Dose-related effects of atorvastatin on mortality and inflammatory responses to endotoxin-induced shock in rats

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Abstract

Purpose - Statins have been prescribed for dyslipidemia for a long time. Till date, several studies have been conducted on effects of atorvastatin on sepsis; however, dose-related effects have never been investigated. This study evaluated dose-related effects of atorvastatin on endotoxin-induced shock in rats.

Methods - Fifty-six male Sprague-Dawley rats were randomly assigned to 1 of the 4 different groups (n = 14 per group): Group C, no medication; group S, oral administration of a small dose of atorvastatin (2 mg/kg/day) for 5 days; group M, oral administration of a medium dose of atorvastatin (5 mg/kg/day) for 5 days; and group L, oral administration of a large dose of atorvastatin (10 mg/kg/day) for 5 days. Following the pretreatment, endotoxin shock was induced by intravenous administration of lipopolysaccharide (15 mg/kg). Hemodynamic recordings, arterial blood gas analyses, and plasma cytokine level measurements were performed regularly.

Results - Mortality rates between groups S and C differed significantly (7% and 43%, respectively) at 8 h after lipopolysaccharide injection ($P < 0.05$). In addition, plasma lactate levels between groups S and C differed significantly at 8 h ($P < 0.05$). With respect to hemodynamics, the systolic arterial pressure was considerably higher in group S than in the other groups at 8 h after endotoxin injection.

Conclusions - A small dose of atorvastatin improved mortality rates and inhibited inflammatory responses to endotoxic shock in rats. These effects were not observed in other doses of atorvastatin, suggesting dose-related effects of atorvastatin on endotoxic shock.

Key words atorvastatin, endotoxic shock, anti-inflammatory effect, dose-related effect

Introduction

The 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors, known as “statins,” are widely prescribed for dyslipidemia. Recently, statins have been shown to possess anti-inflammatory and immunomodulatory “pleiotropic” characteristics[1]. Research to clarify clinical advantages of these effects has been avidly conducted lately. For example, statins have been shown to reduce cardiovascular events in patients with coronary disease[2)3)] and decrease serum cytokine levels and reduce neutrophil adhesion in patients undergoing coronary artery bypass grafting[4]. In addition, several animal studies have been conducted to investigate beneficial effects of statins on septic rats[5)6)7)]. However, these reports have mainly focused on effects of statins administered following the induction of sepsis, and few studies have been conducted on dose-related effects of statins administered before the induction of sepsis.

We hypothesized that atorvastatin pretreatment may show dose-dependent beneficial effects in endotoxic rats. This study aimed to evaluate dose-related effects of atorvastatin pretreatment on mortality and inflammatory responses to endotoxin-induced shock in rats.

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Materials and Methods

The experimental protocol was approved by the Animal Care Committee of our institution, and care and handling of the animals were performed in accordance with the National Institutes of Health guidelines.

Experimental design and protocol

Fifty-six male Sprague-Dawley rats, weighing 423 (±26) g {$\bar{x}$ [± standard deviation (SD)]}, were included in this study. The rats were randomly allocated to 1 of the 4 groups (n = 14 per group): group C, no medication; group S, oral administration of a small dose of atorvastatin (2 mg/kg/day) for 5 days; group M, oral administration of a medium dose of atorvastatin (5 mg/kg/day) for 5 days; and group L, oral administration of a large dose of atorvastatin (10 mg/kg/day) for 5 days. These doses were determined on the basis of previous reports[8)9)]. After 5 days of pretreatment, all the rats were anesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg) and ventilated through tracheotomy. The femoral artery was cannulated to monitor the blood pressure and draw blood samples. Lactated Ringer solution containing a muscle relaxant (pancuronium bromide, 0.02 mg/mL) and pentobarbital sodium (0.5 mg/mL) were continuously infused at a rate of 10 mL/kg/h through the femoral vein cannula. The rats were connected to a pressure-controlled ventilator (Servo 900B; Siemens-Elema, Solna, Sweden) that delivered 21% oxygen at a frequency of 28 breaths/min with an inspiratory:expiratory ratio of 1:1. The rats were rested on a table for approximately 15 min for hemodynamic stabilization. Following this, baseline recordings of the heart rate (HR) and systolic arterial pressure (SAP) were obtained. This method of animal preparation was reported previously.[10)]

Endotoxic shock was induced by intravenous injection of endotoxin (15 mg/kg). The rats did not receive any therapy before, during, or following shock induction. The rectal body temperature was maintained between 36℃ and 38℃ with a heating pad on the table.

The survival rate was observed for 8 h after endotoxin injection. Arterial blood samples (0.25 mL) were obtained to measure pH, partial pressure of arterial carbon dioxide, partial pressure of arterial oxygen, and base excess and lactate levels at baseline, 1, 2, 3, 4, 5, and 8 h. Additional arterial blood samples (1.5 mL) were obtained to measure plasma cytokine [tumor necrosis factor (TNF)-alpha and interleukin (IL)-6] levels at baseline, 2, 4, and 5 h. Enzyme-linked immunosorbent assay kits (BioSource, Camarillo, CA, USA) were used to measure cytokine (TNF-alpha and IL-6) levels.

Statistical analysis

Data are presented as the mean (± SD). Differences between groups at baseline were analyzed by 1-way analysis of variance followed by a post hoc test (the Dunnett method). Differences between groups at different times were analyzed by 2-way analysis of variance with repeated measures

Table 1. Arterial blood gas analyses at baseline and after endotoxin injection

Group		Baseline	2h	4h	8h
pHa					
	C	7.48±0.05*	7.42±0.06	7.38±0.09	7.23±0.13*
	S	7.42±0.08	7.32±0.05	7.31±0.07	7.13±0.13
	M	7.44±0.08	7.33±0.07	7.31±0.08	7.11±0.13‡
	L	7.51±0.06	7.40±0.09	7.37±0.11	7.10±0.09§
PaO2					
	C	508±33	496±36	480±33	458±165
	S	523±49	513±43	487±58	373±167
	M	518±67	481±50	456±57	404±131
	L	527±49	536±52	469±68	410±65
PaCO2					
	C	35.3±5.7*	36.1±7.2	34.9±10	31.5±10.5*
	S	43.9±10.2	48.7±9.0	47.4±7.7	52.6±14.6
	M	43.2±9.7	50.6±11	45.8±9.8	48.2±17.2‡
	L	32.8±6.8†	33.1±6.2	29.3±7.8	35±15.1†

Values are expressed as $\bar{X}$±SD. pHa = arterial pH, PaO_2 = partial pressure of arterial oxygen, $PaCO_2$ = partial pressure of arterial carbon dioxide, SD = standard deviation. *P<0.05 Group S v.s. Group C, †P<0.05 Group S v.s. Group L, ‡*P*<0.05 Group M v.s. Group MC, §*P*<0.05 Group C v.s. Group L

followed by a post hoc test (the Bonferroni method). Survival rates among the groups were compared using the Kaplan-Meier and Mantel-Cox tests. Significance was defined as P value <0.05. SPSS (version 17.0 for Windows, USA) was used to perform statistical analyses.

Results

Hemodynamics

No significant differences in the baseline HR and SAP were noted among the 4 groups (Fig. 1). Both HR and SAP continuously decreased after the injection in all groups. SAP was significantly higher in group S than in groups C and L at 8 h after endotoxin injection (P<0.05). HRs were significantly higher in group S than in groups C and L at 8 h after endotoxin injection (P<0.05).

Survival rates

Survival rates at 8 h were 57%, 93%, 64%, and 43% for groups C, S, M, and L, respectively (Fig. 2). The survival rate of group S was significantly higher than that of groups C and L at 8h (P < 0.05).

Blood gases

Results of blood gas analyses are shown in Table 1. pHa was significantly lower in group S compared the other 3 groups at baseline (P<0.05). At 8h, it was higher in groups C compared with other 3 groups (P<0.05). PaO_2 did not differ significantly among all groups at baseline and 8h. $PaCO_2$ was significantly higher in group S compared with the other 3 groups at baseline and 8h (P<0.05).

Lactate levels and base excess levels

No significant difference was observed in the baseline lactate and base excess levels among the 4 groups (Fig. 3). Metabolic acidosis, as indicted by the increase in serum lactate levels and decrease in base excess levels, developed in all the groups during the experiment; however, base excess levels in group S were significantly higher than those in the other groups at 8h after the injection (Fig. 3, A). Lactate levels in group S were significantly lower than those in the other groups at 8h (P < 0.05) (Fig. 3, B).

Plasma cytokine levels

Plasma cytokine levels were measured in groups C, S, and L (Fig. 4). Baseline values of the 3 groups were similar. TNF-alpha and IL-6 levels increased in all the 3 groups after endotoxin injection. However, there were no significant differences in any of these cytokines among the 3 groups at 5h.

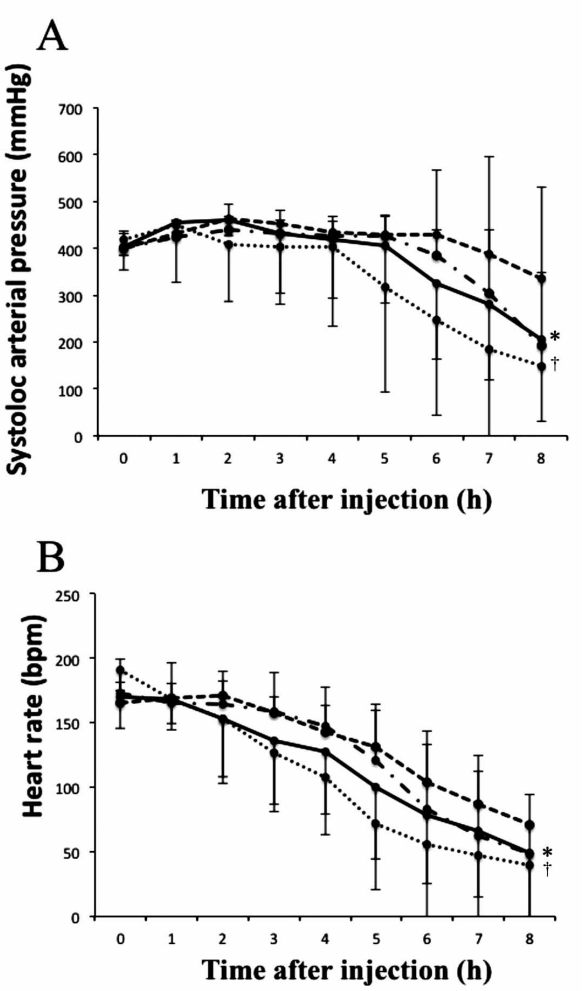

Figure 1. Hemodynamic changes in systolic arterial pressure (A) and heart rates (B) from baseline (0h) up to 8 h after endotoxin injection. —— group C (control), ····· Group L (large dose), —·— group M (medium dose), — — group S (small dose)
A: At 8h, * P<0.05 Group S v.s. Group C, † P<0.05 Group S v.s. Group L
B: At 8h, * P<0.05 Group S v.s. Group C, † P<0.05 Group S v.s. Group L

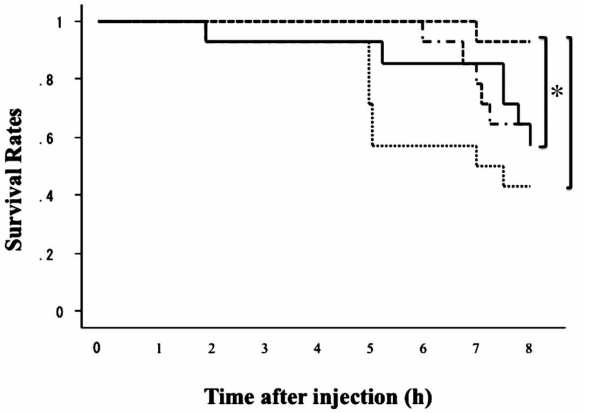

Figure 2. Survival rates after endotoxin injection. —— group C (control), ····· Group L (large dose), —·— group M (medium dose), — — group S (small dose)
At 8h, * P<0.05 Group S v.s. Group C, † P<0.05 Group S v.s. Group L

Discussion

Endotoxemia causes hypotension and metabolic acidosis in addition to triggering increases in plasma proinflammatory cytokine levels, which lead to high mortality rates at 8 h after endotoxin injection. In the present study, oral administration of a small dose of atorvastatin inhibited the development of severe hypotension and metabolic acidosis, resulting in lower mortality rates. However, this beneficial effect was not observed with a medium or large dose of the same agent. Of note, the anti-inflammatory effect was exerted only with a small dose and not with larger doses.

Several studies have recently demonstrated that statins possess anti-inflammatory and immunomodulatory "pleiotropic" characteristics. Ando et al. showed that pretreatment with cerivastatin reduced mortality rates in endotoxic mice[5]. Merx et al. revealed that pretreatment with simvastatin improved survival in septic mice[6]. They also showed that post-treatment with statins improved survival the following year[7]. However, there are few studies on dose-related effects of atorvastatin pretreatment on sepsis. The present study demonstrated that a small dose of atorvastatin exerts anti-inflammatory effects in endotoxic rats; however, a medium or large dose does not. These findings suggest that atorvastatin may have dose-related anti-inflammatory effects on sepsis.

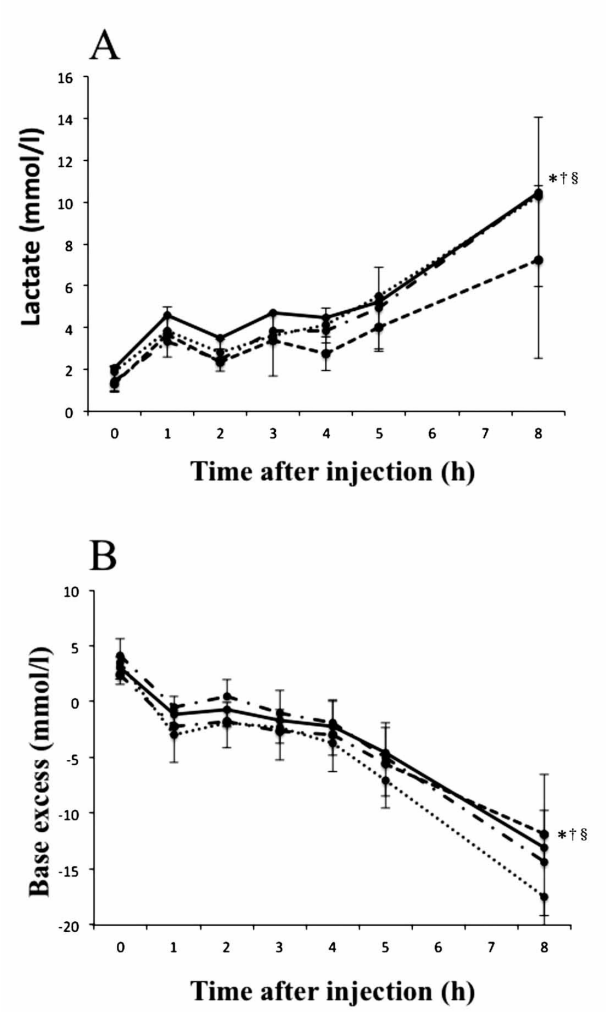

Figure 3. Baseline values of plasma lactate concentrations (A) and base excess (B), and their changes after endotoxin injection. —— group C (control), ····· Group L (large dose), —·— group M (medium dose), — — group S (small dose)
A: At 8h, * P<0.05 Group S v.s. Group C, † P<0.05 Group S v.s. Group L, § P<0.05 Group S v.s. Group M
B: At 8h, * P<0.05 Group S v.s. Group C, † P<0.05 Group S v.s. Group L, § P<0.05 Group S v.s. Group M

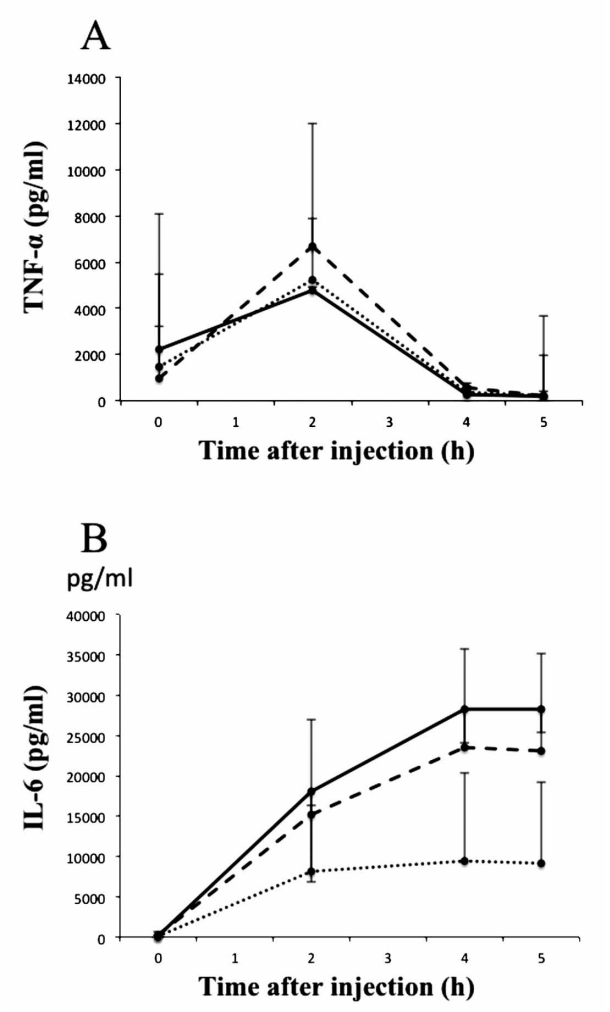

Figure 4. Serum cytokine levels measured after endotoxin injection. —— group C (control), ····· Group L (large dose), — — group S (small dose)

Many studies have shown that various types of statins exert different degrees of anti-inflammatory effect; however, few studies have been conducted to investigate dose-related effects of statins using animal models. Medeiros et al. showed that a small dose of atorvastatin reduced mucosal damage and inflammation in 5-fluorouracil-induced oral mucositis and that higher doses of atorvastatin were associated with hepatotoxicity and amplified leukopenia[11]. Our findings suggest that an appropriate dose of atorvastatin may improve mortality rates and inhibit inflammatory responses in sepsis, however, higher doses may not have the same effect. This may be due to the fact that deleterious effects of atorvastatin, such as rhabdomyolysis, manifest themselves in higher serum levels and therefore negating the beneficial effects of atorvastatin.

Recent studies have demonstrated that statins inhibit the elevation of proinflammatory cytokines such as TNF-alpha and IL-1-beta in vitro and in vivo[12)-14)]. One possible mechanism of these effects is that statin reduces inducible nitric oxide synthase expression by blocking the transcription factors nuclear factor kB and signal transducer/activator of transcription-1[15)]. However, the present study failed to show a dose-dependent anti-inflammatory effect of atorvastatin with respect to TNF-alpha and IL-6 levels. However, in this study, we did not evaluate other types of cytokines, such as IL-1 beta, which might have contributed to the findings of this study. Further investigations are necessary to determine the optimal dosage and timing of administration of atorvastatin that will reduce cytokine levels.

Whether atorvastatin shows the same degree of anti-inflammatory effects in different sepsis models, including cecal ligation and puncture as well as intravenous injection of bacteria, remains to be elucidated. Endotoxic shock manifests itself differently depending on the clinical situation; therefore, further studies are required to understand these situations.

Several clinical studies have demonstrated that atorvastatin pretreatment inhibits systemic inflammatory responses after major surgery. Chello et al.[16)] and Pan et al.[17)] showed that statin pretreatment improves perioperative mortality and decreases cytokine levels following coronary bypass surgery. Raux et al. reported that oral administration of statin inhibits the development of an aneurysm sac after elective endovascular aneurysm repair[18)]. These findings and those of the present study suggest that statins have beneficial effects not only intraoperatively but also postoperatively on patients undergoing major surgery.

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