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Research Paper

# Dose-dependent effects of sildenafil on post-ischaemic left ventricular function in the rat isolated heart

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## Abstract

**Objectives** Sildenafil may be beneficial during myocardial ischaemia/reperfusion, but this effect may be dose-dependent, accounting for previous conflicting results. We have explored the effects of two acute and one chronic administration regimen on left ventricular function.

**Methods** The study was conducted on 36 Wistar rats (290 ± 7 g). Sildenafil was administered 30 min before ischaemia at a low (0.7 mg/kg, $n = 8$) or high (1.4 mg/kg, $n = 8$) dosage. The chronic treatment arm ($n = 8$) consisted of two daily injections of sildenafil (0.7 mg/kg) for three weeks. The control group was formed by 12 rats. Ischaemic contracture, post-ischaemic recovery and hypercontracture were measured in isolated, Langendorff-perfused preparations.

**Key findings** Ischaemic contracture tended to be lower after high-dose sildenafil, while remaining unchanged after low-dose or chronic sildenafil administration. Compared with controls (62.9 ± 2.0% of baseline developed pressure), post-ischaemic recovery was higher ($P = 0.0069$) after low dose (75.1 ± 2.4%), unchanged ($P = 0.13$) after high dose (69.1 ± 2.1%), but lower ($P < 0.001$) after chronic (42.9 ± 4.5%) sildenafil administration. Compared with controls (71.8 ± 3.9 mmHg), hypercontracture was higher ($P = 0.0052$) after chronic sildenafil administration (89.5 ± 4.1 mmHg), but similar after acute low dose (65.7 ± 3.3 mmHg, $P = 0.33$) or high dose (67.1 ± 4.7 mmHg, $P = 0.43$).

**Conclusions** The effects of sildenafil after ischaemia/reperfusion were strongly dose-dependent. Beneficial actions on left ventricular function were evident after acute pretreatment with a low dosage, but were lost after doubling the dose. Chronic sildenafil administration deteriorated left ventricular function during ischaemia and reperfusion.

**Keywords** ischaemia; left ventricular function; reperfusion; sildenafil

## Introduction

Myocardial infarction often leads to heart failure, a primary cause of morbidity and mortality worldwide. The widespread use of reperfusion strategies has improved the prognosis of acute myocardial infarction, but the value of this therapy is hampered by reperfusion injury, defined as myocardial cell death after restoration of blood flow.[1] During the past decade, a vast amount of research has been devoted to interventions that can limit this untoward effect of reperfusion. Although the precise pathophysiological mechanisms of reperfusion injury are not fully understood, cellular cyclic guanosine monophosphate (cGMP) levels have been shown to be inversely related to the severity of ischaemia/reperfusion injury, resulting in calcium overload and cell death.[2–5]

Sildenafil citrate is a water soluble aromatic compound, structurally resembling the guanosine base of cGMP. Its main pharmacological action is through the inhibition of phosphodiesterase-5 (PDE-5) in the corpus cavernosum.[6] Sildenafil increases cGMP levels, which in turn lower intracellular calcium, resulting in venous and arterial vasodilatation. Although PDE-5 was initially thought to be present only in corpus cavernosum, systemic vasculature, platelets and skeletal muscle, subsequent reports have

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confirmed the presence of PDE-5 in the ventricular myocardium.[7,8] In light of those findings, experimental studies have suggested that sildenafil improves intracellular calcium handling, diminishes post-ischaemic ventricular dysfunction and confers cardioprotection during ischaemia–reperfusion.[9–13] Despite these promising observations, current data concerning the effects of sildenafil on the ischaemic heart are controversial. In an in-vivo rabbit model of regional ischaemia, pretreatment with sildenafil failed to decrease infarct size.[14] The reasons for these contradictory results are unclear, but may be related to dosage of the drug and timing of delivery before the induction of ischaemia.[10,15] These postulated dose-dependent effects are likely to correspond to variable actions on intracellular cGMP levels, which may differ after long-term use.[2,10,16,17]

In this study, we have explored the effects of different sildenafil dosing regimens on indices of left ventricular (LV) function after ischaemia–reperfusion. For this purpose, we examined two different acute drug dosages, but also a more clinically applicable chronic sildenafil administration scheme. Post-ischaemic LV recovery was measured *in vitro* using the isolated, Langendorff-perfused rat heart model, which can assess LV function independently of haemodynamic conditions (preload and afterload). To shed more light into possible cardioprotective mechanisms, we evaluated hypercontracture, which reflects intracellular calcium handling during reperfusion.

## Materials and Methods

### Animal population

The study was conducted in 36 male Wistar rats (purchased from the National Research Centre for Natural Sciences 'Democritus', Athens, Greece) of similar age (20–22 weeks) and weight (250–350 g). The animals were housed two to three per cage in our animal facilities, under optimal laboratory conditions (controlled humidity, temperature and light/dark cycles), with water and standard rat chow freely available. All animals received humane care, in accordance with the recommendations in the Declaration of Helsinki, as well as with the 'Position of the American Heart Association on Research Animal Use'. The experimental protocol conformed to institutional standards and national legislation. The study was approved by the local state authority (approval number 5687/08-01-2008).

### Study protocol

Two treatment arms were examined, namely acute and chronic. For this purpose, the animal population was randomly assigned to one of the following groups: acute low-dose sildenafil, acute high-dose sildenafil, acute saline, chronic sildenafil, or chronic saline. The end-points of the study included the following: assessment of pre- and post-ischaemic LV function; and assessment of hypercontracture, a process consisting of sustained shortening and stiffening of the myocardium, due to uncontrolled calcium influx into myocytes after reperfusion. Since this process profoundly affects not only systolic but primarily diastolic function, hypercontracture was expressed as the peak value of LV diastolic pressure during reperfusion. The protocol of the study is shown in Figure 1 and the study end-points in Figure 2.

### Drug administration

In the acute treatment arm, sildenafil was given at a dosage of either 0.7 mg/kg ($n$ = 8) or 1.4 mg/kg ($n$ = 8), as a single intraperitoneal injection (solution volume 0.5 ml), delivered 30 min before ischaemia. Drug administration at low (0.7 mg/kg) or high (1.4 mg/kg) dosages was based on previous studies examining the cardioprotective actions of sildenafil during ischaemia–reperfusion.[11,14,16] In the acute treatment arm, the control group was formed by six rats that received 0.5 ml normal saline (0.9%). The chronic treatment arm ($n$ = 8) consisted of two daily intraperitoneal injections of sildenafil for three weeks, the last injection being administered 30 min before the experiment. Each injection consisted of 0.7 mg/kg sildenafil (solution volume 0.5 ml). In the chronic treatment arm, the control group was formed by six rats that received 0.5 ml normal saline (0.9%). The small volume of the solutions and the intraperitoneal route of administration precluded any effects on heart function in our animal model.

### Heart perfusion

Heart perfusion was performed as described previously.[18] In brief, following the induction of anaesthesia with isoflurane, the hearts were rapidly excised via thoracotomy and were submerged in ice-cold Krebs–Henseleit buffer, before being mounted on the aortic cannula of a Langendorff apparatus (ADInstruments Ltd, Oxfordshire, UK). Perfusion was carried out using Krebs–Henseleit buffer (composition

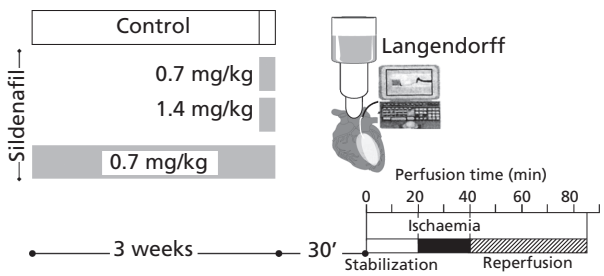


**Figure 1** Study protocol

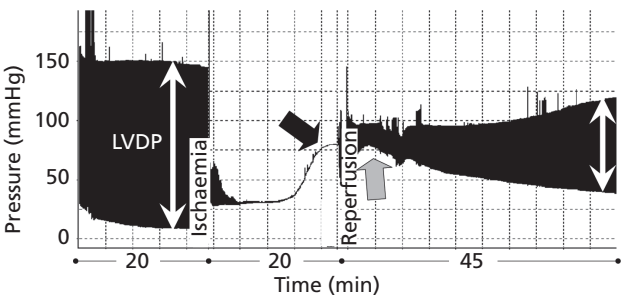


**Figure 2** End-points of the study. Study end-points were left ventricular developed pressure (LVDP) at the end of reperfusion and immediately before the induction of ischaemia (white arrows), ischaemic contracture (black arrow) and hypercontracture (grey arrow)

in mmol/l: sodium chloride 118, potassium chloride 4.7, potassium phosphate monobasic 1.2, magnesium sulfate 1.2, sodium bicarbonate 25 and glucose 11). As ischaemic contracture and hypercontracture depend on calcium concentration in the perfusate, we chose a low calcium chloride concentration of 1.4 mmol/l.[19] The perfusate was bubbled continuously with a gas mixture of 95% $O_2$ and 5% $CO_2$, which equilibrates the buffer at a pH of 7.4, and the perfusion apparatus was heated to a temperature of 37°C. Atrial pacing was performed at a rate of 250 beats/min (2–ms pulse duration, amplitude 30% above the pacing threshold) throughout the experiment.

### Ischaemia–reperfusion protocol

During an initial 20-min stabilisation period, the perfusion was adjusted to maintain a constant coronary perfusion pressure of 60 mmHg. Subsequently, normothermic, zero-flow, global ischaemia was induced by turning off the aortic cannula for 20 min, followed by a 45-min reperfusion period.

### Assessment of left ventricular function

A fluid-filled balloon was inserted in the LV via the left atrium. The balloon was connected to a pressure transducer, allowing measurements of contractility under isovolumic conditions. All parameters were recorded with the PowerLab system (ADInstruments Ltd, Oxfordshire, UK). The intraventricular balloon volume was adjusted to provide a LV end-diastolic pressure of approximately 6 mmHg. This value was kept constant throughout the experiment, ensuring a stable preload. Coronary pressure was observed continuously and was held constant. Coronary blood flow was monitored by effluent measurements.

### Ischaemic contracture

As a measure of LV function during ischaemia, we recorded the increase in the minimal value of LV pressure during ischaemia, a variable frequently referred to as ischaemic contracture.

### Post-ischaemic left ventricular function

The left ventricular developed pressure (LVDP) was measured as an index of LV systolic function. LVDP pressure was defined as the difference between LV peak systolic pressure and LV end-diastolic pressure. LVDP was measured at the end of the stabilisation period and 45 min after reperfusion. Post-ischaemic recovery was defined as the ratio (expressed as a percentage) of LVDP at the end of reperfusion divided by LVDP immediately before the induction of ischaemia.

### Hypercontracture

Hypercontracture was defined as the peak value of LV diastolic pressure during the first 5 min of reperfusion. This is considered a reliable indirect index of intracellular calcium handling during reperfusion.[5]

### Statistical analysis

Data were presented as mean ± SEM. Baseline data and indices of post-ischaemic LV function in the experimental groups were compared using one-way analysis of variance. When significant effects were identified, post-hoc individual comparisons were performed using the Duncan's multi-stage test. A value of $P < 0.05$ was considered significant in all tests.

## Results

The five experimental groups (two acute treatment groups, one chronic treatment group and two control groups) were comparable in terms of age, body weight, or heart weight (all $P > 0.10$). Comparison between the two control groups in the acute and chronic treatment arms revealed no differences in any variable. Therefore, for presentation purposes, we report the results from the three active treatment groups and a single control group.

### Left ventricular developed pressure before ischaemia

There was a significant variance in LVDP before the onset of ischaemia in the four groups ($F = 4.76$, $P = 0.0073$). This variance was due to lower values in all three treatment groups, compared with controls (124.9 ± 2.6 mmHg). Values were 113.9 ± 2.1 mmHg ($P = 0.0037$) in the acute low-dose sildenafil group, 111.5 ± 2.2 mmHg ($P = 0.00076$) in the acute high-dose sildenafil group and 116.1 ± 1.4 mmHg ($P = 0.014$) in the chronic sildenafil group.

### Coronary blood flow

At the end of the 20-min stabilisation period, coronary blood flow displayed a significant variance ($F = 3.39$, $P = 0.029$) between groups. This was due to higher ($P = 0.016$) values in the low-dose sildenafil group (11.3 ± 0.9 ml/min), compared with controls (9.1 ± 0.3 ml/min). Coronary blood flow in the high-dose group (9.8 ± 0.6 ml/min, $P = 0.41$) and in the chronic group (8.8 ± 0.4 ml/min, $P = 0.75$) did not differ from that in controls.

### Ischaemic contracture

There was a trend (defined as $0.05 < P < 0.10$) towards a significant variance in ischaemic contracture in the four groups ($F = 2.3$, $P = 0.094$). This was secondary to lower ($P = 0.029$) values in the high-dose sildenafil group (52.7 ± 5.2 mmHg), compared with controls (66.4 ± 3.1 mmHg). Ischaemic contracture in the low-dose (60.8 ± 3.8 mmHg) and chronic (63.3 ± 3.4 mmHg) sildenafil groups was comparable with that in controls. Ischaemic contracture is illustrated in Figure 3.

### Post-ischaemic recovery

There was a significant variance in post-ischaemic recovery in the four groups ($F = 21.7$, $P < 0.0001$). This was due to higher ($P = 0.0069$) values in the low-dose sildenafil group (75.1 ± 2.4), compared with controls (62.9 ± 2.0). In contrast, post-ischaemic recovery was lower ($P < 0.001$) in the chronic sildenafil group (42.9 ± 4.5) than in controls. Post-ischaemic recovery was comparable ($P = 0.13$) in the high-dose sildenafil (69.1 ± 2.1) and the control groups. All values are depicted in Figure 4.

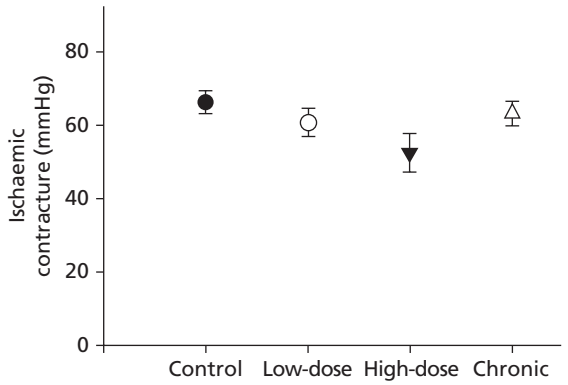


**Figure 3** Ischaemic contracture. Ischaemic contracture was similar in the four groups, albeit marginally lower in the high-dose sildenafil group

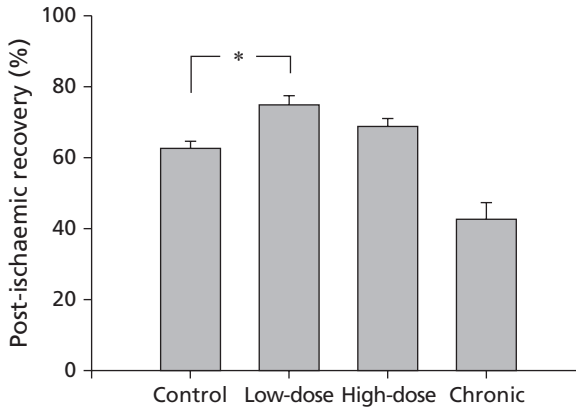


**Figure 4** Post-ischaemic recovery. Post-ischaemic recovery was significantly ($^*P < 0.05$) higher in the low-dose sildenafil group

### Hypercontracture

There was a significant variance in hypercontracture in the four groups ($F = 6.35$, $P = 0.0016$). This variance was due to higher ($P = 0.0052$) values in the chronic sildenafil group (89.5 ± 4.1 mmHg), compared with controls (71.8 ± 3.9 mmHg). Hypercontracture was similar in the acute low-dose (65.7 ± 3.3 mmHg, $P = 0.33$) and high-dose (67.1 ± 4.7 mmHg, $P = 0.43$) sildenafil groups, when compared with controls.

### End-diastolic pressure at the end of reperfusion

At the end of reperfusion, there was a significant variance in LV end-diastolic pressure ($F = 13.6$, $P = 0.00001$). This variance was due to lower ($P = 0.045$) values in the low-dose sildenafil group (25.6 ± 1.8 mmHg), compared with controls (36.4 ± 3.5 mmHg). In contrast, end-diastolic pressure at the end of reperfusion was higher ($P = 0.00032$) in the chronic sildenafil group (57.3 ± 4.8 mmHg) than in controls. End-diastolic pressure at the end of reperfusion was comparable ($P = 0.54$) between the high-dose sildenafil (33.4 ± 2.2 mmHg) and the control groups. Figure 5 illustrates hypercontracture and LV end-diastolic pressure at the end of reperfusion.

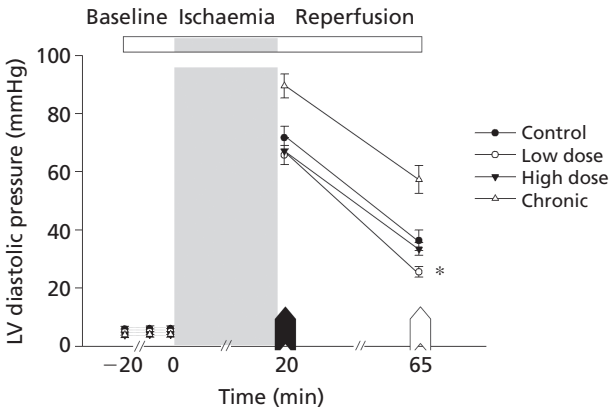


**Figure 5** Left ventricular diastolic pressure. Hypercontracture (black arrow) was higher in the chronic sildenafil group. At the end of reperfusion (white arrow), left ventricular end-diastolic pressure was higher in the chronic sildenafil group, but lower in the low-dose sildenafil group ($^*P < 0.05$) than in controls

## Discussion

This study has investigated the effects of sildenafil on post-ischaemic LV function and hypercontracture after global myocardial ischaemia followed by reperfusion. The Langendorff-perfused working rat heart model was utilised, which allows precise measurements of contractile indices, independent of haemodynamic conditions. Both acute and chronic sildenafil administration decreased systolic blood pressure, confirming the pharmacological efficacy in the rat model used in our experiments. The main findings presented here indicated that the effects of sildenafil on the myocardium differed depending on the dosage and duration of administration before the onset of ischaemia.

### Acute sildenafil administration

We found that low-dose (0.7 mg/kg) but not high-dose (1.4 mg/kg) sildenafil administered acutely before the onset of ischaemia improved post-ischaemic recovery. Moreover, acute low-dose sildenafil administration increased coronary blood flow before the induction of ischaemia. These data were in accordance with previous results, signifying the dose-dependent effects of acute sildenafil administration.[11,14] In an in-vivo rabbit model, a reduction in myocardial infarct size was demonstrated after sildenafil administration 30 min before coronary artery ligation, when given as a bolus dose of 0.7 mg/kg.[11] In contrast, another study in the same model reported that 1.45 mg/kg sildenafil, administered intravenously at the same time interval before the induction of ischaemia, failed to produce any cardioprotective effects after ischaemia/reperfusion.[14]

The results of this study, together with previous findings, have underscored the importance of sildenafil dosage in eliciting its effects on the ischaemic myocardium.[11,14] This conclusion is supported by further studies that have demonstrated that sildenafil was effective in improving post-ischaemic ventricular dysfunction only in a relative narrow dose range.[10,13] In the isolated, Langendorff-perfused rat

heart model, pretreatment with a very low dose of sildenafil (0.05 mg/kg) decreased infarct size and improved LV function after ischaemia/reperfusion.[13] This dosage corresponded to the concentrations previously found to improve post-ischaemic LV function in the same model.[10] In that study, very low concentrations (50 nM) of sildenafil improved LV function during reperfusion and reduced infarct size, while doubling the concentration removed the cardioprotective effect of the drug.[10] Further doubling of sildenafil concentration led to poorer mechanical function during reperfusion and to exacerbation of reperfusion injury.

### Chronic sildenafil administration

To the best of our knowledge, our study has been the first to evaluate the effects of prolonged pretreatment with sildenafil on post-ischaemic LV function. Such administration may be clinically applicable, given recent reports demonstrating beneficial effects of sildenafil in the treatment of pulmonary hypertension and heart failure.[20,21] However, in our experiments, chronic pretreatment with sildenafil actually deteriorated LV post-ischaemic recovery after ischaemia–reperfusion by approximately 30%.

### Hypercontracture

It has been known for decades that following reperfusion, excessive contractile activation occurs, a phenomenon previously known as 'stone heart' and more recently referred to as hypercontracture. This sustained shortening and stiffening of the myocardium has been clearly demonstrated in experimental studies and is considered an important mediator of cell death following reperfusion.[4,5] Histological studies have previously established contraction band necrosis as a prominent feature in reperfused infarcted tissues.[22] Importantly, this phenomenon is established during the first minutes of reperfusion and is thought to occur due to uncontrolled calcium influx into cardiomyocytes.[5]

In this study, we hypothesized that the intracellular calcium lowering effects of sildenafil might decrease reperfusion-induced hypercontracture. However, in our experiments, both acute sildenafil dosing regimens failed to decrease hypercontracture upon reperfusion. Nonetheless, low-dose sildenafil lowered end-diastolic pressure at the end of reperfusion, while high dose was ineffective. In contrast, chronic pretreatment with sildenafil augmented hypercontracture after ischaemia–reperfusion. Moreover, end-diastolic pressure at the end of reperfusion was higher after chronic sildenafil administration than in controls.

### Effects of sildenafil on intracellular cAMP

The explanation for the dose-dependent cardioprotective effects of sildenafil is uncertain. The 3′,5′-cyclic nucleotide phosphodiesterases are a class of enzymes that are capable of cleaving the phosphodiester bond in either cAMP or cGMP to yield 5′-cyclic nucleotides and are therefore involved in second messenger signalling pathways.[23] To date, 11 different isoforms of phosphodiesterases have been classified, of which at least five have been observed in the heart. Among the various types of phosphodiesterases, PDE-5 selectively hydrolyses cGMP relative to cAMP, while PDE-3 is primarily a cAMP-hydrolysing enzyme. Increased intracellular cAMP levels contribute to cytosolic calcium overload and to ischaemia/reperfusion injury.[24] In an experimental setting similar to ours, sildenafil dose-dependently increased not only cGMP, but also cAMP levels, possibly by inhibition of PDE-3 by the accumulated cGMP, as suggested by previous studies.[16,24]

Another potential explanation for the concomitant increase in cAMP levels at high sildenafil concentrations is the attenuation of sildenafil selectivity for PDE-5, which in turn explains the dose-dependent effects of sildenafil and the loss of cardioprotection at high dosages. Previous findings in the rat isolated heart lend further support to our hypothesis; in that study, a differential effect of sildenafil on intracellular cAMP levels was observed, depending on the drug concentration.[10] Low sildenafil concentrations increased intracellular cGMP levels and attenuated the ischaemia-induced elevation in myocardial cAMP levels, with beneficial effects on ischaemia/reperfusion injury. In contrast, high concentrations of sildenafil further increased myocardial cGMP levels, but this was accompanied by an equally large elevation in cAMP levels, that may have accounted for the detrimental effects observed in those experiments.[10] Nonetheless, although our findings were consistent with those considerations, more data are required before firm conclusions can be drawn.

### Chronic sildenafil administration

The deterioration in left ventricular function during ischaemia and reperfusion, observed in our experiments after chronic sildenafil administration, was difficult to explain, but the reasons may have been twofold. First, increased cGMP concentration may have exerted a negative inotropic effect mediated by a protein kinase-G-dependent reduction in myofilament responsiveness to calcium ions.[24] Second, attenuation of sildenafil pharmacological properties may have occurred, as reported previously in patients using sildenafil regularly for erectile dysfunction or chronically for pulmonary arterial hypertension.[17,25]

### Strengths and limitations of the study

We feel that this work has added important information on the effects of sildenafil on LV function after ischaemia/reperfusion. The inclusion of a chronic sildenafil pretreatment group, in addition to the two acute sildenafil groups, and the accuracy of measurements associated with the use of isolated working heart preparations represent major strengths of the study. The measurement of hypercontracture after reperfusion has increased further the value of our work. However, two limitations may be apparent. First, we examined only two acute and one chronic sildenafil dosages. The addition of more dosing regimens, including both lower and higher doses, would have improved this work, albeit at the cost of decreased statistical power. Second, the discussion on the effects of sildenafil on cAMP levels has been based on previous findings and not on the results of this study.

## Conclusions

The effects of sildenafil on LV function after ischaemia/reperfusion depended on the dose and the duration of

administration before the induction of ischaemia. Beneficial effects were evident after acute pretreatment with low dosage, but were lost after doubling the dose. Chronic sildenafil administration deteriorated LV function during ischaemia and during reperfusion.

## Declarations

### Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

### Funding

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### Acknowledgements

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