Cerivastatin Improves Survival of Mice with Lipopolysaccharide-Induced Sepsis

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ABSTRACT
Development of severe sepsis is thought to result from the overproduction of cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and nitric oxide. Recently, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, which are antihypercholesterolemic agents, have been reported to inhibit lipopolysaccharide (LPS)-induced production of cytokines and nitric oxide in vitro. In this study, we tested these effects in vivo. After LPS administration (15 mg/kg i.p.) to CD-1 mice, serum levels of both TNF-α and IL-1β transiently increased, and peaked at 2 h. After the peak responses of TNF-α and IL-1β, serum levels of nitrite and nitrate increased until at least 8 h. Pretreatment of the mice with cerivastatin (20 mg/kg i.p. 12 and 1 h before LPS injection) reduced serum levels of TNF-α and IL-1β at 2 h, and nitrite and nitrate at 8 h, by 93, 60, and 44%, respectively. In this model of sepsis, cerivastatin significantly (P = .016) improved the rate of 7-day survival from 26.7 to 73.3%. These results cast new light on the usefulness of cerivastatin in preventing severe sepsis.

Materials and Methods
Treatment of Mice. Male CD-1 mice (Charles River, Japan Inc., Kanagawa, Japan) were given a standard laboratory diet and water ad libitum and housed under controlled environmental conditions. They were 5 to 6 weeks of age at the start of experiments. After a minimum 7-day acclimation period, the mice were divided into two groups and given saline with or without cerivastatin sodium (20 mg/kg i.p., donated by Bayer Pharmaceuticals, Osaka, Japan) at 10 ml/kg 12 and 1 h before LPS administration under sterile conditions. Cerivastatin sodium is an entirely synthetic compound and produced sterilely. In addition, cerivastatin itself neither increased serum levels of TNF-α nor affected the health of mice, such as their dietary intake or body weight (data not shown). LPS (Difco, Detroit, MI) was administered i.p. at a dose of 15 mg/kg. Survival of mice was monitored at intervals of 12 h for 7 days. All animal procedures were in

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ABBREVIATIONS: LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; iNOS, inducible nitric-oxide synthase; NO, nitric oxide; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; NOx, nitrite and nitrate; NF-κB, nuclear factor-κB.
accordance with the standards set forth in the Guidelines for the Care and Use of Laboratory Animals of the Takara-machi campus of Kanazawa University.

**Serum TNF-α and IL-1β Measurement.** Mice were anesthetized with ether, and blood was collected by cardiac puncture. Serum levels of TNF-α and IL-1β were determined by an enzyme-linked immunosorbent assay kit (Endogen, Woburn, MA) with a polyclonal rabbit anti-mouse TNF-α or IL-1β antibody. Lower limits of quantification of TNF-α and IL-1β were 10 and 3 pg/ml, respectively. Their intra- and interassay coefficients of variation were all <10%.

**Serum Nitrite and Nitrate (NOx) Measurement.** The serum samples were deprived of protein by filtration through molecular cut-off filters, microconcentrators 10 (Amicon, Beverly, MA), and assayed for NOx by the Griess method (Green et al., 1982) with a commercial kit (Dojindo, Kumamoto, Japan).

**Statistical Analysis.** Differences in serum TNF-α, IL-1β, and NOx levels between cerivastatin-pretreated and control mice were determined with the nonparametric Mann-Whitney test. The overall difference in survival rate of LPS-treated mice was determined by survival analysis, and P values were determined by the log-rank (Mantel-Cox) test. Values are presented as mean ± S.E., and significance of differences was assumed at \( P < .05 \). All calculations were performed with the computer program Statview, version 4.5, for Macintosh (Abacus Concepts, Berkeley, CA).

### Results

**Effects of Cerivastatin on Serum TNF-α and IL-1β Levels in LPS-Treated Mice.** Expression of inflammatory cytokines, such as TNF-α and IL-1β, is known to be induced by LPS in various types of cells in vitro (Pahan et al., 1997). Therefore, we first investigated the changes over time in serum levels of these mediators after LPS administration (15 mg/kg i.p.) in CD-1 mice. As shown in Fig. 1A, serum TNF-α levels, which were not detectable before LPS injection, rose at 1 h, peaked at 2 h, and dropped to near basal levels by 4 h. Serum IL-1β levels also peaked at 2 h, but remained high longer than serum TNF-α levels (Fig. 1B).

To investigate whether HMG CoA reductase inhibitor suppresses LPS-induced cytokines in vivo, cerivastatin (20 mg/kg) was administrated i.p. 12 and 1 h before LPS injection. Cerivastatin significantly reduced the peak levels of TNF-α and IL-1β by 93 and 60%, respectively, without affecting their basal levels (Fig. 2).

**Effect of Cerivastatin on Serum NOx Levels in LPS-Treated Mice.** As reported previously (Tracey et al., 1995), CD-1 mice injected with 15 mg/kg LPS time dependently accumulated high serum levels of NOx, which reached about 16 times basal level at 8 h. Preadministration of cerivastatin (20 mg/kg i.p. 12 and 1 h before LPS injection) significantly reduced serum NOx level at 8 h by 44% without affecting basal NOx level (Fig. 3).

**Effect of Cerivastatin on Survival Rate of Mice Treated with LPS.** Based on the above-mentioned findings, we examined whether cerivastatin could prevent LPS-induced death. Eleven of the 15 mice pretreated with saline alone (73.4%) died within 1 to 4 days after LPS injection (15 mg/kg; Fig. 4). Only 26.7% of mice pretreated with cerivastatin (20 mg/kg i.p. 12 and 1 h before LPS administration) died after LPS challenge. The overall difference in survival rate between groups with and without cerivastatin was significant \( (P = .016) \).

### Discussion

HMG CoA reductase catalyzes the formation of mevalonate from acetyl-CoA, and regulates cholesterol biosynthesis. Mevalonate metabolites, particularly farnesyl pyrophosphate, are involved in post-translational modification of several important proteins such as cellular membrane G proteins and Ras proteins (Goldstein and Brown, 1990; Maltese, 1990). In addition, inhibitors of HMG CoA reductase have recently been shown to reduce LPS- and TNF-α-induced nuclear factor-κB (NF-κB) activation in mesangial cells, vascular smooth muscle cells, and mononuclear cells (Gujarro et al., 1996; Ortego et al., 1999). Furthermore, in rat macrophages, the HMG CoA reductase inhibitor lovastatin inhibited LPS-induced expression of cytokines and iNOS, which is thought to be mediated via NF-κB activation (Pahan et al., 1997). Because these effects were not reversed by cholesterol and ubiquinone, which are end products of the mevalonate pathway, but were reversed by mevalonate and farnesyl pyrophosphate, the mevalonate pathway is thought to play an important role in controlling NF-κB-mediated expression of cytokines and iNOS. In this study, we demonstrated for the
first time in vivo that cerivastatin inhibits elevation of inflammatory cytokine and NO levels, although the molecular mechanisms underlying these effects remain to be investigated.

In concordance with inhibition of induction by LPS of cytokines and NO, cerivastatin actually improved survival of mice with LPS-induced sepsis. Sepsis frequently affects cholesterol metabolism, and LPS has been reported to provoke an increase in hepatic cholesterol synthesis in rodents that is accompanied by increase in HMG CoA reductase synthesis (Feingold et al., 1993). In addition, Memon et al. (1993) have shown that TNF-α and IL-1β increase hepatic HMG CoA reductase activity and cholesterol synthesis in mice. These findings suggest that LPS-induced overproduction of cytokines and NO is strongly associated with activation of the mevalonate pathway, which can be blocked by HMG CoA reductase inhibitors. Whether cerivastatin prevents LPS-induced death via inhibition of the mevalonate pathway remains to be elucidated.

Serum lipoproteins are known to protect against endotoxin-induced death by binding and inactivating endotoxin (Harris et al., 1990). In this study, the administration of cerivastatin 12 and 1 h before measurement did not affect serum levels of low-density lipoprotein cholesterol and high-density lipoprotein cholesterol (data not shown). Therefore, cerivastatin does not seem to prevent LPS-induced sepsis by modifying serum lipoprotein profiles.

Severe sepsis unfortunately still has a high mortality rate because no single treatment has been found to be completely effective for it (Parrillo, 1996). Although overproduction of cytokines, such as TNF-α and IL-1β, is thought to play an important role in the development of sepsis (Hesse et al., 1988; Cannon et al., 1990), recent clinical trials of agents directed against TNF-α or IL-1 have failed to improve survival of patients with sepsis (Fisher et al., 1993, 1994). In addition, overexpression of iNOS induced by LPS and/or cytokines in sepsis has been reported to cause hypotension and blood flow abnormality. However, it is still unclear whether iNOS-deficient mice are resistant to LPS-induced death (Laubach et al., 1995; MacMicking et al., 1995; Wei et al., 1995), and whether NOS inhibitors are effective in LPS-induced sepsis models (Kilbourn et al., 1990; Nava et al., 1994).
1992; Minnard et al., 1994). Because in this study cerivastatin inhibited not only the production of TNF-α and IL-1β but also the production of NO, resulting in prevention of LPS-induced death, it might be very useful for prophylaxis of severe sepsis. However, the agent does not seem to be so effective against sepsis after the onset of severe inflammation because administration of cerivastatin 2 and 13 h after LPS injection did not protect mice from LPS-induced death (data not shown).

Excessive production of NO by iNOS, together with overproduction of cytokines, is thought to contribute to the pathogenesis of various diseases, including infections, cancers, and autoimmune diseases (Kröncke et al., 1998; Takamura et al., 1998). Therefore, cerivastatin might be useful for the prevention of some of these diseases. Further experimental and clinical studies will be needed to clarify such protective effects of cerivastatin, in fact, of HMG CoA reductase inhibitors.

In conclusion, cerivastatin, an HMG-CoA reductase inhibitor, inhibited overproduction of cytokines and NO and improved survival of mice with LPS-induced sepsis. These findings suggest that cerivastatin may be useful for preventing sepsis.

References


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