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SHORT COMMUNICATION

A single bout of exercise promotes sustained left ventricular function improvement after isoproterenol-induced injury in mice

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Abstract We have investigated whether acute (swimming) exercise is sufficient to have sustained beneficial effects against cardiac functional decline observed after high-dose isoproterenol administration. Mice were subjected to one bout of swimming for 30 min ("swim" group). Twenty-four hours later, they were given isoproterenol (160 mg/kg) to cause injury. Two control groups were included, a shallow "water" group, for which no swimming took place, and a "cage" group; they were both given isoproterenol as in the "swim" group. Cardiac function was assessed by tissue Doppler imaging (TDI) 24 h, 2 weeks, and 4 weeks post-isoproterenol. Left ventricular (LV) systolic function including endocardial velocity and radial strain rate declined significantly in all groups at all time points after isoproterenol, compared with their pre-isoproterenol treatment values. The "swim" group, however, had significantly higher LV systolic function compared with either of the control groups at 24 h, and this improvement persisted 2 and 4 weeks post-treatment. There were no significant differences between the control groups at any time point. In conclusion, a single bout of swimming has sustained beneficial effects against injury, as measured by TDI, after administration of isoproterenol.

Keywords Brief exercise · Swim · Mice · Isoproterenol · Cardioprotection · Tissue Doppler imaging

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Introduction

It has long been established that exercise reduces the risk of cardiovascular disease and promotes the prevention and/or improved management of chronic diseases including diabetes [1–4]. There is also evidence that a single bout of exercise can increase resistance to cardiac injury. Studies reporting on the positive effect of acute exercise have been conducted on dogs and rats [5–10]. These studies focussed on early events, within 24 h post-injury, and have not looked at function of the heart in vivo beyond this time point.

A high dose of the β-adrenergic agonist isoproterenol has been used to induce myocardial injury in rabbits, rats, and mice [11–15]. Our own previous studies have shown that isoproterenol induces myocardial lesions in mice [12]. Isoproterenol is believed to cause myocardial damage though transient ischemic events, and has been called an oxidative stress model of myocardial injury [16]. The combined effect on the myocardium and vasculature induces myocardial ischemic events that result in focal lesions similar in wound morphology to that from the blockage of coronary vessels [17, 18]. The isoproterenol model of myocardial injury has been used in previous studies assessing the cardioprotective effects of exercise in rats [10].

There is no report on the effect of a single bout of exercise on isoproterenol-induced cardiac injury and/or

functional decline. Here we have used a mouse model and assessed the effect of acute swimming exercise on cardiac function in vivo, 24 h, and 2 and 4 weeks after high-dose isoproterenol treatment. Tissue Doppler imaging (TDI), which has been validated for mice [19, 20] was used; this method can detect changes in regional left ventricular (LV) systolic function, including at the level of the cardiomyocyte [19, 21]. Our findings document for the first time for the mouse that a single bout of swimming exercise confers sustained protection from isoproterenol-induced adverse effects on cardiac systolic function.

Materials and methods

Mice

All CD-1 mice were housed and treated according to standards and guidelines set by the Canadian Council for Animal Care. The investigation conforms to 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).

Exercise

The swimming procedure was essentially as previously reported with minor modifications, with at least four mice per group [22–24]. Briefly, mice (maximum $n = 10$) were placed in a water bath (50 × 30 × 20 cm) filled with fresh water (32–37°C) for a maximum of 30 min with continuous monitoring. To control for the stress of being placed in water, a group of mice ("water" group) was placed in an identical water bath but containing only shallow (1–2 inches) water (32–37°C) so that swimming was not required. All mice were individually identified by use of toe tattoos and coloured marks made with a non-toxic permanent marker. When removed from the water bath, mice were placed under a heat lamp on fluffed towels to help shorten drying time and to help keep their body temperature from dropping. When animals were dry, they were maintained, as with the control ("cage") group, for 24 h before treatment with or without isoproterenol. Chronic application of the swimming procedure over 28 days (5 days per week for 4 weeks) resulted in significant 1.14-fold ($p < 0.001$, $n = 9$) and 1.15-fold ($p < 0.01$, $n = 4$–9) increases in heart weight-to-body weight ratio compared with the "water" and "cage" groups, respectively, but there were also no significant, and thus detrimental, changes in body weight when assessed in a chronic swimming experiment over an extended period (data not shown). Mouse weights were recorded immediately before euthanasia by cervical dislocation, at which point hearts were rapidly excised and atria were removed. Cardiac ventricles were blotted on paper towels to remove excess blood, and then weighed.

Isoproterenol treatment

Mice (8–14 weeks) were weighed on the day of drug delivery. Pre-treatment with the analgesic ketoprofen (Anafen®; Merial Canada, Baie d'Urfé, QC, Canada) at a dose of 5 mg/kg was given subcutaneously 1 h before isoproterenol administration. (−)-Isoproterenol hydrochloride (Sigma, St Louis, MO, USA) was diluted in sterile saline to stock solutions varying between 30 and 50 mg/ml, depending on the average weight of the group of animals, so that the final volume administered is between 100 and 180 µl. Isoproterenol was given intraperitoneally (i.p.) at a final dose of 160 mg/kg, because histological assessment has shown this results in measurable left ventricular (LV) pathology scores in CD-1 mice one day after injection [12].

Morphological assessment

Ventricular tissue was fixed in formalin and embedded in paraffin. Paraffin-embedded tissue was cut into 7-µm sections and stained with haematoxylin and eosin. Tissue morphology was assessed by bright-field microscopy.

Echocardiographic analysis

Transthoracic echocardiography was performed using a 13-Mhz probe (Vivid 7; GE Medical Systems, Milwaukee, WI, USA) in awake mice at baseline, and 24 h and 2 and 4 weeks post-injection as previously described [25]. Hearts were imaged in the 2D parasternal short-axis view and three different frames of an M-mode echocardiogram were recorded. LV end-diastolic diameter (LVEDD) and LV fractional shortening (FS) were measured. The LV ejection fraction (EF) was calculated using the prolate ellipsoid geometric model and parasternal long-axis views for measurement of LV end-systolic and diastolic volumes [25, 26]. TDI was acquired on a parasternal short-axis view at the level of the papillary muscles, at a rate of 483 frames per second, for unanaesthetized mice at baseline, and 24 h and 2 and 4 weeks post-injection [19]. For peak systolic endocardial velocity (V_{endo}), a region of interest (0.2 × 0.2 mm) in the posterior wall was analysed. The rate of fractional tissue deformation in response to applied force, or radial strain rate (SR) per second (s^{-1}) was measured over a distance of 0.6 mm (using an Echopac PC; GE Medical Systems) [19, 20]. The temporal smoothing filters were turned off for all measurements. The values obtained in five consecutive cardiac cycles were averaged.

Statistical analysis

Statistical analysis of data, mean and standard error, was performed using GraphPad InStat® and GraphPad Prism® software. The student *t* test was used when comparing two groups, as in Fig. 2. All other data were analysed using two-way ANOVA with the Bonferroni post-test. A value of $p < 0.05$ was considered statistically significant.

Results

Previously, an i.p. bolus injection of 160 mg/kg isoproterenol was shown to result in measurable left ventricular (LV) pathology scores one day after injection [12]. This model of cardiac injury was used for this work. As expected [12], isoproterenol induced increased cellular infiltration 24 h after treatment, indicative of myocardial damage; areas of myocardial lesions (scar) were more clearly discerned 4 weeks after isoproterenol administration (Fig. 1).

In pilot experiments isoproterenol was administered to mice and myocardial function assessed 24 h later by M-mode echocardiography and by TDI. Data from male and female mice were not significantly different and so results from both sexes were pooled. M-mode echocardiography showed no change in left ventricular end diastolic diameter, fractional shortening (FS), or ejection fraction (EF) at 24 h post-injection (Table 1). However, significant changes in TDI results were observed. Endocardial velocity (V_{endo}) and strain rate per second (SR (s^{-1})) decreased to 48 ± 7.9 and 53 ± 4.2%, respectively, of those for their non-isoproterenol-treated counterparts (Fig. 2). TDI was therefore used to evaluate myocardial function in our subsequent studies.

We examined the effect of one 30-min bout of swimming on cardiac function in mice that were subsequently subjected to isoproterenol injury. Two sets of control mice were used for comparison with this "swim" group. These included a "water" group, which was treated in an identical manner as the "swim" group except the water was too shallow for swimming, and a "cage" group, where mice were not subjected to swimming or exposed to water

Table 1 Echocardiographic analysis of mice subjected or not to isoproterenol (IsP) treatment

Treatment	−IsP ($n = 10$)	+IsP ($n = 9$)
LVEDD (mm)	3.0 ± 0.2	3.1 ± 0.2
FS (%)	55.0 ± 4.0	55.0 ± 5.0
EF (%)	81.0 ± 3.0	80.0 ± 3.0

LVDD left ventricular end diastolic diameter, *FS* fractional shortening, *EF* ejection fraction

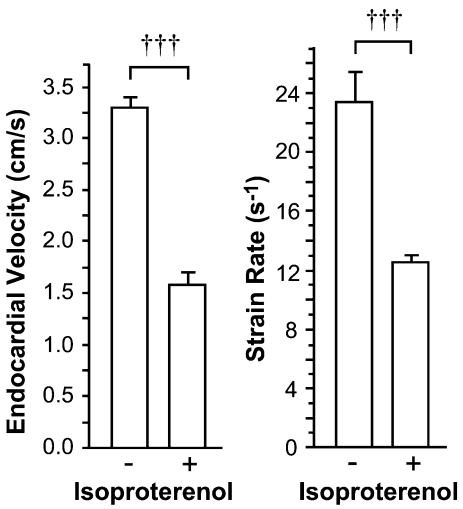

Fig. 2 Significant decreases in TDI results are seen 24 h after a single high dose of isoproterenol. Mean values of left ventricular endocardial velocity (V_{endo}) and radial strain rate (SR) per second (s^{-1}) are shown for isoproterenol-treated (+) and untreated mice (−), as indicated. Significant differences between treated and untreated mice, as assessed by use of the *t* test, are denoted by $^{\dagger\dagger\dagger}(p < 0.001)$. *Error bars* indicate standard error of the mean ($n = 7$)

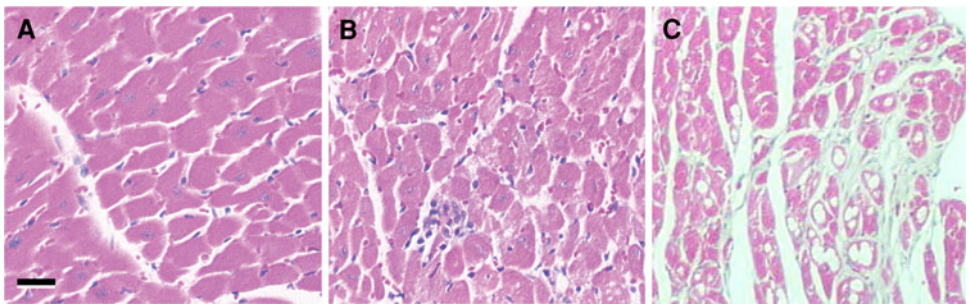

Fig. 1 Isoproterenol-induced histological changes. Sections of paraffin-embedded hearts from **a** untreated, control mice and from mice **b** 24 h and **c** 4 weeks after isoproterenol injection were stained with haematoxylin and eosin for nuclei and cytoplasm, respectively. Myocardial injury is suggested by increased cellular infiltration reflected in nuclear aggregation, as in (**b**), and the presence of disrupted myocardial structure, as in (**c**). *Bar* 10 μm

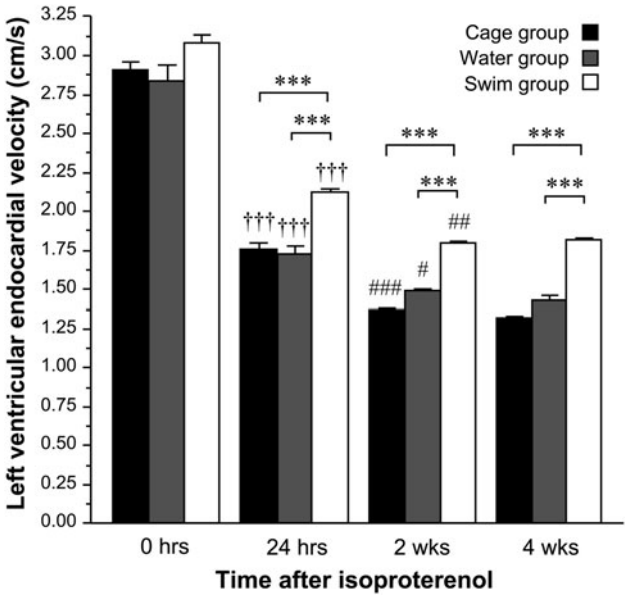

Fig. 3 Left ventricular endocardial velocity (V_{endo}) is improved significantly in the swimming exercise group up to 4 weeks post-injury. Mice were subjected to one 30-min bout of swimming ("swim" group; *white column*); placement in shallow water ("water" group, *grey column*), or being left in a cage ("cage" group, *black column*). Cardiac function assessed immediately before isoproterenol injection is shown at $t = 0$. Cardiac function for each group is also shown at 24 hours (*hrs*) and 2 and 4 weeks (*wks*) post-isoproterenol, as indicated. Significant differences between groups at each time point are indicated by *brackets*; $p < 0.001$ is denoted by ***. For the same group, significant differences between values at 24 h compared with those at $t = 0$, are indicated by $^{\dagger\dagger\dagger}(p < 0.001)$; significant differences between values at 2 weeks compared with 24 h are denoted by $^{\#\#\#}(p < 0.001)$, $^{\#\#}(p < 0.01)$, and $^{\#}(p < 0.05)$. *Error bars* indicate standard error of the mean ($n = 4$)

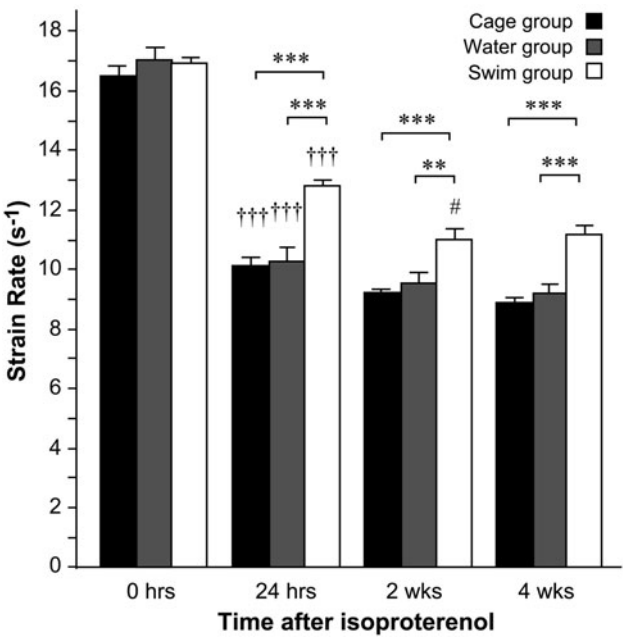

Fig. 4 Strain rate (SR) values (s^{-1}) are improved significantly in the swim exercise group up to 4 weeks post-injury. Mice were subjected to one 30-min bout of swimming ("swim" group; *white column*); placement in shallow water ("water" group, *grey column*), or being left in a cage ("cage" group, *black column*). Cardiac function assessed immediately before isoproterenol injection is shown at $t = 0$. Cardiac function for each group is also shown at 24 hours (*hrs*) and 2 and 4 weeks (*wks*) post-isoproterenol, as indicated. Significant differences between groups at each time point are indicated by *brackets*; $p < 0.01$ and $p < 0.001$ are denoted by ** and ***, respectively. For the same group, significant differences between values at 24 h compared with those at $t = 0$, are indicated by $^{\dagger\dagger\dagger}(p < 0.001)$; significant differences between values at 2 weeks compared with 24 h are denoted by $^{\#}(p < 0.05)$. *Error bars* indicate standard error of the mean ($n = 4$)

during the duration of the exercise period. Twenty-four hours after the swimming exercise, experimental and control groups were subjected to isoproterenol administration. Baseline cardiac function was assessed before and after swimming exercise (or the equivalent time spent in water or a cage), but before isoproterenol treatment. There were no significant differences between the "before" and "after" V_{endo} or SR baseline values within each group (data not shown); we have used the TDI values obtained after swimming and before isoproterenol for comparisons with subsequent time points.

As seen in Fig. 3, isoproterenol induced a 41–43% decrease in V_{endo}, compared with pre-treatment values, in the control "cage" and "shallow water" groups, at the 24 h time point. By 2 and 4 weeks post-isoproterenol treatment, V_{endo} declined even further, by 56% in the "cage" and 51% in the "shallow water" group. The "swim" group V_{endo} values also declined, compared with their corresponding pre-isoproterenol values, by 31% at 24 h, and 40% at 2 and 4 weeks post-isoproterenol treatment (Fig. 3). Nevertheless, V_{endo} values for the "swim" group were significantly higher than corresponding control values at all time points (Fig. 3). V_{endo} values were not significantly different between "cage" and "water" control groups at any time point (for example, values at 24 h were 1.76 ± 0.08 and 1.73 ± 0.09, respectively). However, V_{endo} for the "water" group showed a trend towards some improvement compared with the "cage" group at the later time points of 2 and 4 weeks; pooling of data for the 2 and 4 weeks time points indicated a small (8%) but significant ($p < 0.05$, $n = 8$) increase in V_{endo} of the "water" group compared with the "cage" group.

Compared with pre-isoproterenol values, SR declined significantly, by 40% in the control groups and by 25% in the "swim" group, at 24 h after isoproterenol treatment (Fig. 4). SR values (s^{-1}) in the "swim" group showed a further decline at 2 weeks post-isoproterenol treatment compared with the 24 h value; no further decline in SR values was observed at the 4 week time point. SR values of the "swim" group were significantly higher than the corresponding values for either control group, at all time

points post-isoproterenol (Fig. 4). SR values were not significantly different between control "cage" and "water" groups at 24 h (10.13 ± 0.29 vs. 10.33 ± 0.44) or 4 weeks (8.91 ± 0.15 vs. 9.20 ± 0.31) post-injury.

Discussion

Our main findings are that TDI can detect the adverse effects of high-dose isoproterenol on cardiac function at an early stage, and that a single bout of swimming is sufficient to elicit improved cardiac outcome for at least 4 weeks post-isoproterenol, as measured by TDI.

While M-mode echocardiography did not detect changes in FS and EF, TDI provided evidence of significant left ventricular functional deterioration 24 h after isoproterenol administration. FS and EF are highly dependent on haemodynamic conditions and as such fail to detect early, subtle alterations in regional LV systolic function. TDI, a modification of conventional blood-flow Doppler that images tissue-derived, high amplitude, and low velocity Doppler signals, is less sensitive than LVEF to alterations in loading conditions [20, 27]. Thus, V_{endo} and SR would be less load-dependent compared with FS and EF, and enable quantitative assessment of cardiac function for specific regions of the heart in real time, even before any irregularities can be measured in the whole organ by standard M-mode echocardiography [19, 20, 25, 27]. For example, in a canine model of Duchenne's muscular cardiomyopathy, results from TDI were affected before any changes to conventional fractional shortening were observed [21]. Thus, TDI was used for all subsequent functional assessments of the effect of a single bout of swimming on cardiac function after isoproterenol administration.

Previous studies by us and others have shown that high-dose isoproterenol injection causes myocardial injury, indicated by increased cellular infiltration at early time points; this was also confirmed in this study. Isoproterenol-induced myocardial injury is likely to contribute to the functional decline detected by TDI. It is logical to suggest, although it remains to be documented directly, that the acute swimming exercise may have prevented or reduced the isoproterenol-induced myocardial injury and would be expected to contribute to the observed improvements in function both at the early (24 h) and later time points (2–4 weeks). This would be concordant with previous studies in which acute exercise (running on a treadmill, for example) was shown to prevent myocardial tissue damage measured by histological approaches in dog and rat models [5, 6, 10].

Acute exercise is reported to be capable of eliciting both early and late preconditioning type cardiac responses [5, 9]. In our system, isoproterenol was administered 24 h after the swimming exercise, so the beneficial effects of the latter are presumed to reflect a late-preconditioning type of mechanism. It is also likely that activation of the protein kinase C (PKC) pathway is involved. The PKC pathway is central to both early and late preconditioning responses [28–30], and PKC activation has been linked to exercise-induced preconditioning [7, 9].

Interestingly, one of our control groups, the "water" group, showed a slight improvement in (pooled, 2–4 weeks) V_{endo} values compared with the "cage" group. This would suggest that simply being placed in water and outside the normal "cage" environment might have been "stressful" enough for some activation of beneficial signalling pathways.

In conclusion, a single bout of (swimming) exercise is sufficient to increase resistance to isoproterenol-induced myocardial dysfunction within 24 h post-treatment and enable prediction of improved functional outcome. Furthermore, this initial benefit to cardiac function as assessed by TDI in vivo will persist for at least 4 weeks. The time frame and nature of the benefit is consistent with exercise late preconditioning.

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