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The Progression of Isoflurane-induced Malignant Hyperthermia and Its Attenuation by Cisatracurium in a Pre-clinical Porcine Model of Heart Transplant

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Abstract

Background: Even after several years of study, the intra-operative diagnosis of Malignant Hyperthermia (MH) and the approach to anesthesia in MH-susceptible individuals has remained a challenge. In this study we present the pre-operative and intra-operative findings of development and progression of MH in a porcine model that was assigned for heart transplants.

Methods: Female Yorkshire swine were assigned as either donor or recipient and anesthetized with inhalational isoflurane with or without cisatracurium. The inadvertent development of signs indicating MH, including, alterations required in ventilator/bypass machine settings, arterial and venous blood tests, gross- and histo-pathology, were followed through the surgeries that lasted for variable durations between donors and recipients.

Results: Both donors and the recipients were apparently MH-susceptible, and showed systemic intraoperative signs of variable severity, that were greater in longer duration recipient surgeries, and in those in which cisatracurium was not administered. These included features of hypermetabolism such as elevated pCO_2 and serum lactate, increased requirements for O_2 (FIO_2), and multi-organ changes, including pulmonary congestion, features of intestinal pseudo-obstruction, and inflammatory infiltrates in livers and hearts.

Conclusions: The isoflurane-induced MH progressed temporally in severity with the duration of surgery. Use of cisatracurium was directly beneficial in attenuating and/or delaying the progress of MH.

Keywords: Malignant hyperthermia; Isoflurane; Cisatracurium; Hypermetabolism

Abbreviations: MH: Malignant Hyperthermia; FIO2: Fractional Inspiration of Oxygen; RR: Respiratory Rate; PEEP: Positive End-Expiratory Pressure; CVP: Central Venous Pressure; ABP: Arterial Blood Pressure; CPB: Cardio-Pulmonary Bypass; CK: Creatine Kinase; AST: Aspartate Amino Transferase; ALT: Alanine Amino Transferase; GGT: Gamma Glutamyl Transferase; SpO_2: Peripheral O_2 Saturation; BUN: Blood Urea Nitrogen; Hb: Hemoglobin; Hct: Hematocrit; iCa^{2+}: Ionic Calcium; BP: Blood Pressure; HR: Heart Rate; $ETCO_2$: End-Tidal CO_2; SIRS: Systemic Inflammatory Response Syndrome; DIC: Disseminated Intravascular Coagulation

Introduction

Malignant hyperthermia (MH), a hypermetabolic syndrome, afflicts an estimated 1:10,000 patients undergoing surgery in the United States [1]. In the majority of mutational defects, hypersensitivity of the ryanodine receptor results in excessive leakage of calcium from sarcoplasmic reticulum resulting in muscle contractures, increased O_2 and ATP consumption, CO_2 and lactic acid production, heat generation and release of intracellular contents such as creatine kinase and myoglobin [2]. In the swine, MH occurs as the porcine stress syndrome attributed to physical and/or environmental stress and credited to mutation in the ryanodine receptor as well; in human beings, while excessive exercise and heat stroke have been associated with MH [3], this condition has largely been reported in intra- and post-operative setting with the use of depolarizing muscle relaxants such as succinylcholine or volatile inhalational anesthetics like halothane and its analogs including isoflurane, for anesthesia.

Although, the skeletal muscle is most dramatically affected in MH, severe affliction of other organs/organ systems and multi-organ failure has been reported [4-7]. While investigating the role of our novel heart storage solution in a porcine orthotopic heart transplant model, we observed the development of unusual signs of hypermetabolism and multi-systemic involvement in both the donors and recipients [8]. Anesthetic used in these surgeries was inhalational isoflurane and the severity of observed signs was found to be proportional to duration of anesthesia. Inadvertently, we also observed partial attenuation of isoflurane-dependent development of clinical signs with the use

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of the paralytic agent, cisatracurium, a non-depolarizing skeletal muscle relaxant. Interestingly, due to the nature of this study, we had the animal on anesthesia, either for only 1-1.25 hours (in donors) or approximately 5-6 hours (in recipients), thus allowing a comparison of the MH-related effects of short-term and long-term use of isoflurane for anesthesia and the MH-attenuating ability of cisatracurium thereof.

Methods

Anesthesia and surgery

Female Yorkshire swine of 45 ± 5 Kg weight were used as the pre-clinical model of heart transplant. All experiments were performed according to protocols approved by our institutional animal committee. Animals were transported to our research facility and were allowed to rest and acclimatize for at least 48 hours and kept nil per oral (NPO) for the night before being taken up for surgery. A total of three heart transplant surgeries (thus involving three donors and three recipients) were performed. While no paralytic agent was used during tracheal intubation, atropine (0.05 mg/kg), telazol (4-6 mg/kg), and xylazine (1-2 mg/kg) i.m. were given during pre-anesthetic sedation in all surgeries. Upon intubation, animals were connected to ventilator (FIO_2: 60%; Tidal Volume: 500 mL; RR: 15-20/min; PEEP-5 cm H_2O). In the first two donor-recipient surgeries, inhalational isoflurane (2%) was used for maintenance anesthesia and no paralytic agent given. In the third donor-recipient surgeries, isoflurane (1.5%) was used for anesthesia, but cisatracurium (0.2-0.4 mg/kg i.v) was given 10-15 mins before midsternal skin incision, to achieve paralysis.

During surgeries the peripheral oxygen saturations were monitored using saturation probe attached either on pinna or the tongue. Core body temperature was recorded using a rectal probe. Femoral vein and artery were catheterized for Central Venous (CVP) and Arterial Blood Pressure (ABP) measurement respectively. All surgeries were performed through mid-sternal approach, and the end of surgery was marked by completion of heart extraction (in donor surgeries) or by approximate observation period of 30-60 mins after donor heart anastomosis into the recipient (in recipient surgeries). The average duration for donor surgeries from the beginning of ventilation to the end of surgery was approximately 1-1.25 hours, and that for recipient transplant surgeries (including 30-60 mins post-transplant observation) was approximately 5-6 hours. In the recipient surgeries, hypothermic cardio-pulmonary bypass (CPB; target core temperature of 32°C) was initiated upon aortic cross clamp of recipient heart, and continued till after orthotopic transplant was completed. During surgery, the respiratory rate and the fractional inspiration of O_2 (FIO_2) levels were manually altered, as required, based upon peripheral O_2 saturations (SpO_2) and pCO_2. Fluid resuscitation, sodium bicarbonate and insulin-dextrose infusions were given as deemed necessary.

Blood sample collection and investigations

In all cases, the first (baseline) samples were collected from femoral vessels approximately 20-30 minutes after start of ventilation, once the arterial access had been achieved. The final samples for donors were collected at the end of surgery (total 1-1.25 hours of surgery) before the donor heart was extracted. In recipient surgeries, the samples obtained prior to heart extraction (2-2.5 hours through surgery), are reported here as mid-surgery samples while the final blood samples were obtained after thirty minutes of observation upon heart transplant (total 5-6 hours of surgery). Both arterial and venous blood samples were analyzed for blood gases including pH, pO_2, pCO_2, HCO_3^- and base excess using i-Stat blood analyzer system (Abaxis Ltd). Venous blood samples were also analyzed using Vetscan VS2 blood analyzer system (Abaxis Ltd) for lactate levels, serum enzymes (CK, AST, ALT and GGT), electrolytes (Ca^{2+}, K^+, P, iCa^{2+}) and other blood parameters including total bilirubin, creatinine, blood urea nitrogen (BUN), hemoglobin (Hb) and hematocrit (Hct).

Postmortem analysis

The thoracic and abdominal cavities were explored for evidence of gross abnormalities. Liver biopsies from donors 1 and 2 at end of donor surgeries and heart biopsies from donors 1 and 2 after completion of recipient transplant surgery experiments were obtained for histopathology. The biopsies were fixed in 10% formalin and then processed for Hematoxylin and Eosin (HE) staining for histopathology analysis.

Data collection and analysis

The CVP and ABP data including heart rate and blood pressure were acquired using iWorx data acquisition system and analyzed using Labscribe software (Dover, NH). The blood samples were processed by i-Stat (Abaxis Ltd.; Union City, CA) and Vetscan (VS2; Abaxis Ltd.; Union City, CA) for blood gases and other blood parameters. Photographic data was acquired using Canon digital camera (Powershot G12). Data was analyzed using Sigmaplot 11.0 software. The normal physiological values for Yorkshire swine were derived from the available literature [9-13].

Results

Pre-operative assessment

On the day of operation, all animals were apparently healthy, and there was no evidence of muscle atrophy or rigidity on gross examination.

Intraoperative observations

Fluctuations in BP and HR were observed during both donor and recipient surgeries. The FIO_2 levels had to be increased to as high as 60-100% due to fall in peripheral SpO_2. All cases developed cyanosis towards the end of operation. These parameters and observations were more difficult to manage with increased duration of surgery in recipients and especially in surgeries in which cisatracurium was not given (Table 1).

	---------- Donors -------			--------- Recipients ---------		
	1*	2*	3†	1*	2*	3†
BP Fluctuations (not responsive to i.v. fluids)	+	+	+	+++	+++	+
Tachycardia	+	+	+	++	+++	++
Evident diaphoresis	++	++	+	++++	++++	+
Cyanosis	++	+	-	+++	+++	+
Intestinal engorgement and edema	++	++	+	++++	++++	++
Pulmonary congestion	+	+	+	++	+++	+
Inflammatory infiltrates in heart	+	+	+	+++	++++	++

Note that due to the difference in durations of donor and recipient surgeries (1-1.25 and 5-7 hours respectively), the observations made within donor and recipient groups although are in the same time range, observations made in donors were made at earlier time points than those in recipients.
*Isoflurane (inhalation).
†Isoflurane (inhalation) and Cis-atracurium (i.v.).

Table 1: Intra- and post-operative observations in donors and recipients.

Blood gas analysis

All donors (except number 3) and recipients demonstrated a high blood pH, low pCO_2 and high-normal HCO_3^- levels in baseline samples (Tables 2A and 2B). A high arterial-venous pCO_2 gradient (>6 mmHg) was observed in all blood samples from donors 1 and 3 and the final sample from recipient 3. The baseline lactate levels were higher than normal in all cases (Tables 2A and 2B). During surgery, in recipients 1 and 2, the fractional change in values over baseline levels, in serum lactate and fall in base excess were approximately 4-5 times and 3-4 times, respectively, greater than that in donors 1 and 2. In contrast, the alterations in serum lactate and base excess in donor and recipient 3 (both of which received cisatracurium) were relatively similar (Figures 1A and 1B).

Serum enzymes

The baseline CK levels were markedly elevated in both donors and recipients with the highest levels noted in recipient 2. The serum AST and ALT levels were within normal range at baseline. Although mild elevations in ALT levels were evident intraoperatively, the AST levels were markedly raised in the final blood samples of all recipients. The GGT levels were normal at baseline and not altered during surgery (Table 3).

Serum electrolytes and other investigations

Intraoperatively, serum K^+ levels in mid-surgery samples were higher in the longer duration recipient surgeries that did not receive cisatracurium (recipients 1 and 2). Serum Ca^{2+} levels were not altered during donor surgeries but were decreased and increased above normal, respectively, in recipients 1 and 2. Ionic calcium (iCa^{2+}) was as high as 2.24 and 1.49 mM/L (normal range 1.12-1.32 mM/L) at the end of recipient 1 and 2 surgeries, respectively. Serum phosphorus levels were dramatically decreased in recipient 1 and 2, especially in the final blood samples. Mild to moderate elevation of BUN at baseline was noted in all the donors and recipients, and either remained unaltered or marginally increased during surgery. Serum creatinine levels were stable throughout all surgeries with good urine output. The color of urine was darker in the final hours of recipient 1 and 2 surgeries. The total bilirubin levels remained stable in all of the surgeries. Recipient 2 demonstrated an enhanced coaguability initially (observed during

		Sample	Ph [A: 7.40-7.53]; [V: 7.38-7.48]	pO_2 (mmHg) [A: 73-92]; [V: 32-45]	pCO_2 (mmHg) [A: 35-44]; [V: 44-55]	HCO_3^- (mM/L) [22-33]	Lactate (mM/L) [0.5-1.5]
Donor 1 (Isoflurane only)	Arterial	Baseline	7.634	172	26.6	28.2	-
		Final	7.573	221	28.9	26.7	-
	Venous	Baseline	7.494	31	34.3	26.4	2.38
		Final	7.469	51	35.5	25.8	3.8
Donor 2 (Isoflurane only)	Arterial	Baseline	7.659	198	25.3	28.5	-
		Final	7.445	219	33.6	23.1	-
	Venous	Baseline	7.542	59	28.2	26.5	2.82
		Final	7.404	42	38.9	24.3	4.26
Donor 3 (Isoflurane+cis-atracurium)	Arterial	Baseline	7.476	219	43.6	32.1	-
		Final	7.543	190	31.8	27.4	-
	Venous	Baseline	7.398	45	51.7	31.9	1.92
		Final	7.411	36	46.9	29.8	2.97

Table 2A: Arterial and venous blood gases (donors).

	Sample	pH	pO_2	pCO_2	HCO_3^-	Lactate	
Recipient 1 (Isoflurane only)	Arterial	Baseline	7.573	180	32.6	30.1	-
		Mid-Surgery	7.219	206	45.9	18.7	-
		Final	7.236	241	30.1	12.8	-
	Venous	Baseline	7.485	42	37.6	28.3	1.26
		Mid-surgery	7.282	50	50.1	23.7	3.69
		Final venous	7.159	47	39.0	13.9	3.82
Recipient 2 (Isoflurane only)	Arterial	Baseline	7.655	257	26.3	29.3	-
		Mid-surgery	7.245	235	53.0	23	-
		Final	7.285	255	41.1	19.2	-
	Venous	Baseline	7.59	57	29.5	28.3	1.9
		Mid-surgery	7.234	27	54.1	22.9	6.85
		Final	7.201	27	45.3	17.8	5.28
Recipient 3 (Isoflurane+cis-atracurium)	Arterial	Baseline	7.67	200	25.2	29.1	-
		Mid-surgery	7.515	186	32.4	26.1	-
		Final	7.509	162	33.9	27	-
	Venous	Baseline	7.605	71	26.7	26.5	2.39
		Mid-surgery	7.427	46	35.5	23.4	3.65
		Final	7.465	38	37.5	26.9	4.66

Arterial and venous blood gases were analyzed both before the start of surgery (baseline) and at the end of surgery (1-1.25 hrs for donors and 5-7 hrs for recipients). An additional mid-surgery sample (2-2.5 hrs) was also sent for analysis in the case of recipients. Lactate levels were measured only in the venous samples. Normal values for Yorkshire swine are provided in parentheses (A: Arterial; V: Venous) in the top row of Table 2A.

Table 2B: Arterial and venous blood gases (recipients).

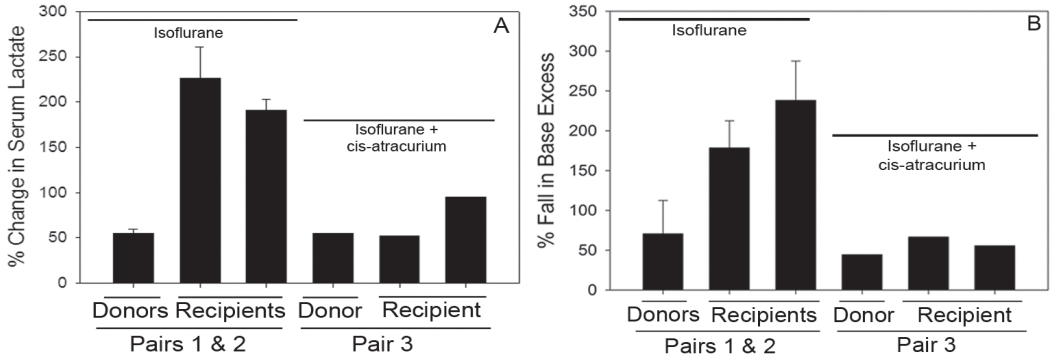

Figure 1: Alterations in blood lactate and base excess levels in donors and recipients on isoflurane with or without cisatracurium.Graph shows percentage changes in the venous blood lactate levels (A) and Base Excess in ABG's (B) from baseline in the final samples collected from donors (1-1.25 hours) and the mid-surgery (2-2.5 hrs; 1st of recipient bars) and final samples (5-6 hrs; 2nd of recipient bars) in the recipients during anesthesia using Isoflurane without or with cis-atracurium. (A) In donor-recipients pairs 1 and 2, the average venous lactate levels in final samples were increased from baseline levels by 55.36% and 190.53% respectively. The average mid-surgery lactate levels in recipients 1 and 2 demonstrated 226.68% increase from baseline levels. The donor-recipient pair 3 demonstrated an increase in lactate levels of 54.68% and 94.97% in the final samples and a mid-surgical increase of 52.7% in the recipient 3. (B) In donors-recipients pairs 1 and 2, the base excess in ABG final samples was decreased from baseline levels by an average of 70.53% (taken at 1-1.25 hours of surgery) and 238.19% (5-6 hours after surgery) respectively. The average mid-surgery base excess (2-2.5 hours of surgery) in recipients 1 and 2 was decreased by 178.47% from baseline. The donor and recipient 3, that received cis-atracurium in addition, demonstrated a decrease in base excess of 44.44% and 55.55% in the final samples and a mid-surgical decrease of 66.66% in the recipient 3.

	Sample	CK (U/L) [25-35]	AST (U/L) [17-45]	ALT (U/L) [9-35]	GGT (U/L) [23-61]
Donor 1	Baseline	478	23	22	38
	Final	630	27	24	42
Donor 2	Baseline	492	25	30	27
	Final	542	24	27	27
Donor 3	Baseline	867	30	37	47
	Final	862	25	35	44
Recipient 1	Baseline	886	24	29	36
	Mid-surgery	1587	46	31	27
	Final	7962	594	46	19
Recipient 2	Baseline	2709	25	30	34
	Mid-surgery	3530	30	24	23
	Final	6945	857	41	16
Recipient 3	Baseline	922	26	25	41
	MID-surgery	2130	107	24	31
	Final	3200	1346	84	27

The levels of creatine kinase (CK), aspartate aminotransferases (AST), alanine aminotransferases (ALT) and gamma glutamyl transferase (GGT) were checked both before the start of surgery (baseline) and at the end of surgery (1-1.25 hrs for donors and 5-7 hrs for recipients). An additional mid-surgery sample (2-2.5 hrs) was also sent for analysis in the case of recipients. Normal values for Yorkshire swine are provided in parentheses in the first column.

Table 3: Serum enzymes levels in donors and recipients.

central line insertion and midline skin incision), but high activated clotting time (355 seconds) after routine heparinization (5000 IU i.v.), prior to aortic cannulation (Table 4).

Postmortem analysis and histopathology

Irrespective of the time taken for the donor-recipient surgeries, congestion of lungs, gross intestinal swelling with fluid engorgement and edema of intestinal walls (Figure 2; indicating progressive intestinal pseudo-obstruction/paralytic ileus), were apparent at the end of all surgeries. Postmortem histopathology showed hemorrhages and mild to moderate inflammatory infiltration in all the donor livers and transplanted hearts, (Figure 3). These were more severe in extent in heart biopsies taken at the end of transplantation surgery in recipients.

Discussion

Although we have not done genetic analysis or halothane contracture test, the induction of MH in our surgical cohort is obvious especially in the backdrop of a possible homozygosity due to in-breeding. The 4-5 fold elevation in CK levels over pre-transit values (25-35 U/L) in MH-susceptible swine after 16-18 hours of transportation is consistent with our finding of even higher CK levels (478-2709 U/L) after 48 hours of transportation [14]. Though surgical incisions can result in post-operative elevation in blood CK, the rapid intra-operative exponential rise in CK levels, particularly in surgeries with isoflurane anesthesia without cisatracurium, indicate underlying skeletal muscle pathology. While, CK, AST, ALT may remain normal in MH , elevations in AST, ALT, GGT and bilirubin have been described in heat stroke, a close correlative of MH [14-16]. Although, heart, liver, skeletal muscle or RBC's could be the source of aminotransferases, the rapid intra-operative elevations in AST in our surgeries, without evident acute liver damage or peripheral RBC lysis, could only be explained by ongoing skeletal muscle damage with dark colored urine (recipients 1 and 2) due to myoglobinuria. Moreover, despite the intraoperative maintenance of high peripheral SpO_2, the serum lactate levels were elevated (peak 6.85 mM/L in the 2nd recipient) indicating non-hypoxic glycolysis. This is consistent with reported pro-lactate metabolic shift due to rapid depletion of high energy phosphates, in a hypermetabolic state such as that of MH and the raised baseline BUN levels despite good renal function [10,17]. Taken together, these observations suggest a hypermetabolic state with skeletal muscle damage and support the notion of stress-susceptibility in the surgical cohort. Nevertheless, while considering MH, other conditions such as chronic muscle diseases need to be kept in mind [18]. Interestingly, while aminotransferases are elevated in chronic muscle diseases the AST elevations, due to its longer half-life, are more marked than CK [19]. Our finding of elevations only in baseline CK is thus, more in favor of MH-susceptibility.

While MH-susceptible swine are reported to have compensated respiratory acidosis, baseline samples in our study suggested, uncompensated respiratory alkalosis [20]. This is consistent with the development of respiratory alkalosis reported in MH-susceptible swine

	Sample	K^+ (mM/L) [3.9-4.9]	P (mg/dL) [6.9-7.2]	Ca^{2+} (mg/dL) [10.2-11.9]	BUN (mg/dL) [1.4-4.2]	Creatinine (mg/dL) [1-3]	T. Bil. (mg/dL) [0-0.7]	Albumin (g/dL) [1.8-3.4]	Hemoglobin (g/dL) [7.3-10.2]	Hematocrit (%) [23.5-33.0]
Donor 1	Baseline	3.9	8.5	10.7	5	1.3	0.3	4.9	8.5	25
	Final	4.7	9.1	10.3	7	1.4	0.3	3.8	7.8	23
Donor 2	Baseline	3.7	7.4	10.7	5	1.1	0.3	4.5	9.5	28
	Final	3.4	7.3	10	5	1.1	0.2	4	8.5	26
Donor 3	Baseline	4	8.7	11.3	7	1.3	0.3	5	10.5	31
	Final	4.1	8.7	11.9	9	1.4	0.3	4.3	9.9	29
Recipient 1	Baseline	4.3	7.1	10.4	8	1.3	0.3	5.4	10.9	32
	Mid-Surgery	6.2	5.9	7.5	9	0.8	0.3	3.8	12.6	28
	Final	4.1	4.3	7	9	0.9	0.3	1.9	8.8	26
Recipient 2	Baseline	3.9	7.6	11.5	5	1.1	0.3	5.2	9.9	29
	Mid-Surgery	5.1	6.3	8.5	6	1.1	0.3	3.5	10.2	30
	Final	3.5	3.3	14.6	6	0.9	0.3	1.8	9.2	27
Recipient 3	Baseline	4.4	8.1	9.8	10	1.6	0.3	3.9	9.2	27
	Mid-Surgery	3.5	6.7	8	9	1.2	0.2	2.4	8.5	26
	Final	4	8.5	11.7	10	1.3	0.2	2.9	8.2	24

Venous blood was analyzed for electrolytes potassium (K^+), phosphorus (P), calcium (Ca^{2+}), blood urea nitrogen (BUN), creatinine, total bilirubin (T. Bil.), albumin, hemoglobin and hematocrit. Samples were sent both before the start of surgery (baseline) and at the end of surgery (1-1.25 hrs for donors and 5-7 hrs for recipients). An additional mid-surgery sample (2-2.5 hrs) was also sent for analysis in the case of recipients. Normal values for Yorkshire swine are provided in parentheses in the top row.

Table 4: Serum electrolytes and other blood tests.

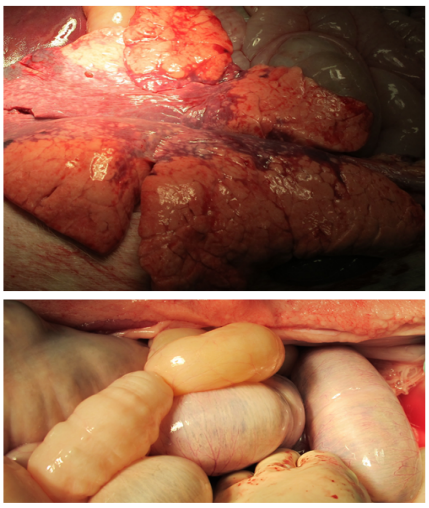

Figure 2: Gross morphology of internal organ (lungs and intestines) at the end of recipient surgeries.Images show the gross appearances of lungs (above) and intestines (below) from recipient 1, at the end of surgery. The lungs were congested while the intestinal loops were dilated and engorged with fluid and air.

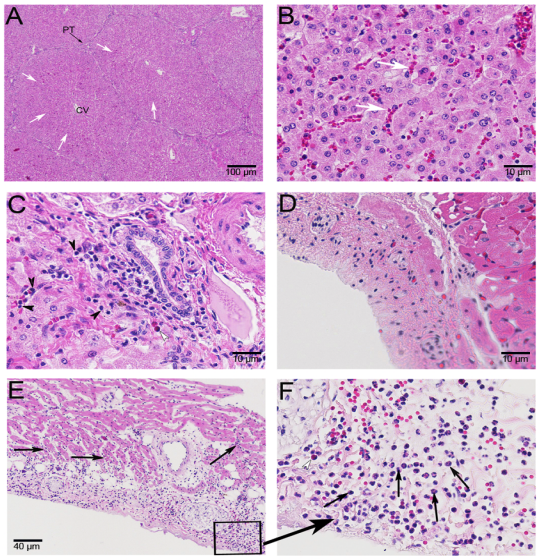

Figure 3: Histopathology of internal organs (liver and heart) in donors and recipients exposed to Isoflurane alone. Images show histopathology (HE staining) of a donor liver (A, B and C; magnifications 40X, 400X, 400X) and transplanted heart (D, E and F; magnifications 400X, 100X; Inset magnification) in animals that developed malignant hyperthermia. Figures 3A and 3B show congestion of liver sinusoids with greater severity observed in the zone 2 (white arrows) of liver lobules. Higher magnification also revealed the presence of lymphocytic infiltrates (3C; black arrowheads) with occasional eosinophils (3C; white arrowhead) in the region of portal triad. The endocardium of transplanted heart (3D) was devoid of any inflammatory infiltration but the epicardial side revealed infiltrates creeping into the myocardium (3E; black arrows) at several places. Magnified region shows neutrophilic infiltration (3F; black arrows) with occasional eosinophils (3F; white arrowhead). PT: Portal Triad; CV: Central Vein.

enduring heat-stress and mixed metabolic acidosis with compensatory respiratory alkalosis of Canine stress syndrome [21-23]. In our study, a mild CO_2 washout could have contributed to alkaline pH as the baseline samples were collected during ventilation. Intraoperatively, despite non-dramatic pCO_2 elevations, the percentage increase in blood pCO_2 was comparable to that in MH [24-26]. Additionally, in contrast to halothane, that causes rapid MH-dependent rise in blood pCO_2, with newer volatile anesthetic agents, this is more gradual and can be masked by adjustments of ventilator settings during surgery [27,28] On the contrary, a prolonged time in the building up of intraoperative end-tidal CO_2 ($ETCO_2$), an adjunct to arterial pCO_2 (elevated in recipients 1 and 2), and a high venous-arterial pCO_2 gradient (as in donors 1, 3 and recipient 1), are independent predictors of poor surgical outcomes [29,30]. Furthermore, contrary to its name malignant hyperthermia, the elevated body temperature in MH is not necessarily seen immediately in the intra-operative period, but can be post-operative or may not occur at all [31]. The core body temperatures in our surgeries were not elevated possibly due to the shorter durations of donor surgeries and the use of hypothermic CPB in recipients. Furthermore, in our surgeries, we manually altered the ventilator or CPB settings to adjust the minute volume and/or FIO_2 to maintain pCO_2 and SpO_2 within

acceptable limits and with an added hypothermia of CPB, could have masked the frank changes of MH, though the covert underlying progress of MH was not halted, regardless. This is consistent with the reported progression of MH, despite CPB-dependent hypothermia, with another halogenated anesthetic gas, sevoflurane and that the reversal of hypothermic-CPB by itself can exacerbate metabolism and induce MH [24,32].

Of all the volatile anesthetics, isoflurane has shortest induction period (median of 30 minutes) which explains the rapidity with which MH-dependent signs developed [33]. While non-depolarizing skeletal muscle relaxants are vastly used during surgeries, their role as MH-triggers is not well understood [34-36]. In our studies, the addition of cisatracurium to the protocol attenuated and delayed the apparent multi-systemic involvement and progression of MH despite comparable durations of surgery. While blood gases and blood lactate levels were more severely deranged, the postmortem findings of lung congestion and gross features of intestinal pseudo-obstruction were also greater in severity, in the absence of cisatracurium administration. Additionally, the elevations in total creatinine kinase in the blood, and fluctuations in serum electrolytes including K^+, Ca^{2+} and P were more evident in surgeries devoid of cisatracurium. Furthermore, inflammatory infiltration of solid organs and coagulation disturbances (in recipient 2) were consistent with a developing SIRS (Systemic Inflammatory Response Syndrome) and DIC (Disseminated Intravascular Coagulation) respectively, other but rare features of MH [5]. These findings are also consistent with previously reported congestion of lungs, cardiac hemorrhages and myofibrolysis in MH [37]. Apparently, mild inflammatory infiltration (in liver/heart) was also evident in donors 1 and 2, suggesting that the progress of SIRS started rapidly with isoflurane in the absence of cisatracurium. Nevertheless, the attenuated systemic and clinical signs during recipient 3 surgery, endorse the beneficial effect of cisatracurium in isoflurane-induced porcine MH. It is probable that, in the absence of cisatracurium and with extended exposures to isoflurane, all the donors eventually would have exhibited an MH-like scenario as well. The present study suggests that cisatracurium (and not necessarily the whole class of anti-paralytic agents), is not only a non-trigger of MH but could in fact attenuate the progression of MH during surgery. Further studies to corroborate our present findings, especially in clinical settings, are called for.

Conclusions

To the best of our knowledge, this is the first report where cisatracurium has been shown to be of direct benefit in MH by attenuating/delaying its progress. Compared to net blood pCO_2 levels, the fractional alterations in pCO_2 and base excess, and the intraoperative development of intestinal pseudo-obstruction, could be more sensitive early indicators of underlying progression of isoflurane-induced MH. Finally, due to the evident cellular infiltration of livers and hearts, as seen in our study, histopathologies in MH-susceptible research models should be interpreted with caution.

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