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Protective Effect of Decursinol on Mouse Models of Sepsis: Enhancement of Interleukin-10

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The effects of decursinol on various models of sepsis were investigated. Intra-peritoneal pretreatment of mice with various doses of decursinol (1~100 mg/kg) effectively suppressed lethality induced in three mouse models of experimental sepsis, i.e., lipopolysaccharide (LPS)/D-galactosamine (GalN), high-dose LPS (20 mg/kg), and cecal ligation and puncture (CLP). Intra-peritoneal pretreatment of mice with decursinol (50 mg/kg) markedly enhanced the LPS/GalN-induced increase of plasma interleukin-10 (IL-10) levels, without affecting plasma TNF-α, IL-6 and IL-12 levels. These results suggest that decursinol could be effective for prevention or treatment of sepsis.

Key Words: Decursinol, Experimental sepsis, Interleukin-10

INTRODUCTION

Severe sepsis is a serious disease accounting for the major of death in intensive care unit. Until now, treatment of severe sepsis is unsatisfactory (Nguyen and Smith, 2007). In an attempt to find a potential anti-septic agent from plants, we screened crude extracts of oriental medicinal herbs for protecting lipopolysaccharide (LPS)-induced-lethality in D-galactosamine-sensitized mice, and found that orally administered methanol extract of *Angelica* significantly inhibited the LPS-induced- lethality (unpublished observation). As decursinol (Fig. 1) is a known constituent of *Angelica* (Ryu et al., 1967), the effect of decursinol on various mouse models of sepsis was investigated in the present study.

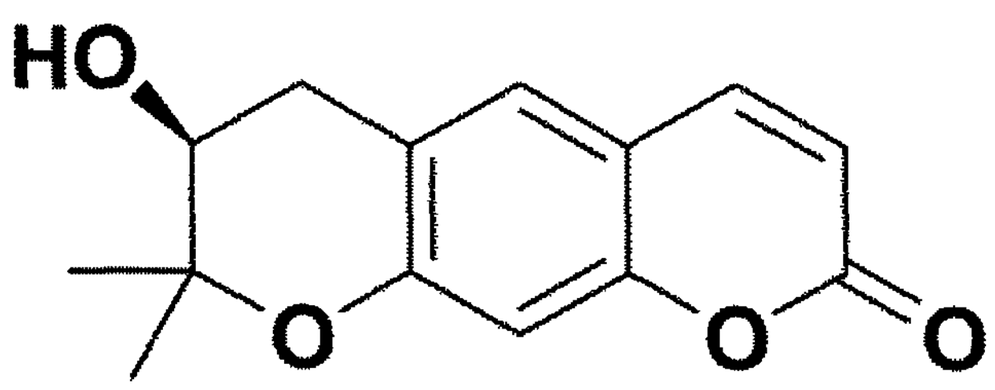

Fig. 1. Structure of decursinol.

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METHODS

Animals and models of sepsis

Five male ICR mice, weighing 23~28 g, were housed per cage in a room maintained at 22±1°C with an alternating 12 hour light-dark cycle. Food and water were available ad libitum. Procedures for animal experiments were approved by the Animal Experimentation Committee at Hallym University. For lipopolysaccharide (LPS)/D-galactosamine (GalN)-induced lethality (Galanos et al., 1979), LPS (*Escherichia coli* 055 : B5, Sigma, USA) was dissolved in phosphate-buffer saline (PBS) at 1 μg/μl and stored at −80°C until use. GalN (ICN, USA) was dissolved in PBS at 0.16 g/ml and added to 7.2 μl LPS. The LPS/GalN mixture was used immediately. Each mouse received LPS/GalN (LPS 36 μg/kg, GalN 0.8 g/kg) intra-peritoneally (i.p.) at a volume of 1 ml/100 g of body weight. Decursinol was dissolved in 10% DMSO, and was administered i.p. 30 min prior to i.p. injection of LPS/GalN. For high dose LPS-induced lethality, various doses (1~100 mg/kg) of decursinol were administered i.p. 30 min prior to i.p. injection of LPS (20 mg/kg). For cecal ligation & puncture (CLP) (Yan et al., 2004), mice were anaesthetized with pentobarbital (50 mg/kg, i.p.), and a small abdominal midline incision was made and the cecum was exposed. The cecum was mobilized, ligated below the ileocecal valve, and punctured through both surfaces two with a 22-gauge needle, and the abdomen was closed. Mice subjected to sham-CLP underwent the same procedure as above except for ligation and puncture of the cecum. Various doses (2, 10, 50 mg/kg) of decursinol were administered i.p. at 2 h and 4 h after CLP.

ABBREVIATIONS: CLP, cecal ligation and puncture; GalN, D-galactosamine; IL, interleukin; LPS, lipopolysaccharide.

Cytokine measurements

Mice were i.p injected with decursinol (50 mg/kg) or vehicle (10% DMSO) at 30 min before i.p. injection of LPS/GalN. Blood was collected from the retro-orbital venous plexus at 1.5 h after LPS/GalN administration and centrifuged at 4,000 g at −4°C for 15 min. Plasma sample was stored at −20°C until assayed. Plasma levels of TNF-α, IL-6, IL-10, and IL-12 were measured with an enzyme-linked immunoassay kit (Genzyme, USA). Assays were performed exactly as described by manufacturers.

Statistical analysis

Statistical analysis of survival data was performed by the log-rank test. Cytokine data were evaluated by one-way analysis of variance (ANOVA). Bonferroni and Newman-Keuls tests were used for *post-hoc* comparisons. p values less than 0.05 were considered to indicate statistical significance.

RESULTS

Protection of mive against LPS/ GalN-, high dose LPS-, and CLP-induced lethality by decursinol

To examine the protective effect of decursinol against LPS/GalN-induced lethality, mice were pretreated with decursinol 30 min before LPS/GalN injection, and lethality was observed for 3 days. As shown in Fig. 2a, treatment of mice with LPS/GalN (i.p.) induced 100% death rate within 24 h. However, intra-peritoneal pretreatment of mice with decursinol (1~100 mg/kg) dose-dependently protected the animals from LPS/GalN-induced lethality; the lethality began to be significantly improved with the dose of 3 mg/kg, and was completely inhibited at the dose of 100 mg/kg.

Next, we examined the effect of decursinol on the high-dose LPS-induced lethality. As shown in Fig. 2b, treatment of mice with high dose of LPS (20 mg/kg, i.p.) induced 71% (5 from 7) death rate in 3 days. However, intra-peritoneal pretreatment of mice with decursinol (100 mg/kg) significantly inhibited the high-dose LPS-induced lethality.

Next, we examined the protective effect of decursinol on lethality which was induced by CLP, a model of septic peritonitis. As shown in Fig. 2c, CLP induced 71% (5 from 7) death rate in 3 days. However, intra-peritoneal treatment of mice twice with decursinol at 2 h and 4 h after CLP significantly inhibited CLP-induced lethality at the dose of 10 mg/kg. Interestingly, decursinol was less effective at the dose of 50 mg/kg than at 10 mg/kg, suggesting that the optimal effective dose of decursinol is lower in CLP model than in LPS model. However, when given twice at 6 h and 8 h after CLP, decursinol showed no protective effect on CLP-induced lethality (data not shown).

Effects of decursinol on LPS/ GalN-induced plasma cytokine levels

Intra-peritoneal injection of LPS/GalN into mice resulted in a marked elevation of plasma TNF-α, IL-6, IL-10, and IL-12 levels, when measured at 1.5 h after LPS/GalN

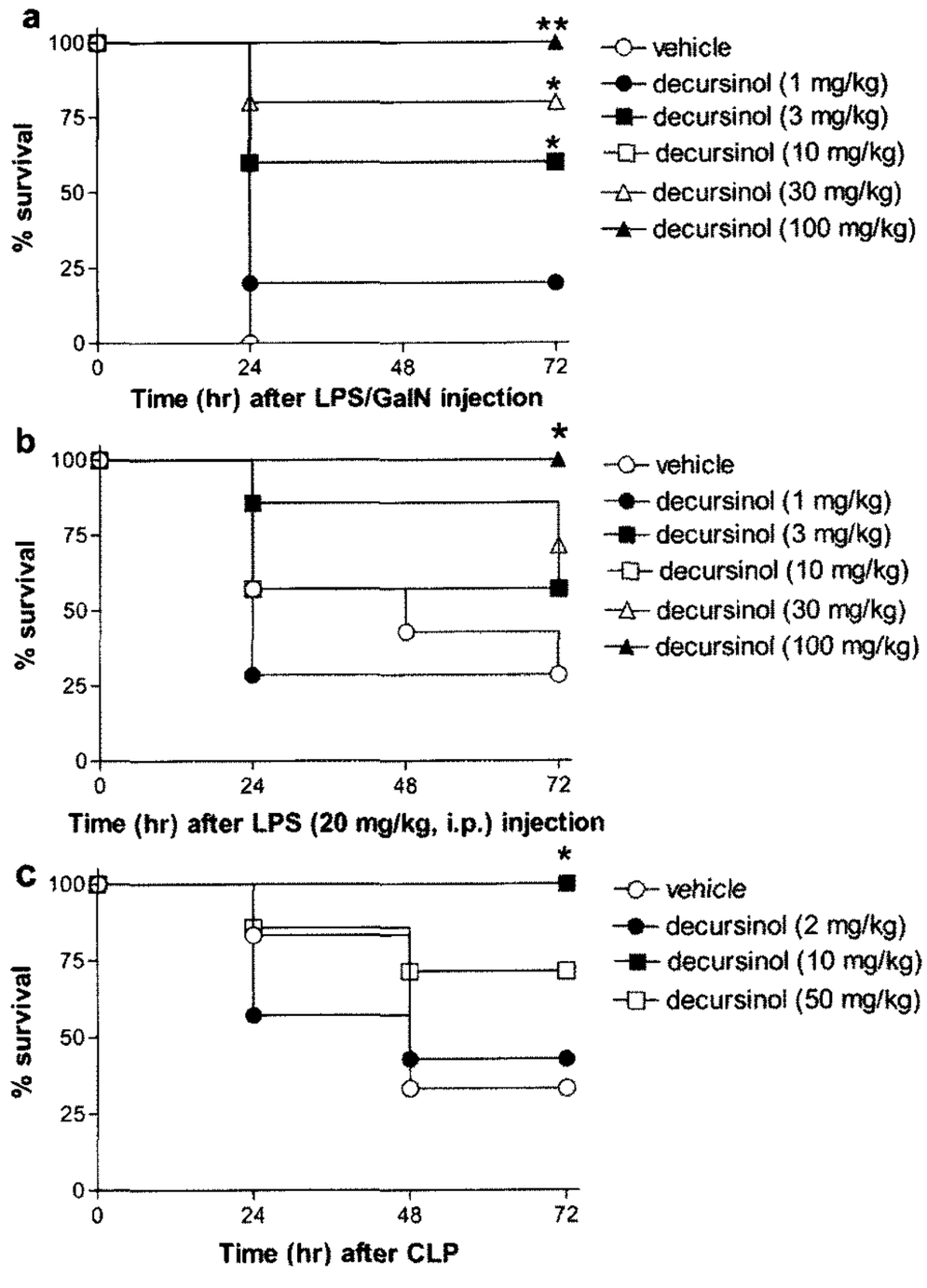

Fig. 2. Protective effect of decursinol on lethality which was induced by LPS/GalN (a), high dose LPS (b), and CLP (c). Decursinol was injected intraperitoneally (i.p.) 30 min prior to the i.p. administration of LPS/GalN (a), and high dose LPS (b). Decursinol was i.p. administered twice at 2 h and 4 h after CLP. Number of mice for each group was five (a), seven (b), and seven (c). *$p<0.05$, **$p<0.01$ significantly different from vehicle-treated group.

injection. Pretreatment with decursinol (50 mg/kg i.p.) 30 min prior to LPS/GalN injection resulted in a marked augmentation of LPS/GalN-induced plasma levels of IL-10, without affecting LPS/GalN-induced plasma TNF-α, IL-6, and IL-12 levels (Fig. 3).

DISCUSSION

Decursinol has been shown to have neuroprotective and analgesic effects (Yan et al., 2004; Choi et al., 2003; Lee et al., 2003). In the present study, decursinol was shown to have a protective effect on various mouse models of sepsis, i.e. lethality induced by LPS/GalN, high dose-LPS, and CLP. Among various cytokines, decursinol markedly enhanced LPS/GalN-induced plasma IL-10 levels.

IL-10 is an important anti-inflammatory cytokine (Pestka et al., 2004). Endogenous IL-10 reportedly protects mice from death during septic peritonitis (van der Poll et al., 1995). Administration of recombinant IL-10 inhibits LPS toxicity in mice (Howard et al. 1993). Therefore, augmentation of IL-10 levels can be an effective way of treatment or prevention of sepsis (Scumpia and Moldawer, 2005). In the present study, decursinol was found to markedly augment

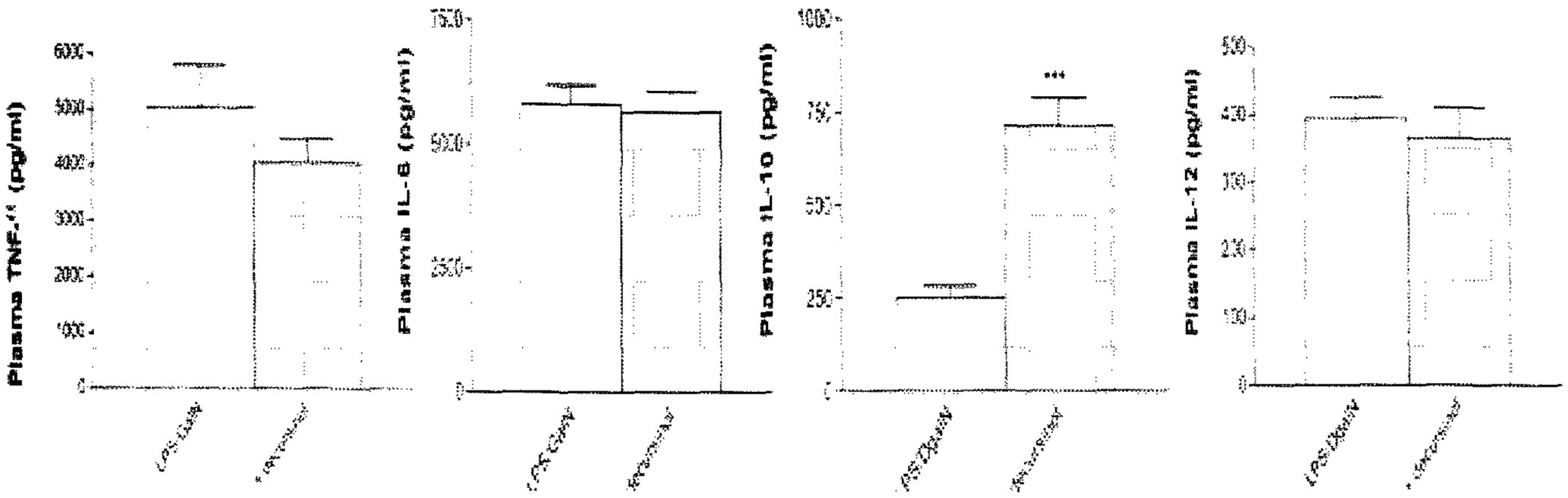

Fig. 3. Enhancement of LPS/GalN-induced plasma IL-10 levels by pretreatment with decursinol. Decursinol was i.p. administered 30 min prior to the i.p. administration of LPS/GalN, and plasma levels of cytokines were measured 1.5 h after i.p. administration of LPS/GalN. Data represent means±SEM of five to seven mice. ***$p<0.001$ significantly different from LPS/GalN-treated group at the time point.

LPS/GalN-induced plasma IL-10 levels. Therefore, the action of decursinol could be ideal for this purpose. In addition to sepsis, IL-10 has been reported to be beneficial in various other inflammatory diseases, including inflammatory bowel disorders, multiple sclerosis and rheumatoid arthritis (Pestka et al., 2004). Boosting endogenous IL-10 production has been suggested as an important strategy for treating various inflammatory disorders (Zhou et al. 2005). Thus, the IL-10 elevating activity of decursinol could be potentially effective in these disorders.

The mechanism of decursinol involved in the increase of IL-10 remains presently unclear. Several classes of agents are known to increase IL-10 levels, including cAMP elevating agents [such as isoproterenol (Suberville et al., 1996)] and pyrrolidine dithiocarbamate (PDTC), an antioxidant (Nemeth et al., 1998). However, these agents decrease TNF-α and IL-6, while augmenting of IL-10. Thus, decursinol is different from the above cited agents in that it specifically increases IL-10 levels without altering TNF-α and IL-6. Further studies are needed for elucidating mechanisms involved.

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