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Circulating cyclic adenosine monophosphate concentrations in milrinone treated paediatric patients after congenital heart surgery

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Abstract

Background: Milrinone is a phosphodiesterase type 3 inhibitor that results in a positive inotropic effect in the heart through an increase in cyclic adenosine monophosphate. The purpose of this study was to evaluate circulating cyclic adenosine monophosphate and milrinone concentrations in milrinone treated paediatric patients undergoing congenital heart surgery. *Methods:* Single-centre prospective observational pilot study from January 2015 to December 2017 including children aged birth to 18 years. Milrinone and circulating cyclic adenosine monophosphate concentrations were measured at four time points through the first post-operative day and compared between patients with and without low cardiac output syndrome, defined using clinical and laboratory criteria. *Results:* Fifty patients were included. Nine (18%) developed low cardiac output syndrome. For all patients, 22% had single ventricle heart disease. The density and distribution of cyclic adenosine monophosphate concentrations varied between those with and without low cardiac output syndrome but were not significantly different. Milrinone concentrations increased in all patients. Paired t-tests demonstrated an increase in circulating cyclic adenosine monophosphate concentrations during the post-operative period among patients *without* low cardiac output syndrome. *Conclusions:* In this prospective observational study, circulating cyclic adenosine monophosphate concentrations increased in those without low cardiac output syndrome during the first 24 post-operative hours and milrinone concentrations increased in all patients. Further study of the utility of cyclic adenosine monophosphate concentrations in milrinone treated patients is necessary.

Milrinone is a phosphodiesterase type 3 inhibitor with sites of action in the cardiac and vascular smooth muscle. Phosphodiesterase type 3 hydrolyzes the critical second messenger cyclic adenosine monophosphate and phosphodiesterase type 3 inhibition results in a positive inotropic effect in the heart through an increase in cyclic adenosine monophosphate.[1] Phosphodiesterase type 3 inhibitors cause relaxation of vascular smooth muscle and induce vasodilation thereby reducing ventricular afterload without an effect on oxygen consumption.[2] Milrinone is prescribed in paediatric and adult patients for the treatment of acute decompensated heart failure. It is also prescribed for the prevention of low cardiac output syndrome in children after congenital heart surgery.[3] Low cardiac output syndrome is defined in general as a decline in cardiac output 6–18 hours after cardiac surgery[4,5] and is characterised by tachycardia, hypotension, and end-organ dysfunction. The definition of low cardiac among published studies varies, resulting in variable incidence ranging from 25 to 80%.[3,5–7]

While standard dosing of milrinone is routinely used in children after cardiac surgery, the actual dose–response relationship is unknown. Early studies demonstrated a sigmoidal relationship between plasma milrinone concentration and percent cardiac index improvement across a range of 100–300 ng/ml. No additional improvement in cardiac index has been appreciated at concentrations above 500 ng/ml but there is an increased risk of adverse side effects including hypotension and systemic vasodilation.[8,9] Some paediatric and adult centres have the ability to measure milrinone concentrations for clinical use. However, significant variability in serum milrinone concentrations has been noted between patients despite similar weight-based dosing strategies.[10] This uncertainty in dosing is confounded by differences in patient age, size, and ontological maturation of the kidneys, which can be confounded by the fact that

cardiopulmonary bypass results in a significant proportion of patients experiencing acute kidney injury, whereby oliguria and decreased filtration results in elevated milrinone concentrations.[11–13] In the absence of the ability to identify the optimal dose, children are at risk for clinically relevant over or under-dosing of milrinone that can lead to haemodynamic compromise and end-organ dysfunction. In the landmark study that assessed the efficacy and safety of milrinone in preventing low cardiac output syndrome in infants and children after corrective surgery, there was a 64% relative risk reduction in the development of low cardiac output syndrome with prophylactic use of high-dose milrinone (0.75 μg/kg/min).[3] Given the reported known variability in milrinone concentrations in patients treated with milrinone, we sought to evaluate if cyclic adenosine monophosphate differed between those with and without low cardiac output syndrome. Cyclic adenosine monophosphate concentration increases in rabbit and human platelets in response to milrinone treatment, and milrinone increases cyclic adenosine monophosphate in rabbit myocardium and rabbit coronary artery smooth muscle cells.[14] Thus, we surmised that circulating cyclic adenosine monophosphate would serve as a surrogate for myocardial cyclic adenosine monophosphate in children undergoing congenital heart surgery.

The purpose of this prospective observational study was to evaluate circulating cyclic adenosine monophosphate and milrinone concentrations in milrinone treated paediatric patients undergoing congenital heart surgery. We hypothesised that cyclic adenosine monophosphate concentrations would depend on the presence of low cardiac output syndrome and that patients with and without low cardiac output syndrome would experience differences in circulating cyclic adenosine monophosphate concentrations over time.

Methods

We performed a single-centre prospective study of children up to the age of 18 years who underwent cardiac surgery with cardiopulmonary bypass and who had a Society of Thoracic Surgeons-European Association for Cardio-Thoracic Surgery Congenital Heart Surgery score ≥3[15] from January 2015 to December 2017. The study was approved by the University of Colorado Multiple Institutional Review Board and informed consent was obtained for each patient. Patients were excluded if they received milrinone pre-operatively.

Clinical data were collected pre-, peri-, and post-operatively. Society of Thoracic Surgeons-European Association for Cardio-Thoracic Surgery Congenital Heart Surgery score categories were included, and patients were further classified into the following categories: severe disease that included single ventricle patients at any stage of palliation, transposition of the great arteries; shunt – included atrioventricular septal defects and tetralogy of Fallot; valve defects – including repair or replacement of the tricuspid, mitral, pulmonary, or aortic valves; pulmonary vein repair; and abnormalities of the aorta including root dilation, and isolated arch hypoplasia with coarctation. Clinical outcomes included duration of ventilation, length of intensive care unit and hospital stay, and operative mortality. All patients underwent zero-balance/continuous ultrafiltration during the operation. Vasoactive inotrope score was calculated at each of the measured time points as previously described, and included milrinone.[16] The presence of low cardiac output syndrome was evaluated from 4 to 24 hours after intensive care unit admission by two independent reviewers with expertise in the management of critically ill children after cardiac surgery and was blinded to the patient clinical status and history. Reviewers reported either presence or absence of low cardiac output syndrome and were not required to provide criteria. Low cardiac output syndrome was defined as at least two of the following: an arterial-venous oxygen content difference >30% where the systemic saturation was determined using pulse oximetry and the venous oxygen saturation by cerebral near-infrared spectroscopy; metabolic acidosis as measured by an increase in base deficit by greater than 4; elevated lactate >2 mg/dl for 2 consecutive measures; tachycardia (>90% for age) for 2 consecutive hours based on the centre for disease control normative data for children[17]; systolic hypotension for 2 consecutive hours per the paediatric advanced life support criteria[18]; escalation of vasoactive medications over 2 consecutive hours; addition of new vasoactive medications; cardiac arrest. These criteria were adapted from the Prophylactic use of milrinone after cardiac operation in a paediatrics study.[3] Patients were assessed for arrhythmias (atrial, ventricular, or supraventricular) while on milrinone therapy and their temporal relationship to the onset of low cardiac output syndrome. A clinically significant arrhythmia was defined by the initiation of either short-term or long-term anti-arrhythmic treatment. Cardiac arrest was defined as that which occurred during the study period. Mortality was defined as that which occurred during the operative hospitalisation or within 30 days of discharge.

A loading dose of milrinone (25–75 μg/kg) was administered at the discretion of the surgeon and anesthesiologist simultaneously with the initiation of a milrinone infusion ranging from 0.25 to 0.75 μg/kg/min in the operating room. The milrinone infusion was continued in the cardiac intensive care unit and the dose was adjusted by the care team based on routine clinical care. Milrinone dosing was not standardised between patients.

Blood samples were collected pre-operatively and at four time points after cardiac surgery: cardiac intensive care unit admission, post-operative day 0 at 16:30, post-operative day 1 at 07:30, and post-operative day 1 at 12:00. Samples were not collected if the milrinone infusion had been discontinued. Timing of sample collection from milrinone initiation was also not standardised as sample processing had to be performed immediately, and we were constrained by the availability of qualified personnel. Blood samples were centrifuged at 200 × *g* for 10 minutes at 18°C and 120 μl of platelet-rich plasma was aliquoted into a cryovial and frozen for later quantification of milrinone concentration. The light blue top tube was then again centrifuged at 800 × *g* for 15 minutes at 18°C, and the platelet-poor plasma was extracted and frozen. Platelet poor plasma was stored at −80°C for future analyses. Plasma milrinone quantification was performed on the platelet-rich plasma using liquid chromatography/mass spectrometry-mass spectrometry (LC/MS-MS) as previously described.[12] 'Baseline' patient milrinone concentration was assessed from the ICU admission sample. Quantification of circulating cyclic adenosine monophosphate was performed using the platelet-rich plasma and a commercially available assay (cyclic adenosine monophosphate Parameter assay ELISA, R&D Systems, Minneapolis, MN). Quantification of circulating cyclic adenosine monophosphate was also performed using the platelet-poor plasma and it was not different (data not shown). Authors were blinded to low cardiac output syndrome status for the quantification of circulating cyclic adenosine monophosphate.

Given the pilot nature of this study, we did not perform a power calculation. Descriptive analyses (means, standard deviations (SD), median, interquartile range (IQR), and frequency distributions) were used to describe patient demographics in those with and

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without low cardiac output syndrome. Comparisons of demographics, operative and outcome characteristics by low cardiac output syndrome were done using Chi-square or Fisher's exact tests, two-sample independent t-tests, or Wilcoxon sum rank tests as appropriate. A violin plot was generated to show the distribution of cyclic adenosine monophosphate and milrinone over time in patients with and without low cardiac output syndrome. Paired t-tests were used to compare cyclic adenosine monophosphate between each time point in patients with low cardiac output syndrome and repeated for those without low cardiac output syndrome. The outcome was milrinone concentration, and besides known confounders, variables that had p-values <0.2 in unadjusted models were included in the final adjusted model. The following covariates were included in the model: milrinone dose, weight, age at surgery (dichotomized by the median, 3.9 months), kidney function (difference in glomerular filtration calculated using the paediatric Schwartz formula[19] between the pre-operative assessment and the lowest post-operative value coincident with other laboratory measures), circulating cyclic adenosine monophosphate, and LCOS. Interactions between circulating cyclic adenosine monophosphate and LCOS, and time and LCOS were tested. Milrinone concentration and cyclic adenosine monophosphate were both log-transformed due to skewness. Results were back-transformed and reported along with 95% confidence intervals and p-values. The significance level was set at 0.05 Statistical analyses were performed using SAS version 9.4 (Carey, NC) and R version 3.6.1. The Strobe guideline checklist was used in manuscript preparation (Supplemental Material I).

Results

A total of 50 patients were included in the study. The incidence of low cardiac output syndrome in this cohort was 18% (n = 9). There was no significant difference in patient demographics, diagnosis, and operative characteristics between patients with and without low cardiac output syndrome. Patients with low cardiac output syndrome had a significantly lower glomerular filtration rate compared to those without low cardiac output syndrome (p = 0.02). Mortality was significantly higher among those with low cardiac output syndrome (n = 2; 22%) compared to those without low cardiac output syndrome (n = 0; p = 0.03). Four (44%) patients with low cardiac output syndrome developed a post-operative arrhythmia while on milrinone compared to 10% (n = 4) of patients without low cardiac output syndrome (p = 0.03); however, arrhythmia was not the cause or a major contributing factor to low cardiac output syndrome in any case. Arrhythmia onset was after the onset of low cardiac output syndrome. Arrhythmias were characterised as ectopic atrial tachycardia, junctional ectopic tachycardia, or reentrant type supraventricular tachycardia. No patients experienced ventricular arrhythmias. Patient demographics, operative and post-operative characteristics, and outcomes are summarised in Table 1.

Figure 1a depicts a violin plot demonstrating the distribution of milrinone concentrations over time. There was not a significant difference in the median milrinone concentrations between those with and without low cardiac output syndrome over time. Figure 1b depicts a violin plot demonstrating the distribution of cyclic adenosine monophosphate over time among patients with and without low cardiac output syndrome. In patients without low cardiac output syndrome, there was an increase in the median and distribution of circulating cyclic adenosine monophosphate over time as demonstrated by the change in the width of the plot. In patients with low cardiac output syndrome, the median and probability density (width) did not change over each of the measured time points. Figure 2 demonstrates a heat map of the corresponding p-values for pair-wise changes of cyclic adenosine monophosphate levels within each group (low cardiac output syndrome and without low cardiac output syndrome) at each of the measured time points. The pair-wise differences were significantly different for patients without low cardiac output syndrome, whereas none of the pair-wise comparisons were different in the low cardiac output syndrome group.

In the multi-variable mixed model, milrinone concentration over time was found to be associated with milrinone dose, age, kidney function, and time since surgery. After adjusting for all variables in Table 2, for every 0.1 mcg/kg/minute increase in milrinone dose, the log milrinone concentration increased by 6% (95% CI: 0.1–11%; p = 0.046). Compared to intensive care unit admission, milrinone concentration was significantly higher on a post-operative day 1 at both time points (p < 0.0001). The milrinone concentration on a post-operative day 1 at 7:30 am was 55% higher than admission (95% CI: 37–75%, p < 0.001), and the milrinone concentration on a post-operative day 1 at 12:00 pm was 43% higher than admission (95% CI: 25–62%, p < 0.001). Compared to neonates, the milrinone concentration was 38% lower (95% CI: 23–50%; p < 0.001) in infants, and 49% lower in children (95% CI: 31%, 62%; p < 0.001). There was no association between log milrinone concentration and circulating cyclic adenosine monophosphate concentration, nor log milrinone concentration and low cardiac output syndrome.

Discussion

To our knowledge, this is the first study to assess circulating cyclic adenosine monophosphate concentrations in paediatric patients treated with milrinone after paediatric heart surgery. Despite the fact that milrinone concentrations were generally within the previously described therapeutic range of 100–300 ng/ml, some patients still experienced low cardiac output syndrome. There are several possible explanations for the presence of low cardiac output syndrome in the setting of 'therapeutic milrinone concentrations': a failure of cyclic adenosine monophosphate to increase in response to milrinone or the influence of phosphodiesterase type 3A polymorphisms in limiting milrinone efficacy,[20] or, that factors independent of phosphodiesterase type 3 were responsible for low cardiac output syndrome events. Importantly none of the patients who experienced low cardiac output syndrome had ventricular dysfunction or residual anatomic lesions that may have contributed, although echocardiograms were not performed at the time of low cardiac output syndrome.

The quantity of myocardial cyclic adenosine monophosphate needed to enhance myocardial contraction and relaxation and prevent low cardiac output syndrome is unknown. There is likely a ceiling effect of milrinone therapy once there is complete inhibition of phosphodiesterase type 3A enzyme activity, such that no additional milrinone will be beneficial; where, in fact, increasing milrinone may be detrimental due to loss of phosphodiesterase type 3A selectivity. In early studies evaluating milrinone concentration and percent improvement in cardiac index, the desired range of milrinone concentration for cardiac index improvement was 100–300 ng/ml. In these studies there was no improvement in the cardiac index above milrinone concentrations of 500 ng/ml, despite continued vasodilation and hypotension.[8,9] This ultimately

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Table 1. Patient demographics, operative and post-operative characteristics and outcomes

Variable	All (n = 50)	Without LCOS (n = 41)	LCOS (n = 9)	p-value
Demographics				
Age at surgery (months)	3.89 (0.23, 14.8)	4.04 (0.66, 17.36)	0.23 (0.2, 4.28)	0.25
Age categories				
Neonate (<1 month)	18 (36%)	13 (32%)	5 (56%)	0.43
Infant (≥1 month–1 year)	19 (38%)	17 (41%)	2 (22%)	
Child (≥1 year)	13 (26%)	11 (27%)	2 (22%)	
Weight (kg)	4.06 (3, 8.54)	4.27 (, 9.2)	3.08 (2.81, 3.79)	0.24
Male	28 (56)	21 (51)	7 (78)	0.27
Single ventricle	11 (22)	9 (22)	2 (22)	1.00
Diagnosis				
Coarctation/VSD or IAA/VSD	4 (8)	3 (7)	1 (11)	0.20
Pulmonary valve disease	6 (12)	4 (10)	2 (22)	
TGA	7 (14)	5 (12)	2 (22)	
Left sided obstructive lesions (single ventricle)	7 (14)	5 (12)	2 (22)	
Right sided obstruction (single ventricle)	4 (8)	4 (10)	0 (0)	
AVSD	10 (20)	9 (22)	1 (11)	
TAPVR	1 (2)	1 (2)	0 (0)	
Truncus Arteriosus	1 (2)	0 (0)	1 (11)	
Other	10 (20)	10 (24)	0 (0)	
STAT category				
3	23 (46)	18 (44)	5 (56)	0.18
4	22 (44)	20 (49)	2 (22)	
5	5 (10)	3 (7)	2 (22)	
Subclassifcation based on operation type				
Severe disease	16 (32)	12 (29)	4 (44)	0.87
Shunt	17 (34)	14 (34)	3 (33)	
Valve disease	5 (10)	5 (12)	0 (0)	
Pulmonary vein repair	3 (6)	3 (7)	0 (0)	
Aortic arch repair	9 (18)	7 (17)	2 (22)	
Operative Characteristics				
Cross clamp time (minutes)	88.06 ± 42.96	91.22 ± 45.31	73.67 ± 27.49	0.14
Cardiopulmonary bypass time (minutes)	161.4 ± 63.07	161.66 ± 62.59	160.22 ± 69.12	0.96
Deep hypothermic circulatory arrest time (minutes)	0 (0, 0)	0 (0, 0)	0 (0, 4)	0.52
Milrinone loading dose mcg/kg	49.94 (24.32, 50.12)	50 (25.45, 50.12)	42.22 (0, 51.11)	0.61
Milrinone starting infusion rate (mcg/kg/min)				
0.3 mcg/kg/min	1 (2)	0 (0)	1 (11)	0.33
0.5 mcg/kg/min	48 (96)	40 (98)	8 (89)	
0.7 mcg/kg/min	1 (2)	1 (2)	0 (0)	
Post-Operative Characteristics				
Milrinone Concentrations (ng/ml)				
CICU Admission	111.68 ± 36.77	112.67 ± 39.5	105.92 ± 12.58	0.43
POD 0 1630	120.93 ± 51.9	120.25 ± 55.75	124.9 ± 19.86	0.71
POD 1 0730	187.02 ± 108.05	183.38 ± 116.95	203.24 ± 54.85	0.45

(*Continued*)

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Table 1. (*Continued*)

Variable	All (n = 50)	Without LCOS (n = 41)	LCOS (n = 9)	p-value
POD 1 1200	170.82 ± 88.63	167.3 ± 95.34	186.08 ± 51.54	0.42
Absolute cAMP Concentrations				
CICU Admission	87.3 (74.6, 97.5)	87.4 (76.7, 100.8)	75.7 (68.2, 91.2)	0.30
POD 0 1630	91.5 (72.0, 101.5)	90.2 (72.0, 100.9)	94.9 (81.2, 101.4)	0.70
POD 1 0730	102.8 (87.9, 129.2)	102.7 (87.8, 127.6)	105.8 (94.8, 137.0)	0.66
POD 1 1200	104.9 (87.7, 130.4)	105.4 (88.3, 130.7)	101.2 (84.9, 129.0)	0.59
Highest AVDO2	35.05 ± 12.89	34.38 ± 11.73	37.86 ± 17.58	0.59
Highest Lactate	3.51 ± 2.19	3.11 ± 2.04	5.33 ± 1.96	0.01
Highest VIS	12.14 ± 5.45	11.15 ± 5.13	16.67 ± 4.69	0.008
Baseline GFR	69.14 ± 28.97	70.26 ± 27.32	64.01 ± 37.03	0.64
Lowest GFR	59.89 ± 25.39	62.97 ± 26.24	45.86 ± 15.31	0.02
Arrhythmia (yes)	8 (35%)	4 (10%)	4 (44%)	0.03
Outcomes				
CICU LOS (days)	3 (2, 5)	3 (2, 5)	3 (2, 4)	0.78
Ventilation duration (days)	1.96 (0.98, 2.9)	1.96 (0.96, 2.25)	2.21 (1.04, 3.33)	0.38
Mortality	2 (4)	0 (0)	2 (22)	0.03
Cardiac arrest	0 (0)	0 (0)	0 (0)	1
ECMO	1 (2)	0 (0)	1 (11)	0.18

Normally distributed continuous variables are summarised as mean with standard deviation and compared using t-tests. Non-normally distributed variables are summarised as median with interquartile range and compared using Wilcoxon rank-sum test. Categorical variables are summarised as number with percent and were compared using Chi-square or Fisher exact tests. AVDO2 = arterial-venous oxygen content difference; AVSD = atrioventricular septal defect; CICU = cardiac intensive care unit; ECMO = extracorporeal membrane oxygenation; GFR = glomerular filtration rate; IAA = interrupted aortic arch; kg = kilograms; LCOS = low cardiac output syndrome; LOS = length of stay; mcg/kg/min = micrograms per kilogram per minute; POD = post-operative day; TAPVR = total anomalous pulmonary venous return; TGA = transposition of the great arteries; VIS = vasoactive inotrope score; VSD = ventricular septal defect.

Table 2. Unadjusted and adjusted associations for log milrinone concentration

Milrinone concentration (n = 50)	Unadjusted exponential coefficient (95% CI)	p-value	Adjusted exponential coefficient (95% CI)	p-value
Milrinone dose, 0.1 mcg/kg/min	1.07 (1.01, 1.13)	0.02	1.06 (1.001, 1.11)	0.046
Milrinone concentration time, POD 0 1630 (Reference level = Admission)	1.06 (0.95, 1.18)	0.31	1.03 (0.92, 1.15)	0.57
Milrinone concentration time, POD 1 0730 (Reference level = Admission)	1.59 (1.41, 1.79)	<0.001	1.55 (1.37, 1.75)	<0.001
Milrinone concentration time, POD 1 1200 (Reference level = Admission)	1.48 (1.30, 1.69)	<0.001	1.43 (1.25, 1.62)	<0.001
Age at surgery (Ref:Neonate: <1 month)			0.62 (0.50,0.77)	<0.001
Infant (1 month to 1 year) Child (≥1 year)			0.51 (0.38,0.69)	<0.001
Log circulating cAMP			1.12 (0.88, 1.41)	0.35
Kidney function (baseline minus lowest GFR)			1.01 (1.00, 1.02)	0.001
Weight (kilograms)			1.00 (0.99, 1.01)	0.82
LCOS (Reference level = No LCOS)			0.91 (0.71, 1.18)	0.50

cAMP = cyclic adenosine monophosphate; CI = confidence interval; GFR = glomerular filtration rate; mcg/kg/min = micrograms per kilogram per minutes; POD = post-operative day.

resulted in a shift of the milrinone dose-cardiac index response curve to the right, where the negative effects were enhanced.

A possible explanation of the detrimental effects of milrinone as it relates to ventricular arrhythmias in adults may be hyperphosphorylation of the ryanodine receptor, even when milrinone concentrations are reportedly within the 'therapeutic range'.[21] Furthermore, at higher milrinone dosing, there may be an 'off-target' effect with inhibition of some phosphodiesterase type 4 isoforms that may promote heart failure and arrhythmias.[22,23] Arrhythmias after paediatric cardiac surgery are common, and

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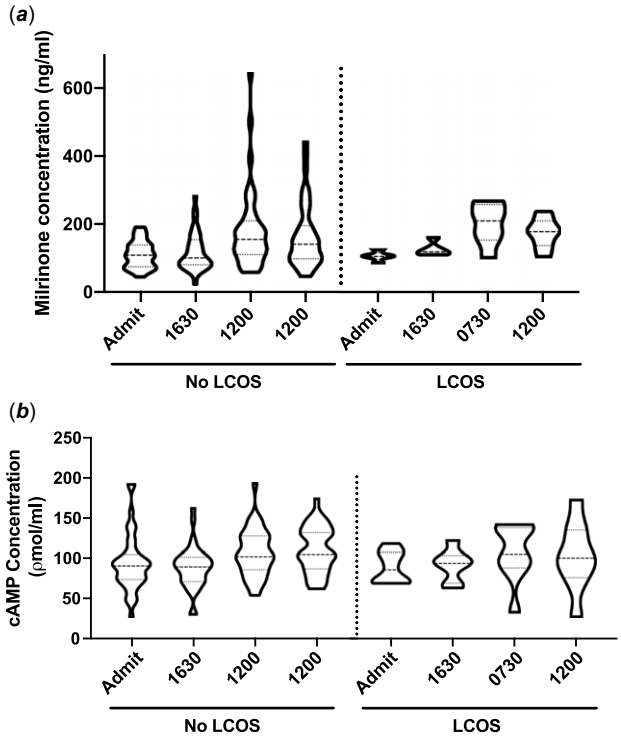

Figure 1. Violin plot demonstrating the probability density of milrinone (***a***) and circulating cyclic adenosine monophosphate (***b***) at different time points in patients with and without low cardiac output syndrome. (***a***) In this violin plot, a greater width corresponds to a greater density of data. The line in the centre represents the median, and the adjacent lines represent the interquartile range. The x-axis details the specific time points and the y-axis details the milrinone concentration (ng/ml). In patients without low cardiac output syndrome, there is a greater distribution of concentrations over time, while the density (width) does not change. In patients with low cardiac output syndrome, the density and distribution change most dramatically for the 2 measures on post-operative day 1. (***b***) In this violin plot, The line in the centre represents the median, and the adjacent lines represent the interquartile range. The x-axis details the specific time points and the y-axis details the circulating cyclic adenosine monophosphate (cAMP) concentration (pmol/ml). In patients without low cardiac output syndrome (left), there is an increase in the median and distribution of circulating cyclic adenosine monophosphate over time as demonstrated by the change in width. In patients with low cardiac output syndrome (right), the median and probability density (width) do not change over each of the measured time points.

in some reports occur at a rate of 15–50%.[24–26] In the study by McFerson et al, a higher milrinone dose in neonates after the Norwood operation was associated with tachyarrhythmia development, although both epinephrine and conduit type also contributed.[26] Similarly, Smith et al reported an association between milrinone and clinically significant arrhythmias following congenital heart disease surgery.[27] Nearly 20% of subjects in our study experienced atrial or supraventricular arrhythmias despite the fact that milrinone concentrations were predominantly within the 'therapeutic range' of 100–300 ng/ml.[8,9] A greater proportion of arrhythmias occurred in those with low cardiac output syndrome. Based on the temporal relationship between the onset of low cardiac output syndrome and arrhythmia onset, we do not believe that the arrhythmia was the cause of low cardiac output syndrome but could have resulted in its prolongation. Certainly, however, assessment of cyclic adenosine monophosphate concentrations and even milrinone concentrations may be less relevant in patients who develop low cardiac output syndrome as a consequence of incessant arrhythmia.

Our study found an increase in milrinone concentrations across time points among patients with and without low cardiac output syndrome and a difference in circulating cyclic adenosine monophosphate concentration across post-operative time points in those *without* low cardiac output syndrome. The increase in both circulating cyclic adenosine monophosphate and milrinone concentration over time, particularly in those without low cardiac output syndrome group may be related to a prolonged redistribution process that occurs during the first 12 hours of milrinone administration (context-sensitive half time, where the effective half-life increases as the length of time the administration increases).[28] In addition, we have identified a promoter polymorphism in the phosphodiesterase 3A gene that encodes myocardial phosphodiesterase type 3 and regulates cardiac myocyte cyclic adenosine monophosphate concentration. Patients homozygous for a phosphodiesterase 3A deletion polymorphism respond to phosphodiesterase 3 inhibition by increasing phosphodiesterase 3A mRNA expression and phosphodiesterase type 3 activity, effectively producing tolerance to the effects of phosphodiesterase type 3 inhibition.[20] Thus, this polymorphism is thought to contribute to heterogeneity in response to phosphodiesterase type 3 inhibitors. This may be relevant as we did not demonstrate any pair-wise differences in cyclic adenosine monophosphate concentrations across several time points in patients with low cardiac output syndrome (Fig 2), but there were differences in those without low cardiac output syndrome. The frequency of the deletion polymorphism in the myocardium of children has not been evaluated, but the role of this genetic variation certainly warrants further investigation. Furthermore, our study suggests that personalization of care could additionally be achieved by identifying a biomarker (e.g. circulating cyclic adenosine monophosphate) representative of the biologic response to milrinone. Indeed, future studies ideally would include comprehensive pharmacokinetic analysis, assessment of cardiac index (as a physiological marker of milrinone efficacy), and measurement of circulating cyclic adenosine monophosphate levels to further inform the optimal dosing of milrinone for individual paediatric patients. Importantly, if this poloymorphism is a contributing factor for 'non-responders', an optimal milrinone dose may not be found despite elevated milrinone concentrations, or cyclic adenosine monophosphate leading to the possibility of a greater side effect profile.

This study has several strengths and limitations. This was a prospective study that included a heterogeneous cohort of paediatric patients undergoing congenital heart surgery. There are several limitations: First, patient heterogeneity may impact milrinone dose and concentration and may have introduced type 2 error in view of the relatively small sample size of the study. The number of patients with low cardiac output syndrome was small ($n = 9$) and it is possible that circulating cyclic adenosine monophosphate concentration is more related to low cardiac output syndrome and not milrinone concentration. In addition, milrinone loading and maintenance dosing were not standardised, nor did we perform a milrinone pharmacokinetic evaluation. While only one patient in this study was on milrinone >0.5 mcg/kg/min, it has been demonstrated that at higher doses milrinone is a non-selective PDE inhibitor. Therefore, changes in circulating cyclic adenosine monophosphate in patients on higher doses of milrinone cannot be definitively attributed to PDE3-inhibition alone. The distribution of milrinone and cyclic adenosine monophosphate may be related to the small study sample size. Finally, it is important to acknowledge that the biology of circulating cyclic adenosine monophosphate is highly complex and that plasma cyclic adenosine monophosphate could (and probably does) come from tissues other than just the myocardium.

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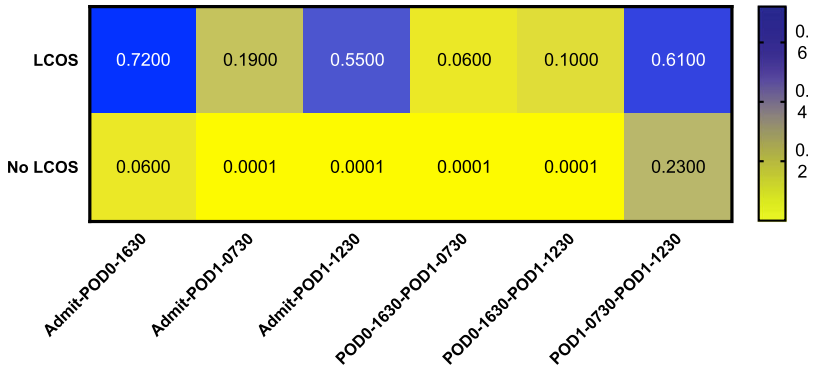

Figure 2. Heat map of paired t-test p-values comparing the difference in circulating cyclic adenosine monophosphate between time points in patients with and without low cardiac output syndrome. Labels on the x-axis represent the comparison groups. The top row is the p-value for comparisons made in those with LCOS. The bottom row is the p-value for the comparisons made in those without LCOS. In this figure, there is no difference in circulating cAMP across any of the compared time points in patients with LCOS. There was a significant increase in circulating cAMP in patients without LCOS except when comparison was made between admission and POD 0 1630 ($p = 1.00$) and between the two time points on POD 1 ($p = 0.91$).

In conclusion, in this prospective observational study, milrinone concentrations increase across post-operative time points in all patients, and circulating cyclic adenosine monophosphate concentrations differ between those *without* low cardiac output syndrome. Further study of cyclic adenosine monophosphate concentrations as a biomarker of milrinone efficacy, coupled with a pharmacokinetic analysis and an assessment of cardiac index as a physiological marker of milrinone efficacy in paediatric heart disease patients is warranted.

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Conflicts of interest. None of the authors have any relevant conflicts of interest.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on research in children and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the Colorado Multiple Institutional Review Board.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S1047951121000251

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