation. Fluid maintenance was provided by keeping the central venous pressure (CVP) value at 12-15 mmHg levels. Intravenous isolyte-S (20-30 mL/kg/h) was used for fluid replacement. A continuous insulin infusion was initiated if a high potassium level condition was encountered (higher than 5 mmol/L). On the other hand, sodium bicarbonate was used to treat severe acidosis (pH <7.10). Body temperature was maintained at 36 °C and the partial pressure of carbon dioxide ($PaCO_2$) level was 30-35 mmHg. Paracetamol and morphine (intravenous/intramuscular) were administered for postoperative pain control. Intravenous Catecholamine (μg/kg/min) usage was also recorded.

Besides, corrected QT (QTc) interval (in D2 derivation) were calculated based on Bazett's formula (corrected QT interval = QT/RR1/2) to evaluate ECG results recorded at three separate times; i) immediately after anesthesia induction (pre-perfusion), ii) right after reperfusion phase, and iii) at the end of the surgery (postperfusion)[9].

Animal Studies

Animal studies were performed on six (three-month-

Table 1: The contents of the University of Wisconsin solution (UWS) and Histidine-Tryptophan-Ketoglutarate solution (HTKS).

University of Wisconsin solution (SERVATOR B SALF®; S.A.L.F S.p.A., Cenate Sotto, Italy)		Histidine-Tryptophan-Ketoglutarate solution (Bretschneider modificata®; Galenica Senede, Siena, Italy)	
pH (20 ^{0}C)	7.1-7.5	pH (25 ^{0}C)	7.02-7.2
Osmolality	320 mOsm/kg	Osmolarity	320 mOsm/L
Poly (o-2-hydroxyethyl) startch	50 g/l	Sodium Chloride	15 mmol/l (0.8766 g)
Lactobionic acid	105 mmol/l	Potassium Chloride	9 mmol/l (0.6710 g)
Potassium hydroxide	100 mmol/l	Magnesium chloride hexahydrate	4 mmol/l
Sodium Hydroxide	27 mmol/l	Histidine hydrochloride monohydrate	18 mmol/l
Adenosine	5 mmol/l	Histidine	180 mmol/l
Allopurinol	1 mmol/l	Tryptophan	2 mmol/l
Potassium dihydrogen phosphate	25 mmol/l	Mannitol	30 mmol/l
Magnesium sulphate heptahydrate	5 mmol/l	Calcium chloride bihydrate	0.015 mmol/l
Raffinose pentahydrate	30 mmol/l	Potassium hydrogen 2-ketoglutarate	1 mmol/l (0.1842 g)
Glutathione	3 mmol/l		
Sodium hydroxide or Hydrochloric acid for pH adjustment		Potassium hydroxide for pH adjustment	
Water	1000 ml	Water	1000 ml
Total Na ions	29 mEq/l		
Total K ions	125 mEq/l		

old) male Wistar rats. All procedures were approved by Akdeniz University Animal Experiments Local Ethics Committee (No 771/2018.10.14/24, date: 10/10/2018). Ventricular myocytes were isolated by enzymatic digestion of the myocardium, as described previously[10,11]. Briefly, rats were anesthetized with intraperitoneal administration of pentobarbital sodium (50 mg/kg). Subsequently, their hearts were excised quickly and cannulated through the aorta to the Langendorff apparatus, where they were retrogradely perfused with Ca^{2+}-free solution containing 137 mM NaCl, 5.4 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 6 mM HEPES, and 20 mM glucose (pH adjusted to 7.2). Then, an enzymatic solution containing 0.8 mg/mL collagenase (Collagenase A) and 0.07 mg/mL protease (type XIV) was passed through for approximately 20 minutes and the minced ventricles were filtered through the mesh. Following Ca^{2+} adaptation of cell suspension, data was recorded at 36 ± 1 °C. Ventricular myocytes were perfused either with the UWS or HTKS.

Electrophysiological experiments

In an experimental setting, we assessed the effects of these two GPS on membrane potential and ionic currents of ventricular myocytes to investigate the mechanism of asystole or diastolic arrest during electrical stimulation.

Myocyte Contractility

Isolated cells were continuously perfused with Tyrode solution containing 137 mM NaCI, 5.4 mM KCI, 0.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 11.8 mM HEPES, 10 mM glucose (pH: 7.35), and then stimulated (5–10 V) with a platinum electrode at 1 Hz frequency. The changes in sarcomere length in response to electrical stimulus represent myocyte shortening and contractility[12]. Sarcomere shortening traces of ventricular myocytes with stable responses were recorded with a video-based myocyte contractility system of Ion Optix (IonOptix LLC, Milton USA) for at least two minutes and then washed in either with UWS (SERVATOR B SALF) or HTKS (Bretschneider modificata).

Action Potential and Membrane Current Recordings

Action potential (AP) traces were obtained using the patch-clamp amplifier's current-clamp mode (Axon 200B, Molecular Devices, USA). A solution containing 120 mM potassium aspartate, 20 mM KCl, 10 mM NaCl, 10 mM K-HEPES, 5 mM MgATP (pH =7.2) was used to fill the patch pipettes. APs were evoked with 1 Hz frequency, and we used the Clampfit 10.2 software (Molecular Devices, USA) to analyze the recordings.

For current recordings, a whole-cell configuration of the patch-clamp amplifier was used, and the holding potential was set to -70 mV. The continuous gap-free protocol was initiated at this potential, and then either UWS or HTKS were applied by a fast perfusion system (Harvard Apparatus MA, USA). However, ramp protocols were used for the recording of total K+ currents. The descending ramp from +80 to -120 mV with a two-second duration was applied while the holding potential was -40 mV. This protocol allows us to assess the values of both transient outward (I_{to}) and inward rectifier (I_{K1}) currents[12,13].

Statistics

Analyses were conducted through the IBM SPSS Statistics for Windows, Version 23.0. (IBM Corp., Armonk, NY, USA). The normal distribution hypothesis was analyzed through the Shapiro-Wilk test, Q-Q plot graphics, skewness, and kurtosis coefficients. The independent t-

test was performed to compare the patient characteristics, ABG analysis with laboratory results. The numeric comparison of recorded heart rate and inotropic interventions conducted by Fisher's exact test in case of a small cell rate with an expected value of less than five was above 20 %. Also, the Pearson chi-square test was conducted in other cases. Relations between digital data were determined through the parametric Pearson or non-parametric Spearman test.

Additionally, repeated Measures ANOVA was used to assess the differences between pre-perfusion, reperfusion, and postperfusion QTc values groups in terms of groups. Paired comparisons were followed by the Bonferroni post hoc test. Paired t-test was performed to compare the results of in vitro electrophysiological data. A p-value of less than 0.05 was considered statistically significant.

Results

Retrospective patient screening

The files of 100 living donor kidney transplant recipients were browsed for the study. Twenty patients were excluded due to the specified exclusion criteria. Forty patients were included in Group UW, and 40 patients were included in Group HTK. Patient preoperative dialysis type and durations, characteristics, warm and cold ischemia duration, and solution amounts were analyzed, and no statistically significant difference was detected among the groups (Table 2). According to the comparison of ABG and other laboratory analyses between the two groups, there was no clinically significant difference detected at designated times (Table 3). On the other hand, the reperfusion and postperfusion potassium concentrations in both groups were significantly increased con-

Table 2: Preoperative dialysis type and duration, patient characteristics and perfusion data of the eighty renal transplant patients divided into two groups: Group UW used the University of Wisconsin solution, and Group HTK used Histidine-Tryptophan-Ketoglutarate solution.

		Group UW (n =40)	**Group HTK** (n =40)
Preemptive		17	16
Hemodialyses		20	20
Peritoneal dialysis		3	4
Dialysis duration (month)		57.24 ± 64.46	40.42 ± 57.20
Patient Characteristics	Sex (Male/Female)	24/16	28/12
	Age (year)	43.85 ± 13.50	39.15 ± 2.29
	BMI (kg/m^2)	23.86 ± 4.42	25.04 ± 4.90
	WI	5.45 ± 3	4.11 ± 2
Perfusion	CI	74.50 ± 31.03	66.51 ± 16.50
	GPS (mL)	269.36 ± 81.90	283.92 ± 75.70

Data are presented as mean ± standard deviation. BMI: body mass index, WI: Warm ischemia = the period before organ retrieval in the living donor, CI: Cold ischemia = the period wherein the graft is transported from donor to recipient under hypothermic regulation, GPS: graft preservation solution.

Table 3: Pre-perfusion, reperfusion and postperfusion arterial blood gas and other laboratory analysis between group UW (used the University of Wisconsin solution) and group HTK (used Histidine-Tryptophan-Ketoglutarate solution).

Parameters	**Preperfusion**		**Reperfusion**		**Postperfusion**	
	Group UW (n =40)	Group HTK (n =40)	Group UW (n =40)	Group HTK (n =40)	Group UW (n =40)	Group HTK (n =40)
pH	7.44 ± 0.10	7.41 ± 0.10	7.39 ± 0.10	7.38 ± 0.10	7.37 ± 0.06	7.36 ± 0.08
PCO_2 mmHg	29.75 ± 5.22	30.11 ± 4.24	32.56 ± 4.20	31.65 ± 4.20	34.15 ± 3.43	32.61 ± 3.62
PO_2 mmHg	245.90 ± 105.3	248.05 ± 104.3	206.61 ± 69.31	195.16 ± 58.59	192.25 ± 56.79	215.69 ± 72.03
HCO_3^- mmol/L	19.56 ± 3.85	19.19 ± 4.30	19.89 ± 2.95	18.64 ± 3.22	19.60 ± 2.95	18.77 ± 3.30
BE (B) mmol/L	-3.49 ± 4.30	-4.60 ± 4.90	-4.28 ± 3.59	-5.67 ± 4.01	-4.91 ± 3.44	-5.75 ± 3.90
HCT %	28.40 ± 5.12	28.42 ± 5.20	27.37 ± 5.28	27.42 ± 4.43	29.03 ± 5.12	27.84 ± 4.80
HB g/dL	9.70 ± 1.73	9.70 ± 1.74	9.31 ± 1.81	9.31 ± 1.53	9.92 ± 1.71	9.49 ± 1.62
O_2 SAT %	99.31 ± 0.10	99.33 ± 0.60	99.09 ± 0.91	99.09 ± 0.64	99.02 ± 0.67	99.23 ± 0.58
Na^+ mmol/L	136.14 ± 2.84	135.59 ± 3.70	134.29 ± 3.31	133.26 ± 3.02	133.09 ± 2.97	133.10 ± 2.41
K^+ mmol/L	4.15 ± 0.83*	3.89 ± 0.84¶	4.52 ± 0.89	4.20 ± 1.03	4.61 ± 0.82	4.03 ± 0.84
Cl^- mmol/L	106.41 ± 4.94	107.10 ± 5.96	105.51 ± 5.60	106.02 ± 4.95	104.34 ± 4.94	105.23 ± 4.53
Ca^{++} mmol/L	0.80 ± 0.20	0.90 ± 0.15	0.77 ± 0.15	0.89 ± 0.14	0.81 ± 0.13	0.87 ± 0.14
Glucose mg/dL	93 ± 18.04	90.40 ± 25	106.10 ± 23.79	104.39 ± 30.03	119.81 ± 30.62	116.40 ± 32.34
Lactate mmol/L	0.81 ± 0.30	0.80 ± 0.32	1.03 ± 0.35	0.97 ± 0.31	1.29 ± 0.50	1.16 ± 60

Data are presented as mean ± standard deviation. BE: base excess, HCT: hematocrit, HB: haemoglobin. *: p <0.01 vs. Group UW reperfusion and Group UW postperfusion. ¶: p <0.01 vs. Group HTK reperfusion and Group HTK postperfusion.

cerning pre-perfusion concentrations (p <0.01). Potassium levels were below 4.62 mmol/l in all measurements.

The basal and perfusion heart rate of both groups was significantly slower after reperfusion. On the other hand, cardiac problems were observed with reperfusion in Group UW. Those were sinus bradycardia (n =19), (p <0.01) and short-term (≥ 3 s pause) asystole (n =8), (p <0.01). While the intervention was not required for some cases, intravenous Atropine was administered to seven patients. When this was observed not to be sufficient, catecholamine infusion therapy was initiated (p <0.01). On the other hand, no cardiac problems were encountered in Group HTK (Table 4).

In the comparison made in different time intervals within the Group UW; the difference between pre-perfusion QTc (467.08 ± 59.95 ms) and reperfusion QTc (523.04 ± 47.93 ms) was -55.96 ms (p <0.01), while the difference between pre-perfusion QTc and postperfusion QTc (526.88 ± 48.27 ms) was -59.80ms (p <0.01). However, the difference between reperfusion QTc and postperfusion QTc was -3.84 ms.

Likewise, in the comparison made in different time intervals within the Group HTK, the difference between pre-perfusion QTc (467.42 ± 37.63 ms) and reperfusion QTc (488.29 ± 42.98 ms) was -20.88 ms (p =0.019). Additionally, the difference between pre-perfusion and postperfusion QTc (489.83 ± 53.38 ms) was -22.42 ms (p =0.026). On the other hand, the difference between reperfusion and postperfusion QTc was -1.54 ms.

The QTc comparison between groups revealed that Group UW's reperfusion (p =0.01) and postperfusion (p =0.014) QTc intervals were longer than Group HTK in the same periods (Table 5), while no significant QTc differences were observed in the comparison of pre-perfusion periods.

Table 4: Bradycardia, asystole and inotrope infusion frequencies between group UW (used the University of Wisconsin solution) and group HTK (used Histidine-Tryptophan-Ketoglutarate solution).

	Group UW (n =40)		Group HTK (n =40)	
	n	percent	n	percent
Bradycardia	19	48.7 %*	0	0.0 %
Asystole	8	20.5 %*	0	0.0 %
Inotropic Treatment	7	17.9 %*	0	0.0 %

*: p <0.01 vs. Group HTK (used Histidine-Tryptophan-Ketoglutarate solution).

Table 5: Differences in corrected QT (QTc) interval values at pre-perfusion, reperfusion, and postperfusion times of both groups (group UW used the University of Wisconsin solution; group HTK used Histidine-Tryptophan-Ketoglutarate solution).

	Group UW	Group HTK
Preperfusion QTc (ms)	467.08 ± 59.95**	467.42 ± 37.63*
Reperfusion QTc (ms)	523.04 ± 47.9[3] Ɨ	488.29 ± 42.98
Postperfusion QTc (ms)	526.88 ± 48.2[7] Ɨǂ	489.83 ± 53.38

Data are presented as mean ± standard deviation. QTc: corrected QT interval. *: p <0.05 vs. Group HTK reperfusion QTc and Group HTK postperfusion QTc, **: p <0.01 vs. Group UW reperfusion QTc and Group UW postperfusion QTc, Ɨ: p <0.05 vs. Group HTK at reperfusion QTc, ǂ: p <0.05 vs. Group HTK at postperfusion QTc.

In vitro electrophysiological data

The change in sarcomere length of isolated ventricular myocytes in response to different concentrations of UWS and HTKS was examined to understand the potential effect of GPS on the contractile function of the myocardium. Neither the amplitude of shortening nor contraction/relaxation rates changed during perfusion of ventricular myocytes with 1 % v/v concentration of either UWS or HTKS (Figure 2A). However, 5 % v/v concentration of UWS resulted in apparent pause (asystole) episodes, despite persisting electrical pulses terminated with the washout of myocytes with the Tyrode solution (Figure 2B). There was an apparent increase in myocyte's contractile response after washout of UWS, which can be attributed simply to Ca^{2+} loading during GPS. Interestingly, perfusion with an equal amount of HTKS did not cause any change in contractile activity of ventricular myocytes. Increasing the stimulus amplitude (from 6 V to 8 V) during UWS perfusion reversed the ventricular myocyte's contractile activity, although it was a bit irregular (Figure 2C). However, the washout of the UWS completely restored myocyte's contractility immediately after switching to Tyrode solution.

As it is shown in Figure 3, 5 % v/v concentration of UWS caused a fast and significant depolarization in the resting membrane potential along with a decrease in the peak of AP while the same amount of HTKS achieved only a modest but insignificant change both in resting membrane potential and peak value of AP which were all reversible after washout (Figure 3A and Figure 3B).

Then the impact of GPS was also examined using continuous recording protocol, and ionic currents of the membrane in response to either solution were determined. Consistent with the membrane potential recordings, UWS elicited a significant inward current at -70 mV, which is the likely underlying mechanism of GPS-induced membrane depolarization, most probably due to the elevating concentrations of extracellular K^+ (Figure 4A). However, HTKS activated only a modest current, which may not change the membrane potential remarkably. The stimulated currents were inactivated after the washout of GPS.

Furthermore, to verify the contribution of K^+ currents to the measured inward currents, we applied a descending ramp protocol to the ventricular myocytes. As suggested in the previous reports, the outward current recorded under these conditions is defined as I_{to}, while the inward component is defined as I_{K1} [13,14]. As shown in Figure 4B, UWS elicited a steep increase in I_{K1}, while no remarkable

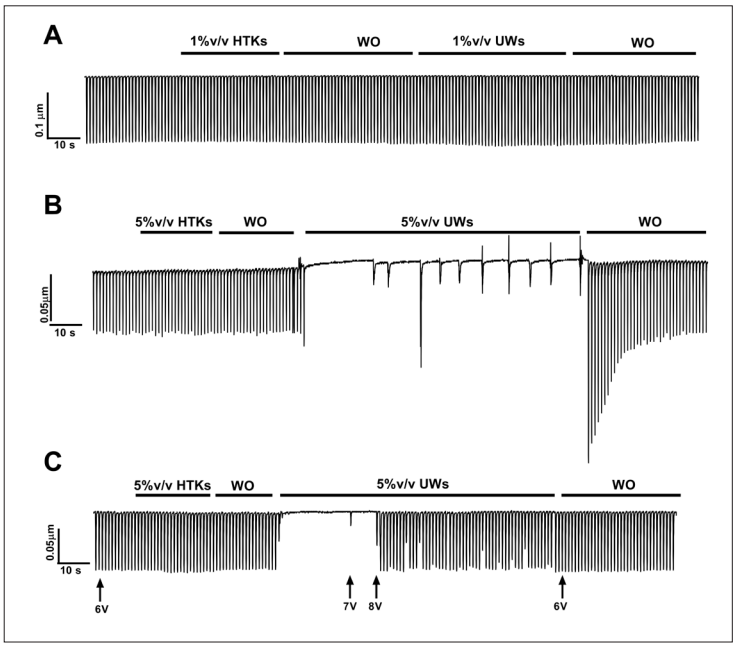

Figure 2: The effect of UWS and HTKS on the contractile activity of ventricular myocytes during electrical field stimulation with 1 Hz pulses. A) Fractional shortening of ventricular myocytes did not change during perfusion with 1 % v/v concentration of either University of Wisconsin solution (UWS) or Histidine-Tryptophan-Ketoglutarate solution (HTKS). B) 5 % v/v concentration of UWS resulted in apparent pause (asystole) episode, whereas the same amount of HTKS did not elicit any change in contractile activity of myocytes. (C) The contractile activity of myocytes stimulated by 6 V pulses stopped upon perfusion with UWS, and it was restored only by increasing the amplitude of electrical stimulus to 8 V while the responses were irregular. However, washout of the UWS fully reversed the contractility of ventricular myocytes even at 6 V stimulus amplitude (n =24 myocytes/2 rats). WO: washout.

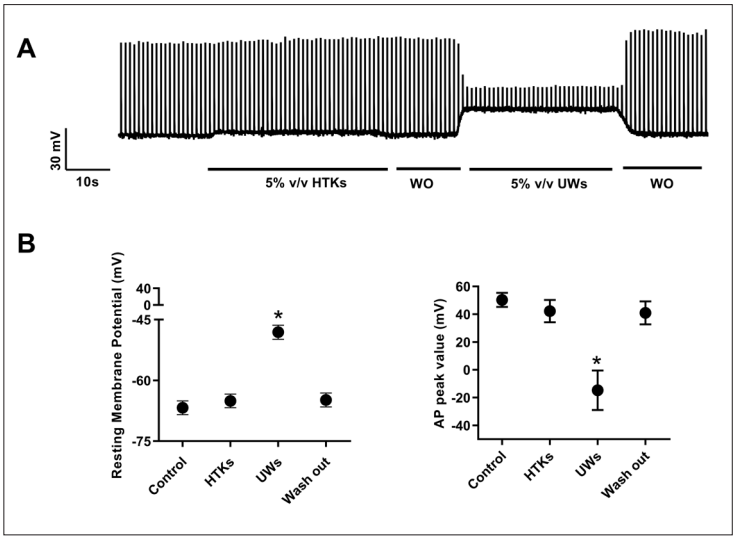

Figure 3: Effects of University of Wisconsin solution (UWS) and Histidine-Tryptophan-Ketoglutarate solution (HTKS) on action potential and resting membrane potential of rat ventricular myocytes. A) and B) 5 % v/v concentration of UWS depolarized the membrane potential significantly and decreased the peak value of action potential (AP) reversibly. Whereas neither the resting membrane potential nor the peak value of AP change during perfusion of ventricular myocytes with HTKS.
Data are presented as mean ± standard deviation. WO: washout, UWS: University of Wisconsin solution, HTKS: Histidine-Tryptophan-Ketoglutarate solution. *: p <0.01. Control versus UWS or UWS (n =11 myocytes/2 rats).

change was observed in the outward component (n =3). Conversely, neither outward nor inward K^+ currents significantly changed during HTKS perfusion.

Discussion

UWS has become the reference standard solution among GPSs. The use of HTKS, on the other hand, as an equivalent alternative to UWS, is a matter of preference[15]. It was presented in the literature that GPSs have beneficial effects in preventing or decreasing ischemia-reperfusion injuries of the transplanted graft [16,17]. As it is well known, the contents of GPS, particularly electrolyte concentrations, were remarkably different. To understand this difference's likely mechanism in the clinic course, we retrospectively scanned patient files, conducted an experimental study, and determined rat ventricular myocyte's response to these two solutions. We believe the research contributes to the literature to demonstrate the change in QTc with the reperfusion phase.

The clinical part of the study revealed that, although the heart rate slowed down with reperfusion in both GPS groups, short-term asystole, which may require intervention, was encountered in the Group UW. Similarly, although the QTc intervals were prolonged with reperfusion in both groups, it was noted that the QTc intervals were longer in Group UW than Group HTK. Arrhythmia, prolonged QT, or QTc interval dispersion are more frequently encountered in patients with chronic renal failure who received frequent hemodialysis treatments[18,19]. In addition to this clinical picture, the impact of GPS used in transplantation on the myocardium is an issue that needs to be investigated. Our study statistically revealed that both GPSs have different effects on QTc. The comparison of ABG and electrolyte analysis of both groups resulted in no significant difference. However, the potassium concentration increased with reperfusion in both groups. Ac-

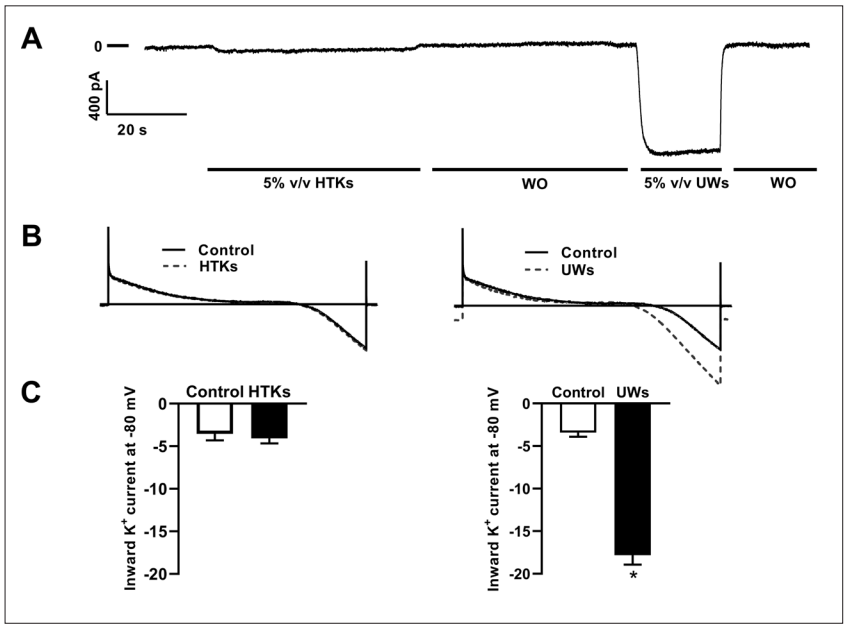

Figure 4: Effects of graft preservation solution on membrane currents. A) Consistent with action potential (AP) recordings, the University of Wisconsin solution (UWS) elicited a significant inward current at -70 mV holding potential, which was inactivated after washout. However, Histidine-Tryptophan-Ketoglutarate solution (HTKS) activated only a modest current which may not change the membrane potential remarkably. B) K^+ currents were recorded during voltage ramp protocol from +80 to −120 mV with 2 s duration. Total K^+ currents in the absence and presence of either UWS or HTKS were plotted against the voltage. C) Graphical presentation of inward K^+ current values measured at -80 mV.

Data are presented as mean ± standard deviation. WO: washout, UWS: University of Wisconsin solution, HTKS: Histidine-Tryptophan-Ketoglutarate solution. *: p <0.05. Control versus HTKS or UWS (n =3 myocytes/2 rats).

cordingly, perfusion of cardiomyocytes with the K^+-rich (total concentration of K^+ 21.4 mM) Tyrode solution has been shown to induce significant membrane depolarization[20]. Besides, Maruyama et al[21] have demonstrated that roughly 10 mmol/L elevations of extracellular K^+ concentration can lead to a 20 mV shift in the resting membrane potential of myocytes, which is capable of inactivating voltage-dependent sodium channels and thereby blocks conduction of the myocardial AP, and thus inducing "depolarized arrest". Consistent with these findings, we measured a depolarized resting membrane potential and defective AP, which were resulted in a contractile arrest. These effects were more pronounced in UWS than HTKS, most likely due to its exceedingly higher K^+ content. Our membrane current recordings have clearly presented that large inward currents induced by UWS are the underlying mechanism of this highly significant depolarization in membrane potential. The inward current might increase because of high extracellular K^+ in UWS, identified by a ramp protocol. Hyperkalemia has also been suggested to induce cytosolic Ca^{2+} overload; and, thus, contributes to ventricular dysfunction, which is a well-known adverse effect of GPS. This is because once the resting membrane potential depolarizes to the threshold level of the L-type Ca^{2+} channel, its activation will lead to Ca^{2+} influx into the myocyte, eventually resulting in cytosolic Ca^{2+} loading[20]. Similarly, the reason for incomparable contractile amplitude that we observed by wash out of UWS following pause episode (Figure 2B) was probably the rising of cytosolic Ca^{2+} during myocyte's perfusion with hyperkalemic UWS. Hereby, considering the findings of rat myocytes, the underlying cause of QTc prolongation with reperfusion may associate with this mechanism. QT interval shows the duration of repolarization and depolarization times of the ventricular myocardium shortens with increasing heart rate and lengthens with decreasing heart rate. The likely reason for this change is the modification of ions in the myocardial cells[22, 23]. On the other hand, Monfared and Ghods[24] concluded that there was no correlation between the maximum QTc and potassium concentration, even though kidney allograft receivers had a significant relation with serum Mg^{2+}, Ca^{2+}, bicarbonate, and pH. However, there was no significant difference between the two groups in terms of electrolyte (Na^+, K^+, Ca^{2+}, Cl^-) and bicarbonate levels in ABG's taken at different stages in the presented study.

Besides, Zukowski et al[25] conducted a study on deceased donor kidney transplants to investigate whether warm and cold ischemia duration affects QTc interval. They could not demonstrate that ischemia duration influencing the prolongation of QTc. In any case, there are no significant differences found in analyzing the grafts' warm and cold ischemia times between the two groups in the conducted study.

Additionally, UWS (SERVATOR B SALF®) contains 1.34 g/L (5 mmol/L) adenosine. Adenosine is known to be used in the treatment of supraventricular tachycardia. This feature is due to interfering AV node, which results

in heart and conduction rate reduction[26]. The benefits of adenosine in GPSs were also presented in some studies. McAnulty et al[27] suggested that having adenosine in UWS facilitates adenosine triphosphate (ATP) synthesis for hypothermic kidney perfusion. Consistently, Fremes et al[28] preserved isolated rodent hearts within adenosine-containing GPSs. They reported that these solutions did not change graft results; however, adenosine treatment before the operation positively affected the graft's recovery, preserved in a hypothermic solution for a long time. Additionally, Lanir et al[29] suggested that ATP synthesis may increase after transplantation if substrates as ATP precursors, especially adenosine are found in GPS.

On account of all mentioned, it can be essential to revise the GPS content, especially for patients with cardiogenic comorbidity. In the present study, we revealed that QTc prolongation might be linked with the feature of GPS. We presume that the clinical symptoms with the reperfusion phase may be caused particularly by potassium and adenosine. Although K^+ concentrations in analyzed ABG do not differ significantly between groups, its effect on QTc interval cannot be ignored. More prospective trials are required to understand its etiology comprehensively.

However, some limitations should be noted in this study. Analyzing the blood samples taken directly from the renal vein after perfusion could better reflect the electrolyte and adenosine levels entering the systemic circulation. Therefore, a prospective study with renal vein blood samples might be conducted for more conclusive results. On the other hand, the consistency between the concentration of the flush solution perfused on cardiomyocytes in the experimental study and that get into the circulation during graft reperfusion was another limitation.

Conclusion

Although kidney transplantation is performed under appropriate hemodynamic and metabolic conditions, critical ECG changes may occur, particularly during and after the reperfusion phase. Today, many GPSs with differing contents are available for clinical use. Some of these have been suggested to be superior to others in different aspects. Therefore, we aimed to reveal the impact of two commonly used GPSs on the QTc interval at the intraoperative period. However, it should be noted that hemodynamic and metabolic changes that occur with reperfusion in kidney transplantations may be due to many different processes. Consequently, it is noteworthy to consider the properties of GPS in transplantation surgeries.

Conflicts of Interest

Authors declare no commercial associations, contractual relations, or proprietary considerations that might pose a conflict of interest related or unrelated to the submitted manuscript. Authors have had no involvements that might raise the question of bias in work reported or in the conclusions, implications, or opinions stated.

References

1. Beaubien ER, Pylypchuk GB, Akhtar J, Biem HJ. Value of corrected QT interval dispersion in identifying patients initiating dialysis at increased risk of total and cardiovascular mortality. Am J Kidney Dis. 2002; 39: 834-842.
2. Dilaveris PE. Molecular predictors of drug-induced prolongation of the QT interval. Curr Med Chem Cardiovasc Hematol Agents. 2005; 3: 105-118.
3. Monfared A, Ghods AJ. Improvement of maximum corrected QT and corrected QT dispersion in electrocardiography after kidney transplantation. Iran J Kidney Dis. 2008; 2: 95-98.
4. Kim ED, Watt J, Tereshchenko LG, Jaar BG, Sozio SM, Kao WHL, et al. Associations of serum and dialysate electrolytes with QT interval and prolongation in incident hemodialysis: the Predictors of Arrhythmic and Cardiovascular Risk in End-Stage Renal Disease (PACE) study. BMC Nephrol. 2019; 20: 133.
5. Monfared A, Atrkar Roshan Z, Salari A, Asadi F, Lebadi M, Khosravi M, et al. QT intervals in patients receiving a renal transplant. Exp Clin Transplant. 2012; 10: 105-109.
6. Malik M, Batchvarov VN. Measurement, interpretation and clinical potential of QT dispersion. J Am Coll Cardiol. 2000; 36: 1749-1766.
7. Malhis M, Al-Bitar S, Farhood S, Zaiat KA. Changes in QT intervals in patients with end-stage renal disease before and after hemodialysis. Saudi J Kidney Dis Transpl. 2010; 21: 460-465.
8. Brisson H, Arbelot C, Monsel A, Parisot C, Girard M, Savier E, et al. Impact of graft preservation solutions for liver transplantation on early cytokine release and postoperative organ dysfunctions. A pilot study. Clin Res Hepatol Gastroenterol. 2017; 41: 564-574.
9. Bazett HC. An Analysis of the Time-Relations of Electrocardiograms. Ann Noninvasive Electrocardiol. 1997; 2: 177-194.
10. Olgar Y, Ozturk N, Usta C, Puddu PE, Ozdemir S. Ellagic acid reduces L-type Ca2+ current and contractility through modulation of NO-GC-cGMP pathways in rat ventricular myocytes. J Cardiovasc Pharmacol. 2014; 64: 567-573.
11. Ozturk N, Yaras N, Ozmen A, Ozdemir S. Long-term administration of rosuvastatin prevents contractile and electrical remodelling of diabetic rat heart. J Bioenerg Biomembr. 2013; 45: 343-352.
12. Kucuk M, Celen MC, Yamasan BE, Kucukseymen S, Ozdemir S. Effects of prasugrel on membrane potential and contractile activity of rat ventricular myocytes. Pharmacol Rep. 2018; 70: 156-160.
13. Kucuk M, Celen MC, Yamasan BE, Olgar Y, Ozdemir S. Effects of Ticagrelor on Ionic Currents and Contractility in Rat Ventricular Myocytes. Cardiovasc Drugs Ther. 2015; 29: 419-424.
14. Chorvatova A, Hussain M. Effects of caffeine on potassium currents in isolated rat ventricular myocytes. Pflugers Arch. 2003; 446: 422-428.
15. Mohr A, Brockmann JG, Becker F. HTK-N: Modified Histidine-Tryptophan-Ketoglutarate Solution-A Promising New Tool in Solid Organ Preservation. Int J Mol Sci. 2020; 21: 6468.
16. Jing L, Yao L, Zhao M, Peng LP, Liu M. Organ preservation: from the past to the future. Acta Pharmacol Sin. 2018; 39: 845-857.
17. Parsons RF, Guarrera JV. Preservation solutions for static cold storage of abdominal allografts: which is best? Curr Opin Organ Transplant. 2014; 19: 100-107.
18. Kim ED, Watt J, Tereshchenko LG, Jaar BG, Sozio SM, Kao WHL, et al. Associations of serum and dialysate electrolytes with qt interval and prolongation in incident hemodialysis: the Predictors of Arrhythmic and Cardiovascular Risk in End-Stage Renal Disease (PACE) study. BMC Nephrol. 2019; 20: 133.
19. Familoni OB, Alebiosu CO, Ayodele OE. Effects and outcome of haemodialysis on QT intervals and QT dispersion in patients with chronic kidney disease. Cardiovasc J S Afr. 2006; 17: 19-23.
20. Alekseev AE, Jovanović A, López JR, Terzic A. Adenosine slows the rate of K(+)-induced membrane depolarization in ven-

tricular cardiomyocytes: Possible implication in hyperkalemic cardioplegia. J Mol Cell Cardiol. 1996; 28: 1193-1202.
21. Maruyama Y, Chambers DJ, Ochi M. Future perspective of cardioplegic protection in cardiac surgery. J Nippon Med Sch. 2013; 80: 328-341.
22. Li W, Bai Y, Sun K, Xue H, Wang Y, Song X, et al. Patients with metabolic syndrome have prolonged corrected QT interval (QTc). Clin Cardiol. 2009; 32: E93-E99.
23. Straus SM, Kors JA, De Bruin ML, van der Hooft CS, Hofman A, Heeringa J, et al. Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. J Am Coll Cardiol. 2006; 47: 362-367.
24. Monfared A, Ghods AJ. Improvement of maximum corrected QT and corrected QT dispersion in electrocardiography after kidney transplantation. Iran J Kidney Dis. 2008; 2: 95-98.
25. Zukowski M, Biernawska J, Kotfis K, Kaczmarczyk M, Bohatyrewicz R, Blaszczyk W, et al. Factors influencing QTc interval prolongation during kidney transplantation. Ann Transplant. 2011; 16: 43-49.
26. Curtis AB, Belardinelli L, Woodard DA, Brown CS, Conti JB. Induction of atrioventricular node reentrant tachycardia with adenosine: differential effect of adenosine on fast and slow atrioventricular node pathways. J Am Coll Cardiol. 1997; 30: 1778-1784.
27. McAnulty JF, Southard JH, Belzer FO. Improved maintenance of adenosine triphosphate in five-day perfused kidneys with adenine and ribose. Transplant Proc. 1987; 19: 1376-1379.
28. Fremes SE, Zhang J, Furukawa RD, Mickle DA, Weisel RD. Adenosine pretreatment for prolonged cardiac storage. An evaluation with st. Thomas' hospital and university of Wisconsin solutions. J Thorac Cardiovasc Surg. 1995; 110: 293-301.
29. Lanir A, Jenkins RL, Caldwell C, Lee RG, Khettry U, Clouse ME. Hepatic transplantation survival: correlation with adenine nucleotide level in donor liver. Hepatology. 1988; 8: 471-475.