[¹¹C]ACETATE REST-STRESS PROTOCOL TO ASSESS MYOCARDIAL PERFUSION AND OXYGEN CONSUMPTION RESERVE IN A MODEL OF CONGESTIVE HEART FAILURE IN RATS

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Abstract

This study describes an [¹¹C]acetate rest/stress method to obtain an indirect estimate of myocardial blood flow (MBF) and myocardial oxygen consumption (MVO₂) in rats. Doxorubicin cardiotoxicity was used to test the usefulness of this approach for the assessment of congestive heart failure.

Methods—[¹¹C]acetate rest-stress have been used in clinical research to assess the capacity of the coronary arteries to respond to stress. In this article we used this approach to assess the cardiotoxicity of doxorubicin in a rat model. The method was first validated in a group of healthy rats and then used to follow the effect of doxorubicin chemotherapy on cardiac function. The effect of doxorubicin on myocardial perfusion and oxygen consumption reserve was measured at rest and under dobutamine stimulation.

Results—Validation of the protocol showed a good correlation between the MBF and MVO₂ ($r^2 = 0.68$). The doxorubicin treated group showed a significant ($p=0.04$) decrease in cardiovascular perfusion reserve at 1.3 ± 0.2 compared to the control animals at 1.6 ± 0.2 . Similar results were obtained for the myocardial oxygen consumption reserve (treated 1.8 ± 0.4 vs controls 2.3 ± 0.3 ; $p=0.02$).

Conclusions—We describe an [¹¹C]acetate PET rest/stress protocol for the assessment of cardiotoxicity in rat and its application to the follow-up of doxorubicin chemotherapy. This is a

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Conflict of Interest

The authors have declared relevant conflicts of interest.

rapid and reliable approach to the measurement of cardiac perfusion and oxygen consumption reserve that could be applied to the development of new strategies to reduce the cardiotoxicity of anthracycline.

Keywords

[¹¹C]Acetate; Cardiovascular Reserve; Myocardial Perfusion; Myocardial Oxygen Consumption; Rest/Stress Study; Small Animal PET Imaging; Dobutamine; Congestive Heart failure

Introduction

To sustain its contractile function, the healthy heart relies mainly on the aerobic oxidation of fatty acid substrates via the tricarboxylic acid cycle (TCA). However when the energy supply is modified by diet, exercise, stress or other factors, myocardial cells can modulate their metabolic activity, or favor alternative sources of energy such as glucose, amino acids or ketone bodies.

Due to the important role of acetate in energy metabolism, [¹¹C]acetate can be useful to explore myocardial metabolism [1–8]. When used with the proper kinetic models, [¹¹C]acetate PET imaging has the advantage of providing information on both myocardial blood flow (MBF) and myocardial oxygen consumption (MVO₂) simultaneously. In myocytes, [¹¹C]acetate enters the tricarboxylic cycle (TCA) where it is metabolized into acetyl-coA (beta-oxidation) yielding [¹¹C]CO₂, a process requiring the presence of NADH H⁺ and FADH₂ from TCA and leading to the production of ATP, which is the primary source of energy necessary for heart contraction [7–9]. Since the clearance rate of [¹¹C]acetate and its metabolites are correlated with oxygen consumption independently of the substrate type or of the physiological state of the subject [10,11], [¹¹C]acetate can be used to indirectly estimate myocardial MVO₂.

This promising method has already been used with success in clinical research for the assessment of ischemia, viability studies, dilated (non-ischemic) cardiomyopathy, aortic stenosis and heart transplantation evaluation [8]. Doxorubicin chemotherapy is known to induce a decrease in mitochondrial oxidative phosphorylation [12]. By providing an index of myocardial blood flow and oxidative metabolism, [¹¹C]acetate can therefore be used to detect changes linked to the pathophysiology of myocardial dysfunction by monitoring oxygen consumption and concomitant changes in myocardial perfusion.

In this article, we first confirmed the validity of [¹¹C]acetate rest/stress PET imaging protocol in conjunction with a three compartment kinetic model to measure MBF, MVO₂ and cardiac reserve in a rat model. We then applied this approach in a model of congestive heart failure induced by doxorubicin cardiotoxicity.

Materials and Methods

Animal model

The animal experiments were conducted in accordance with the recommendations of the Canadian Council on Animal Care and of the in-house Ethics Committee for Animal

Experiments. The [^{11}C]acetate rest/stress PET protocol was validated in a group of nine male Sprague-Dawley rats (Validation group) (Charles River) weighing 370 ± 30 grams. Five of these rats received dobutamine to increase their cardiac work and four rats received the beta-blocker metoprolol. With reference to previous studies, we experimentally selected doses of dobutamine or metoprolol sufficient to increase or decrease the basal heart function by 10%. Metoprolol is a negative chronotropic agent that decreases cardiac work and was selected to increase the range of cardiac workload values.

The application of the method to the assessment of doxorubicin cardiotoxicity was performed in a group of Fischer rats, weighing 235 ± 28 grams. Six rats received doxorubicin weekly, for six weeks (i.v. 2 mg/kg) (Doxorubicin group) before being imaged [13] while the six other rats were treatment free (Control group) weighing 230 ± 20 grams. The chemotherapy treatment dosage was established from previous studies that showed cardiac mitochondrial damage and histopathology and the weekly evaluation of an alteration in the left ventricular ejection fraction (LVEF). The decrease in LVEF, as measured from the cardiac gated [^{11}C]acetate image, was used as an index of doxorubicin induced myocardial dysfunction [12].

PET imaging

Isoflurane anesthesia (1.5%, 1 L/min oxygen, Abbott Laboratories) was delivered through a nose cone, and two venous catheters were installed into the caudal veins, one for the injection of the pharmacological agent and the other for the administration of [^{11}C]acetate. In the validation group, the arterial blood pressure and heart rate were monitored throughout the experiment with a pressure analyzer (Blood Pressure Analyzer 400, Micro-Med Inc.) connected to a membrane probe transducer attached to a PE50 polyethylene catheter (Becton Dickinson) inserted into the femoral artery. Two of the systolic pressure measurements could not be completed due to technical problems. For all groups, the body temperature, respiration and heart rates were monitored using external probes, and recorded for the entire duration of the experiment (SA Instruments Inc., NY, USA).

The validation of the protocol was done in healthy rats on the Sherbrooke Small Animal PET Scanner [14] capable of achieving an in-plane resolution of 2.1 mm. The application of the protocol to the assessment of doxorubicin cardiotoxicity was performed on a LabPET4 scanner [15,16] with a transaxial and axial field of view (FOV) of 11 cm and 3.75 cm, respectively, and a nearly isotropic resolution of 1.35 mm. The LabPET4 scanner was used in these latter experiments as it replaced the older Sherbrooke small animal PET scanner. A new control group was thus measured using the LabPET4 scanner to ensure that technical differences between the two scanners would not affect the data.

Rest/stress protocol

For the validation study, a 10-min PET list mode was used with an intravenous (i.v.) administration of 90 ± 10 MBq of [^{11}C]acetate. The pharmacological stress was induced using dobutamine (Novopharm) or metoprolol (Novartis Pharma). Following the completion of the rest list-mode acquisition, dobutamine administration was performed with three stepwise increases in the infusion rate (5 $\mu\text{g/kg/min}$ for 2 min, 10 $\mu\text{g/kg/min}$ for 2 min and

20 µg/kg/min for 9 min). The second injection of [^{11}C]acetate (90 ± 10 MBq) was initiated 5 minutes before the end of the dobutamine infusion and the stress dynamic PET imaging data were acquired for 10 minutes thereafter. Metoprolol stimulation was achieved by a constant infusion, 0.2 mg/kg over 10 minutes with the second injection of [^{11}C]acetate for PET imaging occurring after 5 minutes from the beginning of the administration of the pharmacological agent. For the doxorubicin study (on the LabPET4 scanner), the control and doxorubicin groups had each a 20-min list mode acquisition for the rest and stress conditions with a 10 minutes interval between the two scans.

Image derived input function (IDIF) and myocardium correction

The rest-stress protocol requires the administration of two consecutive injections of [^{11}C]acetate to the same rat, separated by a time interval of at least 20 minutes. Therefore, some residual radioactivity from the first injection still remained in the animal when the second dose of [^{11}C]acetate was injected (see Fig. 1a). The residual radioactivity was subtracted from the second [^{11}C]acetate injection by extrapolating the time activity curve (TAC) from the first [^{11}C]acetate injection. Correction for partial volume and spill-in effects were performed using the left ventricular blood volume coefficient [17]. The coefficients were evaluated by imaging a cylindrical phantom filled with water and containing spheres of different sizes (2.9, 4.5, 5.6, 6.9 9.5 13.1 and 17.4 mm diameter). For the assessment of spill in, the cylinder was filled with water containing radioactivity, while the spheres were cold. For the determination of partial volume, the spheres contained radioactivity while the cylinder was filled with plain water. Fig. 1b shows typical blood and myocardial time-activity curves corrected for these effects.

Data Analysis

For the validation protocol, a dynamic series of 22 frames was sorted out from the list-mode data, using the following sequence: 12×5 sec, 8×30 sec, 2×150 sec and the images reconstructed using the maximum likelihood expectation maximization (ML-EM) algorithm [18]. Tissue time-activity curves were then generated from a region of interest (ROI) drawn manually on the myocardium in the summed frames of the interval from 1 to 3 minutes and then copied to all frames of the rest/stress image series. The input blood curve was obtained from a ROI drawn on the left ventricular cavity (Fig. 1c). For the doxorubicin study, we selected a time series of 12×10 sec, 6×30 sec and 6×150 sec for the analysis and the images reconstructed using ML-EM algorithm. A three-compartment kinetic model was used for the computation of blood flow and myocardial oxygen consumption using the following equations [19,20]:

$$C_t(t) = [A_1 \cdot \exp(-(k_2 + k_3)t) + A_2 \cdot \exp(-k_4 t)] \otimes C_p(t) + v \cdot C_{tot}(t)$$

$$A_1 = K_1 k_2 / (k_2 + k_3)$$

$$A_2 = K_1 k_3 / (k_2 + k_3)$$

where C_t , C_p and C_{tot} are the tissue, plasma and total arterial time-activity curves, K_1 to k_4 are the rate constants and v is the blood volume. K_1 is related to myocardial blood flow, while k_2 is an index of MVO_2 . The activity due to metabolites in the plasma was estimated as:

$$C_p(t) = [1 - a_0 \cdot (1 - \exp(-t \cdot \log 2 / m))] \cdot C_{tot}(t)$$

where a_0 and m are parameters to be fitted for the presence of $[^{11}\text{C}]\text{CO}_2$ in the blood.

Left ventricular ejection fraction

For the control and doxorubicin groups, the list mode $[^{11}\text{C}]\text{acetate}$ PET data acquired with cardiac gating were analyzed in the interval from 60 to 240 sec with a sequence of eight electrocardiogram (ECG)-gated frames, selected on the basis of the R-R intervals, to extract the left ventricular volumes and ejection fraction. The Corridor4DM software from Segami Oasis (Columbia, MD, USA) was used for reorientation and cardiac data analysis.

Correlation of the index of myocardial oxygen consumption

Among the various parameters that have been proposed to measure the oxygen demand in healthy subjects [8], the heart rate-pressure product (RPP) (heart rate $\times$ systolic pressure) is recognized to be a good index of MVO_2 [8,21–23]. In the validation experiment, we were able to calculate the RPP using the heart rate and the measurements of the systolic pressure obtained with the arterial pressure analyzer. This value was correlated with the MVO_2 derived from the $[^{11}\text{C}]\text{acetate}$ measurements.

Statistical analysis

All data were expressed as mean $\pm$ standard deviation (SD). For the validation study, the determination coefficients (r^2) were used to evaluate the strength of the relationship between the MVO_2 and MBF and the MVO_2 and RPP difference. The results from the rest and pharmacological intervention measurements were compared using a two tail paired Student t -test. The changes induced by pharmacological intervention were calculated as follow: $1 - (\text{R/S}) \times 100$, where R represents the rest (baseline) and S the stimulated (dobutamine or metoprolol) values. The individual values from each animal were averaged and the data were reported as mean $\pm$ standard deviation (SD). For the doxorubicin model, the rest/stress were compared using a two tail paired Student t -test in both groups. To compare the myocardial perfusion and myocardial oxygen consumption reserve, a two tail unpaired Student t -test was used. Statistical analyses were performed with the PRISM 5.0 software package (GraphPad Software). A probability value equal to or less than 0.05 was considered significant.

Results

Myocardial perfusion reserve is a potentially useful indicator of cardiac health status. When derived from $[^{11}\text{C}]\text{acetate}$ PET imaging, indirect estimates of myocardial perfusion, myocardial oxygen consumption and cardiac reserve are obtained. Our first step was to validate this approach in healthy rats. We then evaluated its potential application for the measurement of congestive heart failure induced by doxorubicin treatment.

Validation of the rest/stress protocol in healthy rats

All pharmacological interventions induced a significant change (paired Student *t*-test $p < .05$) in MBF (K_1) and MVO_2 (k_2) (Fig. 2). The administration of the beta-blocker metoprolol ($N=4$) reduced myocardial blood flow ($26.3 \pm 14.0\%$), oxygen consumption ($13.2 \pm 3.7\%$) and heart rate ($11.9 \pm 9.7\%$). On the other hand, the inotropic and the chronotropic positive effect brought about by dobutamine ($N=5$) increased the myocardial blood flow ($60.8 \pm 69.5\%$), oxygen consumption ($107.2\% \pm 90.3\%$) and heart rate ($16.3\% \pm 10.6\%$). Furthermore, both the beta-blocker and dobutamine were found to slightly decrease the systemic blood pressure by $12.3 \pm 11.0\%$ and $9.9 \pm 2.5\%$, respectively (Table 1). In this rat model, we found that the relationship between MVO_2 and RPP remained true over the wide range of cardiac workloads ($r^2 = 0.60$) (Fig. 3). The RPP values decreased from the Rest values with beta-blocker ($-24.0 \pm 13.5\%$) and slightly increased above Rest values during stress ($+6.3 \pm 4.7\%$).

Assessment of cardiotoxicity in the doxorubicin group

Several detrimental effects of doxorubicin on the heart function integrity were highlighted under pharmacological stress. The weekly treatments of doxorubicin ($N=6$), resulted in a trend towards a reduction in MVO_2 (k_2) (1.6 ± 0.5 vs 2.4 ± 0.4 for controls, $p=0.06$) under dobutamine stimulation. The perfusion reserve (1.3 ± 0.2 vs 1.6 ± 0.2 for controls, $p=0.04$) and oxygen consumption reserve (1.8 ± 0.4 vs 2.3 ± 0.3 for controls, $p=0.02$, unpaired *t*-test) were significantly altered (Fig. 4 and Table 2). Similarly, as expected, the left ventricular ejection fraction at baseline was diminished ($38.3 \pm 4.9\%$ vs $58.6 \pm 5.4\%$ for controls).

Discussion

In this paper we describe a rest/stress [^{11}C]acetate PET imaging protocol that can effectively yield measurement of the myocardial blood flow [3,4,6,24–26], oxygen consumption [2,8,10,11,19,24,25,27] and cardiac reserve across many different conditions in the rat. Several rest/stress methods have been developed for myocardial PET studies, including [^{82}Rb]-Rubidium which was used in conjunction with dipyridamole, adenosine or dobutamine [28,29]. [^{15}O]H $_2$ O with adenosine was also used to investigate the nonischemic dilated cardiomyopathy [30]. Another approach was to use [^{13}N]ammonia with dipyridamole to investigate the effect of long-terms inhibition of the angiotensin converting enzyme or Beta blocker [31]. The challenge here was to adapt this procedure to a rat model while retaining a reliable correlation between perfusion and oxygen consumption values under pharmacological stimulation. In developing this model several factors had to be considered such as the faster heart beat of the rat, the transit time of [^{11}C]acetate in the rat myocardium, the effect of the anesthetic agent, the length of the protocol and the residual radioactivity from successive radiotracer injections. We had to be sure that the LVEF, cardiac reserve of MBF and MVO_2 measured in the rat were reliable and amendable to the assessment of the cardiac function in the rat over a wide range of values. This protocol can be applied to the assessment of congestive heart failure induced by doxorubicin. Because they are non invasive, [^{11}C]acetate imaging studies are well suited to perform test-retest experiments in the same subject. With the advent of high resolution small animal PET scanners, this rest/stress technique has the potential to become an important tool in the

evaluation of cardiomyopathy and for the development of new cardioprotective drugs. Furthermore, the strength of the method was demonstrated over a wide range of cardiac workload to validate its suitability for the measurement of congestive heart failure.

PET imaging with [^{11}C]acetate in the rat is challenging because of the short cardiac transit time of [^{11}C]acetate [24] and the rapid heart rate (300 ± 42 BPM) of the rat compared to other animal models such as the dog (122 ± 51 BPM) [11] or to experiments conducted in human subjects (75 ± 14 BPM) [22]. The best target/background ratio in the rat myocardium was reached between the first and third minutes after administration of the radiotracer. Therefore, for kinetic modeling, the use of list mode data acquisition was an important asset to allow a proper selection of time frames over such a short time interval [32]. Despite the small size of the rat heart and ventricular cavity, the high quality images that were obtained using the Sherbrooke Small Animal PET and the LabPET4 scanners made it possible to draw accurate ROI for extracting the tissue and blood input curves in both the rest and stress studies.

In order to assess the potential of [^{11}C]acetate over a wide range of cardiac workload, we used metoprolol to decrease the heart rate and dobutamine to increase it. Metoprolol, an antagonist of the β_1 receptor, is also used to antagonize the tachycardia induced by catecholamines, by decreasing the heart rate, contractility and mean blood pressure [33]. Dobutamine on the other hand, is an inotropic positive drug that increases the frequency and the strength of the contractions by acting on the β_1 -adrenergic receptors while at the same time causing a vasodilatation of the peripheral blood vessels via the β_2 receptors [34]. In this study, we chose to administer low doses of beta-blocker (0.2 mg/kg i.v.) and dobutamine (infused at a slower rate of 20 $\mu\text{g/kg/min}$), unlike other experimental protocols which used up to 0.5 mg/kg [35] and 40 $\mu\text{g/kg/min}$ [34], respectively, in human studies. Although beyond the scope of the present work, it would be of interest to repeat these experiments at higher doses to extend the range of myocardial workload at which the perfusion and oxygen consumption can be measured with [^{11}C]acetate.

A 3-compartment model which takes into account the influence of the [^{11}C]acetate metabolites ([^{11}C]O₂) on the blood curve [19] was used to calculate MBF and the MVO₂. Since the heart was imaged with [^{11}C]acetate before the administration of the drug (rest) and again after the pharmacological intervention (stress), it was necessary to correct the data for residual radioactivity remaining from the first [^{11}C]acetate administration.

A longer delay allowing further physical decay and biological clearance of the residual activity before the second injection would have resulted in a longer period of anesthesia. It has been reported that anesthesia led to a decrease in the systemic blood pressure compared to the values obtained with in conscious cats [36]. We believe that this effect is brought about by the use of isoflurane 1.5% while the animal is under spontaneous breathing conditions [37]. This unavoidable effect of anesthesia, which may affect the baseline values of MBF and MVO₂, did not mask the effects of the drugs used in this study with the observation of a significant impact of pharmacological action on MBF and MVO₂ values determined with [^{11}C]acetate.

The measurement of MBF and MVO_2 were highly correlated ($r^2 = 0.68$) and followed the heart rate and rate-pressure product over a wide range of cardiac workload (Fig. 3). The indirect estimates of the myocardial perfusion obtained in this work using the [^{11}C]acetate method were similar to those previously reported in the rat with [^{13}N]ammonia at 3.8 ± 0.9 mL/g/min (N=10) and with [^{11}C]acetate at 4.0 ± 0.8 mL/g/min (N=12) under similar isoflurane anesthesia [38].

The heart rate, the arterial blood pressure and the rate pressure product (RPP) index were used as reference values to assess the effect of pharmacological interventions. A close correlation was found between these physiological parameters and the MBF and MVO_2 values measured at rest as well as during adrenergic stimulation and inhibition, with the exception of the arterial blood pressure (Table 1). We noticed that either positive or negative stimulation of the beta-adrenergic receptor led to a decrease in mean systemic blood pressure, although with dobutamine, this effect did not reach statistical significance ($p = 0.08$). Nevertheless, the RPP, which is known to be well correlated with the oxygen consumption [23], was found to hold true even when the blood pressure was decreased through pharmacological manipulation. In the current study, we were therefore able to confirm the relationship between the RPP and myocardial oxygen consumption.

Chemotherapy treatment with doxorubicin is well known to have a detrimental impact on cardiac health. Overtime, doxorubicin induces a cardiomyopathy resulting from myocytes apoptosis [12,39]. The damage is linked to oxidative stress at the level of the mitochondria that will interfere with oxidative phosphorylation. The [^{11}C]acetate rest-stress protocol described here thus allowed us to directly and non-invasively monitor mitochondrial metabolism and at the same time to measure the myocardial perfusion reserve.

Conclusion

The measurement of myocardial blood flow (MBF) and oxygen consumption (MVO_2) in the rat using [^{11}C]acetate was found to be sensitive and appropriate for the evaluation of myocardial perfusion. This method, although developed for human applications, can find several applications in small animal research, such as the evaluation of therapeutic angiogenesis promoting revascularization in ischemic tissues, the remodeling after a myocardial infarction, the inflammation in the coronary arteries or the assessment of cardioprotective drugs. The small animal PET imaging rest/stress protocol presented here makes it is possible to reliably measure myocardial perfusion and the rate of oxidative metabolism over a wide range of cardiac workload. This method was also found suitable for the measurement of the impact of doxorubicin chemotherapy on cardiac myocytes.

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Fig. 1.

(a) Correction of the second $[^{11}\text{C}]$ acetate injection for the residual radioactivity from the first injection. The correction was made by subtracting the remaining extrapolated radioactivity of the first $[^{11}\text{C}]$ acetate injection from that of the second injection using a compartmental model. (b) $[^{11}\text{C}]$ acetate tissue and blood time activity curves at rest (left) and under dobutamine induced stress (right). (c) Heart images of a Fisher male rat (197 grams) treated with doxorubicin (2 mg/kg/week), for 6 weeks, following i.v. administration of $[^{11}\text{C}]$ acetate under rest (105 MBq) or dobutamine stimulation (107 MBq). The images were constructed from the 60 to 180 sec time interval of a dynamic study. Horizontal long axis (HLA), Vertical long axis (VLA) and short axis (SA) images for the rest and stress conditions are shown.

Fig. 2.

Box and Whisker Plot of the myocardial perfusion (a) and myocardial oxygen consumption (b) in the validation of the healthy rat group. Metoprolol (N=4), Dobutamine (N=5). *Paired two tail Student *t*-test, $p < .05$ for rest vs. pharmacological intervention.

Fig. 3.

(a) Correlation between the indirect estimate of myocardial blood flow (MBF) and myocardial oxygen consumption (MVO_2). (b) Correlation between the indirect estimate of myocardial oxygen consumption (MVO_2) and the rate pressure product (RPP) difference for negative (Beta-blocker) and positive (Dobutamine) pharmacological stimulation.

Fig. 4. Box and Whisker Plot in the doxorubicin group (2 mg/kg for 6 weeks) (N=6), compared to the control (N=6) rat heart. (a) Myocardial blood flow, (b) myocardial oxygen consumption and (c) cardiac reserve. *Paired two tail Student *t*-test, $p < .05$ for rest vs. pharmacological intervention and unpaired two tail Student *t*-test, $p < .05$ for cardiac reserve doxorubicin group vs. control.

Table 1

Hemodynamics and kinetics of Stress/Rest [¹¹C]acetate protocol validation

Control group (N = 9)	Rest	Beta-Blocker (N=4)	Rest	Stress (N=5)	
MBF (<i>K_f</i>) (min ⁻¹)	3.5 ± 1.0	2.6 ± 0.7	4.2 ± 0.7	6.6 ± 2.4	p=0.08
MVO2 (k2) (min ⁻¹)	1.3 ± 0.5	1.2 ± 0.4	* 1.4 ± 0.5	2.6 ± 0.3	*
Heart Rate (BPM)	304 ± 47	265 ± 23	p=0.12 299 ± 42	345 ± 29	*
Systolic pressure (mm Hg)	86 ± 23	80 ± 6	n=3 94 ± 13	89 ± 13	* [‡]
Diastolic pressure (mm Hg)	64 ± 18	61 ± 14	n=3 68 ± 10	60 ± 10	* [‡]
Mean pressure (mm Hg)	78 ± 3	67 ± 9	p=0.12 78 ± 8	70 ± 7	* [‡]
RPP ^{‡‡}	23576 ± 2541	16463 ± 2648	n=3 29722 ± 4906	31531 ± 4887	[‡] p=0.08

* Paired 2-tailed *t* test, rest vs pharmacological intervention, *p* < 0.05.

[‡] N = 4;

^{‡‡} RPP, rate pressure product

Table 2MPI, MVO₂ and myocardial reserve for the Treated and Control groups

	Control (N=6)	Treated* (N=6)	p value [†]
MPI (<i>K₁</i>)			
Rest (min ⁻¹)	3.3 ±0.4	3.2±0.5	0.83
Stress (min ⁻¹)	5.3±1.4	4.3±1.1	0.28
MVO ₂ (<i>k₂</i>)			
Rest (min ⁻¹)	1.0±0.2	0.9±0.2	0.55
Stress (min ⁻¹)	2.4±0.4	1.6±0.5	0.06
MPI reserve ^{††}	1.6±0.2	1.3±0.2	0.04
MVO ₂ reserve ^{††}	2.3±0.3	1.8±0.4	0.02

* Weekly treatment (2 mg/kg i.v. doxorubicin) for 6 weeks.

[†] Unpaired 2-tailed *t*-test, control vs. treated groups.^{††} Reserve was calculated as the ratio of Stress to Rest condition.