Atorvastatin restores the balance between pro-inflammatory and anti-inflammatory mediators in rats with acute myocardial infarction

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Abstract. – Background: Pro- and anti-inflammatory cytokines play a major role in the development of acute myocardial infarction (AMI). This paper tests the hypothesis that atorvastatin may attenuate the severity of myocardial ischemic injury by restoring the balance between pro-inflammatory and anti-inflammatory mediators.

Materials and Methods: Sixty adult male albino rats were used. Experimental AMI was induced by subcutaneous injection of isoprenaline. Atorvastatin was given for five days, then, it was combined with isoprenaline in the last two days of treatment protocol. Rats without any treatment were used as controls. Rats were subjected to ECG tracing, assessment of Creatine phosphokinase (CPK) and CPK-MB, measurements of C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), and plasminogen activator inhibitor-1 (PAI-1).

Results: Induction of AMI by isoprenaline resulted in a significant elevation of ST segment, elevation of CPK and CPK-MB. CRP, TNF-α and plasma PAI-1 were significantly elevated in the AMI rats compared to the control groups. On the other hand, the level of the anti-inflammatory cytokine IL-10 was significantly reduced. Treatment with atorvastatin prior to induction of AMI was associated with a significant reduction of serum CRP, TNF-α, plasma PAI-1 and an increase of serum IL-10.

Conclusions: This study suggests the usefulness of atorvastatin as an attenuating agent against AMI. Atorvastatin restores the balance between the pro-inflammatory and the anti-inflammatory mediators and modulates the fibrinolysis by reducing the levels of PAI-1.

Key Words:
Experimental acute myocardial infarction, Atorvastatin, Tumor necrosis factor-α, Interleukin-10, Plasminogen activator inhibitor-1.

Introduction

Inflammation plays an integral role in the pathogenesis of cardiovascular events. One of the potential inflammatory markers is C-reactive protein (CRP), an acute phase reactant that reflects different degrees of inflammation and has been shown as an independent risk factor in a variety of cardiovascular diseases. CRP mediates monocyte chemotactic protein-1 induction in endothelial cells and causes the expression of intercellular adhesion molecule-1 and vascular cellular adhesion molecule-1 by the endothelial cells. Furthermore, CRP stimulates the monocyte release of pro-inflammatory cytokines that contribute to the pathogenesis of the cardiovascular diseases. Pro-inflammatory cytokines have been implicated in the initiation and maintenance of systemic and vascular inflammation associated with the coronary artery disease. In addition, during the inflammatory reactions anti-inflammatory cytokines are also produced and tend to modulate the inflammatory cellular signaling pathways. Normally, there is a balance between pro-inflammatory and anti-inflammatory cytokines, as the members of the anti-inflammatory class down-regulate the expression of pro-inflammatory cytokines.

Tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) are considered as key regulators of inflammatory responses. TNF-α has a broad spectrum of biological activities and it is known for its powerful pro-inflammatory effects. It critically regulates several cellular functions during the monocytes/macrophages differentiation and it is a cytokine implicated in myocardial dysfunction and remodeling. TNF-α induces cardiac myocytes expression of inter-
leukin-6 (IL-6) that has subsequent deleterious effects on the cardiac myocyte function. Moreover, it is thought to contribute to the progression of the atheroma by augmenting the local inflammatory response. IL-10 is a potent anti-inflammatory cytokine, secreted by lymphocytes of the T-helper 2 (Th2) subtype, monocytes and macrophages. It is considered as a major inhibitor of pro-inflammatory cytokine synthesis and down-regulator of the fibrinogen biosynthesis. Additionally, IL-10 is expressed in the human atherosclerotic plaques and has several anti-atherogenic effects.

The imbalance between pro-inflammatory and anti-inflammatory cytokines is thought to be important for the development of several inflammatory disorders. In coronary heart diseases the marked rise in inflammatory cytokines is not accompanied by the elevation of the anti-inflammatory cytokine IL-10.

It is known that a decrease in fibrinolytic activity is linked to an increase in the risk of myocardial infarction. Tissue plasminogen activator, the key enzyme in the initiation of fibrinolysis, is synthesized in endothelial cells and stored in small dense vesicles. It is secreted both basally and in response to thrombin and several vasoactive agents through a calcium-dependent and G protein-coupled pathway. Plasminogen activator inhibitor-1 (PAI-1) is the primary physiologic inhibitor of plasminogen activation in the blood. Elevated plasma levels of PAI-1 are associated with an increased incidence of acute myocardial infarction AMI.

Growing evidence suggests that statins are more than simple lipid-lowering drugs. Statins have other properties that are collectively referred as pleiotropic effects. These pleiotropic effects have a major role in protecting the myocardium against ischemic injury. Some of the cholesterol-independent or the “pleiotropic” effects of statins involve improving the endothelial function, enhancing the stability of the atherosclerotic plaques, decreasing the oxidative stress and inhibiting the thrombogenic response. It is well known that the pretreatment with statins reduces the myocardial injury after acute ischemia and reperfusion by increasing the expression of endothelial nitric oxide synthase. However, the effects of statins on the cytokines are thus far limited. Therefore, the aim of this work was to study the effect of atorvastatin on pro-inflammatory and anti-inflammatory mediators in experimental AMI.

**Materials and Methods**

**Drugs**

Isoprenaline hydrochloride: was supplied as a white odorless crystalline powder (Sigma Chemical Co., CA, USA). Atorvastatin: was supplied as a white powder (Amoun Pharmaceutical Company, Cairo, Egypt).

**Animals**

Sixty Wistar adult albino rats weighing 150 ± 10 g were used in this study and were purchased from the Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt). They were allowed free access to food and water ad libitum. They were kept under constant conditions with 12/12 h light/dark cycles and left for the acclimatization for one week before the start of the study.

**Induction of AMI**

Experimental acute myocardial infarction was induced by subcutaneous injection of isoprenaline hydrochloride dissolved in normal saline at a dose of 85 mg/kg on two successive days as described by Banerjee et al.

**Experimental Protocol**

Animals were divided into six groups, each group formed of ten rats. All drugs were freshly prepared each time.

**Group 1:** Control untreated group. Received no drugs.

**Group 2:** Saline control group of isoprenaline. Received normal saline 0.2 ml orally through gastric gavage for five days + subcutaneous injection of 0.2 ml saline on the last two days.

**Group 3:** Saline control group of atorvastatin. Received normal saline 0.2 ml for seven days, orally, daily through gastric gavage.

**Group 4:** Atorvastatin control group. Received atorvastatin orally daily for seven days by gastric gavage in a dose of 10 mg/kg.

**Group 5:** Isoprenaline-induced myocardial infarction group. Received normal saline 0.2 ml orally daily for five days + subcutaneous injection of isoprenaline in a dose of 85 mg/kg in the last two successive days.

**Group 6:** Isoprenaline and atorvastatin treated group. Received atorvastatin in a dose of 10 mg/kg orally daily by gastric gavage for five days, then, it is combined with isoprenaline in a dose of 85 mg/kg subcutaneous injection in the last two days.
Twenty-four hours after the last dose, rats were subjected to the following:

**Electrocardiogram (ECG).** Urethane was injected intra-peritoneal in a dose of 1.25 mg/kg (20) before ECG recording. Each rat was subjected to ECG monitoring using the two-channel oscillograph. All leads were recorded; ST segment displacement was measured in millimeters.

**Biochemical Analysis.** Blood was collected through the tail vein; serum and plasma were separated. Serum CPK, CPK-MB were measured using Hitachi auto analyzer (Roche Diagnostics Co., Mannheim, Germany)\(^2\). Serum CRP level was estimated by ELISA\(^2\). Serum TNF-α and IL-10 were measured by ELISA technique using a kit purchased from Biosource International (Camarillo, CA, USA) as described before\(^2\). Plasma PAI-1 was determined by ELISA (Hyphen Bio Med., Paris, France)\(^3\).

**Statistical Analysis**
Data were collected, tabulated and the results were evaluated using SPSS version 11. Means ± SD were used to describe the data. Student’s \(t\)-test was used to test for statistical differences between two groups. \(P\) value \(\leq 0.05\) was considered as statistically significant. We used ANOVA test for comparison between the control groups.

**Results**

**Electrocardiogram (ECG)** (Table I, Figure 1).
ECG tracing of control groups showed a normal ECG pattern in all rats (Figure 1A). Subcutaneous injection of isoprenaline for two successive days induced MI represented by elevation of ST segment and presence of sinus tachycardia compared to its control group (5.3 ± 1.02 mm) \(p\leq0.05\) (Figure 1B). However, there was a significant reduction in the ST segment in rats pretreated with atorvastatin (1.15 ± 0.411 mm) with appearance of a pathological Q wave (Figure 1C) compared to the rats with AMI \(p\leq0.05\).

**Biochemical Analysis**
As shown in Table II, CPK and CPK-MB levels in isoprenaline treated group were significantly higher than its saline control groups. At the same time atorvastatin-treated group showed a statistically significant decrease in CPK and CPK-MB levels when compared with the isoprenaline treated group.

In Table III, serum CRP and TNF-α increased significantly in rats treated with isoprenaline compared to its control group. Pretreatment of rats with atorvastatin showed a significant reduction in serum CRP and TNF-α as compared with the isoprenaline treated group \(p\leq0.05\).

In the isoprenaline treated group, the levels of the anti-inflammatory cytokine IL-10 decreased significantly compared to its levels in the control group. However, pretreatment of rats with atorvastatin revealed significant elevation in IL-10 levels, compared to the isoprenaline treated group \(p\leq0.05\) (Table III).

Animals receiving isoprenaline showed a significant elevation of plasma PAI-1 levels, compared to its control group. Pretreatment of atorvastatin significantly decreased plasma PAI-1 levels in comparison with the isoprenaline treated group (Table III).

Figure 2 shows that the correlation between TNF-α and PAI-1 was \(r=0.731\) (Figure 2A); between CRP and IL-10 was \(r=-0.846\) (Figure 2B); between CRP and TNF-α was \(r=0.836\) (Figure 2C).

**Figure 1.** ECG tracing in control and treated groups. **A.** ECG tracing in control groups. **B.** Isoprenaline treated group. **C.** Atorvastatin treated group.
Table I. ST segment elevation levels (mm) in control and treated groups.

<table>
<thead>
<tr>
<th>Control groups</th>
<th>Treated groups</th>
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<tr>
<td>Normal control</td>
<td>Saline (control of atorvastatin)</td>
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<td></td>
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<tr>
<td>ST segment elevation</td>
<td>0.00</td>
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*Significantly different from the isoprenaline treated group (*p* ≤ 0.05). n = 10.

Table II. Serum CPK (U/L) and CK-MB (U/L) in control and treated groups.

<table>
<thead>
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<th>Control groups</th>
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<td>Normal control</td>
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<td></td>
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<tr>
<td>CPK</td>
<td>209.4 ± 8.66</td>
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<tr>
<td>CK-MB</td>
<td>45.5 ± 10.39</td>
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*Significantly different from the saline control of isoprenaline (*p* ≤ 0.05). n = 10. ‡Significantly different from the isoprenaline treated group (*p* < 0.01).

Table III. Serum CRP (µg/ml), IL-10 (pg/ml), TNF-α (pg/ml) and plasma PAI-1 (ng/ml) in control and treated groups.

<table>
<thead>
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<tbody>
<tr>
<td>Normal control</td>
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</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>1.12 ± 0.28</td>
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<tr>
<td>TNF-α</td>
<td>39.6 ± 9.3</td>
</tr>
<tr>
<td>IL-10</td>
<td>73.6 ± 14.19</td>
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<tr>
<td>PAI-1</td>
<td>1.94 ± 0.54</td>
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*Significantly different from the saline control of isoprenaline (*p* ≤ 0.05). n = 10. ‡Significantly different from the isoprenaline treated group (*p* < 0.01).
Systemic inflammation plays a major role in the myocardial damage and preservation. Therefore, one of the major therapeutic goals of the modern cardiology is to design strategies aimed at minimizing the myocardial necrosis and optimizing the cardiac repair following myocardial infarction. Large evidence supports the pathophysiological role of the pro-inflammatory cytokines in AMI, but the potential protective role of anti-inflammatory cytokines and the possibility that AMI is caused by an inflammatory cell-mediated imbalance in pro and anti-inflammatory cytokine production is still unclear. Therefore, an intensive interest may be generated concerning the novel therapies to modulate this cytokine network. The present work was designed to study the effect of atorvastatin on the pro-inflammatory and anti-inflammatory mediators in isoprenaline-induced experimental AMI.

In this study, AMI was induced by a subcutaneous administration of isoprenaline HCl in a dose of 85 mg/kg for two successive days. Induction of AMI was supported by the elevated ST segment and raised total serum CPK and CPK-MP. One possible mechanism that explains cardiotoxicity from isoprenaline is through the stimulation of the beta-adrenergic receptors, causing a coronary vasoconstriction and increasing the heart rate, contractility and myocardial oxygen consumption. These changes exert biochemical effects on several cellular and subcellular processes, as the oxidative stress, the calcium overload and the mitochondrial injury or dysfunction. Another mechanism was explained by Ferrari et al, who stated that isoprenaline is auto oxidized by the molecular oxygen to produce hydroxyl anion radicals. This leads to an increased peroxidation and loss of high energy phosphates, triggering an inflammatory cascade through the induction of cytokines and the direct inhibitory effect on the myocardial function. The metabolic alterations that occur during the ischemia facilitate the formation of free radicals from the residual molecular oxygen. In addition, Marjomaki et al stated that during myocardial infarction, the cytoplasm of cardiac myocytes becomes more acidic due to the lactate accumulation, and the resulting necrosis is believed to result, at least in part, from the leakage of lysosomal enzymes into the cytoplasm. Following the myocyte death, the cytosolic contents may eventually enter the systemic circulation, where they may be detected as markers of the ischemic heart disease, as, serum levels of CPK, and CPK-MP, are elevated several hours following AMI.

Elevated CRP levels often accompany the acute coronary syndromes and have been identified as an index of further events. In the current study the level of CRP in control groups was consistent with Chen et al. Isoprenaline-induced MI resulted in a significant elevation of serum CRP. Activation of the endothelial cells and the leukocytes within the microcirculation, or within the myocardial necrosis, may contribute to this systemic inflammatory reaction. Moreover, the infarcted region undergoes a local necrosis.
and a myocyte apoptosis resulting in a complement activation, free radicals generation, and an accumulation of cellular debris. The cellular debris is phagocytosed by infiltrating immune cells such as macrophages and neutrophiles triggering the inflammatory response and a cytokine cascade release. 

Cytokine expression is markedly up regulated in healing AMI. Our data clearly showed a significant increase in serum TNF-α in the isoproterenoline treated group. This finding agrees with Satoh et al., who demonstrated the marked elevation of various inflammatory mediators as interleukins and TNF-α in cases of AMI and congestive heart failure. The source of TNF-α following the myocardial ischemia is mainly the cardiac mast cells degranulation. 

Increased TNF-α induces the apoptosis of the cardiac myocytes and exerts a negative inotropic effects, at least potentially, through the increased production of sphingosine. TNF-α also, induces a cardiac myocyte expression of IL-6, which may have subsequent deleterious effects on the cardiac myocyte function. Furthermore, TNF-α induces the production of free radicals, which in turn can further damage the myocardium. 

In addition to the major role that the pro-inflammatory cytokines play in the development of AMI, anti-inflammatory cytokines are also produced and tend to modulate the inflammatory pathways. IL-10 is an anti-inflammatory cytokine, that is initially described as a cytokine synthesis inhibitory factor (CSIF), with potent deactivating properties on the inflammatory cell subpopulations of T helper 1 cells, endothelial cells, granulocytes and monocytes/macrophages. In this study, serum level of IL-10 in control groups was consistent with previous reports. The level of IL-10 was significantly decreased after the induction of AMI by isoproterenol. This higher pro-inflammatory to anti-inflammatory cytokine ratio, suggests that the decreased levels of the anti-inflammatory cytokine IL-10 were associated with the increased risk of clinical instability in AMI. On the other hand, the IL-10 serum level is inversely related to future events in patients with AMI, this highlighting the important role of IL-10 in counterbalancing the pro-inflammatory response. 

The fibrinolytic inhibitor, PAI-1, plays a critical role in vascular pathophysiology both at the intra and extra vascular levels. Our data revealed a significant increase in PAI-1 among the rats with AMI compared to the control groups. It was reported before that TNF-α is the major inducer of PAI-1 synthesis in differentiated cells. Our results agree with this finding. There was a positive correlation between the levels of TNF-α and PAI-1. The crucial role of TNF-α in the cardiovascular disease provides evidence for its direct and pleiotropic effects on the circulation and endogenous fibrinolysis. 

In our study, the pleotropic effect of atorvastatin resulted in reasonable attenuating effects in the form of reduction of the elevated ST segment in the ECG tracing and a significant reduction in the biomarkers of AMI: total CPK and CPK-MB. Moreover, the pre-treatment of rats with AMI with atorvastatin, significantly reduced the pro-inflammatory cytokine TNF-α, and increased the anti-inflammatory cytokine IL-10. This finding was explained by Anguera et al., who stated that IL-10 exerts a negative modulator effect on the immune response through inhibiting cytokine synthesis by T cells indirectly by affecting macrophages and promoting a switch in the immune response from a delayed-type hypersensitivity to an antibody-type. Over expression of IL-10 in AMI may be a homeostatic mechanism leading to the restriction of the ischemic/necrotic zone after AMI, regulating the post-infarction healing and repair processes, and finally targeting to the neutralization of the acute inflammatory reaction. The elevated level of IL 10 might correlate with a better prognosis in cases of AMI. 

This study was extended to evaluate the effect of atorvastatin on PAI-1. Significant decrease in PAI-1 levels was detected in AMI rats pre-treated with atorvastatin as compared with the untreated rats with AMI. This finding was in agreement with Tousoulis et al., who demonstrated that atorvastatin depresses both the fibrinolysis and coagulation systems through the decrease of pro-inflammatory cytokines such as TNF-α. In addition, the pro-fibrinolytic activity of atorvastatin was confirmed by Bruni et al., who demonstrated that atorvastatin was able to reduce the platelet activity and thrombin generation, which can modulate the fibrinolysis by a thrombin activable fibrinolysis inhibitor. Another possible mechanism was through the direct effect of atorvastatin on the hepatocyte function and its biosynthetic ability: it decreases the production of liver derived components of the coagulation system. 

In conclusion, atorvastatin seems to have anti-inflammatory properties, by decreasing the
serum concentration of the pro-inflammatory cytokine TNF-α, increasing the anti-inflammatory cytokine IL-10 and modifying the acute phase reactant CRP. Furthermore, atorvastatin can modulate the fibrinolysis by reducing the levels of PAI-1.

References


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