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ORIGINAL ARTICLE

Atorvastatin administered before myocardial infarction in rats improves contractility irrespective of metabolic changes

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SUMMARY

Statins have a beneficial effect after myocardial infarction, but the relationship between glucose transporters and their use before the event has not yet been studied. We assessed the effects of atorvastatin treatment pre- and post-myocardial infarction on cardiovascular function and glucose transporter 4 (GLUT4) in the heart. Wistar–Kyoto rats were treated with 20 mg/kg atorvastatin or vehicle for 14 days before coronary artery occlusion surgery (myocardial infarction) or sham surgery. Echocardiographic evaluations were carried out 48 h after myocardial infarction (protocol A) and after 7 days (protocol B), when atorvastatin was also administered. Plasma inflammatory markers and GLUT4 in the heart were also evaluated. Animals were divided into the following groups: sham-operated and vehicle (C), myocardial infarction and vehicle (I), sham-operated and atorvastatin (CAt) and myocardial infarction and atorvastatin (IAt). After 48 h, myocardial infarction induced higher left ventricular fractional shortening in IAt versus I (~ 60%, $P = 0.036$), and the ejection fraction was lower (protocol A ~ 37%; protocol B ~ 30%). Myocardial infarction was associated with a rise in plasma membrane GLUT4 after 48 h (~ 40%, $P < 0.001$), and a reduction in GLUT4 after 7 days (I 25%; IAt 49%, $P < 0.001$). Atorvastatin treatment for 48 h after the infarction did not change GLUT4 expression, and after 7 days it had an additional negative effect on GLUT4 content (~ 39%, $P = 0.030$). In conclusion, atorvastatin treatment pre- and post-myocardial infarction improved myocardial contractility after 48 h, but not after 7 days, and was not associated with an increase in GLUT4 expression.

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INTRODUCTION

Acute myocardial infarction is a major consequence of coronary artery disease, leading to high morbidity and mortality. It frequently determines high rates of heart failure, which is characterized by low cardiac output, thus limiting oxygen delivery to the peripheral tissues.[1] The impaired cardiac function is secondary to both decreased contractility and ventricular compliance, followed by ventricular remodeling.

Increased expression of pro-inflammatory cytokines (tumor necrosis factor-α (TNF-α) and interleukin (IL-6) occurs in the heart after an acute myocardial infarction.[2–4] By contrast, IL-10 is considered a major anti-inflammatory cytokine and, as a compensatory mechanism, it might acutely increase after cardiac injury.[4,5] Imbalance between pro- and anti-inflammatory cytokines, with a shift towards pro-inflammatory cytokines, has been linked to insulin resistance, with TNF-α playing a key role.[6] Increased pro-inflammatory cytokines have been related to reduced expression of the glucose transporter 4 (GLUT4) in adipose tissue and plasma.[7,8] Furthermore, nuclear factor-κB (NF-κB), a mediator of several inflammatory effects, has been shown to repress GLUT4 protein expression, thus contributing to insulin resistance.[9]

In steady-state conditions (aerobic perfusion, normal workload), the heart generates its energy mainly by oxidizing non-esterified fatty acids, with a 40% glucose contribution.[10] Glucose utilization by cardiomyocytes becomes more important when energy demand increases; for example, in periods of high workload[10] or imbalance, as in ischemia, hypoxia or pressure overload.[11,12] In such situations, the reduced amount of oxygen available impairs the substrate oxidation, and anaerobic glycolysis becomes an important source of energy. Glucose uptake must increase to maintain cardiomyocyte homeostasis, which is why increased plasma membrane GLUT4 protein is beneficial. In fact, impaired postischemic heart contractile recovery was previously shown in cardiac-specific GLUT4 knockout mice.[13] Furthermore,

in the perfused hearts of insulin-resistant mice, substrate metabolic derangement and contractile dysfunction were restored with GLUT4 overexpression (transgenic *db*/*db*-hGLUT4 mice).[14] This points to the GLUT4 protein as a target for pharmacological interventions seeking to improve myocardial contractility through cardiac metabolism improvement.[15]

Statins are drugs that reduce mortality after myocardial infarction in humans as a result of their lipid-lowering and pleiotropic effects.[16,17] In insulin-resistant conditions, the anti-inflammatory effects of statins have been extensively reported, and involve increased glucose uptake, insulin signaling and GLUT4 expression.[18–22] Furthermore, a 4-week treatment with atorvastatin can also reduce the inflammatory state after myocardial infarction of rats.[19] However, neither the effects of statins given before and immediately after an acute myocardial infarction on cardiac functional changes, nor on the potentially GLUT4-mediated metabolic state, have been investigated. The present study aimed to evaluate the effect of atorvastatin given before and immediately after an acute myocardial infarction on myocardial function and on the potential mechanisms involved, such as plasma inflammatory cytokine markers and GLUT4 expression in heart tissue.

METHODS

Animals

All procedures and experiments involving animals followed the recommendations of the Brazilian College of Animal Experimentation and international standards of animal research set out in the Guide for the Care and Use of Laboratory Animals.[23] The study was approved by the research ethics committee of Institute of Cardiology/University Foundationof Cardiology of Rio Grande do Sul, Porto Alegre, Brazil (protocol 4387/09).

A total of 64 male Wistar–Kyoto (WKY) rats aged ~ 60 days were divided into the following four groups: (i) sham-operated treated with oral vehicle (C); (ii) sham-operated treated with oral atorvastatin (CAt); (iii) left coronary artery ligation treated with oral vehicle (I); and (iv) left coronary artery ligation treated with oral atorvastatin (IAt). The dose of atorvastatin (20 mg/kg bodyweight) was based on the literature, considering its pleiotropic effects.[24,25] We aimed to study the effects of statin treatment applied before and immediately after myocardial infarction, in an attempt to attain cardiac remodeling effects, which generated the two protocols fully detailed here.[26,27]

In protocol A, atorvastatin (20 mg/kg bodyweight) or vehicle was given to 25 animals for 14 days before left coronary artery ligation or sham surgery. Five out of 25 rats died as a result of procedure-related complications. Thus, 20 animals were equally allocated into groups of this protocol (n = 5 per group). A total of 24 h later, an echocardiography was carried out. Then, 48 h after the echocardiography, the animals were anaesthetized again, blood from the aorta was collected, thoracotomy was carried out and the hearts were removed.

In protocol B, atorvastatin or vehicle was given to 39 animals for 14 days before left coronary artery ligation or sham surgery (six out of these rats died from procedure-related complications), and was followed by continued administration of atorvastatin or vehicle for a further 7 days. Thus, 33 rats were allocated to this protocol, as follows: C (n = 8), CAt (n = 7), I (n = 9) and IAt (n = 9). After that, the animals were treated exactly as described in protocol A. These experimental protocols are summarized in Fig. 1.

The animals were bred and kept under conventional conditions in the animal house at the Animal Production and Experimentation Department of the Center for Scientific and Technological Development, State Health Research Foundation of Rio Grande do Sul, Porto Alegre, Brazil. All the animals received standard food and water *ad libitum*, and were kept in cages under a 12-h light/dark cycle (from 0600 to 1800 h).

Coronary artery ligation and sham surgery

Myocardial infarction was induced according to a procedure previously described in the literature.[28,29] Briefly, animals were placed in the dorsal decubitus position and anaesthetized with ketamine (160 mg/kg bodyweight) and xylazine (10 mg/kg bodyweight) administered intraperitoneally. After orotracheal intubation,

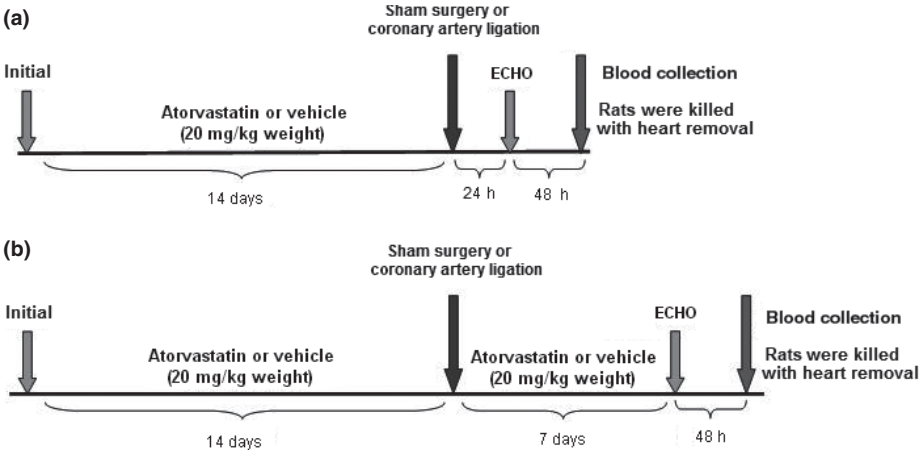

Fig. 1 Experimental design. (a) Protocol A. Atorvastatin or vehicle was given for 14 days. Left coronary artery ligation or sham surgery was then carried out. Echocardiography was carried out 24 h later. Then, 48 h later, blood was collected and the rats' hearts were removed. (b) Protocol B. Atorvastatin or vehicle was administered for 14 days. Left coronary artery ligation or sham surgery was then carried out, followed by continued administration of atorvastatin or vehicle for seven more days and echocardiographic assessment. Blood was collected and the rats' hearts were removed 48 h later.

animals were submitted to mechanical ventilation with a Harvard ventilator, Model 683 (Holliston, MA, USA). A surgical incision was made in the skin along the left sternal margin, and divulsion of the pectoral and transverse muscle was carried out. Thoracotomy was carried out in the second intercostal space, and the thorax was opened without exteriorization of the heart. The left anterior descending coronary artery was identified and occluded with a 6-0 mononylon suture between the left atrial appendage margin and the pulmonary artery. The thoracic cavity was then closed with a 5-0 mononylon thread, the muscles were repositioned and the skin sutured. All animals received analgesic after the surgical procedure (butorphanol 2 mg/kg).

Infarcted animals were considered those with an ejection fraction $\leq 50\%$ and infarcted area (akinetic) $\geq 30\%$.[28,30] Animals that did not develop myocardial infarction were discarded, except three rats in which complete failure of coronary artery ligation occurred. These rats were included as controls, as it was clear that little or no myocardial damage would be expected, as follows: one rat into C, protocol A; one rat into CAt, protocol A; and one rat into CAt, protocol B.[31,32] Animals that were submitted to a similar surgical procedure, but without coronary artery ligation, were used as sham-operated.

Echocardiography

Echocardiography was carried out with the animals placed in the left lateral decubitus position (45°) to obtain cardiac images. The EnVisor HD System, Philips Medical (Andover, MA, USA) was used, with a 12–3-MHz transducer, 2-cm deep, and fundamental harmonic imaging. Images were captured by a trained operator with experience in animal echocardiography.

Left ventricular dimensions

The end-diastolic and end-systolic transverse areas (cm^2) were obtained by tracing the endocardial border at three levels: basal (at the tip of the mitral valve leaflets), middle (at the papillary muscle level) and apical (distal from the papillary muscle, but before the final curve cavity).[33,34] End-diastolic and end-systolic diameters (cm) were measured using the M-Mode, also in the three planes. The final value for each animal was obtained from the average of the planes.

Size of myocardial infarction

On each echocardiographic transverse plane (basal, middle and apical), the arc corresponding to the segments with infarction (regions or segments of the myocardium showing one of the following changes in myocardial kinetics: systolic movement akinetic and/or hypokinetic region) and the total endocardial perimeter were measured at the end-diastole. Infarction size was estimated as percentage of circumference of endocardial perimeter = (akinetic and/or hypokinetic region/endocardial perimeter) $\times$ 100.[35]

Left ventricular systolic function

Left ventricular ejection fraction (EF) was calculated as: (end-diastolic volume − end-systolic volume/end-diastolic volume) $\times$ 100; end-diastolic and end-systolic cavity volumes were calculated using Simpson's rule.[36] Left ventricular fractional shortening (LVFS) was obtained using the equation: LVFS = diastolic diameter − systolic diameter (SD)/diastolic diameter $\times$ 100).[37]

Membrane preparation

Cardiac tissue was prepared by homogenization as described in the literature.[38] In brief, heart tissue was fragmented and processed in a buffer solution (pH 7.4, 10 mmol/L Tris–HCl, 1 mmol/L EDTA and 250 mmol/L sucrose) at a 1 : 4 ratio (weight : volume) using a mechanical tissue homogenizer (Marconi, Piracicaba, Brazil), four to five 30-s pulses at 20 000 rpm. It was then centrifuged at 1000 *g* for 10 min. The supernatant was removed, and the pellet resuspended in the same buffer, at one-third of the original volume, and centrifuged at 1000 *g* for 10 min. The supernatant was mixed with that from the first step and centrifuged at 16 000 *g* for 60 min. The pellet was resuspended in 500 μL buffer, corresponding to the plasma membrane fraction. The supernatant was centrifuged at 150 000 *g* for 75 min, and the pellet was resuspended in 250 μL buffer, corresponding to the microsomal fraction. To determine the total protein concentration of the fractions, a modified Bradford method was used with Coomassie Blue reagent (Bio Rad, Philadelphia, PA, USA).[39]

Western blotting

GLUT4 protein content in the subcellular fractions was determined using western blotting. The samples (150 μg) were separated by electrophoresis on 10% polyacrylamide gel/sodium dodecylsulphate (SDS; Invitrogen, Carlsbad, CA, USA) with a molecular weight marker (BenchMark; Invitrogen, Burlington, VT, USA). NuPAGE sample buffer (Invitrogen) was used to prepare protein samples for denaturing gel electrophoresis. The samples were denatured for 5 min at 100°C, and separated in buffer containing 0.25 mmol/L Tris-base (Invitrogen), 1.92 mmol/L glycine (Merck, Darmstadt, Germany) and 1% SDS

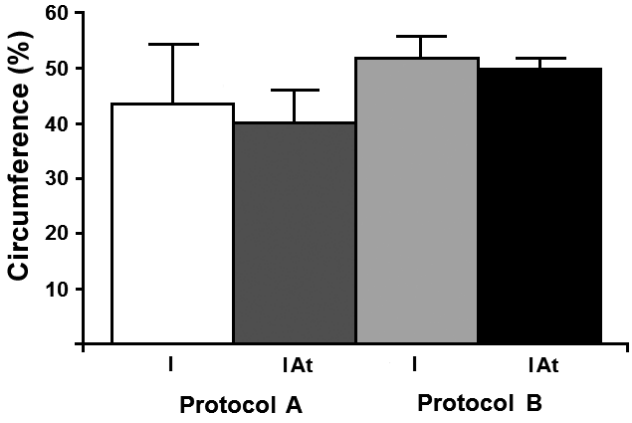

Fig. 2 Representation of the extension of the myocardial infarction caused by coronary artery ligation. Acute myocardial infarction induced by left coronary artery ligation and oral vehicle (I; $n = 5$, protocol A; $n = 9$, protocol B); and acute myocardial infarction induced by left coronary artery ligation and atorvastatin (IAt; $n = 5$, protocol A; $n = 9$, protocol B). The results are shown as mean ± standard deviation. One-way ANOVA; no difference between the groups ($P = 0.362$).

Table 1 Bodyweight (g) of the animals during the study

	C	CAt	I	IAt
Initial	289.6 ± 56.5	275.0 ± 51.0	287.4 ± 47.8	262.3 ± 46.7
7 days	318.1 ± 50.7	310.6 ± 33.1	323.1 ± 43.8	299.5 ± 32.4
14 days	333.8 ± 49.8	325.5 ± 32.8	334.4 ± 42.5	315.9 ± 36.9
21 days	326.8 ± 42.4	329.6 ± 25.1	334.1 ± 31.9	313.2 ± 32.9

The results are shown as mean ± standard deviation. Repeated measures ANOVA ($P = 0.662$). Fourteen-day treatment with oral atorvastatin or vehicle followed by myocardial infarction plus 7 days of treatment with oral atorvastatin or vehicle after myocardial infarction. C, sham-operated rats and oral vehicle; Cat, sham-operated rats and atorvastatin; I, left coronary artery ligation and oral vehicle; IAt, left coronary artery ligation and atorvastatin.

(Invitrogen). Proteins were then transferred to a Hybond ECL nitrocellulose membrane (GE Healthcare, Niskayuna, NY, USA) in a semi-dry transfer system (GE Healthcare). Membrane blocking was achieved in 5% casein for 1 h. The membrane was incubated with 1 : 250 anti-GLUT4 antibodies (#CBL243; Millipore, Billerica, MA, USA) for 16 h at 4°C, followed by additional incubation for 3 h at 37°C. The membrane was washed (in 0.05% phosphate-buffered saline/Tween 20, 1 × 15 min, 3 × 5 min), and incubated with conjugated anti-immunoglobulin G with peroxidase (#AP307P, Millipore) for 1 h. It was washed again in phosphate-buffered saline/Tween 20 (1 × 15 min, 4 × 5 min), incubated with a substrate for peroxidase (ECL kit; GE Healthcare), in the dark, for 3 min and exposed to an ultrasensitive radiographic film (Kodak, Frankfurt, Germany) for 1 h. Band intensity was measured by optical density using a public-domain software program, Scion Image (Scion Corporation, Maryland, USA). For loading controls, densitometric analysis of total protein in the lanes was carried out on Ponceau-stained membranes (based on Page Ruler Prestained Protein Ladder; Thermo Scientific, Marietta, Ohio, USA). These values were used to normalize the respective GLUT4 values.[40] The final results were expressed as arbitrary units (AU).

Enzyme-linked immunosorbent assay

The expression of the inflammatory markers IL-6, TNF-α and IL-10 was only carried out in protocol A, and analyzed by enzyme-linked immunosorbent assay (ELISA). Plasma samples were thawed 1 h before use, and assayed using Platinum ELISA kits available (eBiosciences, San Diego, CA, USA) specifically for the detection of molecules in rats. All measurements were carried out using four-parameter linear regression (Excel; Microsoft, Redmond, WA, USA).

Statistical analysis

The results are described as means and standard deviations. The number of animals (n) in each group is presented in the legends. Differences were assessed using the Student's t-test (myocardial infarction area) and two-way ANOVA followed by the *post-hoc* (Bonferroni) test for the other variables. The factors used in the ANOVA were: sham surgery or induction of acute myocardial infarction, atorvastatin or vehicle, and the interaction between these factors. The level of significance was set at 5% for all tests.

RESULTS

The size of the myocardial infarction area is shown in Fig. 2. There were no differences in the area of myocardial infarction induced by coronary artery ligation in both protocols, a critical condition for the analysis of the biochemical and echocardiographic parameters presented here.

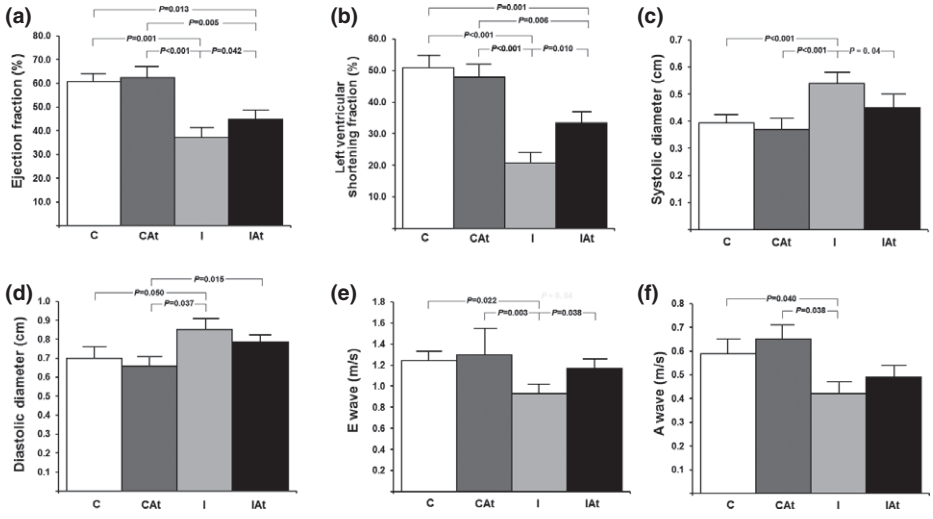

Fig. 3 Effects of atorvastatin on cardiovascular function (echocardiography) in protocol A. The results are shown as mean ± standard deviation, $n = 5$ for all groups. Two-way ANOVA followed by *post-hoc* (Bonferroni) test was used for all assessments of cardiac function. (a) Ejection fraction ($P = 0.025$). (b) Left ventricular shortening fraction ($P = 0.036$). (c) Systolic diameter ($P = 0.007$). (d) Diastolic diameter ($P = 0.044$). (e) E wave ($P = 0.040$). (f) A wave ($P = 0.021$). C, sham-operated rats and oral vehicle; CAt, sham-operated rats and atorvastatin; I, acute myocardial infarction induced by left coronary artery ligation and oral vehicle; IAt, acute myocardial infarction induced by left coronary artery ligation and atorvastatin.

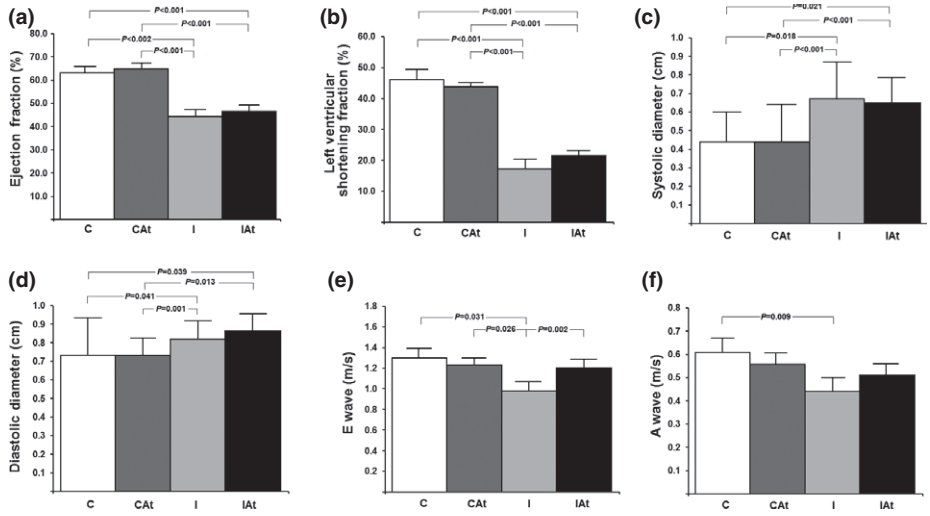

Fig. 4 Effects of atorvastatin on cardiovascular function (echocardiography) in protocol B. The results are shown as mean ± standard deviation. Two-way ANOVA followed by *post-hoc* (Bonferroni) test was used for all assessments of cardiac function. (a) Ejection fraction ($P < 0.001$). (b) Left ventricular shortening fraction ($P < 0.001$). (c) Systolic diameter ($P < 0.001$). (d) Diastolic diameter ($P = 0.007$). (e) E wave ($P = 0.001$). (f) A wave ($P = 0.031$). C, sham-operated rats and oral vehicle ($n = 8$); CAt, sham-operated rats and atorvastatin ($n = 7$); I, acute myocardial infarction induced by left coronary artery ligation and oral vehicle ($n = 9$); and IAt, acute myocardial infarction induced by left coronary artery ligation and atorvastatin ($n = 9$).

No differences were observed in the bodyweights of the rats throughout the experiments (repeated measures ANOVA, $P = 0.662$; Table 1).

Figures 3 and 4 show assessment of cardiac function obtained in the two protocols (A and B, respectively). The left ventricle EF was lowered ~ 37% in response to myocardial infarction (protocol A: C *vs* I, $P = 0.001$; protocol B: C *vs* I, $P = 0.002$). Atorvastatin treatment showed only a transitory beneficial effect that was observed in 48 h (I *vs* IAt, $P = 0.042$), but not after 7 days. Similarly to EF, the myocardial infarction reduced the left ventricular shortening fraction in both protocol A (C *vs* I, $P < 0.001$) and protocol B (C *vs* I, $P < 0.001$). Furthermore, atorvastatin treatment for 48 h increased the left ventricular shortening fraction in 60% (I *vs* IAt, $P = 0.010$) and ~ 25% after 7 days, but there was no statistical significance. Increased SD was seen in the animals after myocardial infarction in both protocols (protocol A: C *vs* I, $P < 0.001$; protocol B: C *vs* I, $P = 0.018$), leading to impaired systolic function; additionally, atorvastatin before myocardial infarction reduced the SD (protocol A: I *vs* IAt, $P = 0.004$) to values similar to those observed in C animals. Curiously, atorvastatin did not have any effect on SD in protocol B. The diastolic diameter increased in response to myocardial infarction (protocol A: C *vs* I, $P = 0.050$; protocol B: C *vs* I, $P = 0.041$), but no change was observed with atorvastatin treatment in either protocol. Myocardial infarction reduced flow velocity waveforms (E wave and A wave) in protocols A (C *vs* I, $P = 0.022$ and C *vs* I, $P = 0.040$, respectively) and B (C *vs* I, $P = 0.031$ and C *vs* I, $P = 0.009$, respectively), and atorvastatin treatment recovered the E wave in protocol A (I *vs* IAt, $P = 0.038$) and B (I *vs* IAt, $P = 0.002$). The E/A ratio, indicating diastolic function, was similar in all groups in both protocols (data not shown).

Plasma cytokines (IL-6, TNF-α and IL-10) were only measured in protocol A, and are shown in Fig. 5. Acute myocardial infarction induced an increase in plasma levels of both pro-inflammatory (IL-6 and TNF-α: C *vs* I, $P < 0.001$ for both) and anti-inflammatory IL-10 (C *vs* I, $P = 0.002$) cytokines. Treatment with atorvastatin did not reverse the effect of myocardial infarction on pro-inflammatory cytokines. Furthermore, it induced a slight decrease in the plasma levels of IL-10, when compared with the I group.

GLUT4 protein content modulation in the rats' hearts varied according to the protocol. In protocol A (Fig. 6a,b), myocardial infarction led to increased GLUT4 expression in both microsomal ~ 30% (C *vs* I, $P = 0.040$) and plasma membrane fractions ~ 40% (C *vs* I, $P = 0.042$); and there was a slight, but statistically insignificant, increase in GLUT4 expression with atorvastatin. Figure 6c,d shows the GLUT4 expression in the heart of animals subjected to protocol B. Conversely to what was observed in protocol A, the I group of protocol B had a lower GLUT4 content than the C group, in both microsomal (C *vs* I, $P = 0.044$) and plasma membrane fractions (C *vs* I, $P = 0.039$), and, surprisingly, atorvastatin treatment induced an additional reduction in plasma membrane GLUT4 content ~ 39% (IAt *vs* I, $P = 0.030$).

DISCUSSION

The present study showed that continuous use of atorvastatin for 14 days before acute myocardial infarction improved cardiac function (systolic and diastolic parameters) in an early phase (first 48 h), but this effect was not maintained 7 days later, despite the fact that atorvastatin had not been discontinued. These beneficial effects were not associated with changes in heart GLUT4 and plasma cytokines (TNF-α, IL-6 and IL-10).

Treatment with atorvastatin did not affect the animals' weights, consistent with previous reports.[41] The effect of myocardial infarction was similar in both protocols, and the data obtained are consistent with those previously reported.[3] This involves reduced myocardial contractility with consequent impairment of cardiac function assessed 48 h and 7 days after the procedure, which is also in accordance with previous findings.[3,28]

There was a beneficial effect of atorvastatin treatment for 14 days before myocardial infarction, evidenced by an improve-

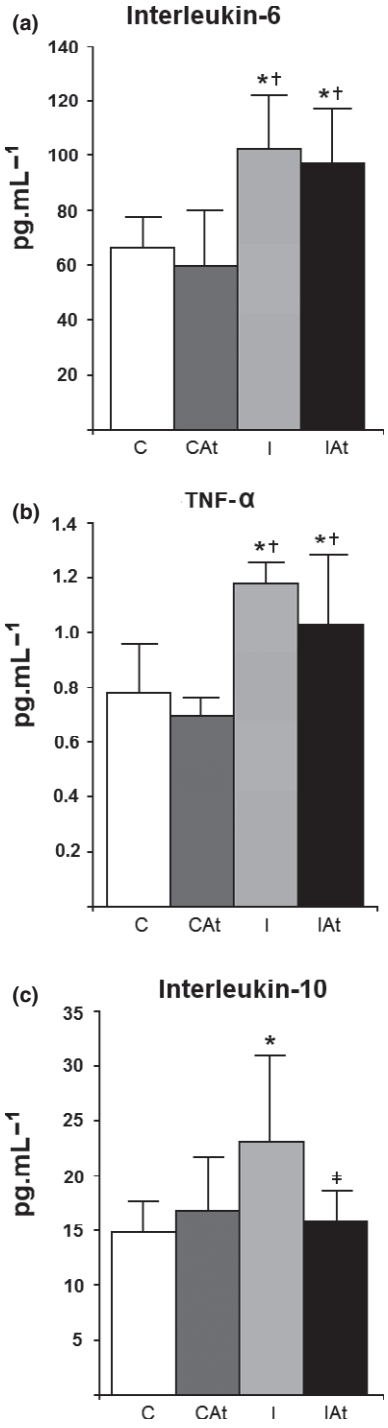

Fig. 5 Plasma levels of (a) interleukin (IL)-6, (b) tumor necrosis factor (TNF)-α and (c) IL-10 after myocardial infarction caused by coronary artery ligation (protocol A). The results are shown as mean ± standard deviation, $n = 5$ for all groups. Two-way ANOVA (IL-6: $P = 0.015$; TNF-α: $P = 0.002$; IL-10: $P = 0.040$) followed by *post-hoc* (Bonferroni) test. *$P < 0.05$ versus sham-operated rats and oral vehicle (C), †$P < 0.05$ versus sham-operated rats and atorvastatin (CAt), ‡$P < 0.05$ versus sham-operated rats and atorvastatin (I). IAt, acute myocardial infarction induced by left coronary artery ligation and atorvastatin.

ment in echocardiographic parameters 48 h after the ischemic insult; however, seven additional days of treatment after the infarction were not associated with these benefits. These findings are probably related to atorvastatin's so-called pleiotropic effects on cardiac function, but they were transitory and did not last more than a few days. The early benefits that we observed with atorvastatin were also reported in animal models of isoproterenol-induced heart failure or volume overload, where treatment with atorvastatin improved the left ventricular function, myocardial contractility and relaxation.[24,42] These improvements were attributed to the anti-oxidant and anti-inflammatory effects of the drug.[24,42] In an animal model of heart failure as a result of myocardial infarction induced by coronary artery ligation, as in the present study, other authors have shown the benefits of atorvastatin on brain natriuretic peptide, which was attributed to reduced myocardial fibrosis as a result of reduced cardiomyocyte apoptosis and reduced inflammatory cytokines associated with increased anti-inflammatory cytokines.[19,43] The present data showed no effects of atorvastatin on the plasma pro-inflammatory markers evaluated, and a slight decrease in the plasma levels of IL-10, but this does not imply that a local anti-inflammatory response and/or other beneficial factors that have not been evaluated (e.g. reduction of oxidative stress) are present.

In the present study, myocardial infarction induced increased GLUT4 expression (microsomal and plasma membrane fractions) after 48 h. Nishino *et al.* showed that GLUT4 mRNA expression increased 150% 3 h after an ischemic insult, and the total GLUT4 protein level increased 107% 24 h after the insult. As previously shown, myocardial ischemia provokes protein kinase C translocation from the cytosol to the membrane, thus increasing the activity of adenosine monophosphate-activated protein kinase, which might contribute to increased translocation of GLUT4 to the cell surface.[44] Additionally, decreased intracellular O_2 tension induced by ischemia activates the hypoxia-inducible factor 1A, a powerful enhancer of GLUT4 transcriptional expression.[45] Curiously, atorvastatin treatment did not change GLUT4 expression, despite its expected enhanced effect, a phenomenon that might be related to the absence of a previously established inflammatory state.[18]

By contrast, 7 days after infarction, GLUT4 content was decreased, a result which might be related to the fact that the GLUT4 content, expressed by g of heart tissue, was measured in the whole left ventricle, including the infarcted area, in which there was less or no GLUT4. Unexpectedly, the extended use of atorvastatin after myocardial infarction resulted in a greater reduction in GLUT4 expression in the plasma membrane, but not in the microsomal fraction, suggesting impaired GLUT4 vesicle mobilization to the cell surface. This unexpected regulation of GLUT4 might be related to the absence of an underlying subclinical inflammation, which is a feature in obesity, insulin resistance and type 2 diabetes,[6] conditions under which statins have been shown to have an anti-inflammatory effect.[18–22] In fact, in 3T3L1 adipocytes, with no latent inflammation, atorvastatin does not induce GLUT4 expression and/or translocation; on the contrary, decreased GLUT4 expression is observed during cell differentiation.[46] Furthermore, in similar conditions (no inflammation), insulin-related decreased GLUT4 translocation to the plasma membrane was reported under statins.[47]

The present results also show that acutely-induced myocardial infarction increases IL-10, perhaps as a compensatory mechanism, as IL-10 is involved in suppressing the acute inflammatory response and in regulating the extracellular matrix metabolism.[4,5]

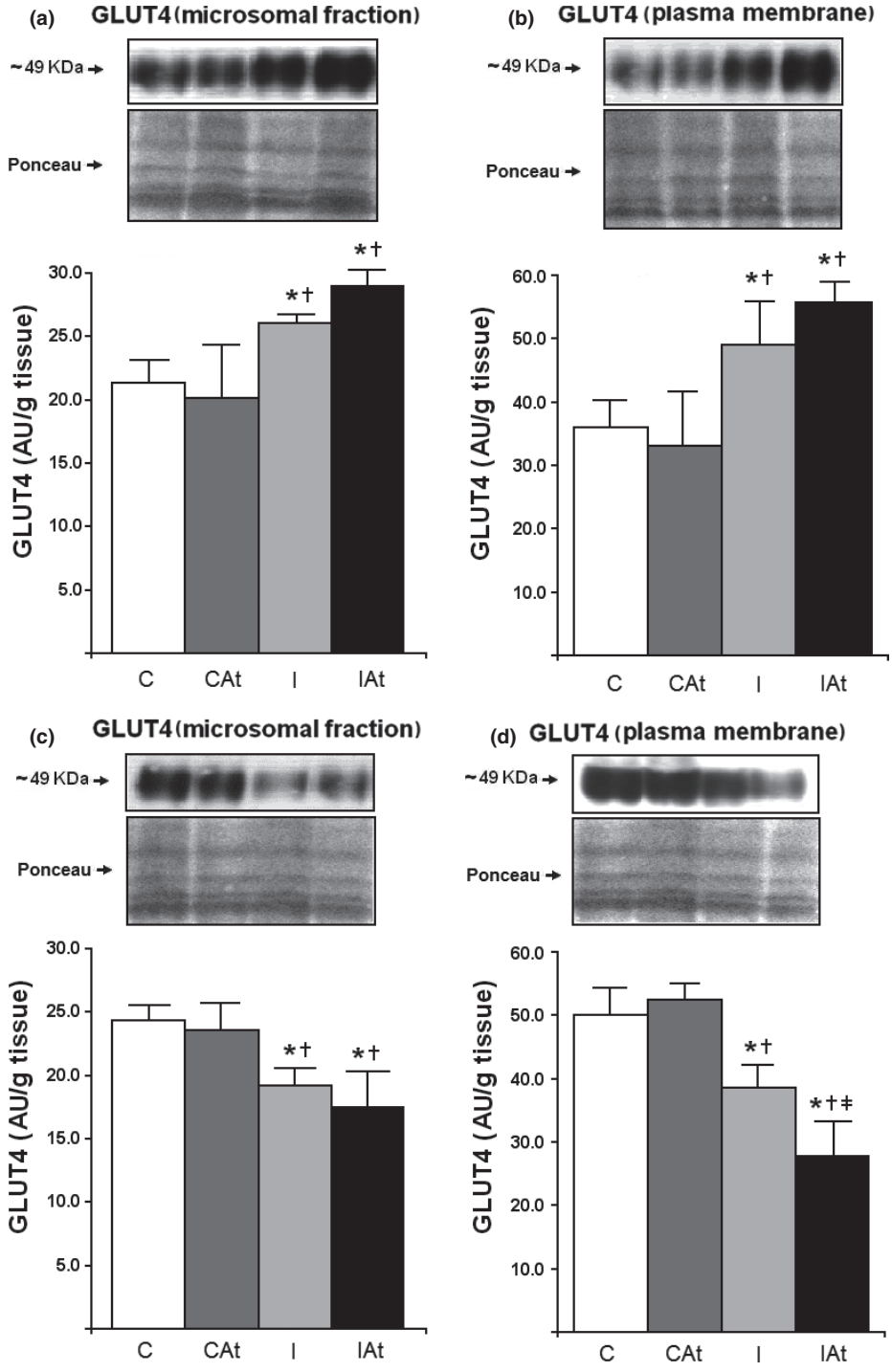

Fig. 6 Expression of glucose transporter 4 (GLUT4) in protocol A and protocol B. (a) Microsomal fraction in protocol A. (b) plasma membrane in protocol A. (c) Microsomal fraction in protocol B. (d) Plasma membrane in protocol B. Quantitative analysis and the corresponding bands in the western blot. The total protein loading was controlled by Ponceau staining. The results are shown as mean ± standard deviation, $n = 5$ for all groups. Two-way ANOVA. (a) $P = 0.003$, (b) $P < 0.001$, (c) $P = 0.036$ and (d) $P = 0.022$ followed by *post-hoc* (Bonferroni) test. *$P < 0.05$ versus sham-operated rats and oral vehicle (C), †$P < 0.05$ versus sham-operated rats and atorvastatin (CAt), ‡$P < 0.05$ versus sham-operated rats and oral vehicle (I). AU, arbitrary units; IAt, acute myocardial infarction induced by left coronary artery ligation and atorvastatin.

In fact, in experimental myocardial ischemia-reperfusion, both TNF-α (pro-inflammatory) and IL-10 (anti-inflammatory) were reported to increase, whereas atorvastatin treatment (at the same dose used here) was shown to prevent increases in both TNF-α and IL-10 levels.[48] In the present study, prior treatment with atorvastatin prevented the myocardial infarct-induced increase in IL-10 levels. Resolution of post-infarction inflammation is likely to involve multiple overlapping regulatory mechanisms controlling various pro- and anti-inflammatory pathways. In the absence of a latent inflammatory condition, an infarct-induced increase in

TNF-α was shown to be transient, peaking within a few hours.[2] As the well-known anti-inflammatory effects of atorvastatin have been related to the presence of a latent inflammatory condition, it is reasonable to suppose that the benefits of atorvastatin use in myocardial infarction might be related to the presence of such a condition and/or longer periods of treatment.[7,19]

The interpretation of the study findings must be tempered with the methodological limitations. Inflammatory markers were measured in plasma, thus conclusions about local cardiac inflammation are limited. Atorvastatin was administered over a short period post-infarct, and there could be additional long-term atorvastatin effects that were not captured during this short timeframe. Despite these limitations, the results are trustworthy and provide some important conclusions – using atorvastatin before ischemia can improve myocardial contractility, but this is a transient effect not associated with GLUT4. Short-term treatment (7 days) with atorvastatin did not produce benefits to myocardial contractility, and seems to have negatively affected the expression of GLUT4 in the plasma membrane, as compared with use of atorvastatin before myocardial infarction.

As a clinical perspective, statins are drugs that have been shown to reduce mortality after myocardial infarction in humans as a result of their lipid-lowering and pleiotropic effects, including anti-inflammatory effects. However, the effects of statins administered before and immediately after an acute myocardial infarction on cardiac functional changes have never been investigated. Despite the methodological limitations of the study, we showed that atorvastatin treatment for 14 days before ischemic insult improved myocardial contractility, evidenced by echocardiographic parameters 48 h after the ischemic insult. However, seven additional days of treatment after the infarction were not associated with these benefits.

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