# Atorvastatin-induced Cardioprotection of Human Myocardium Is Mediated by the Inhibition of Mitochondrial Permeability Transition Pore Opening *via* Tumor Necrosis Factor-α and Janus Kinase/Signal Transducers and Activators of Transcription Pathway

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## ABSTRACT

**Background:** The role of tumor necrosis factor-α (TNF-α), Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway, and mitochondrial Permeability Transition Pore in atorvastatin-induced cardioprotection were examined in human myocardium, *in vitro*.

**Methods:** Isometric force of contraction of human right atrial trabeculae was recorded during 30-min hypoxia and 60-min reoxygenation (control) and in the presence of atorvastatin (0.1 μM, 1 μM, 10 μM). In early reoxygenation, the TNF-α inhibitor, AG490 (inhibitor of JAK/STAT), or atractyloside (mitochondrial Permeability Transition Pore opener), were administered. Cyclosporine A (inhibitor of mitochondrial Permeability Transition Pore opening) was administered during the first minute of reoxygenation alone or in presence of atorvastatin and TNF-α inhibitor or AG490. The force of contraction (percentage of baseline) at the end of reoxygenation period was compared (mean ± SD; n = 6 in each group). Protein expression of JAK/STAT pathway was measured using Western immunoblotting.

**Results:** Atorvastatin 0.1 μM (70 ± 9%), 1 μM (85 ± 5%), 10 μM (89 ± 5%), and Cyclosporine A (87 ± 10%) improved the recovery of force of contraction at the end of reoxygenation, as compared with control (50 ± 3%). Atorvastatin 1 μM (4.64 ± 2.90 ng · ml$^{-1}$ · g$^{-1}$ of tissue) decreased the release of troponin Ic after hypoxia-reoxygenation (control: 26.34 ± 19.30 ng · ml$^{-1}$ · g$^{-1}$; $P < 0.001$). The enhanced recovery of force of contraction after atorvastatin administration was abolished by TNF-α inhibitor (53 ± 8%), AG490 (56 ± 7%), atractyloside (48 ± 8%). Cyclosporine A restored the atorvastatin-induced cardioprotection abolished by TNF-α inhibitor (87 ± 6%) and AG490 (83 ± 9%). Atorvastatin significantly increased the phosphorylation of JAK-2 and STAT-3, TNF-α inhibitor abolished the enhanced phosphorylation of JAK-2 and STAT-3 by atorvastatin.

**Conclusions:** Atorvastatin-induced cardioprotection involved the inhibition of the mitochondrial Permeability Transition Pore opening via the activation of TNF-α and the JAK/STAT pathway in early reoxygenation.

### What We Already Know about This Topic

- Statins have been suggested to decrease perioperative morbidity in patients undergoing major vascular surgery and have been shown to reduce perioperative myocardial ischemia
- This study investigated the cardioprotective effect of continuous atorvastatin exposure on isolated human myocardium submitted to hypoxia-reoxygenation

### What This Article Tells Us That Is New

- Atorvastatin-induced cardioprotection involved inhibition of mitochondrial Permeability Transition Pore opening *via* the activation of tumor necrosis factor-α and janus kinase/signal transducers and activators of transcription pathways in early reoxygenation

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IT has been shown that 3-hydroxy-3-methylglutaryl CoA reductase inhibitors (statins) provide cardiovascular benefits, through both lipid-lowering and non–lipid-lowering effects, and their use for primary and secondary prevention in patients with cardiovascular disease is established.[1] Statins have been suggested to decrease perioperative morbidity in patients undergoing major vascular surgery[2] and have been shown to reduce perioperative myocardial ischemia.[3]

Experimental studies confirmed that statins exert cholesterol-independent pleiotropic effects including cardioprotection against ischemia-reperfusion injury.[4–8] However, the mechanisms of statins-induced cardioprotection remain incompletely understood and could involve multiple signaling pathways known to be associated with pre- and postconditioning.[9] Recently, the activation of cytokine tumor necrosis factor-α (TNF-α), and the prosurvival Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway have been shown to play a key role in myocardial postconditioning (fig. 1), and have been identified as part of the prosurvival "Survivor Activating Factor Enhancement" pathway.[10,11] Increasing evidence suggests that the cardioprotective signaling pathways converge on the mitochondria. In early reperfusion, the opening of the mitochondrial permeability transition pore (mPTP) appears to be the end effector of ischemia-reperfusion injury (fig. 1).[12] We have previously shown that pravastatin administered during reoxygenation prevented the opening of mPTP in human myocardium, *in vitro*.[13] However, the relationship among TNF-α, JAK/STAT pathway, and mPTP has never been investigated.

The aim of the current study was to investigate the cardioprotective effect of a continuous exposure to atorvastatin in isolated human myocardium submitted to hypoxia-reoxygenation. We also examined the mechanisms involved in the cardioprotective effect of atorvastatin focusing on TNF-α, JAK/STAT signaling pathway, and the mPTP.

## Materials and Methods

Right atrial appendages were obtained during cannulation for cardiopulmonary bypass from patients scheduled for coronary artery bypass surgery or aortic valve replacement, after approval of the local medical ethics committee (Comité de Protection des Personnes Nord Ouest III, Caen, France) and written informed consent. All patients received total intravenous anesthesia with propofol, remifentanil, and pancuronium. Patients with chronic atrial arrhythmia and diabetes mellitus treated with insulin or oral hypoglycemic agents were excluded from the study, because of major change in structure and function of the myocardium reported and[14,15] because diabetes and hyperglycemia may interfere with signaling pathways involved in cardioprotection.[15]

### *Human Atrial Trabeculae Model of Hypoxia-Reoxygenation Injury*

**Experimental Conditions.** Right atrial trabeculae (one or two per appendage) were dissected and suspended vertically between an isometric force transducer (MLT0202;

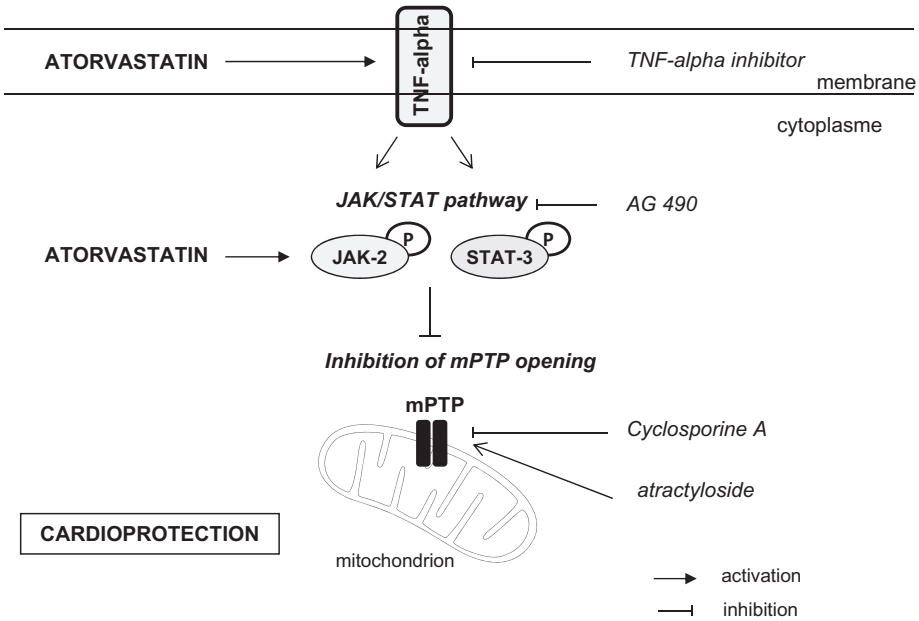


**Fig. 1.** Proposed schematic representation of the signaling pathways leading to atorvastatin-induced cardioprotection of the human myocardium, *in vitro*. Continuous administration of atorvastatin during hypoxia-reoxygenation induced tumor necrosis factor (TNF)-α activation, phosphorylation/activation of signal transducers and activators of transcription (STAT)-3 and Janus kinase (JAK)-2, and the inhibition of mitochondrial permeability transition pore (mPTP) mediate the cardioprotective effect. In atorvastatin-induced cardioprotection, TNF-α activation occurred before and could trigger the JAK-2 and STAT-3 activation/phosphorylation; and inhibition of mPTP opening appears to be downstream of activation of TNF-α, JAK-2, and STAT-3.

ADInstruments, Sydney, Australia) and a stationary stainless clip in a 200-ml jacketed reservoir filled with daily prepared Tyrode's modified solution containing 120 mM NaCl, 3.5 mM KCl, 1.1 mM $MgCl_2$, 1.8 mM $NaH_2PO_4$, 25.7 mM $NaHCO_3$, 2.0 mM $CaCl_2$, and 5.5 mM glucose. The jacketed reservoir was maintained at 34 ± 0.5°C by a thermostatic water circulator (Polystat micropros; Bioblock, Illkirch, France). The bathing solution was insufflated with carbogen (95% $O_2$–5% $CO_2$), resulting in a pH of 7.40 ± 0.02 and a partial pressure of oxygen of 600 ± 50 mmHg. Isolated muscles were field-stimulated at 1 Hz by two platinum electrodes with rectangular wave pulses of 5-ms duration 20% above threshold (CMS 95107; Bionic Instrument, Paris, France).

Trabeculae were equilibrated for 60–90 min to allow stabilization of their optimal mechanical performance at the apex of the length-active isometric tension curve (Lmax). The measurements of force developed were measured continuously, digitized at a sampling frequency of 400 Hz, and recorded and saved in a computer (PowerLab; ADInstruments, Oxford, United Kingdom).

At the end of experiment, the muscle cross-sectional area was calculated from its weight and length assuming a cylindrical shape and a density of 1. To avoid core hypoxia, trabeculae included in the study must have a cross-sectional area less than 1.0 $mm^2$, a force of contraction (FoC) normalized per cross-sectional area greater than 5.0 mN/$mm^2$, and a ratio of resting force/total force less than 0.50; otherwise they were excluded *a posteriori*.[16]

**The Hypoxia-Reoxygenation Protocol.** At the end of the stabilization period, trabeculae were randomly assigned to one of the experimental groups (fig. 2). In all groups, hypoxia-reoxygenation was performed by replacing 95% $O_2$–5% $CO_2$ with 95% $N_2$–5% $CO_2$ in the buffer for 30-min, followed by a 60-min oxygenated recovery period.

**Effects of Atorvastatin on Contractile Force of Human Right Atrial Trabeculae.** In the control group (control; n = 6), trabeculae were exposed to the hypoxia- reoxygenation protocol alone. In separate groups, atorvastatin (Sigma Aldrich, Saint Quentin Fallavier, France) at 0.1 μM, 1 μM, and 10 μM (n = 6 for each concentration) was administered 5 min before hypoxia-reoxygenation protocol and throughout the experiment (fig. 2). The concentrations of atorvastatin administered were based on those measured in volunteers receiving 40 mg atorvastatin.[17]

**Effect of TNF-α Inhibitor, AG490, and Atractyloside on Atorvastatin-induced Cardioprotection.** The mechanisms involved in atorvastatin-induced cardioprotection were studied in the presence of atorvastatin 1 μM because we have shown that 1 μM was the optimal concentration to induce cardioprotection in human myocardium, *in vitro* (fig. 3).

The effect of TNF-α, JAK/STAT pathway, and mPTP was examined using specific inhibitors administered 5 min before and until the first 15 min of reoxygenation in the presence of atorvastatin 1 μM. The TNF-α inhibitor (Atorva + TNF-α Inh; n = 6), AG490 a specific JAK/STAT inhibitor (Atorva + AG490; n = 6), and atractyloside the opener of the mPTP (Atorva + Atract; n = 6) were administered at 10 μM, 100 nM, and 50 μM, respectively. The effect of inhibitors alone was examined in separate groups (TNF-α Inh, AG490, and Atract groups, n = 6 in each group).

It has been shown that 100 nM AG490 abolished cardioprotection in isolated perfused rat hearts.[10] We have previously shown that atractyloside 50 μM abolished statin-induced cardioprotection without effect in control conditions.[13] The concentration of TNF-α inhibitor was based on Calbiochem recommendations.

**Effect of Cyclosporine A on Atorvastatin in the Presence of the TNF-α Inhibitor and AG490.** The relationship among TNF-α, JAK/STAT pathway, and mPTP were examined with Cyclosporine A (inhibitor of mPTP opening) at 0.2 μM administered during the first 15 min of reoxygenation in the presence of atorvastatin 1 μM and TNF-α inhibitor (Atorva + CsA + TNF-α Inh; n = 6) or in the presence of atorvastatin 1 μM and AG490 (Atorva + CsA + AG490; n = 6) (fig. 2). The effect of concomitant administration of cyclosporine A and the TNF-α inhibitor and AG490 on atorvastatin-induced cardioprotection was performed, to determine whether the inhibition of mPTP opening by cyclosporine A could restore the cardioprotection attenuated by inhibition of TNF-α or JAK/STAT pathway, and examine the possible link between mPTP inhibition and activation of TNF-α and JAK/STAT pathway in atorvastatin-induced cardioprotection.

In additional groups, cyclosporine A 0.2 μM was administered alone during the first 15 min of reoxygenation (CsA; n = 6), in presence of TNF-α inhibitor 10 μM (CsA +TNF-α Inh; n = 6), in presence of AG490 100 nM (CsA + AG490; n = 6), in presence of atractyloside 50 μM (CsA + Atract; n = 6), and in presence of atorvastatin 1 μM (CsA + Atorva; n = 6; (fig. 2). Cyclosporine A 0.2 μM has been used to close mPTP in human myocardium *in vitro*.[18]

Atorvastatin and TNF-α inhibitor were dissolved in dimethyl sulfoxide (DMSO), AG490, atractyloside and cyclosporine A were dissolved in ethanol. The final volume of DMSO and ethanol did not exceeded 0.01% of the total bath volume. The effect of DMSO 0.01% (DMSO; n = 6) and ethanol 0.01% (ethanol; n = 6) were examined in separate groups.

The TNF-α inhibitor was purchased from Calbiochem, EMB Millipore, Molsheim, France, atrovastatin, AG490 and atractyloside were purchased from Sigma Aldrich.

### *Measurements of Troponin Ic Release*

The right atrial appendage was pinned in a chamber (20 ml) containing Tyrode's modified solution, oxygenated with 95% $O_2$–5% $CO_2$, maintained at 37 ± 0.5°C (Polystat micropros; Bioblock), and stimulated at a frequency of 1 Hz. Control and atorvastatin 1 μM groups (n = 6 for each) were subjected to 30 min of hypoxia followed by 60 min of reoxygenation.

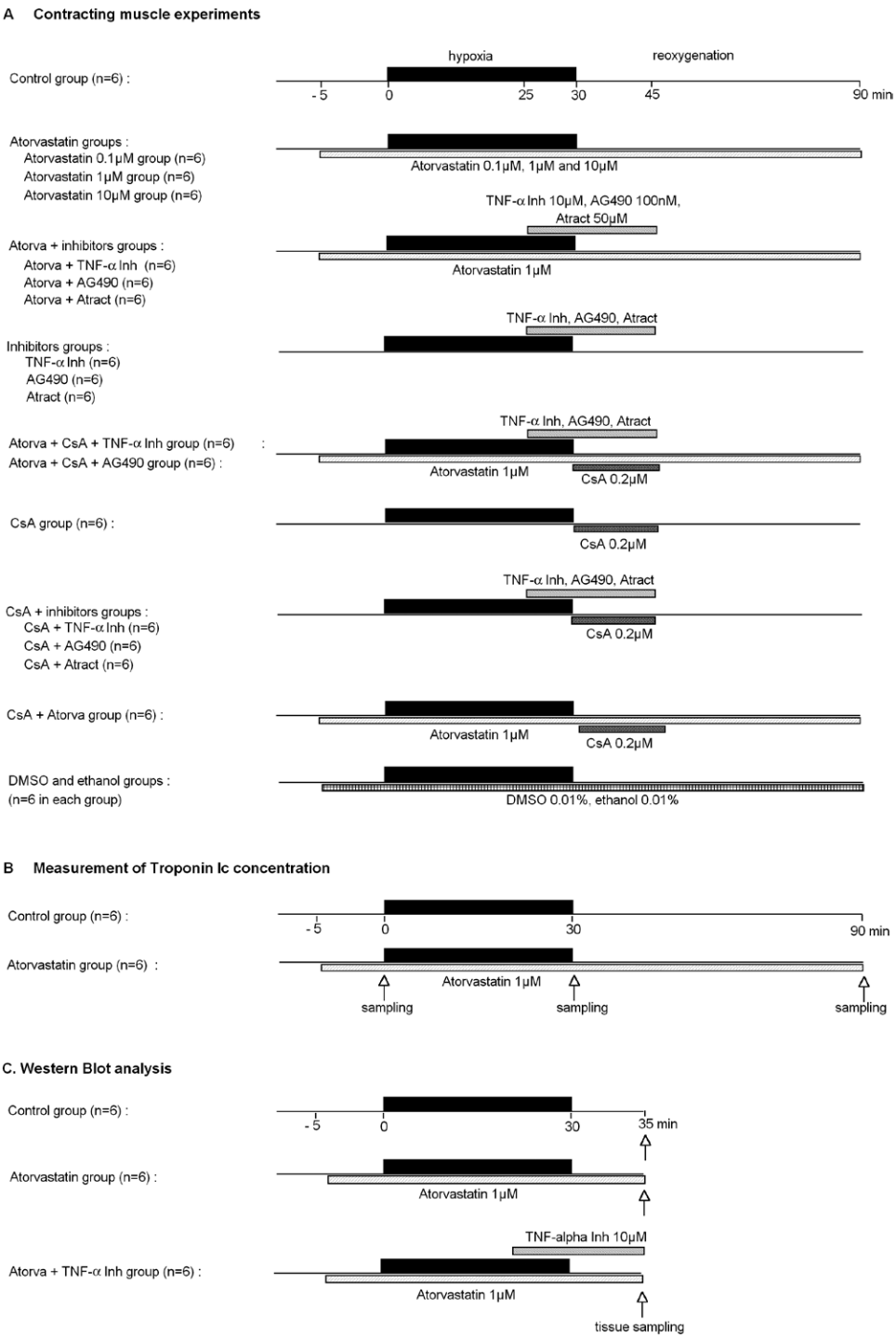


**Fig. 2.** Schematic diagram depicting the experimental protocol. (*A*) Contracting muscle experimental protocols. (*B*) Measurements of Troponin Ic concentration. (*C*) Western blot experimental protocols. Atorva = atorvastatin; atract = atractyloside; CsA = cyclosporine A; DMSO = dimethyl sulfoxide, TNF-α Inh = tumor necrosis factor-α inhibitor.

Atorvastatin 1 µM was administered 5 min before hypoxia-reoxygenation and throughout the experiment (fig. 2).

Samples (700 µl) of Tyrode's solution were collected before hypoxia, at the end of hypoxia, and at 60 min of reoxygenation. Troponin Ic (TnIc) concentration was analyzed with a sensitive and highly specific immunoenzymometric assay (AccuTnI, Access Immunoassay Systems, Beckman Coulter, Villepinte, France) that detects both free and complex bound troponin. The assay allows the detection of troponin Ic within the range of 0.01–100 ng/ml with appropriate dilutions. Concentrations of troponin Ic were normalized taking the weight of the appendage into account.

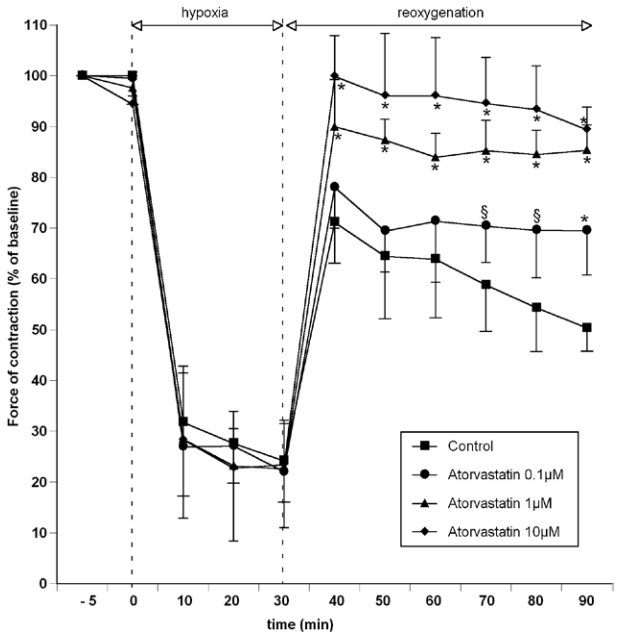


**Fig. 3.** Effect of administration of atorvastatin on the time course of force of contraction of isolated human right atrial trabeculae during a 30-min hypoxic challenge followed by a 60-min reoxygenation period (n = 6 in each group). Atorvastatin were administered continuously from 5 min before hypoxia to the end of the reoxygenation. Data are presented as mean ± SD. *$P$ < 0.001 *vs.* control group. §$P$ < 0.05 *vs.* control group.

### Western Blot Analysis

The right atrial appendage was pinned in a chamber (25 ml) containing Tyrode's modified solution, oxygenated with 95% $O_2$–5% $CO_2$, maintained at 34 ± 0.5°C (Polystat micropros; Bioblock), and stimulated at a frequency of 1 Hz.

Control or atorvastatin group (n = 6 for each) were subjected to a 60-min stabilization 95% $O_2$–5% $CO_2$ period followed by 30 min of hypoxia and 5 min of reoxygenation. In atorvastatin group, atorvastatin at 1 μM was administered 5 min before hypoxia-reoxygenation protocol and throughout the experiment. In an additional group, atorvastatin at 1 μM was administered with TNF-α inhibitor, administered 5 min before and during the first 5 min of reoxygenation (Atorva + TNF-α Inh; n = 6) (fig. 2).

Atrial samples were then frozen in liquid nitrogen and stored at −80°C before protein extraction and Western blot analysis. Frozen tissue samples were extracted into extraction buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml leupeptin-pepstatin A-aprotinin and homogenized with a polytron. Homogenates were centrifuged at 10,000*g* for 15 min, the supernatant was decanted, and protein concentration was determined using the bicinchoninic acid assay (Bradford colorimetric method; Bio-Rad, Marnes-la-Coquette, France). Extracted protein samples were reduced with 100 mM threo-1,4-dimercapto-2,3-butanediol and denatured at 95°C for 3 min. Denatured proteins (30 μg/lane) from human atrial tissues were separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred on nitrocellulose. Membranes were blocked for 1 h in Tris-buffered saline Tween buffer (0.02 M Tris-HCl, pH 7.5, 0.15 M NaCl, and 0.05% Tween 20) containing 5% nonfat dry milk at room temperature.

The membranes were incubated with eight different rabbit polyclonal antibodies recognizing phospho-STAT-3 (Tyr705), STAT-3 total, phospho-JAK-1 (Tyr1022/1023), JAK-1 total, phospho-JAK-2 (Tyr1007/1008), JAK-2 total, phospho-JAK-3 (Tyr980/981), and JAK-3 total (1/1000 dilution each; Cell Signaling Technology, Ozyme, Saint Quentin Yvelines, France), one night (approximately 14 h) at 4°C. After washing in Tris-buffered saline Tween, the blots were incubated with a secondary antibody (goat anti-rabbit, 1/1000 dilution) coupled to peroxidase (Santa Cruz Technology, Tebu-Bio, Le Perray en Yvelines, France) for 1 h at room temperature. The blots were washed again in Tris-buffered saline Tween, and the bands were detected using chemiluminescence reagent (Pierce Perbio Science, Brebieres, France) before exposure to photography film. The Western blots of each group were stripped and probed again with an antibody against Glyceraldehyde 3-phosphate dehydrogenase (1/1000 dilution; Santa Cruz Technology) to ensure equivalent loading.

The developed films were scanned, and the band densities were quantified using National Institutes of Health Image J software (Research Service Branch, National Institutes of Mental Health, Bethesda, MD).

### Statistics

The primary endpoint of the study was the recovery of FoC at 60 min of reoxygenation ($FoC_{60}$, expressed as percent of baseline). Data are expressed as mean ± SD. All $P$ values were two-tailed and a $P$ < 0.05 was considered significant.

Baseline values of main mechanical parameters, age, preoperative left ventricular ejection fraction, and FoC were compared by univariate analysis of variance with group factor as the independent variable. Statistical analysis has been performed with Statview v5.0 software (Deltasoft, Meylan, France). If the $P$ < 0.05, a Bonferroni *post hoc* analysis was performed. Data were analyzed over time using a two-way analysis of variance for repeated measures and Bonferroni *post hoc* analysis with group factor and time (baseline, hypoxia 5, 10, 20, 30 min, and reoxygenation 5, 10, 20, 30, 40, 50, and 60 min) as independent variables. Concentrations of troponin release were compared using a two-way analysis of variance with time and group as independent variables.

In Western blotting experiments, band densities for protein of interest were normalized to that of the band for Glyceraldehyde 3-phosphate dehydrogenase in the same sample. The ratio phosphorylated-STAT-3/STAT-3 total, the ratio phosphorylated-JAK-1/JAK-1 total, the ratio phosphorylated-JAK-2/JAK-2 total, and the ratio phosphorylated-JAK-3/JAK-3 total, were compared by univariate analysis of

**Table 1.** Patients Demographic Data, Preoperative Drug Treatments, and Preoperative Left Ventricular Ejection Fraction

| | | Preoperative Drug Treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Groups and Heart Disease | Age, yr | ACE | bAB | BZD | CA | COR | STA | NT | LVEF, % |
| Control AVR (n = 4)<br>CABG (n = 2) | 66 ± 13 | 2 | 2 | 1 | 0 | 0 | 5 | 0 | 64 ± 9 |
| Atorvastatin 0.1 μM<br>AVR (n = 3); CABG (n = 3) | 72 ± 10 | 3 | 4 | 0 | 0 | 0 | 3 | 0 | 70 ± 7 |
| Atorvastatin 1 μM<br>AVR (n = 2); CABG (n = 4) | 51 ± 12 | 1 | 5 | 1 | 0 | 0 | 3 | 0 | 65 ± 15 |
| Atorvastatin 10 μM<br>AVR (n = 3); CABG (n = 3) | 71 ± 6 | 3 | 2 | 0 | 0 | 0 | 3 | 0 | 64 ± 19 |
| Atorva + TNF-α Inh<br>AVR (n = 3); CABG (n = 3) | 61 ± 17 | 3 | 4 | 0 | 2 | 0 | 3 | 0 | 61 ± 11 |
| Atorva + AG490<br>AVR (n = 4); CABG (n = 2) | 59 ± 17 | 1 | 2 | 1 | 0 | 1 | 4 | 1 | 60 ± 11 |
| Atorva+ atract<br>AVR (n = 5); CABG (n = 1) | 75 ± 8 | 0 | 1 | 0 | 2 | 1 | 4 | 1 | 72 ± 10 |
| Atorva + CsA +TNF-α Inh<br>AVR (n = 3); CABG (n = 3) | 71 ± 11 | 2 | 3 | 0 | 1 | 0 | 2 | 0 | 57 ± 19 |
| Atorva + CsA +AG490<br>AVR (n = 1); CABG (n = 5) | 71 ± 10 | 0 | 4 | 0 | 0 | 0 | 5 | 0 | 69 ± 4 |
| CsA<br>AVR (n = 3); CABG (n = 3) | 74 ± 8 | 4 | 2 | 1 | 1 | 1 | 5 | 0 | 61 ± 8 |
| CsA + Atorva<br>AVR (n = 3); CABG (n = 3) | 70 ± 16 | 1 | 2 | 2 | 2 | 0 | 3 | 0 | 65 ± 10 |
| CsA + TNF-α Inh<br>AVR (n = 5); CABG (n = 1) | 76 ± 12 | 3 | 5 | 1 | 0 | 0 | 2 | 0 | 72 ± 8 |
| CsA + AG490<br>AVR (n = 4); CABG (n = 2) | 75 ± 3 | 1 | 3 | 0 | 0 | 0 | 5 | 0 | 68 ± 19 |
| CsA + atract<br>AVR (n = 4); CABG (n = 2) | 73 ± 7 | 3 | 2 | 3 | 1 | 0 | 5 | 0 | 78 ± 6 |
| TNF-α Inh<br>AVR (n = 1); CABG (n = 5) | 69 ± 14 | 4 | 5 | 0 | 1 | 0 | 5 | 0 | 60 ± 15 |
| AG490<br>AVR (n = 3); CABG (n = 3) | 70 ± 13 | 3 | 3 | 0 | 0 | 0 | 4 | 1 | 61 ± 15 |
| Atract<br>AVR (n = 3); CABG (n = 3) | 65 ± 13 | 2 | 3 | 0 | 0 | 0 | 4 | 2 | 73 ± 3 |
| DMSO<br>AVR (n = 1); CABG (n = 5) | 73 ± 6 | 3 | 2 | 0 | 1 | 0 | 4 | 0 | 60 ± 18 |
| Ethanol<br>AVR (n = 2); CABG (n = 4) | 70 ± 10 | 3 | 2 | 0 | 2 | 0 | 4 | 0 | 55 ± 10 |
| Troponin measurement<br>Control AVR (n = 3); CABG (n = 3)A | 69 ± 14 | 3 | 3 | 1 | 0 | 0 | 2 | 0 | 59 ± 9 |
| Atorvastatin<br>AVR (n = 2); CABG (n = 4) | 76 ± 7 | 3 | 3 | 2 | 0 | 0 | 4 | 2 | 65 ± 14 |
| Western blot: Control-5 min reox<br>AVR (n = 2); CABG (n = 4) | 62 ± 16 | 1 | 2 | 0 | 0 | 1 | 3 | 1 | 62 ± 12 |
| Western blot: Atorva-5 min reox<br>AVR (n = 3); CABG (n = 3) | 71 ± 8 | 2 | 2 | 0 | 1 | 0 | 4 | 0 | 67 ± 12 |
| Western blot: Atorva + TNF-α Inh -5 min reox<br>AVR (n = 2); CABG (n = 4) | 71 ± 10 | 2 | 3 | 0 | 1 | 0 | 3 | 0 | 57 ± 13 |

Age, and LVEF are expressed as mean ± SD.

ACE = angiotensin-converting enzyme inhibitors; Atorva = atorvastatin; atract = atractyloside; AVR = aortic valve replacement; bAB = β-adrenergic blocking drugs; BZD = benzodiazepine; CA = calcium channel antagonists; CABG = coronary artery bypass graft; CsA = cyclosporine A; COR = amiodarone; DMSO = diméthylsulfoxyde; LVEF = preoperative left ventricular ejection fraction; NT = nitroglycerin; STA = statins; TNF-α Inh = tumor necrosis factor-α inhibitor.

**Table 2.** Control Values of Main Mechanical Parameters of Human Right Atrial Trabeculae

| Experimental Groups | $L_{max,}$ mm | CSA, $mm^2$ | FoC, $mN \cdot mm^{-2}$ | RF/TF |
|---|---|---|---|---|
| Control (n = 6) | 7.3 ± 3.1 | 0.42 ± 0.16 | 26 ± 18 | 0.36 ± 0.08 |
| Atorvastatin 0.1 μM (n = 6) | 6.7 ± 0.9 | 0.35 ± 0.14 | 31 ± 18 | 0.29 ± 0.10 |
| Atorvastatin 1 μM (n = 6) | 7.6 ± 2.1 | 0.49 ± 0.21 | 31 ± 15 | 0.19 ± 0.09 |
| Atorvastatin 10 μM (n = 6) | 5.7 ± 2.1 | 0.34 ± 0.14 | 34 ± 18 | 0.32 ± 0.11 |
| Atorva + TNF-α Inh (n = 6) | 5.8 ± 1.2 | 0.29 ± 0.06 | 29 ± 13 | 0.36 ± 0.10 |
| Atorva + AG490 (n = 6) | 5.3 ± 0.5 | 0.40 ± 0.20 | 29 ± 12 | 0.28 ± 0.09 |
| Atorva + atract (n = 6) | 5.3 ± 1.4 | 0.42 ± 0.16 | 23 ± 9 | 0.33 ± 0.06 |
| Atorva + CsA + TNF-α Inh (n = 6) | 6.0 ± 1.5 | 0.45 ± 0.23 | 35 ± 17 | 0.26 ± 0.07 |
| Atorva + CsA + AG490 (n = 6) | 4.4 ± 1.0 | 0.29 ± 0.08 | 35 ± 19 | 0.34 ± 0.13 |
| CsA (n = 6) | 5.5 ± 1.8 | 0.32 ± 0.15 | 32 ± 26 | 0.30 ± 0.05 |
| CsA + Atorva (n = 6) | 5.8 ± 2.2 | 0.43 ± 0.20 | 33 ± 19 | 0.24 ± 0.07 |
| CsA +TNF-α Inh (n = 6) | 6.8 ± 1.8 | 0.45 ± 0.18 | 32 ± 13 | 0.23 ± 0.08 |
| CsA + AG490 (n = 6) | 6.9 ± 1.7 | 0.44 ± 0.16 | 20 ± 9 | 0.40 ± 0.08 |
| CsA + atract (n = 6) | 5.8 ± 1.8 | 0.30 ± 0.07 | 19 ± 6 | 0.44 ± 0.03 |
| TNF-α Inh (n = 6) | 5.8 ± 1.0 | 0.49 ± 0.09 | 20 ± 7 | 0.34 ± 0.13 |
| AG490 (n = 6) | 7.2 ± 1.6 | 0.42 ± 0.05 | 24 ± 5 | 0.28 ± 0.10 |
| Atract (n = 6) | 6.8 ± 2.0 | 0.33 ± 0.13 | 26 ± 15 | 0.40 ± 0.08 |
| DMSO (n = 6) | 7.0 ± 2.7 | 0.58 ± 0.20 | 25 ± 12 | 0.35 ± 0.14 |
| Ethanol (n = 6) | 6.3 ± 0.8 | 0.37 ± 0.08 | 26 ± 19 | 0.34 ± 0.12 |

Data are mean ± SD.

Atorva = atorvastatin; atract = atractyloside; CSA = cross-sectionnal area; CsA = cyclosporine A; DMSO = diméthylsulfoxyde; FoC = isometric force of contraction normalized per cross-sectionnal area; $L_{max}$ = maximal length at the apex of the length-active force curve; TNF-α Inh = tumor necrosis factor-α inhibitor; RF/TF = ratio of resting force on total force.

variance with group factor as the independent variable. If the $P < 0.05$, a Bonferroni *post hoc* analysis was performed.

## Results

Patients' characteristics and left ventricular ejection fraction were not different between groups (table 1). One hundred and fourteen human right atrial trabeculae and 30 right atrial appendages were studied. There were no significant differences between the groups for trabecular length at the apex of the length-active isometric tension curve, cross-sectional area, and ratio of resting-to-total force (table 2).

### Effects of Hypoxia and Reoxygenation on Contractile Force of Human Right Atrial Trabeculae

In the control group, hypoxia induced a marked decrease in FoC; after 30 min of hypoxia, FoC was 24 ± 7% of baseline. Reoxygenation induced a partial recovery of FoC (50 ± 3% of baseline) at end of reoxygenation period in the control group (fig. 3).

### Effects of Atorvastatin on Contractile Force of Human Right Atrial Trabeculae

After 30 min of hypoxia the FoC in atorvastatin-treated groups was 22 ± 11% of baseline in atorvastatin 0.1 μM group; 23 ± 10% of baseline in atorvastatin 1 μM group; and 22 ± 9% of baseline in atorvastatin 10 μM group (respectively $P = 0.71$, $P = 0.78$, $P = 0.90$ *vs.* control group) (fig. 3).

Atorvastatin significantly increased the $FoC_{60}$ as compared with the control group (atorvastatin 0.1 μM: 70 ± 9% of baseline; atorvastatin 1 μM: 85 ± 5% of baseline; atorvastatin 10 μM: 89 ± 5% of baseline; $P < 0.001$ *vs.* control group) (fig. 3).

### Effect of TNF-α Inhibitor, AG490, and Atractyloside Treatment on Atorvastatin-induced Cardioprotection

Administration of TNF-α inhibitor ($FoC_{60}$: 53 ± 8% of baseline), AG490 ($FoC_{60}$: 56 ± 7% of baseline), and atractyloside ($FoC_{60}$: 48 ± 8% of baseline) during the reoxygenation period inhibited the atorvastatin-induced cardioprotection ($P < 0.001$ *vs.* atorvastatin 1 μM group) (fig. 4).

As compared with the control group ($FoC_{60}$: 50 ± 3% of baseline), administration of TNF-α inhibitor alone ($FoC_{60}$: 51 ± 7% of baseline; $P = 0.86$), AG490 alone ($FoC_{60}$: 50 ± 11% of baseline; $P = 0.90$), and atractyloside alone ($FoC_{60}$: 49 ± 6% of baseline; $P = 0.82$) did not modify $FoC_{60}$ (fig. 4).

### Effect of Cyclosporine A during Early Reoxygenation on Atorvastatin, TNF-α Inhibitor and AG490 Treatment

In the presence of cyclosporine A, TNF-α inhibitor and AG490 did not modify the enhanced recovery of $FoC_{60}$ resulting from atorvastatin 1 μM administration (Atorva + TNF-α Inh + CsA: 87 ± 6% of baseline; Atorva + AG490+ CsA: 83 ± 9% of baseline; respectively $P = 0.73$ and $P = 0.62$ *vs.* Atorvastatin 1 μM group). As compared with the control group, cyclosporine A alone increased the $FoC_{60}$ (CsA: 87 ± 10% of baseline; $P < 0.001$). There was no difference

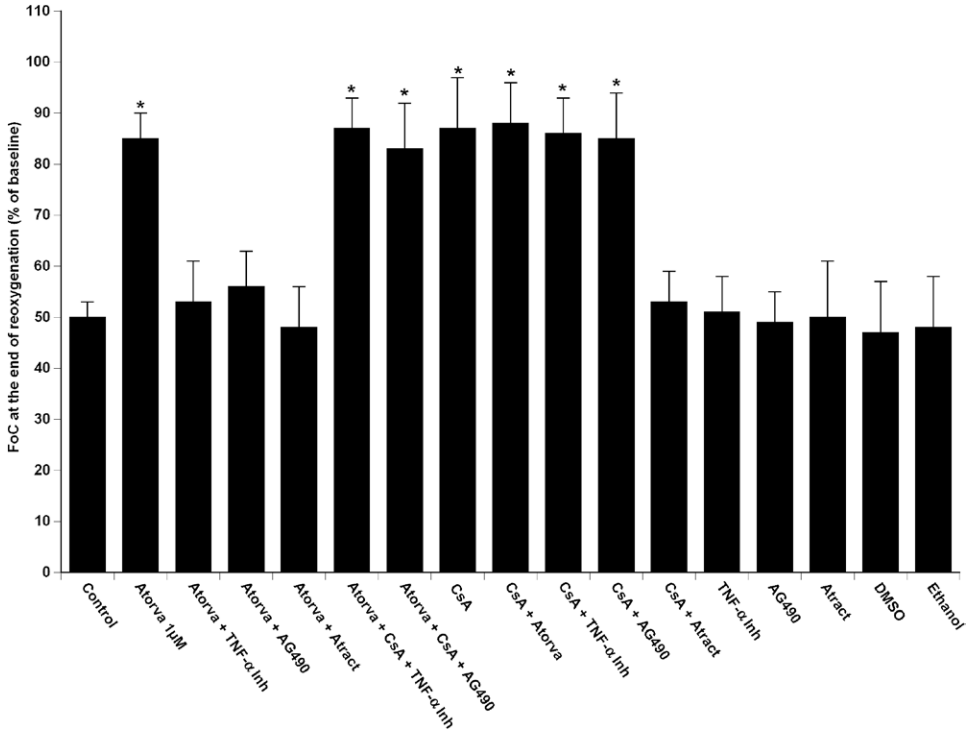


**Fig. 4.** Recovery of force of contraction of isolated human right atrial trabeculae at the end of the 60-min reoxygenation period after the 30-min hypoxic challenge in groups exposed to atorvastatin (atorva) 1 μM alone (n = 6) and in the presence of tumor necrosis factor (TNF)-α inhibitor 10 μM (atorva + TNF-α Inh, n = 6), AG490 100 nM (atorva + AG490, n = 6), atractyloside (atract) 50 μM (atorva + atract, n = 6), in groups exposed to Cyclosporine A 0.2 μM and atorva 1 μM in the presence of TNF-α inhibitor 10 μM (atorva + cyclosporine A [CsA] + TNF-α Inh, n = 6) or AG490 100 nM and (atorva + CsA + AG490, n = 6), in groups exposed to cyclosporine A 0.2 μM alone (CsA, n = 6), in the presence of TNF-α inhibitor 10 μM (CsA + TNF-α Inh, n = 6), in the presence of AG490 100 nM (CsA + AG490, n = 6), in the presence of atract 50 μM (CsA + atract, n = 6), in the presence of atorva 1 μM (CsA + atorva, n = 6), in groups exposed to TNF-α inhibitor 10 μM (TNF-α Inh, n = 6), AG490 100 nM alone (AG490, n = 6), atract 50 μM alone (atract, n = 6), in groups exposed to diméthylsulfoxyde (DMSO) 0.01% (DMSO, n = 6), ethanol 0.01% (Ethanol, n = 6). Data are mean ± SD. *$P < 0.001$ *vs.* control, atorva + AG490, atorva + atract, CsA + atract, AG490, atract, DMSO, ethanol groups.

in the $FoC_{60}$ between atorvastatin 1 μM group (85 ± 5% of baseline) and atorvastatin 1 μM + CsA group (88 ± 8% of baseline; $P = 0.65$) (fig. 4).

### Effect of TNF-α Inhibitor, AG490, and Atractyloside on Cyclosporine A Treatment

The enhanced recovery of $FoC_{60}$ after cyclosporine A administration was abolished in the presence of atractyloside (CsA + Atract: 53 ± 6% of baseline; $P < 0.001$ *vs.* CsA group), but was not modified in the presence of TNF-α inhibitor (CsA + TNF-α Inh: 86 ± 7% of baseline; $P = 0.89$ *vs.* CsA group) and AG490 (CsA + AG490: 85 ± 9% of baseline; $P = 0.79$ *vs.* CsA group) (fig. 4). As compared with control group, DMSO ($FoC_{60}$: 47 ± 10% of baseline; $P = 0.57$ *vs.* control group) and ethanol ($FoC_{60}$: 48 ± 10% of baseline; $P = 0.69$ *vs.* control group) did not modify $FoC_{60}$.

### Effect of Atorvastatin on Concentration of Troponin Ic

The release of troponin Ic was significantly different over time ($P = 0.001$) and between groups ($P = 0.01$). Baseline concentration of troponin Ic released was not different between control (0.90 ± 0.29 ng · $ml^{-1}$ · $g^{-1}$ of tissue) and atorvastatin 1 μM (1.06 ± 0.41 ng · $ml^{-1}$ · $g^{-1}$ of tissue; $P = 0.45$) groups (fig. 4). Atorvastatin 1 μM (9.94 ± 5.16 ng · $ml^{-1}$ · $g^{-1}$ of tissue) significantly decreased the concentration of troponin Ic at the end of reoxygenation as compared with the control (47.96 ± 17.98 ng · $ml^{-1}$ · $g^{-1}$ of tissue; $P < 0.001$) group (fig. 5).

### Effect of Atorvastatin on the Phosphorylation of STAT-3, JAK-1, JAK-2, and JAK-3

At 5-min reoxygenation, atrial samples exposed to atorvastatin 1 μM showed a significant increase in the ratio phospho-STAT-3/STAT-3 total (+171% in atorvastatin *vs.* control; $P < 0.001$). Total STAT-3 expression was not significantly different between control group and atorvastatin group ($P = 0.41$). TNF-inhibitor abolished the increased phosphorylation of STAT-3 induced by atorvastatin ($P = 0.23$ *vs.* control) (fig. 6A). Atrovastatin 1 μM did not modify the ratio of phospho-JAK-1/JAK-1 total (control group ratio: 0.50 ± 0.08 *vs.* atorvastatin group ratio: 0.54 ± 0.12; $P = 0.58$; fig. 6B).

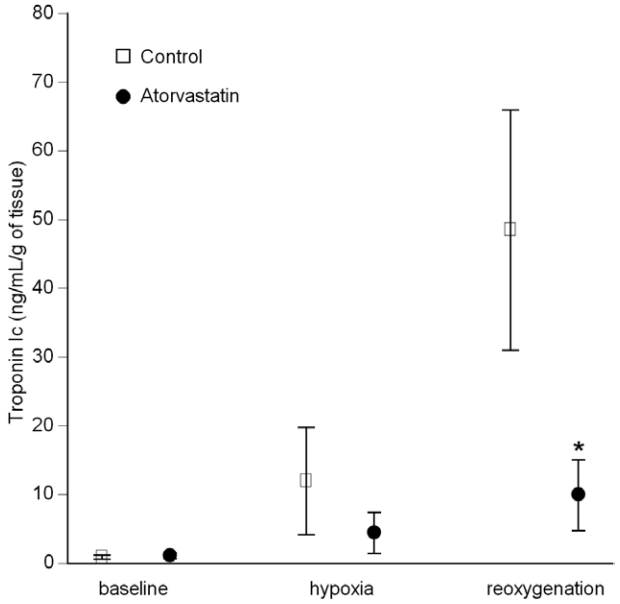


**Fig. 5.** Troponin Ic concentration (ng · ml$^{-1}$ · g$^{-1}$ of tissue) of human atria exposed to atorvastatin 1 μM, before hypoxia (baseline), at the end of hypoxia (hypoxia), and at 60 min of reoxygenation (reoxygenation). Data are mean ± SD. *$P$ < 0.001 *vs.* control group.

At 5 min of reoxygenation, the ratio of phospho-JAK-2/JAK-2 total was enhanced in the presence of atorvastatin 1 μM (+72% in atorvastatin *vs.* control; $P$ < 0.001). Total JAK-2 expression was not significantly different between control group and atorvastatin groups ($P$ = 0.54). TNF inhibitor abolished the increased phosphorylation of JAK-2 induced by atorvastatin ($P$ = 0.66 *vs.* control) (fig. 6C). Atrovastatin 1 μM did not modify the ratio of phospho-JAK-3/JAK-3 total (control group ratio: 0.57 ± 0.10 *vs.* Atorvastatin group ratio: 0.60 ± 12; $P$ = 0.66; fig. 6D).

## Discussion

The current results showed that, in isolated human myocardium, (1) atorvastatin during hypoxia-reoxygenation improved the recovery of force of contraction after hypoxia-reoxygenation, (2) activation of TNF-α, phosphorylation of JAK-2 and STAT-3, and the inhibition of mPTP opening were involved in atorvastatin-induced cardioprotection. Furthermore, the current data suggested that the inhibition of mPTP opening occurred via the activation of TNF-α and JAK/STAT pathway in the early reoxygenation period.

Beyond the lipid-lowering effect, statins have been shown to improve endothelial function, decrease platelet activation, decrease vascular and myocardial remodeling, inhibit vascular inflammation, and stabilize atherosclerotic plaques. Altogether, the pleiotropic effects of statins may be of importance in the perioperative period, and clinical studies suggested that they may decrease perioperative cardiovascular morbidity.[2,3,19] Additionally, experimental studies have shown that statins may trigger myocardial pre- and postconditioning. A single administration of atorvastatin (10 mg/kg equivalent at 0.1 μM plasmatic concentration),[20] and 3 days pretreatment (10 mg · kg$^{-1}$ · day$^{-1}$) have been shown to decrease myocardial infarct volume in rat submitted to 30-min ischemia and 4 h reperfusion, *in vivo*.[4–6] Additionally, 50 μM atorvastatin administered during reperfusion provided cardioprotection in isolated perfused mouse hearts subjected to 35 min ischemia and 30 min reperfusion.[8] In contrast, pretreatment with atorvastatin at 2 mg · kg$^{-1}$ · day$^{-1}$ did not modify the infarct size of rat heart, *in vivo,* suggesting a concentration-dependent cardioprotective effect.[7] However, these studies examined the effect of short-term exposure to statins in healthy animals whereas in patients statins are chronic treatments, which should not be interrupted during the perioperative period. The current results showed that atorvastatin, administered throughout hypoxia-reoxygenation, significantly enhanced the recovery of force of contraction and decreased troponin Ic release of isolated contracting human myocardium at the end of the reoxygenation period. There was a trend toward a lower recovery of force of contraction at the end of the reoxygenation in the presence of atorvastatin 0.1 μM as compared to 1 μM and 10 μM suggesting a concentration relationship effect. Nevertheless, specifically designed studies are required to precisely examine this result.

Although the role of the "Reperfusion Injury Salvage Kinases" and anti-apoptotic pathways in statins-induced cardioprotection has been suggested,[13,21] no data exist on the involvement of prosurvival "Survivor Activating Factor Enhancement" pathway. The "Survivor Activating Factor Enhancement" pathway requires the activation of TNF-α and the JAK-STAT protein families, and has been implicated in the prevention of reperfusion injury. Our results showed that administration of a TNF-α inhibitor during the first minutes of reoxygenation abolished the recovery of force of contraction resulting from atorvastatin administration. This suggests that atorvastatin-induced cardioprotection was mediated, at least in part, through TNF-α activation in early reoxygenation. The role of TNF-α in myocardial ischemia-reperfusion injury was demonstrated by Lacerda *et al.*[10] showing that ischemic postconditioning was ineffective in TNF-α receptor knockout mice, and that exogenous TNF-α given during early reperfusion protected the isolated mouse hearts against ischemia-reperfusion injury. The JAK-STAT signaling pathway involves the JAKs and the STATs protein families. When JAKs proteins are activated (phosphorylated), they can phosphorylate the tyrosine motifs in the cytoplasmic tail of STATs, allowing them to translocate to the nucleus, resulting in gene transcription.[22] Activation of STAT-3 and JAK-2 has been shown to mediate ischemic and pharmacological postconditioning,[10,11,23] and its key role has been confirmed using STAT-3 knockout mice hearts.[24] In the current study, we showed that AG490, a JAK/STAT pathway inhibitor, administered during the reoxygenation period

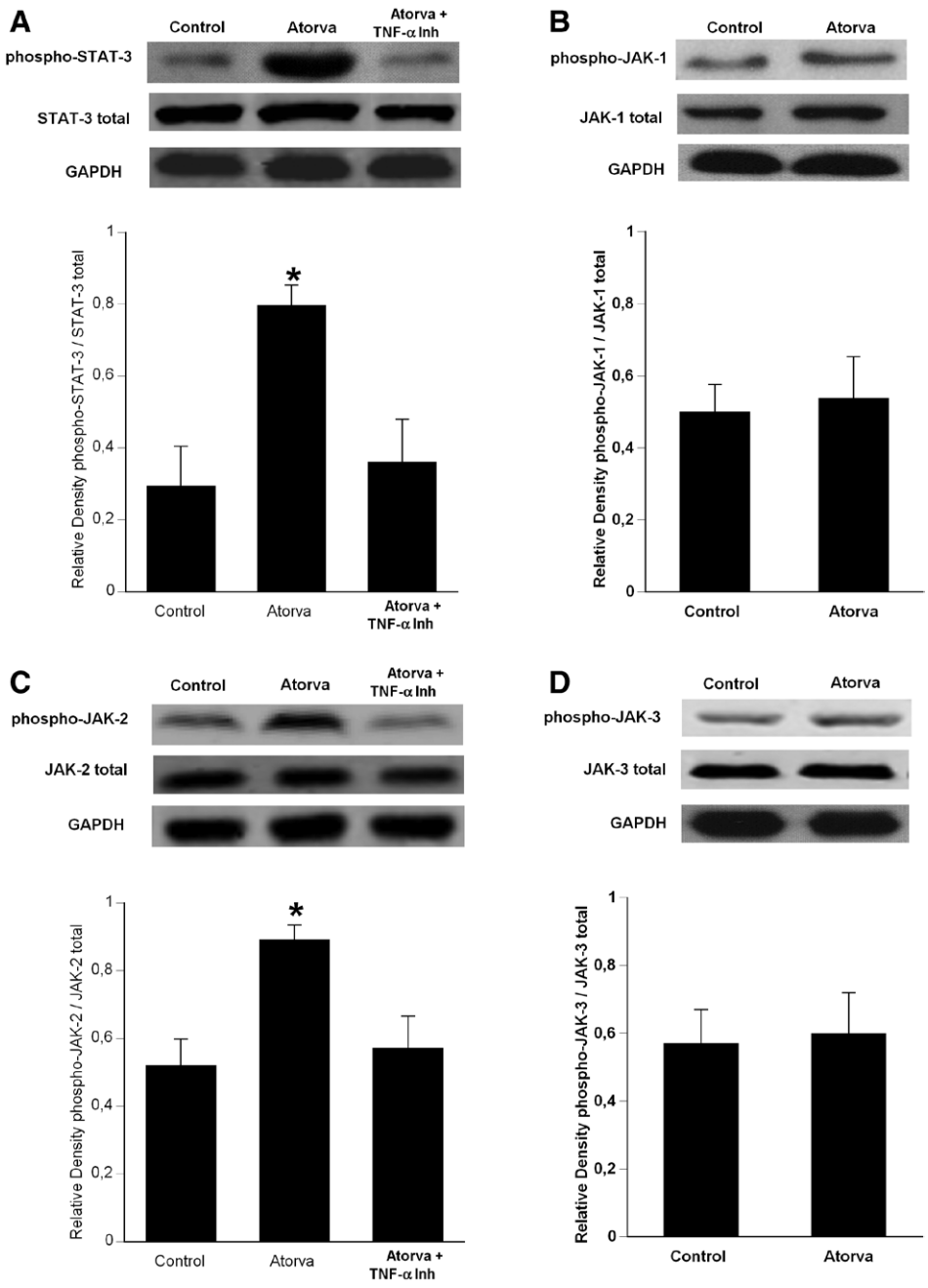


**Fig. 6.** Western blotting and densitometry results. (*A*) Representative Western analysis of tissue samples acquired after 5 min of reoxygenation alone (Control) or in the presence atorvastatin 1 μM (atorva), or in presence of atorvastatin 1 μM and tumor necrosis factor (TNF)-α Inhibitor 10 μM (atorva + TNF-α Inh) and probed for Tyr705 phosphorylation of signal transducers and activators of transcription (STAT)-3 (phospho-STAT-3) and STAT-3 total, respectively. Histogram is depicting the ratio Phospho-STAT-3/STAT-3 total for control and Atorva groups. (*B*) Representative Western analysis of tissue samples acquired after 5 min of reoxygenation alone (control) or in the presence of atorvastatin 1 μM (atorva) and probed for Tyr1022/1023 phosphorylation of Janus kinase (JAK)-1 (phospho-JAK-1) and JAK-1 total, respectively. Histogram is depicting the ratio Phospho-JAK-1/JAK-1 total for control and Atorva groups. (*C*) At the top, representative Western analysis of tissue samples acquired after 5 min of reoxygenation alone (control) or in the presence atorvastatin 1 μM (atorva) or in the presence of atorvastatin 1 μM and TNF-α inhibitor 10 μM (atorva + TNF-α Inh) and probed for Tyr1007/1008 phosphorylation of JAK-2 (phospho-JAK-2) and JAK-2 total, respectively. Histogram is depicting the ratio Phospho-JAK-2/JAK-2 total for control and atorva groups. (*D*) At the top, representative Western analysis of tissue samples acquired after 5 min of reoxygenation alone (control) or in the presence atorvastatin 1 μM (atorva) and probed for Tyr980/981 phosphorylation of JAK-3 (phospho-JAK-3) and JAK-3 total, respectively. Histogram is depicting the ratio Phospho-JAK-3/JAK-3 total for control and Atorva groups. The ratio of phospho-STAT-3/STAT-3 total, phospho-JAK-1/JAK-1 total, phospho-JAK-2/JAK-2 total, and phospho-JAK-3/JAK-3 total were calculated by averaging the results obtained from 6 independent experiments and were normalized to the glyceraldehyde 3-phosphate dehydrogenase (GADPH) in each group. Data are mean + SD. *$P < 0.001$ *vs.* control group.

abolished the enhanced recovery of $FoC_{60}$ resulting from 1 μM atorvastatin administration. Additionally, we showed that, at 5 min of reoxygenation, atorvastatin increased the ratio STAT-3 phosphorylated/STAT-3 total and JAK-2 phosphorylated/JAK-2 total compared with control groups. Taken together, the current results suggest that atorvastatin-induced cardioprotection of human myocardium was mediated by activation/phosphorylation of JAK-2 and STAT-3. In contrast, there was no difference in phosphorylation of JAK-1 and JAK-3 expression between control and atorvastatin groups. Moreover, in the current study, we showed that the TNF-α inhibitor abolished the atorvastatin-induced enhanced phosphorylation of JAK-2 and STAT-3 in the first minutes of reoxygenation suggesting that TNF-α activation was required in atorvastatin-induced activation/phosphorylation of JAK-2 and STAT-3. This is in accordance with previous results showing that myocardial postconditioning with TNF-α was abolished in the presence of AG490, and that STAT-3-deficient mice could not be protected by postconditioning with TNF-α.[10]

We have previously suggested that pravastatin could prevent opening of the mPTP during the reoxygenation period.[13] The current data showed that administration of atractyloside, the mPTP opener, during the first minutes of reoxygenation, abolished atorvastatin-induced cardioprotection. Moreover, coadministration of atorvastatin and cyclosporine A in early reoxygenation period do not confer further cardioprotection as compared to atorvastatin alone or cyclosporine A alone (fig. 4). Taken together, these data suggested that continuous administration of atorvastatin prevents mPTP opening in early reoxygenation period. Little information is available on the relationship between JAK/STAT pathway and mPTP in early reoxygenation. It has been shown that leptin-induced myocardial protection during reperfusion resulted from JAK/STAT signaling pathway activation which was coupled with the inhibition of the mPTP.[25] The current study showed that atorvastatin-induced cardioprotection was abolished by TNF-α inhibitor and AG490 (inhibitor of the JAK/STAT pathway), but that concomitant inhibition of the mPTP opening with cyclosporine A reversed the effect of TNF-α inhibitor and AG490. In addition, we showed that the cardioprotection triggered by cyclosporine A at reoxygenation was not modified by TNF-α inhibitor and AG490. This suggested that TNF-α and JAK/STAT pathway activation occurred before and could trigger the inhibition of mPTP opening. Gao *et al.*[26] have previously shown that TNF-α protects the myocardium against ischemia and reperfusion injury by inhibiting mPTP in isolated rat heart. It has been reported that STAT-3 was present within the mitochondria where it was required for the optimal function of electron transport chain,[25,27] by modulating mPTP function. Nevertheless, the links between the JAK/STAT pathway, mitochondria, and the mPTP would require further study.

Several limitations must be considered in the interpretation of the current results. First, the effects of anesthetic drugs,[28] patient's chronic diseases, and preoperative treatments, including statins, cannot be ruled out. Nevertheless, the control group was also exposed to this limit, cardioprotective signaling pathways have been activated by atorvastatin administration, *in vitro*. Second, age has been shown to impair the effects of cardioprotective strategies. However, it has been suggested that there was no age-related difference in hypoxic preconditioning between myocardium obtained from 60- to 69-yr and 70- to 89-yr-old patients.[29] Importantly, the current result suggests that cardioprotective pathways may be activated in patients in whom these strategies could be applied. Third, the troponin Ic results and force of contraction results must be considered taking the temperature into account (troponin Ic experiments were performed at 37°C whereas other experiments were performed at 34°C to ensure stability of trabeculae over time). It has been shown that hypothermia may modify the mitochondrial calcium sensitivity to mPTP opening.[30] However, during surgical procedures moderate hypothermia may occur. Fourth, the specificity of the antagonist used in the current study must be considered. Our data strongly suggest a key role for mPTP inhibition in atorvastatin-induced cardioprotection. Nevertheless, atractyloside opens mPTP but also inhibits adenosine diphosphate transport by inhibition of adenine nucleotide translocase.[31] Also, AG490 is a general JAK-STAT pathway inhibitor that inhibits all JAK isoforms and STAT-3 without distinction. However, we have tested them individually by western blotting contribution.

In conclusion, the current study showed that cardioprotection afforded by atorvastatin, in human myocardium *in vitro*, depends on activation/phosphorylation of STAT-3 and JAK-2 and the inhibition of mPTP opening, in early reoxygenation. Moreover, our data suggested that activation of JAK/STAT pathway is upstream of inhibition of mPTP opening in atorvastatin-induced cardioprotection (fig. 1).

## References


1. Hindler K, Shaw AD, Samuels J, Fulton S, Collard CD, Riedel B: Improved postoperative outcomes associated with preoperative statin therapy. Anesthesiology 2006; 105:1260–72; quiz 1289–90
2. Le Manach Y, Ibanez Esteves C, Bertrand M, Goarin JP, Fléron MH, Coriat P, Koskas F, Riou B, Landais P: Impact of preoperative statin therapy on adverse postoperative outcomes in patients undergoing vascular surgery. Anesthesiology 2011; 114:98–104
3. Schouten O, Boersma E, Hoeks SE, Benner R, van Urk H, van Sambeek MR, Verhagen HJ, Khan NA, Dunkelgrun M, Bax JJ, Poldermans D; Dutch Echocardiographic Cardiac Risk Evaluation Applying Stress Echocardiography Study Group: Fluvastatin and perioperative events in patients undergoing vascular surgery. N Engl J Med 2009; 361:980–9
4. Birnbaum Y, Lin Y, Ye Y, Martinez JD, Huang MH, Lui CY, Perez-Polo JR, Uretsky BF: Aspirin before reperfusion blunts the infarct size limiting effect of atorvastatin. Am J Physiol Heart Circ Physiol 2007; 292:H2891–7

5. Birnbaum Y, Ye Y, Rosanio S, Tavackoli S, Hu ZY, Schwarz ER, Uretsky BF: Prostaglandins mediate the cardioprotective effects of atorvastatin against ischemia-reperfusion injury. Cardiovasc Res 2005; 65:345–55
6. Atar S, Ye Y, Lin Y, Freeberg SY, Nishi SP, Rosanio S, Huang MH, Uretsky BF, Perez-Polo JR, Birnbaum Y: Atorvastatin-induced cardioprotection is mediated by increasing inducible nitric oxide synthase and consequent S-nitrosylation of cyclooxygenase-2. Am J Physiol Heart Circ Physiol 2006; 290:H1960–8
7. Ye Y, Martinez JD, Perez-Polo RJ, Lin Y, Uretsky BF, Birnbaum Y: The role of eNOS, iNOS, and NF-kappaB in upregulation and activation of cyclooxygenase-2 and infarct size reduction by atorvastatin. Am J Physiol Heart Circ Physiol 2008; 295:H343–51
8. Bell RM, Yellon DM: Atorvastatin, administered at the onset of reperfusion, and independent of lipid lowering, protects the myocardium by up-regulating a pro-survival pathway. J Am Coll Cardiol 2003; 41:508–15
9. Hausenloy DJ, Yellon DM: Survival kinases in ischemic preconditioning and postconditioning. Cardiovasc Res 2006; 70:240–53
10. Lacerda L, Somers S, Opie LH, Lecour S: Ischaemic postconditioning protects against reperfusion injury via the SAFE pathway. Cardiovasc Res 2009; 84:201–8
11. You L, Li L, Xu Q, Ren J, Zhang F: Postconditioning reduces infarct size and cardiac myocyte apoptosis via the opioid receptor and JAK-STAT signaling pathway. Mol Biol Rep 2011; 38:437–43
12. Morin D, Assaly R, Paradis S, Berdeaux A: Inhibition of mitochondrial membrane permeability as a putative pharmacological target for cardioprotection. Curr Med Chem 2009; 16:4382–98
13. Lemoine S, Allouche S, Coulbault L, Cornet V, Massetti M, Galera P, Gérard JL, Hanouz JL: Mechanisms involved in cardioprotective effects of pravastatin administered during reoxygenation in human myocardium *in vitro*. Anesthesiology 2012; 116:824–33
14. Dobrev D, Ravens U: Remodeling of cardiomyocyte ion channels in human atrial fibrillation. Basic Res Cardiol 2003; 98:137–48
15. Amour J, Kersten JR: Diabetic cardiomyopathy and anesthesia: Bench to bedside. Anesthesiology 2008; 108:524–30
16. Paradise NF, Schmitter JL, Surmitis JM: Criteria for adequate oxygenation of isometric kitten papillary muscle. Am J Physiol 1981; 241:H348–53
17. Guo CX, Pei Q, Yin JY, Peng XD, Zhou BT, Zhao YC, Wu LX, Meng XG, Wang G, Li Q, Ouyang DS, Liu ZQ, Zhang W, Zhou HH: Effects of Ginkgo biloba extracts on pharmacokinetics and efficacy of atorvastatin based on plasma indices. Xenobiotica 2012; 42:784–90
18. Shanmuganathan S, Hausenloy DJ, Duchen MR, Yellon DM: Mitochondrial permeability transition pore as a target for cardioprotection in the human heart. Am J Physiol Heart Circ Physiol 2005; 289:H237–42
19. Chopra V, Wesorick DH, Sussman JB, Greene T, Rogers M, Froehlich JB, Eagle KA, Saint S: Effect of perioperative statins on death, myocardial infarction, atrial fibrillation, and length of stay: A systematic review and meta-analysis. Arch Surg 2012; 147:181–9
20. Reddy GD, Reddy AG, Rao GS, Kumar MV: Pharmacokinetic interaction of garlic and atorvastatin in dyslipidemic rats. Indian J Pharmacol 2012; 44:246–52
21. Wolfrum S, Dendorfer A, Schutt M, Weidtmann B, Heep A, Tempel K, Klein HH, Dominiak P, Richardt G: Simvastatin acutely reduces myocardial reperfusion injury *in vivo* by activating the phosphatidylinositide 3-kinase/Akt pathway. J Cardiovasc Pharmacol 2004; 44:348–55
22. Bolli R, Dawn B, Xuan YT: Role of the JAK-STAT pathway in protection against myocardial ischemia/reperfusion injury. Trends Cardiovasc Med 2003; 13:72–9
23. Gross ER, Hsu AK, Gross GJ: The JAK/STAT pathway is essential for opioid-induced cardioprotection: JAK2 as a mediator of STAT3, Akt, and GSK-3 beta. Am J Physiol Heart Circ Physiol 2006; 291:H827–34
24. Hilfiker-Kleiner D, Hilfiker A, Fuchs M, Kaminski K, Schaefer A, Schieffer B, Hillmer A, Schmiedl A, Ding Z, Podewski E, Podewski E, Poli V, Schneider MD, Schulz R, Park JK, Wollert KC, Drexler H: Signal transducer and activator of transcription 3 is required for myocardial capillary growth, control of interstitial matrix deposition, and heart protection from ischemic injury. Circ Res 2004; 95:187–95
25. Smith CC, Dixon RA, Wynne AM, Theodorou L, Ong SG, Subrayan S, Davidson SM, Hausenloy DJ, Yellon DM: Leptin-induced cardioprotection involves JAK/STAT signaling that may be linked to the mitochondrial permeability transition pore. Am J Physiol Heart Circ Physiol 2010; 299:H1265–70
26. Gao Q, Zhang SZ, Cao CM, Bruce IC, Xia Q: The mitochondrial permeability transition pore and the $Ca^{2+}$-activated K+ channel contribute to the cardioprotection conferred by tumor necrosis factor-alpha. Cytokine 2005; 32:199–205
27. Lim HW, Lim HY, Wong KP: Uncoupling of oxidative phosphorylation by curcumin: Implication of its cellular mechanism of action. Biochem Biophys Res Commun 2009; 389:187–92
28. Yu CK, Li YH, Wong GT, Wong TM, Irwin MG: Remifentanil preconditioning confers delayed cardioprotection in the rat. Br J Anaesth 2007; 99:632–8
29. Mariani J, Ou R, Bailey M, Rowland M, Nagley P, Rosenfeldt F, Pepe S: Tolerance to ischemia and hypoxia is reduced in aged human myocardium. J Thorac Cardiovasc Surg 2000; 120:660–7
30. Tissier R, Couvreur N, Ghaleb B, Bruneval P, Lidouren F, Morin D, Zini R, Bize A, Chenoune M, Belair MF, Mandet C, Douheret M, Dubois-Rande JL, Parker JC, Cohen MV, Downey JM, Berdeaux A: Rapid cooling preserves the ischaemic myocardium against mitochondrial damage and left ventricular dysfunction. Cardiovasc Res 2009; 83:345–53
31. Gateau-Roesch O, Argaud L, Ovize M: Mitochondrial permeability transition pore and postconditioning. Cardiovasc Res 2006; 70:264–73