JECT. 2011;43:130–136
The Journal of ExtraCorporeal Technology

Pig Heart Preservation with Antegrade Intracellular Crystalloid versus Antegrade/Retrograde Miniplegia

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Abstract: Both histidine-tryptophan-ketoglutarate (HTK) solution and Braile miniplegia are commercially available and used with high success. The objective of this work was to compare the effects of both strategies in an animal model. Twelve pigs were divided into control, HTK, or Braile groups using a model of controlled global cardiac ischemia/reperfusion under cardiopulmonary bypass with 1 hour heart ischemia followed by 2 hour reperfusion. No significant differences were found over time or between groups for heart rate, arrhythmia, number of defibrillations required, blood gases, myocardial lactate production, myocardial oxygen consumption, nor coronary flow index. The Braile strategy was associated with a lower 120 minute postreperfusion coronary vascular resistance with higher water content, leukocyte infiltration, and oxidative damage compared with controls. Drainage of HTK solution to the venous return was followed by higher potassium and lower sodium blood concentrations. One-hour heart preservation with HTK or Braile systems followed by 2 hour reperfusion both allow for acceptable preservation of the healthy pig myocardium. Maneuvers such as leukocyte filtration or hemofiltration may further improve these conditions. **Keywords:** animal model, cardiopulmonary bypass, histidine-tryptophan-ketoglutarate solution, ischemia/reperfusion, myocardial protection/cardioplegia. *JECT. 2011;43:130–136*

Currently, the most widely accepted methods for cardioplegic preservation are based on blood to which different drugs and crystalloid solutions are added. Perhaps the most popular is so-called miniplegia, which has the advantage of avoiding the use of high-volume solutions and reduces the total quantity of compounds added. A constant flow of tepid or warm oxygenated blood is infused through the aorta and/or coronary sinus while the cardioplegic components are added in-line just before reaching the patient. A miniplegic formulation is commercially available in Brazil, (Braile Biomédica, São José do Río Preto, Brazil) (1,2). This technique requires a formulation for cardioplegic induction and another for cardioplegic maintenance (Table 1).

Another cardioplegic strategy based on histidine-tryptophan-ketoglutarate (HTK) solution is currently used, mainly at European and Latin American centers, as well as at those centers involved in heart transplantation. HTK solution (Hans Köller Chemie, Germany) was designed by Bretschneider (3) in the early 1970s and has had only a minor modification since. It is an intracellular-type crystalloid solution containing an amino acid buffer, potassium, magnesium, calcium, an osmotic agent, a membrane stabilizer, and an energetic precursor (Table 1). It has the advantage of requiring only a single antegrade infusion and no local hypothermia, and provides ultrastructural and energetic preservation for several hours (4–6).

As both methods are commercially available and routinely used by different groups, a protocol was established to compare their effects in a model of controlled global cardiac ischemia/reperfusion in pigs under cardiopulmonary bypass (CPB).

MATERIALS AND METHODS

Twelve male Landrace–Duroc–Holland pigs, 41–52 kg body weight, were used. Anesthesia was induced with ketamine HCl (15 mg/kg intramuscular (i.m.)), midazolam (.3 mg/kg i.m.), and atropine (.05 mg/kg i.m.). After orotracheal intubation, sevoflurane anesthesia was established (minimum alveolar concentration 3% for 5 minutes, and 1% afterward) through a volumetric ventilator (A.D.S. 1000 ventilator, Engler Engineering, Hialeah, FL). Single doses

Received for publication October 6, 2010; accepted May 2, 2011.
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The senior author has stated that authors have reported no material, financial, or other relationship with any healthcare-related business or other entity whose products or services are discussed in this paper.

Table 1. Formulations of both cardioplegic solutions evaluated, according to manufacturers' information.

Component	HTK*	Braile Induction Stock†	Braile Maintenance-Reperfusion Stock†	Braile Induction Final Concentration†‡	Braile Maintenance-Reperfusion Final Concentration†‡
MgCl	–	800 mEq/L	300 mEq/L	9 mEq/L	4 mEq/L
Sodium glutamate	–	600 mMol/L	300 mMol/L	6 mEq/L	3 mEq/L
Sodium aspartate	–	600 mMol/L	300 mMol/L	6 mEq/L	3 mEq/L
KCl	9.0 mEq/L	1500 mEq/L	500 mEq/L	20 mEq/L	10 mEq/L
NaCl	15.0 mMol/L	–	–	–	–
MgCl (6 H_2O)	4.0 mMol/L	–	–	–	–
Mannitol	30.0 mMol/L	–	–	–	–
CaCl (2 H_2O)	.015 mMol/L	–	–	–	–
KH-2-ketoglutarate	1.0 mMol/L	–	–	–	–
Histidine HCl (H_2O)	18.0 mMol/L	–	–	–	–
Histidine	180.0 mMol/L	–	–	–	–
Tryptophan	2.0 mMol/L	–	–	–	–

*HTK is a crystalloid intracellular solution not intended for use mixed with blood.
†Induction solution is administered antegrade and maintenance-reperfusion solution is administered retrograde.
‡Values represent estimated final concentration of the component in coronary-infused blood.

of fentanyl (10 μg/kg intravenous (i.v.)) and vecuronium (100 μg/kg i.v.) were administered. Probes were installed for electrocardiographic and esophageal and rectal temperature monitoring (Mod. 90303B; Spacelabs Healthcare, Issaquah, WA).

Surgical Protocol

After median sternotomy, the left mammary artery was cannulated for arterial pressure monitoring (same Spacelabs monitor). Following pericardiotomy, the ascending aorta was encircled. Heparin was administered (700 IU/kg) and the femoral artery was cannulated, followed by right atrial cannulation with a single double-stage venous cannula or double venous cannulae, according to the experimental group. CPB was established and after ligating the azygos vein, a retrograde coronary infusion cannula was inserted into the coronary sinus through a small right-atrial stab incision. To secure the retrograde infusion cannula, purse-string sutures were applied around the incision and the coronary sinus. Subsequently, an antegrade cardioplegia infusion cannula was inserted into the ascending aorta.

Cardiopulmonary Bypass

The circuit consisted of a Delphin centrifugal pump and coated oxygenator (Terumo Capiox SX-10RX, Somerset, NJ), 3/8″ tubing (Intruvin; Vitalmex, Mexico City, Mexico) and roller pumps for pericardial and cardiotomy suction. Priming included 300 mL 10% hydroxyethyl starch (200 kDa, Fresenius Medical, Mexico), 300 mL Hartmann's solution, 4000 IU heparin, and 20 mL 7.5% sodium bicarbonate. Gases were adjusted with a blender to 100–220 mmHg PaO_2 and 20–45 mmHg $PaCO_2$. Homologous blood was administered to keep the hematocrit between 20–30%. Hartmann's solution was administered as necessary to replace volume losses. Blood flow was 3.0 L/min/m^2 and temperature 36.0–38.0°C at all times. Heparin was added as needed to keep activated clotting times >480 seconds. At the end of the procedure, myocardial biopsies were obtained before exsanguination. Throughout the study, hearts were emptied through a left atrial incision and cardiotomy suction.

Experimental Protocol

Animals were assigned to one of three experimental groups. The HTK solution group ($n = 4$) received a single-dose antegrade coronary infusion of 40 mL/kg HTK solution at 10°C and 60 mmHg, for at least 6 minutes. This infusion pressure is optimal for preservation of myocardial and endothelial function (7). The solution drained into the systemic circulation. The Braile group ($n = 4$) received antegrade normothermic Braile induction miniplegia, followed by continuous normothermic maintenance with 35 mmHg retrograde infusion at the concentrations indicated in Table 1. The control group ($n = 4$) was subject to CPB for a period equal to that for HTK and Braile groups, with no ischemia/reperfusion or cardioplegic infusion.

Coronary flow was controlled at all times as follows: once under CPB with the aorta clamped, blood from the coronary connector of the oxygenator was perfused through the antegrade cardioplegia cannula in the aortic root with a peristaltic pump (Masterflex, L/S 7550; Cole-Parmer Instrument Co., Vernon Hills, IL) at flows adjusted to maintain constant coronary blood pressure (60 mmHg). The Braile solutions were added when needed to this line through a syringe pump (Terufusion TE-331, Terumo Corp., Tokyo, Japan) at the required flows to reach the prescribed component concentrations for induction or maintenance (Table 1). A 15-minute stabilizing period was allowed before base measurements. At this point, coronary flow was interrupted in the HTK group or the syringe pump was started in the Braile group, and cardioplegia was initiated, followed by 60 minutes of protected arrest and 2 hours of controlled reperfusion at 60 mmHg again through the

antegrade cardioplegia cannula in the aortic root. No cold solutions were added to the pericardial pouch at any time.

Measurements

Arrhythmias: Electrocardiogram was continuously monitored for ST segment elevation and arrhythmic events recording.

Energy Charge: Full-thickness left-ventricular biopsies were obtained from the beating heart and immediately stored at −80°C. Tissues were pulverized under liquid nitrogen and homogenized with 5% perchloric acid. The suspension was centrifuged (10,000 rpm/15 minutes, 4°C). The concentration of adenine nucleotides was determined by a standard enzymatic method. Energy charge was calculated as (ATP + .5 ADP)/(ATP + ADP + AMP) (8).

Blood Gases, Hematocrit, Lactate and Electrolytes: Arterial and coronary sinus blood samples were obtained for determination of blood gases, hematocrit, lactate, sodium, potassium, calcium, and glucose in a Gem Premier 3000 system (Instrumentation Laboratory, Lexington, MA). Myocardial lactate production/consumption was estimated as (arterial lactate − coronary sinus lactate)/coronary blood flow. Myocardial oxygen consumption was estimated as:

$$VmO_2 = as_{diff} \times 100Qc,$$
$$as_{diff} = (O_2cont_a - O_2cont_{cs}), \text{ and}$$
$$O_2cont = (SatO_2 \times .0134Hb) + .0031PO_2,$$

where a = arterial; as_{diff} = arterial-coronary sinus oxygen difference; cs = coronary sinus; Hb = hemoglobin; O_2cont = oxygen content; PO_2 = oxygen partial pressure; Qc = coronary blood flow; $SatO_2$ = oxygen saturation; and VmO_2 = myocardial oxygen consumption.

Myocardial Water Content: Left ventricular full-thickness samples were desiccated at 60°C for 7 days. Water content was estimated as 100 − (W2 × 100/W1), where W1 and W2 are the weight at day 0 and 7, respectively.

Neutrophil Infiltration: Myeloperoxidase (MPO) activity, an index of neutrophil infiltration, was measured from 100 mg full-thickness left ventricular biopsies homogenized in 50 mM phosphate buffer with 5% hexadecyltrimethylammonium bromide and .03 mM EDTA (Sigma Chemical Co., St. Louis, MO). Homogenates were frozen–thawed twice and centrifuged (5000 rpm/15 minutes, 4°C). MPO activity was measured from an aliquot of the supernatant in 1 mL of reaction volume with .3% H_2O_2 and 1% O-dianisidine. Absorbance changes were monitored every minute for 10 minutes at 460 nm (Beckman DU 650). One MPO unit is the amount able to degrade 1 μmol H_2O_2/min at 25°C per g tissue (9).

Lipid Peroxidation: Malondialdehyde and 4-hydroxyalkenals concentrations provide an index of lipid peroxidation (LPO). Tissue (1.0–1.5 g) was homogenized in 20 mM Tris buffer, pH 7.4, containing 5 mM butylated hydroxytoluene and centrifuged (5000 rpm/15 minutes, 4°C). Aliquots of the supernatant were used for analysis with a Bioxytech LPO-568 kit (Oxis International, Inc. Portland, OR).

Light Microscopy: Full-thickness biopsies were obtained from the beating left ventricle at the end of the experiments. Hematoxylin-eosin and periodic acid-Schiff-stained slides were evaluated blind by a pathologist and graded from 0–3 for increasing severity of sarcoplasmic vacuolization, dense contraction bands, leukocyte infiltration, interstitial edema, hemorrhage, and glycogen depletion. Grade 0 means no change, grade 1 up to 30%, grade 2 up to 60%, and grade 3 means more than 60% of the observed surface changed.

Statistical Analysis: Results are expressed as mean ± standard deviation. Differences in means within and among groups for all times were analyzed with a one-way analysis of variance with post hoc Tukey tests. For nonnormal distributions (Kolmogorov–Smirnov) or unequal variance (Levene Median), comparisons were done with a Kruskal–Wallis test, followed when needed by a post hoc Dunn test. χ^2 was used for comparisons of severity in light microscopic observations; $p < .05$ was considered significant. The protocol was approved by the institutional board and all animals received humane care in compliance with Mexican regulations (NOM-062-ZOO-1999).

RESULTS

No significant differences were found over time or between groups for heart rate, arrhythmia, number of defibrillations, blood gases, myocardial lactate production (data not shown), nor myocardial oxygen consumption (Table 2). The coronary flow index remained constant without significant differences (Table 2), while in the Braile group, the coronary vascular resistance fell significantly at reperfusion compared with its base value (Figure 1).

A consideration for the use of miniplegia is its ability to reduce systemic concentrations of the cardioplegic components, so systemic calcium, potassium, sodium, and glucose were compared (Table 2). No differences were found for calcium, while systemic potassium concentrations rose compared with the base values in both cardioplegic groups, significantly for the HTK group at 60 minutes reperfusion. HTK solution was allowed to enter the systemic circulation, and this was followed by a significant fall in sodium concentration. Glucose approached similar values for all groups at 120 minutes of reperfusion.

Water content and LPO products were significantly higher in the Braile group than in the control group, with no differences in energy charge and myeloperoxidase (Table 3). Histopathology showed only absent to moderate changes (grades 0–2), with differences for leukocyte infiltration, which was predominantly absent in the control and HTK groups and moderate in the Braile group (Table 4).

Table 2. Differences over time and between groups for coronary flow, myocardial oxygen consumption, and systemic electrolytes and glucose.

	Group	Base	Reperfusion 60′	Reperfusion 120′	*p*
Coronary flow index	Control	.6 ± .19	.6 ± .09	.6 ± .11	.900
(mL/min g^{-1})	HTK	.6 ± .08	.7 ± .11	.7 ± .09	.382
	Braile	.5 ± .06	.7 ± .19	.7 ± .18	.066
	p	.237	.299	.509	
Myocardial VO_2	Control	156.8 ± 88.42	168.7 ± 12.12	158.9 ± 54.75	.957
(mL/min g^{-1})	HTK	126.1 ± 130.00	171.8 ± 41.37	200.1 ± 90.63	.576
	Braile	100.0 ± 111.00	195.5 ± 190.89	244.0 ± 129.13	.410
	p	.767	.941	.487	
Systemic calcium	Control	1.2 ± .13	1.3 ± .06	1.4 ± .07	.191
(mMol/L)	HTK	1.3 ± .07	1.3 ± .09	1.3 ± .06	.659
	Braile	1.4 ± .05	1.4 ± .03	1.4 ± .03	.065
	p	.172	.256	.104	
Systemic potassium	Control	3.9 ± .31	4.3 ± .43	4.4 ± .47	.247
(mMol/L)	HTK	3.9 ± .19	5.1 ± .30*	5.0 ± .34	*<.001 vs. base
	Braile	3.9 ± .47	5.0 ± .79	5.2 ± 1.29	.142
	p	.972	.132	.355	
Systemic sodium	Control	137.3 ± .50	137.0 ± 1.63†	137.0 ± .82‡	NS
(mMol/L)	HTK	137.8 ± .96	128.8 ± .96§	128.8 ± 1.26§	§<.001 vs. base
	Braile	139.0 ± 1.41	139.0 ± .82†	139.5 ± 1.29‡	NS
	p	.096	†<.001 vs. HTK	‡<.001 vs. HTK	
Systemic glucose	Control	108.0 ± 23.82	95.0 ± 41.021	111.3 ± 12.34	.968
(mg/dL)	HTK	141.5 ± 32.30	132.5 ± 31.75	122.5 ± 15.26	.637
	Braile	77.0 ± 23.20¶	69.0 ± 12.36‖	97.8 ± 24.47	.185
	p	¶.019 vs. HTK	‖.041 vs. HTK	.209	

DISCUSSION

We consider that 1 hour heart preservation with either HTK or Braile systems followed by 2 hours reperfusion allows acceptable preservation of healthy pig myocardium; although the Braile strategy was associated with a lower 120 minutes postreperfusion coronary vascular resistance with higher water content, leukocyte infiltration, and oxidative damage. Drainage of HTK solution to the venous return yielded higher potassium and lower sodium blood concentrations.

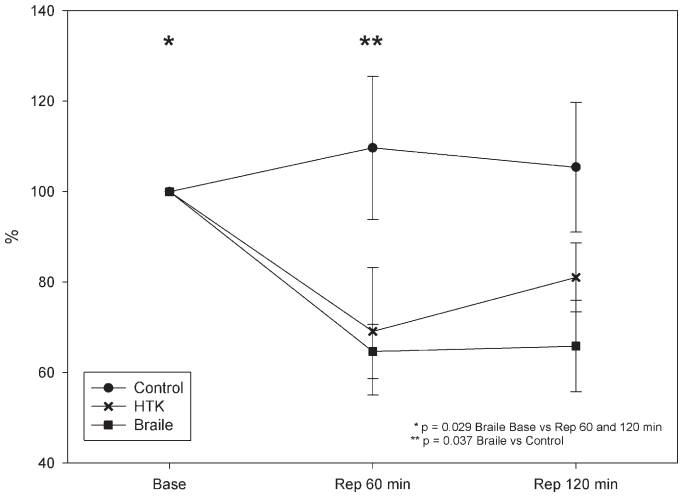

Figure 1. Percent changes in coronary vascular resistance for all groups studied. Rep, reperfusion.

Endothelial dysfunction is an important consequence of ischemia and reperfusion. Despite the theoretical advantage of reducing cell metabolism, cold preservation causes endothelial dysfunction with impaired relaxation and reduced vasodilatation, especially pronounced in distal arteries, with marked impairment of endothelium-dependent microcirculatory responses. These impaired endothelial responses may be caused by blood products or myocardial metabolites released during reperfusion (10,11). In our experiments, hearts from both groups with ischemia/reperfusion had clear falls in coronary vascular resistance compared with controls, but this reached significance only in the Braile group (Figure 1). Shear stress, the frictional force exerted by blood on the endothelium induces endothelial nitric oxide synthase and inducible nitric oxide synthase for nitric oxide production, which is protective from ischemia/reperfusion injury (12). Constant low-flow during miniplegia with a consequent presence of some degree of constant endothelial shear stress in the Braile group could partly explain the reduction in coronary resistance, but whether the degree of shear stress with normothermia and low retrograde flow under oxygenated minicardioplegic conditions is sufficient to exert this effect remains to be determined.

Rises in water content are caused by sodium pump failure during ischemia/reperfusion or rises in hydrostatic pressure during infusion. Values observed were slightly lower than the 81–82% detected in dogs after blood cardioplegia or miniplegia (13). The difference found between groups Braile and control was significant (80.9 ± .98% Braile vs.

Table3. Water content, energy charge, lipoperoxides and myeloperoxidase for all groups studied.

Group	Water Content %	Energy Charge Base*	Energy Charge Rep 120*	LPO mMol/mL	MPO IU/g
Control	79.3 ± .80	.86 ± .22	.70 ± .26	2.94 ± .47	1.11 ± .50
HTK	80.7 ± .26	.89 ± .20	.83 ± .23	4.65 ± 1.91	.75 ± .42
Braile	80.9 ± .98†	.80 ± .12	.91 ± .04	5.96 ± 1.78‡	2.11 ± 1.57
p	†.035 vs. CON	.800	.409	‡.029 vs. CON	.184

*No differences were found between energy charge at rep 120 vs. base in any group.
CON, Control group; MPO, myeloperoxidase.

79.3 ± .80% control; $p = .035$). This may be relevant in view of the demonstration that a small potentially insignificant change in myocardial water content will correspond to a much larger change in left ventricular mass (14) with significant myocardial dysfunction.

Neutrophil infiltration is the main cause of myocardial postischemic damage, low cardiac output, and ventricular ectopy (15). Pigs in the Braile group had higher leukocyte infiltration and a nonsignificant elevation of myocardial myeloperoxidase levels, indicative of neutrophil infiltration. This may be the result of the constant presence of polymorphonuclear cells in the miniplegia throughout the cardioplegic period. Even though neutrophil infiltration peaks after several hours of reperfusion and activation may last for at least 24 hours (16), the participation of activated neutrophils during the cardioplegic period also has significant impacts on clinical and biochemical evolution in coronary artery bypass graft patients (15). Neutrophil-induced injury is mainly dependent on production of reactive oxygen species (17). In our experiments, significantly higher levels of LPO products were also detected in the Braile group (Table 3).

Table 4. Histopathology scoring for all groups studied.

Group	No Change	<30%	30–60%	*p* Value X2
Vacuolization				.558
Control	0	3	1	
HTK	0	3	1	
Braile	1	3	0	
Contraction bands				.900
Control	0	3	1	
HTK	0	3	1	
Braile	0	2	2	
Leukocyte infiltration				.037
Control	3	1	0	
HTK	3	0	1	
Braile	0	4	0	
Interstitial edema				.890
Control	3	1	0	
HTK	0	4	0	
Braile	1	3	0	
Hemorrhage				.091
Control	4	0	0	
HTK	1	3	0	
Braile	2	2	0	
Glycogen depletion				.380
Control	0	3	1	
HTK	0	4	0	
Braile	1	3	0	

Numbers in cells represent total tissue samples for the corresponding severity change

Hemodilution has advantages, but a critical lower value is yet to be determined (18). Some authors have demonstrated a better neurological outcome associated with higher hematocrit values during CPB, while others claim better myocardial preservation and a lower incidence of myocardial infarction with lower systemic hematocrits (19,20). One of the main theoretical advantages of miniplegia is avoiding hemodilution to critical levels. It is important in this context that pigs from the HTK group had hematocrits of 20–30% which is within the acceptable range of hemodilution for this species (21) despite drainage to the systemic circulation and did not require the addition of donor blood to the circuit for hematocrit adjustment (data not shown).

Administration of a combination of antegrade and retrograde cardioplegia is protective against myocardial damage and postoperative complications (22), particularly in patients subject to coronary artery bypass graft. This is presumed to be mediated by the more even distribution of cardioplegic solutions to the coronary circulation distal to an arterial obstruction. Because we used healthy pigs, this condition was not achieved, as there were no obstructed coronary arteries. However, retrograde cardioplegia distributes to subendocardial muscle whether or not coronary arterial stenoses are present, with a preferential distribution to the left ventricle and decreased flow to the right ventricle and septum (23). Myocardial samples were obtained from the left ventricle, which may not reveal the real conditions present in other cardiac structures.

Miniplegia includes heparin, which reduces the paracrine myocardial tumor necrosis factor (TNF)-α protein production and release, and improves the recovery of left ventricular function following global cardiac ischemia in rats (24). Although heparin is used systemically for CPB, this could be a theoretical advantage of the use of heparinized blood-based coronary infusion during ischemia and warrants further study.

As recommended by the manufacturer, the Braile miniplegic strategy should be normothermic. Besides

considerations of rises in viscosity when using undiluted blood, this recommendation is consistent with work with continuously infused diluted blood cardioplegia in dogs, where cardiomyocyte apoptosis and endothelial damage were more intense in cold than tepid conditions (25).

In view of the advantage of needing only a single HTK infusion, the idea of a single-shot normothermic blood cardioplegia is also attractive. Ghazy et al. (26) compared the use of single and repeated Calafiore infusions. The single shot was associated with an elevated need for intraoperative inotropics and increased risk of postoperative dialysis, but a significantly reduced incidence of infarction and no difference in overall mortality. As the study was retrospective, and ischemia times are not indicated, further research should be performed to establish its real value.

Our study may be statistically underpowered due to the small number of animals used. We consider, nevertheless, that the information obtained points to the parameters on which emphasis is required for further studies. Several simple modifications to the cardioplegic strategies evaluated may be advantageous and may warrant future investigation. For example, a reduction in hydrostatic pressures in miniplegia or the use of leukocyte filters would allow for better control of postischemic water content or neutrophil infiltration. Use of hemofilters or avoidance of HTK drainage to the circuit (as recommended by the manufacturer) would reduce significant changes in potassium and sodium systemic levels. HTK preservation can be further improved in terms of endothelial function with the addition of antioxidants (27), which are also useful in humans (28). Formal testing of other additives is necessary, given their potential to improve the preservation already obtained with the strategies evaluated.

Efforts have been made to look for the best evidence-based use of crystalloid or blood cardioplegia. This has been extremely difficult, if not impossible, because of the fact that there is no single crystalloid or blood cardioplegic formula or strategy. This has led to, for example, crystalloid compositions as dissimilar as intracellular and extracellular or single and multiple doses, being grouped as the same for comparisons (29). We consider this an erroneous approach. More work should be done with separate groups to enable objective comparisons of the different strategies available. In this sense, we believe our work contributes to the understanding of different clinically relevant heart preservation strategies.

ACKNOWLEDGMENTS

We thank Socorro Serrano, Ma. de Jesús Delgado and all members from the Surgical Research Division for their support. Also Dr. Ma. Guadalupe Ramírez for statistical assistance. Braile and HTK solutions were kindly donated by the manufacturers. Work supported by grant FOFOI2004087 and the authors' own resources.

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