Poly(ADP-ribose) polymerase regulates myocardial calcium handling in doxorubicin-induced heart failure

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Abstract

Reactive oxygen and nitrogen species are overproduced in the cardiovascular system in response to the exposure to doxorubicin, a cardiotoxic anticancer compound. Oxidant-induced cell injury involves the activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) and pharmacological inhibition of PARP has recently been shown to improve myocardial contractility in doxorubicin-induced heart failure models. The current investigation, by utilizing an isolated perfused heart system capable of beat-to-beat intracellular calcium recording, addressed the following questions: (1) is intracellular calcium handling altered in hearts of rats after 6-week doxorubicin treatment, under baseline conditions, and in response to oxidative stress induced by hydrogen peroxide exposure in vitro; and (2) does pharmacological inhibition of PARP with the phenanthridinone-based PARP inhibitor PJ34 affect the changes in myocardial mechanical performance and calcium handling in doxorubicin-treated hearts under normal conditions and in response to oxidative stress. The results showed a marked elevation in intracellular calcium in the doxorubicin-treated hearts which was normalized by pharmacological inhibition of PARP. PARP inhibition also prevented the myocardial contractile disturbances and calcium overload that developed in response to hydrogen peroxide in the doxorubicin-treated hearts. We conclude that PARP activation contributes to the development of the disturbances in cellular calcium handling that develop in the myocardium in response to prolonged doxorubicin exposure.

Keywords

Peroxynitrite; Poly(ADP-ribose) polymerase (PARP); Adriamycin; Heart failure; Superoxide; Calcium

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1. Introduction

Overactivation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) represents a novel mechanism of cell dysfunction in various pathophysiological conditions associated with the oxidative stress. Evidence is accumulating that in the myocardium, PARP activation is associated with ischemia-reperfusion injury, cardiopulmonary bypass, heart transplantation, circulatory shock, diabetes mellitus and side effects of a variety of drugs overviewed in [1,2].

Cardiotoxicity is a prominent and dose-limiting side effect of the anticancer drug doxorubicin. The cardiotoxic effects of doxorubicin have been extensively investigated. The effects on mechanical performance of the heart have been described; a variety of pathogenetic mechanisms have also been elucidated (including mitochondrial dysfunction, free radical production, changes in iron handling) and the cellular and molecular mechanisms of the myocyte apoptosis and necrosis have been characterized in much detail overviewed in [3-6]. Recent work demonstrated the close relationship between oxidative and nitrosative stress and PARP activation in doxorubicin-treated animals as well as in humans undergoing doxorubicin anticancer therapy [7-9]. Since proper calcium handling is of utmost importance for adequate cardiac mechanical performance, it remains to be explored whether alterations in cardiac myocyte calcium homeostasis are involved in doxorubicin-induced cardiomyopathy.

The current investigation, by utilizing an isolated, perfused heart system capable of beat-to-beat intracellular calcium recording, addresses the following questions: (1) is intracellular calcium handling altered in hearts of rats after 6-week doxorubicin treatment, under baseline conditions, and in response to oxidative stress induced by hydrogen peroxide exposure in vitro; and (2) does pharmacological inhibition of PARP mitigate or prevent the changes in myocardial mechanical performance and calcium handling? To inhibit the catalytic activity of PARP, the potent phenanthridinone-based PARP inhibitor compound, PJ34 was used [10].

2. Materials and methods

2.1. General

Rats were treated with a weekly subcutaneous injection of doxorubicin for six weeks. The PARP inhibitor PJ34 was administered in the drinking water of the animals throughout the experimental period. Six weeks after the onset of treatment, the hearts were excised and perfused according to Langendorff with Krebs–Henseleit solution. Measurement of left ventricular pressure was accomplished via introduction of a balloon into the left ventricle and coronary flow was obtained with an ultrasonic transducer. Indo-1 fluorescent dye was loaded into the heart for the determination of intracellular calcium concentration. Cyclic changes of intracellular free calcium levels (Ca_i^{2+} transient) were measured using surface fluorometry. The hearts were subjected to an oxidative stress with the application of H_2O_2 (600 μM). Hemodynamic performance and functional parameters of intracellular calcium cycling were assessed.

2.2. Animal used—induction of cardiomyopathy

Male Sprague-Dawley rats ranging in body weight from 100 to 120 g were maintained in an animal facility with a 12-h light:12-h dark cycle with free access to food and water. Experiments on animals followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the study protocol was approved by the Laboratory Animals Committee of the Semmelweis University of Budapest.

Animals were allotted to four different experimental groups. Rats were treated with 2.5 mg/kg doxorubicin injection subcutaneously [11] once a week for six weeks (DOX Group: $n = 5$).

Age-matched controls received saline only (Control Group: $n = 6$). In the remaining two experimental groups, doxorubicin-treated rats and their age-matched controls were given 20 mg/BW PJ34, dissolved in water to drink during the treatment period (DOX-PJ Group: $n = 5$, Control-PJ Group: $n = 6$, respectively). There were no differences in the amount of PJ34 intake between groups. The current treatment protocol has previously been shown to achieve maximal inhibition of myocardial PARP activity in vivo [12,13].

2.3. Langendorff heart preparation

The general procedure to measure hemodynamic parameters and fluorescence signals in a beating heart preparation was outlined previously in detail [14].

Six weeks after the initiation of doxorubicin and drug treatments, the animals were anesthetized by 40 mg/kg i.p. pentobarbital (Nembutal–Sanofi, Budapest, Hungary). Hearts were quickly removed and immediately rinsed in cold Krebs–Henseleit solution void of $CaCl_2$ and subsequently mounted on a Langendorff perfusion apparatus (Experimetria Ltd., Budapest, Hungary). Retrograde aortic perfusion was initiated with a modified Krebs–Henseleit solution containing 118 mM NaCl, 4.3 mM KCl, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 0.5 mM NaEDTA, 2.0 mM $CaCl_2$, 11 mM glucose, 5 mM pyruvate (all chemicals purchased from Sigma, Budapest, Hungary) which was equilibrated with 95% O_2 and 5% CO_2. The temperature of the buffer was maintained at 37 °C and pH was adjusted to 7.4. Perfusion pressure was set at 70 mmHg. Throughout the experiments, the hearts were immersed in a bath of Krebs–Henseleit solution kept at 37 °C.

Coronary flow was measured with an ultrasonic transducer (T-106, Transonic Systems Inc., Ithaca NY, USA) inserted into the perfusion line. Left ventricular pressure was measured using a latex balloon inserted through the mitral valve orifice into the left ventricle and connected to a pressure transducer (Electromedics Inc., Englewood, CO, USA). Left ventricular end-diastolic pressure (EDP_{lv}) was set between 5 and 10 mmHg by adjusting the balloon volume. The left ventricular pressure (P_{lv}) was assessed to determine the systolic, diastolic and developed (systolic minus diastolic) pressures and heart rate. The maximal positive and negative first derivative of pressure ($+dP_{lv}/dt_{max}$, $-dP_{lv}/dt_{max}$) were obtained as indices of inotropic and lusitropic state of the heart, respectively.

2.4. Indo-1 AM loading and fluorescence measurement

Hearts were allowed to equilibrate for 30 min before loading of the dye. Two hundred and fifty micrograms of Indo-1 AM was solubilized in a mixture of 0.25 ml dimethylsulfoxide and 0.08 g pluronic F-127 and added to 40 ml Krebs–Henseleit solution containing 118 mM NaCl, 5.5 mM KCl, 1.2 mM $MgSO_4$, 25 mM HEPES, 11 mM glucose, 5 mM Na pyruvate, and 1.5 mM $CaCl_2$. The final concentration of Indo-1 AM was 6.25 μM. The hearts were perfused in a recirculating mode with a peristaltic pump (Cole-Palmer Co., Chicago, IL, USA) connected to the aortic cannula. After 20 min of loading, fluorescence signals reached maximum levels and a washout period started in normal Langendorff mode for 15 min with the modified Krebs–Henseleit solution to eliminate fluorescence originating from Indo-1 AM present in the extracellular compartment.

Fluorescence excitation was provided by a 100 W d.c. mercury arc lamp (Oriel, Stratford, CT, USA). Illumination was filtered at 340 nm and directed through a randomized multifurcated fiber optic light guide onto a circular region of the epicardium of the left ventricular wall. The emitted light was detected by photomultiplier tubes (Electron Tubes Ltd., Ruislip, Middlesex, England). Fluorescence emission was recorded at 400 nm (Ca_i^{2+}-bound dye) and 506 nm (Ca_i^{2+}-free dye). Reflectance was also detected at 340 nm for the estimation of non-specific changes in tissue density (optical artifacts). To minimize UV exposure of the tissue and photo-

bleaching of the intracellular dye, a shutter positioned in front of the excitation light was opened for approximately 1 min in every 5 min. Both the fluorescence and reflectance signals together with the hemodynamic parameters were recorded and stored with a multichannel AD converter (Hemosys, Experimetria, Budapest, Hungary) coupled to an IBM based computer [14].

2.5. Data analysis and interpretation

The ratio of the fluorescence signals at 400 and 506 nm is proportional to the cytoplasmic free calcium level. The individual fluorescence signals were corrected for closed shutter background and tissue autofluorescence of the unloaded tissue. Previously, we have shown that using this loading procedure Indo-1 is not sequestered into the mitochondria, hence, the apparent fluorescence signal is mainly of cytoplasmic origin [14]. Ca_i^{2+} concentration was calculated with the formula determined by Grynkiewicz et al. [15]. The Indo-1 dissociation constant (K_d) for calcium was determined previously at 844 nM [16]. The fluorescent ratios at zero Ca_i^{2+} (R_{min}) and at saturating Ca_i^{2+} (R_{max}) were determined in separate experiments. Two control hearts loaded with Indo-1 were treated with 20 μm; BAPTA-AM to define R_{min} and two other control hearts were infused with 1 μM 4-bromo-calcium-ionophore (A23187) to determine R_{max}. The calcium transient was assessed to determine systolic and diastolic Ca_i^{2+} and Ca_i^{2+} amplitude. Maximal rates of rise of Ca_i^{2+} ($+dCa_i^{2+}/dt_{max}$) and decline of Ca_i^{2+} ($-dCa_i^{2+}/dt_{max}$) were obtained as indices for the rate of calcium release from the sarcoplasmic reticulum (SR) and sequestration back into the SR, respectively.

2.6. Perfusion protocol

After loading of the dye and 15 min washout, the fluorescence signals were recorded in the "basal" situation. Thereafter, the hearts were subjected to an oxidative stress by adding 600 μM H_2O_2 to the perfusion medium. To this end, the H_2O_2 solution was infused into the aortic line via a side port. Hemodynamic parameters and fluorescence signals were recorded about 2 min after the start of infusion. H_2O_2 is an oxidative agent and as such, it also may react directly or indirectly with the calcium tracer, Indo-1, causing deterioration of the fluorescence signal. As a consequence of this, a few hearts not suitable for analysis of the fluorescence signal due to low signal/noise ratios were omitted from the groups subjected to H_2O_2 challenge because in these hearts Indo-1 fluorescence quenched within the first 2–3 min of H_2O_2 infusion (Control, $n = 4$; DOX, $n = 5$; Control-PJ, $n = 3$; DOX-PJ, $n = 4$). The hearts were removed from the cannula at the end of the experiments, wiped off with a paper towel and weighed to determine heart mass.

2.7. Reagents

All reagents were obtained from Sigma–Aldrich (St. Louis, MO and Budapest, Hungary), unless indicated otherwise. The potent, novel, water-soluble phenanthridinone derivative PARP inhibitor, PJ34—the hydrochloride salt of *N*-(-oxo-5,6-dihydro-phenanthridin-2-yl)-*N,N*-dimethylacetamide—was synthesized as described [10].

2.8. Statistics

All data are expressed as mean value ± S.D. Statistical analysis was carried out using one-way analysis of variance followed by Fisher post hoc test. A statistically significant difference of $p < 0.05$ was accepted throughout. Effects of H_2O_2 infusion were analyzed by Student's *t*-test and Fisher post hoc test as appropriate ($p < 0.05$).

3. Results

Six weeks of DOX treatment induced the expected changes in the animals [9,17]. As indicated in Table 1, the body and heart mass was markedly reduced as compared to their age-matched counterparts. Heart weight/body mass ratio of the animals was unaffected by DOX treatment.

3.1. Cardiac mechanical performance

Treatment with DOX for six weeks did not induce significant changes in the hemodynamic parameters of the Langendorff-perfused hearts under basal conditions (Fig. 1, Table 2).

Two minutes of oxidative stress (600 μM H_2O_2) impaired hemodynamic performance of the hearts. The effects of the oxidant were found to be more pronounced in the hearts of the animals that had previously received doxorubicin (Fig. 1). Pre-treatment of the animals with PJ34 provided protection against the deleterious effect of H_2O_2. The protective effects of PJ34 were apparent against all hemodynamic parameters measured in the current study; both inotropic and lusitropic states of the heart were maintained close to control levels after pharmacological inhibition of PARP (Fig. 1).

3.2. Intracellular calcium handling

Typical Ca_i^{2+} tracings are shown in Fig. 2. It can be observed that DOX treatment increased both the end-diastolic level and amplitude of the Ca_i^{2+} transient with a simultaneous reduction of peak systolic left ventricular pressure.

One of the most prominent findings in the current study is the profound elevation of end-diastolic Ca_i^{2+} in the hearts of the DOX-treated animals. The end-diastolic calcium concentration doubled from the control 109 ± 22 to 222 ± 73 nM in the hearts of the animals subjected to DOX (Fig. 3). Pharmacological inhibition of PARP prevented this elevation of end-diastolic Ca_i^{2+} (DOX-PJ group). The oxidative challenge elicited by H_2O_2 triggered a marked additional elevation in the end-diastolic Ca_i^{2+} in the hearts of the DOX-treated animals. This increase was significantly mitigated by pre-treatment of the animals with PJ34 (DOX-PJ versus DOX group, end-diastolic Ca_i^{2+} level, 226 ± 75 nM versus 548 ± 298 nM, respectively).

The analysis of the Ca_i^{2+} transients revealed that the systolic levels and, hence, the amplitude increased in the hearts of DOX-treated rats (Fig. 4A,B). Also, the release of calcium from SR was accelerated in the DOX group, as indicated by the enhanced $+dCa_i^{2+}/dt_{max}$ (Fig. 4C). Oxidative stress by H_2O_2 did not cause any significant alterations in the dynamic parameters of the Ca_i^{2+} transient in any of the experimental groups.

When sensitivity of the contractile machinery was estimated from the ratio of peak systolic pressure (P_{lv}) and peak systolic Ca_i^{2+} there was a statistically significant ($p < 0.05$) difference suppressive effect of doxorubicin which was prevented by the PARP inhibitor (Fig. 5).

4. Discussion

There are multiple mechanisms of doxorubicin-induced myocardial functional disturbances leading to cardiomyopathy overviewed in [7,8]. However, it appears that oxidative and nitrosative stress plays a clear role in this process. Doxorubicin undergoes redox cycling to generate free radicals that are responsible for mediating the various cytopathologies associated. Pharmacological neutralization of oxidant and free radical actions (via iron chelators, antioxidant vitamins, peroxynitrite decomposition catalyst and inducible nitric oxide synthase inhibitors) have been shown to improve cardiac function and protect against doxorubicin-induced cardiomyopathy [7,8,17]. The downstream intracellular mechanisms of doxorubicin-induced cell death and myocardial contractile dysfunction are less understood. Matrix

metalloproteinase activation, due to reactive oxidants such as peroxynitrite, may be involved [18]. There is also evidence that doxorubicin-triggered oxidants promote mitochondrial dysfunction and activate a variety of cell death pathways including cytochrome c release, caspase-3 activation [19,20], DNA strand breakage [9,19] as well as up-regulation of various pro-inflammatory signal transduction pathways and transcriptional regulation of multiple regulatory and inflammatory proteins [21].

One of the more recently identified pathways of doxorubicin cardiotoxicity is related to PARP activation [9]. PARP is an abundant nuclear enzyme of eukaryotic cells with multiple regulatory functions [1]. When activated by DNA single-strand breaks, PARP initiates an energy consuming cycle by transferring ADP ribose units from NAD^+ to nuclear proteins resulting in rapid depletion of the intracellular NAD^+ and ATP pools, slowing the rate of glycolysis and mitochondrial respiration eventually leading to cellular dysfunction and death [1,2]. Overactivation of PARP represents an important mechanism of tissue damage in various pathophysiological conditions associated with increased oxidative stress including myocardial reperfusion injury [1,2] as well as drug-induced cardiomyopathy [9,22].

It is well established that doxorubicin induces profound disturbances in myocardial calcium handling [8,21,23]. The focus of the current study was to investigate whether the PARP pathway regulates the alterations in calcium handling provoked by doxorubicin treatment. We have utilized a 6-week doxorubicin treatment protocol which represents a relatively early stage in the sequel of doxorubicin-mediated pathophysiological events. At this point, contractile function has not yet significantly declined in the isolated perfused hearts ex vivo under basal conditions but calcium handling was already impaired (as evidenced by increased intracellular end-diastolic calcium concentration), indicative of early-stage myocardial calcium overload. While this early-stage increase in myocardial intracellular calcium does not necessarily or immediately impair contractile function, it has been shown to contribute to the late-stage morphological and functional alterations associated with doxorubicin cardiomyopathy [21, 23]. Furthermore, the estimated Ca_i^{2+} sensitivity of the contractile machinery is significantly decreased in the doxorubicin-treated hearts. In Fig. 5, the steady-state relationship between Ca_i^{2+} and force (peak systolic pressure) was characterized by calculating the ratio of peak systolic P_{lv} and peak systolic Ca_i^{2+}. Even without manifest signs of cardiac failure, doxorubicin treatment reduced the calcium sensitivity of the myocardium by about 50% which was almost completely reversed by PJ34 treatment. The exact pathological mechanism of the doxorubicin-induced reduction in Ca_i^{2+} sensitivity has not yet been elucidated.

Our results show that in the early stage of doxorubicin-induced heart failure investigated in the current study, "basal" contractile function of the isolated Langendorff-perfused heart was not overtly impaired. These findings do not corroborate the in vivo observations showing significant depression of cardiac function (see also [17,24,25]). The lack of decline in contractile performance under basal conditions in our study may be explained by the fact that these Langendorff-perfused heart preparations are less challenged than the in vivo hearts (no external work performed), hampering a proper comparison between the in vitro and in vivo setting. However, the doxorubicin-treated hearts in our study became markedly sensitive to the deleterious effect of hydrogen peroxide. A possible explanation for this finding is that the prolonged production of reactive oxidants and free radicals in the doxorubicin-treated heart diminishes the oxygen free radical scavenging capacity, i.e., superoxide dismutase (SOD), glutathione peroxidase (GSHpx) and catalase activity, and hence suppresses endogenous antioxidant levels (such as glutathione) [26,27]. Therefore, it is conceivable that in the heart, the vital antioxidant system is depressed and, therefore, the hearts became more sensitive to an acute oxidative insult. Similar mechanisms may also contribute to the increased susceptibility of patients with doxorubicin therapy to cardiovascular events.

The current results demonstrate that the PARP system plays a regulatory role in the alterations in doxorubicin-induced cellular calcium handling. Both the "basal" intracellular calcium elevation as well as the hydrogen peroxide induced acute enhancement of the intracellular calcium levels were mitigated in the hearts of animals that have received the potent, competitive inhibitor of PARP activity (PJ34) during the in vivo doxorubicin treatment. PARP has two fundamental mechanisms of actions influencing both cellular function and pathophysiological events as overviewed in [1]. The first set of mechanism interferes with the increased catalytic activation due to DNA strand breaks followed by enhanced consumption of cellular NAD^+ and ATP. By doing so, it unfavorably competes with other cellular ATP-requiring processes. The second mechanism relates to the regulatory action of PARP on the expression of a variety of genes and gene products. With respect to the current results (ability of PARP to promote the disturbances in cellular calcium handling), either of the above two sets of mechanisms or the combination of the two may be involved. It is well established that doxorubicin impairs SERCA2a-mediated calcium re-sequestration into the SR which is also an energy-requiring process. It is also known that doxorubicin leads to depletion in cellular NAD^+ and ATP levels [28]. Thus, it is conceivable that diminished myocardial energetic status induces enhanced calcium influx into the intracellular space through "leaky" cell membrane and/or impairs the ability of the cardiac muscle cells to re-sequester calcium. This way, PARP inhibition may exert its beneficial effects through restoration of calcium re-sequestration via normalization of cellular energetic pools and restoration of the function of calcium pumps. It is also conceivable that PARP, on a transcriptional level, is involved in the regulation of proteins essential for myocardial calcium handling in the doxorubicin-treated hearts. For instance, there is evidence that the transcriptional regulation and the expression of multiple proteins involved in myocardial calcium handling is altered in response to doxorubicin, including down-regulation of the SR Ca^{2+}-ATPase (SERCA2a), and the ryanodine receptor (RYR2), and up-regulation of the sarcolemmal calcium channel (dihydropyridine receptor, DHPR) [29-31].

It is important to stress that the extent to which administration of a PARP inhibitor over a longer time period prevents the development of doxorubicin cardiotoxicity is currently unknown. The longest in vivo studies so far have been several weeks up to a month of DOX in the presence of PARP inhibitors [9,17]. These studies primarily investigated functional alterations such as myocardial contractility (as opposed to morphological alterations and pathological signs of cardiomyopathy which tend to develop over longer periods of time). Clearly, longer term studies are required. Nevertheless, a number of studies indicate that PARP inhibitors beneficially affect remodeling in chronic models of heart failure (even though these were induced by various other interventions such as LAD ligation or ascending aortic banding) [12,32]. Overall, it is conceivable that PARP inhibition would also beneficially affect chronic morphological and functional alterations in DOX hearts, but this issue remains to be directly tested in future studies.

The most important novel findings of the present study include: (1) the demonstration of an increase in end-diastolic calcium levels in doxorubicin-treated hearts; (2) development of a decline in calcium sensitivity of the contractile machinery in the diseased hearts; (3) the enhanced susceptibility of the doxorubicin-treated hearts towards a subsequent acute oxidative stress; and (4) the protective effects of PJ34, strongly suggesting that the activation of nuclear enzyme PARP is involved in the functional alterations of the doxorubicin-treated heart. Overall, the results of the current study are consistent with the notion that reactive oxidant species and the PARP pathway plays a pathogenetic role in the development of doxorubicin-induced cardiac dysfunction. Normalization of cellular calcium handling appears to be an additional mode of the cardioprotective effects of PARP inhibitors.

Acknowledgments

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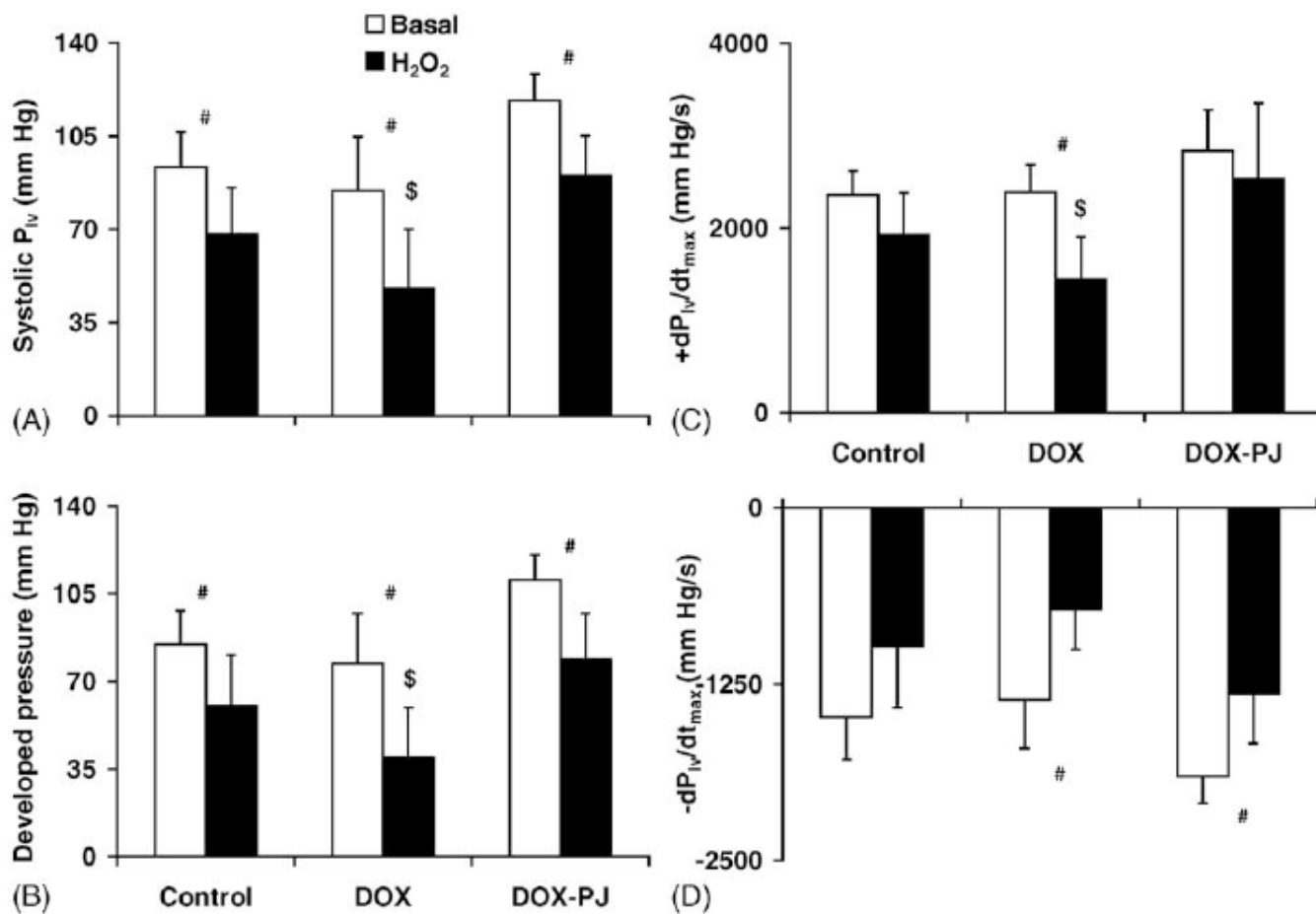
Fig. 1.
Hemodynamic changes in hearts of DOX-treated rats. The figure shows the contractile activity of the hearts in the different experimental groups: (A) peak systolic left ventricular pressure; (B) developed pressure; (C) $+dP_{lv}/dt_{max}$; and (D) $-dP_{lv}/dt_{max}$. [#]Indicates statistically significant ($p < 0.05$) difference between the basal state and the oxidative stress (H_2O_2). [$]Indicates statistically significant ($p < 0.05$) difference between DOX and DOX-PJ groups during oxidative stress. PJ34 treatment per se induced no change in the hemodynamic parameters of the perfused heart (Control vs. Control-PJ, data not shown).
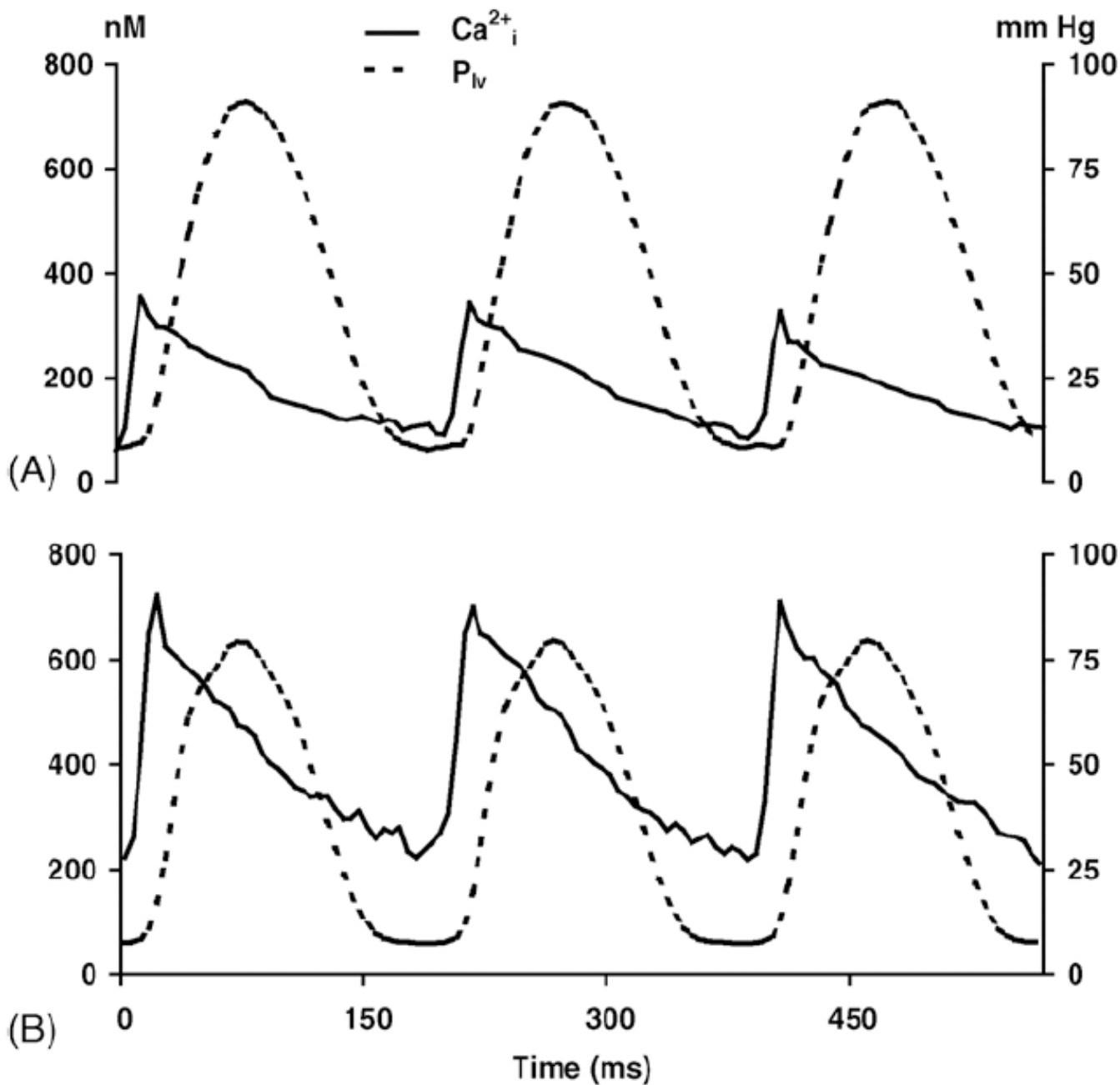
Fig. 2.
Cyclic changes of Ca_i^{2+} and left ventricular pressure. Representative tracings of Ca_i^{2+} transient and left ventricular pressure (P_{lv}) of (A) an intact, perfused control rat heart and (B) DOX-treated heart, both under basal conditions. Note that the peak of the calcium transient precedes the peak of the left ventricular pressure by 30–40 ms.
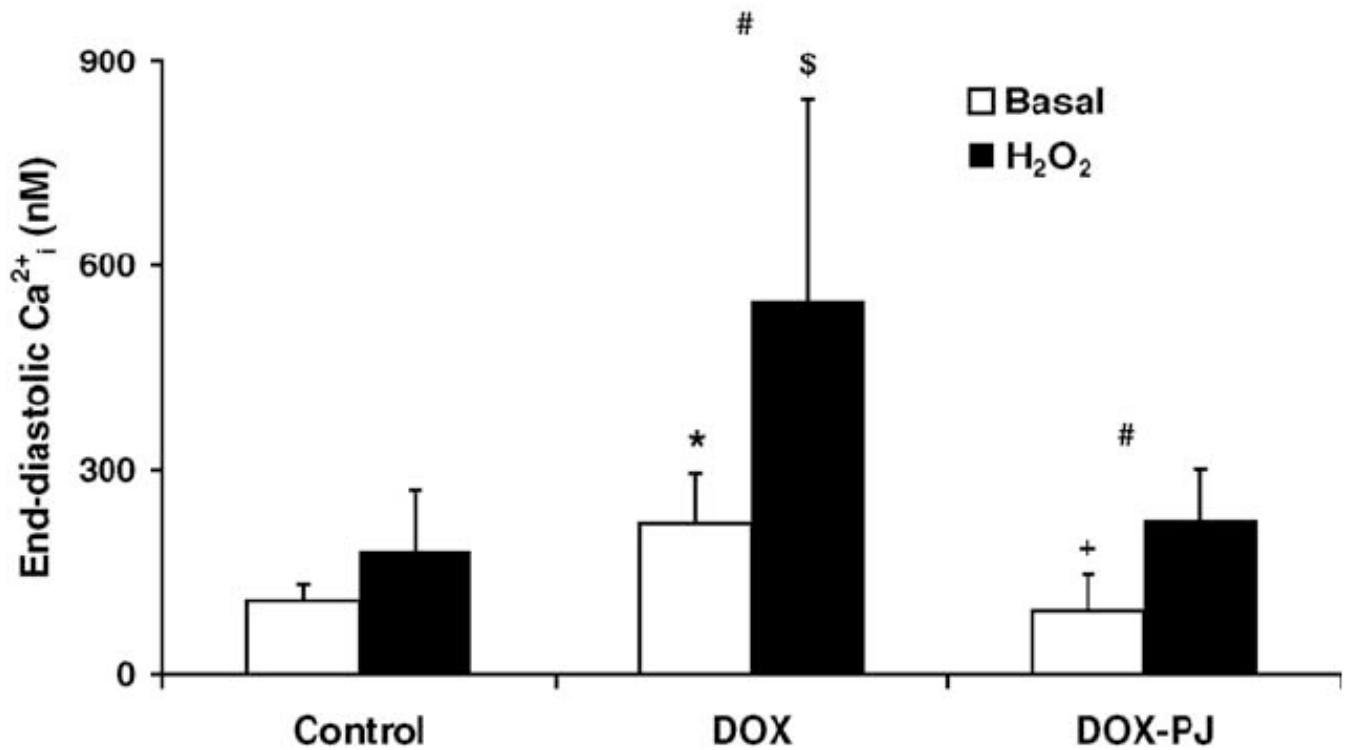
Fig. 3.
Changes in end-diastolic Ca_i^{2+} in DOX-induced cardiomyopathy. The figure shows changes in end-diastolic Ca_i^{2+} levels, prior to (basal) and during oxidative stress (H_2O_2) in the different experimental groups. *Indicates statistically significant ($p < 0.05$) difference between DOX and Control groups under basal condition. +Indicates statistically significant ($p < 0.05$) difference between DOX and DOX-PJ groups under basal condition. #Indicates statistically significant ($p < 0.05$) difference between basal state and oxidative stress (H_2O_2). $Indicates statistically significant ($p < 0.05$) difference between DOX and Control, or DOX-PJ groups during oxidative stress. PJ34 treatment per se did not alter the end-diastolic level of Ca_i^{2+} in the perfused heart (Control vs. Control-PJ, data not shown).
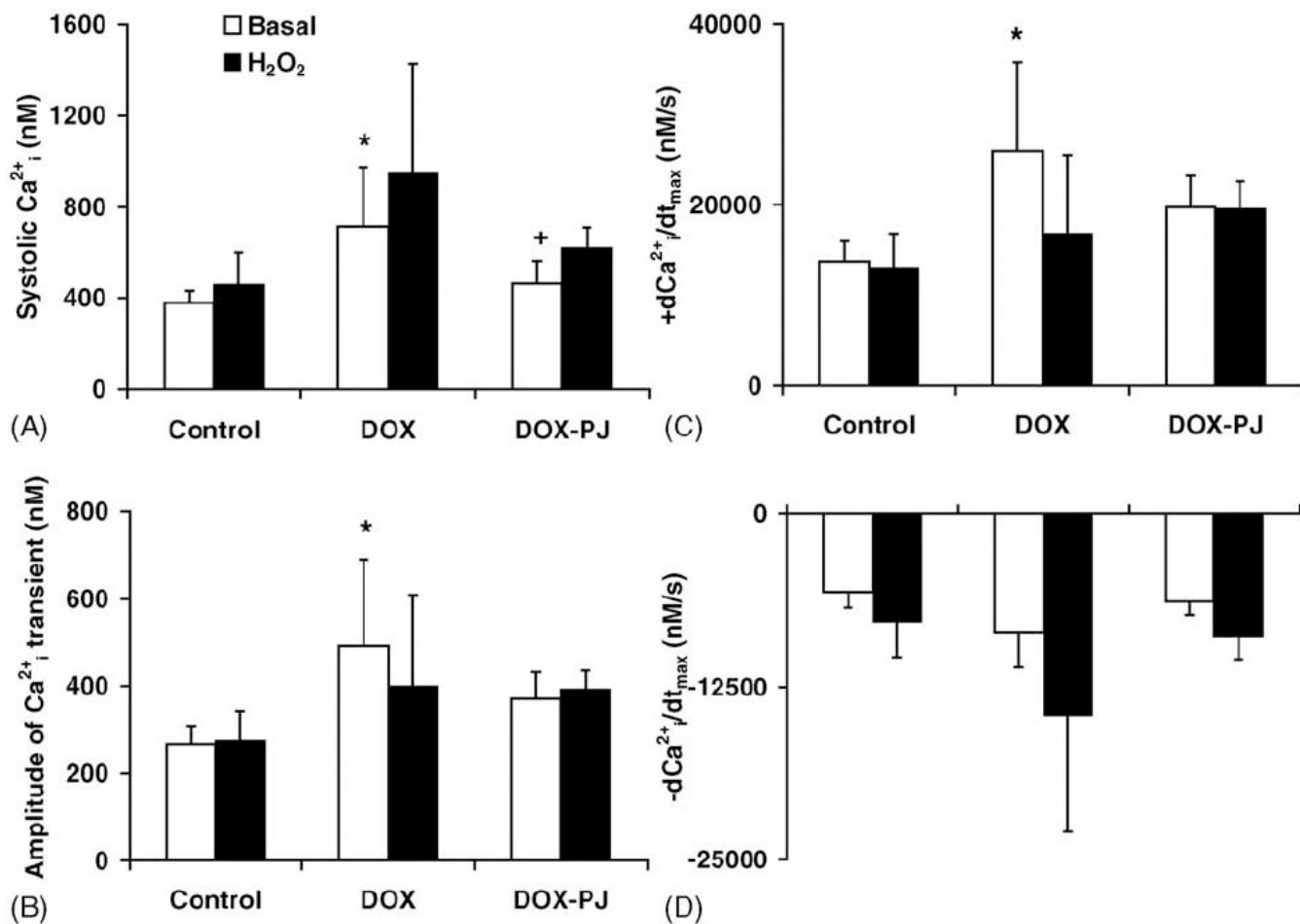
Fig. 4.
Changes in the dynamic parameters of Ca_i^{2+} transient in DOX-induced cardiomyopathy. The figure shows parameters calculated from the Ca_i^{2+} transients of the hearts in the different experimental groups: (A) peak systolic Ca_i^{2+}; (B) amplitude of Ca_i^{2+}; (C) $+dCa_i^{2+}/dt_{max}$; and (D) $-dCa_i^{2+}/dt_{max}$. *Indicates statistically significant ($p < 0.05$) difference between DOX and Control groups under basal condition. +Indicates statistically significant ($p < 0.05$) difference between DOX and DOX-PJ groups under basal condition. PJ34 treatment per se induced no change in the dynamic parameters of the Ca_i^{2+} transient in the perfused heart (Control vs. Control-PJ, data not shown).
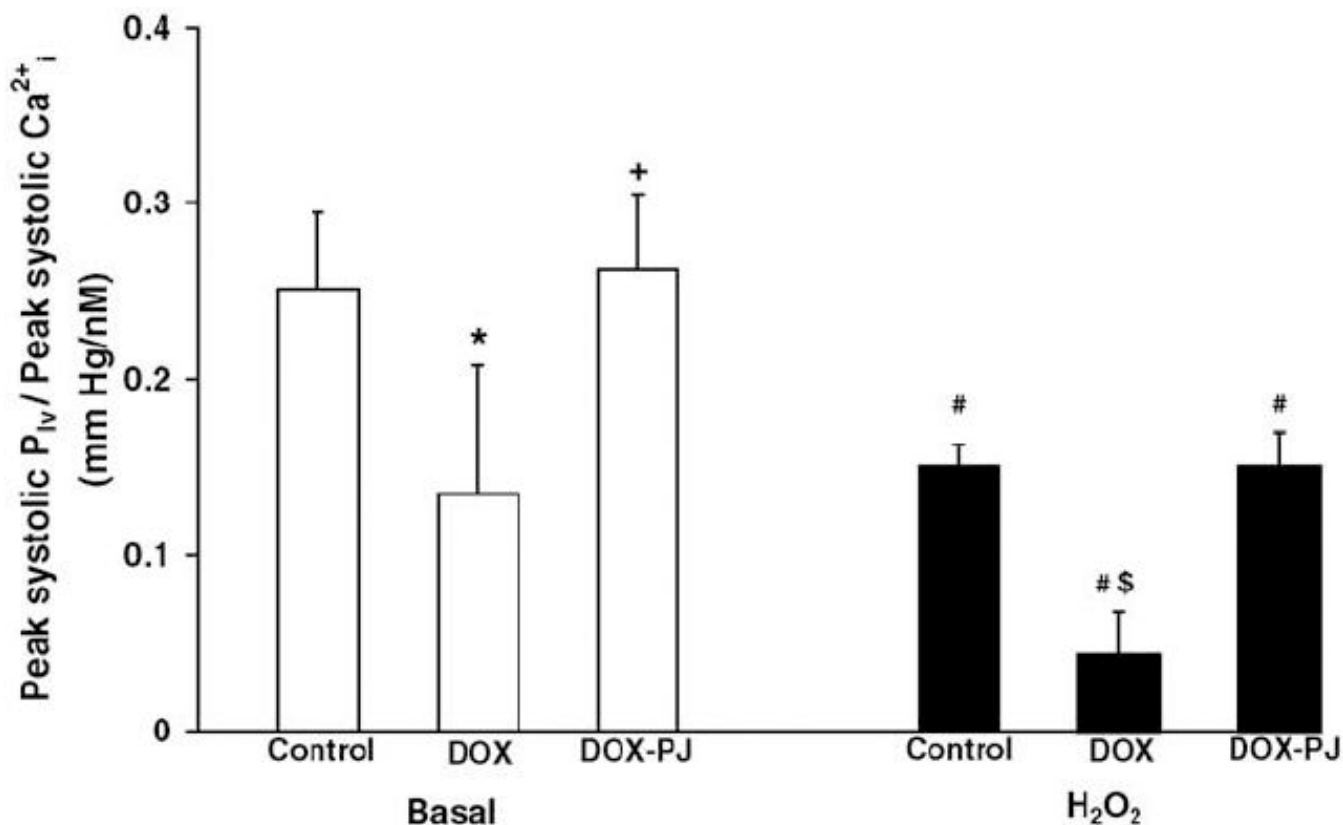
Fig. 5.
Calcium sensitivity of the myocardium during systole. Sensitivity of the contractile machinery was calculated as the ratio of peak systolic pressure (P_{lv}) and peak systolic Ca_i^{2+}. *Indicates statistically significant ($p < 0.05$) difference between DOX and Control groups under basal condition. [+]Indicates statistically significant ($p < 0.05$) difference between DOX and DOX-PJ groups under basal condition. [#]Indicates statistically significant ($p < 0.05$) difference between basal state and oxidative stress (H_2O_2). [$]Indicates statistically significant ($p < 0.05$) difference between DOX on the one hand and Control and DOX-PJ groups on the other during oxidative stress. PJ34 treatment per se induced no change in the Ca_i^{2+} sensitivity of the myocardium (Control vs. Control-PJ, data not shown).

Table 1

General effects of DOX treatment

	Control (*n* = 6)	6-week DOX (*n* = 5)	6-week DOX-PJ (*n* = 5)
Body mass (BM) (g)	366 ± 30	285 ± 36[*]	270 ± 18[*]
Heart mass (HM) (g)	1.33 ± 0.16	1.05 ± 0.19[*]	1.05 ± 0.16[*]
HM/BM (g/g)	0.0036 ± 0.0005	0.0037 ± 0.0005	0.0039 ± 0.0004

Control vs. DOX or DOX-PJ. Data refer to mean and S.D., PJ34 treatment per se did not affect the above parameters (Control vs. Control-PJ, data not shown).

[*] *p* < 0.05.

Table 2

Hemodynamic parameters in isolated Control, DOX and DOX-PJ hearts under basal conditions

	Control ($n = 6$)	6-week DOX ($n = 5$)	6-week DOX-PJ ($n = 5$)
Diastolic P_{lv} (mmHg)	8.4 ± 0.6	7.4 ± 0.2	7.9 ± 0.5
Systolic P_{lv} (mmHg)	93 ± 13	84 ± 20	118 ± 10
Developed pressure (mmHg)	85 ± 13	77 ± 20	110 ± 10
Heart rate (bpm)	291 ± 21	297 ± 18	285 ± 12
Coronary flow (ml/min)	18 ± 3	13 ± 2[*]	16 ± 2
CF/HM (ml/min/g)	13.4 ± 2.2	12.8 ± 1.6	15.2 ± 1.3
$+dP_{lv}/dt_{max}$ (mmHg/s)	2363 ± 255	2387 ± 301	2837 ± 442
$-dP_{lv}/dt_{max}$ (mmHg/s)	-1483 ± 303	-1363 ± 344	-1900 ± 194

Control vs. DOX or DOX-PJ. Data refer to mean and S.D. CF refers to coronary flow. P_{lv} and HM refers to left ventricular pressure and heart mass, respectively. PJ34 treatment per se did not affect the above hemodynamic parameters (Control vs. Control-PJ, data not shown).

[*] $p < 0.05$.