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European Journal of Pharmacology 391 (2000) 317–320

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Short communication

A novel dual regulator of tumor necrosis factor-α and interleukin-10 protects mice from endotoxin-induced shock

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Received 21 January 2000; accepted 28 January 2000

Abstract

A pyrimidylpiperazine derivative, *N*-[1-(4-{[4-(pyrimidin-2-yl)piperazin-1-yl]methyl}phenyl)cyclopropyl]acetamide (Y-39041), is a dual cytokine regulator of tumor necrosis factor (TNF)-α and interleukin-10 production. Lipopolysaccharide-induced TNF-α release in BALB/c mice was inhibited by the oral treatment with the compound at 10–100 mg/kg (about 80% suppression) while interleukin-10 release was augmented (about 10-fold increase at 30 mg/kg). In addition, Y-39041 (30 mg/kg, p.o.) completely protected mice from lipopolysaccharide-induced death by the treatment before and after lipopolysaccharide injection. The finding that Y-39041 suppresses TNF-α production and stimulates interleukin-10 production at the same time provides new insights for the treatment of septic shock, rheumatoid arthritis and Crohn's diseases.

Keywords: Interleukin-10; TNF-α; tumor necrosis factor-α; Lipopolysaccharide

1. Introduction

Endotoxin elicits tumor necrosis factor (TNF)-α release in animals and human volunteers, and the excessive production of TNF-α contributes to the pathology of septic shock. In patients with septic shock, several therapies using soluble TNF receptor and anti-TNF-α chimera antibody have been tried but were not effective (Newton and Decicco, 1999). Therefore, preventing the elevation of pro-inflammatory cytokine by only one anti-cytokine agent may be insufficient to improve septic shock. Interleukin-10 is known as a potent anti-inflammatory cytokine that acts by inhibiting the production of TNF-α and interleukin-6 by macrophages (Fiorentino et al., 1991); therefore, it is expected that new agents capable of regulating both TNF-α and interleukin-10 at the same time would have therapeutic effects in the treatment of septic shock. We screened synthetic compounds that possessed anti-inflammatory activities not only to inhibit TNF-α production but also to augment interleukin-10 production in lipopolysaccharide-stimulated mice in vivo.

According to the above hypothesis we found *N*-[1-(4-{[4-(pyrimidin-2-yl)piperazin-1-yl]methyl}phenyl)cyclopropyl]acetamide (Y-39041). We have shown that the compound is a potent up-regulator and down-regulator of lipopolysaccharide-induced interleukin-10 production and TNF-α production, respectively, by the oral administration route. Phosphodiesterase-IV inhibitors and adenosine derivatives possess regulatory activity on TNF-α and interleukin-10 production (Newton and Decicco, 1999). The compound showed neither inhibitory activity for phosphodiesterase-IV nor binding affinity for the adenosine A_{2A} receptor at 10^{-5} M in vitro. Furthermore, oral treatment with Y-39041 completely protected mice from lipopolysaccharide-induced death not only by the administration prior to lipopolysaccharide injection but also by the administration after lipopolysaccharide injection.

2. Materials and methods

Female BALB/c mice were purchased from Japan Charles River (Kanagawa, Japan). Mice were used at 6–7 weeks of age. The compound was synthesized in our

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00096-0

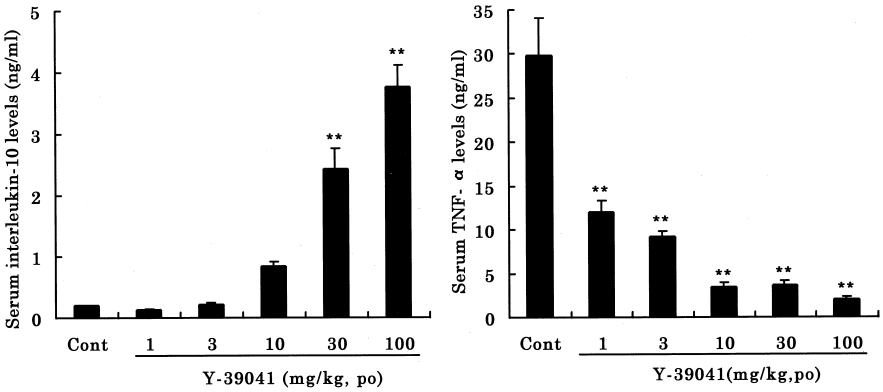

Fig. 1. Y-39041 increases lipopolysaccharide-induced interleukin-10 production and inhibits TNF-α production in mice. Groups of five mice were treated orally with Y-39041 30 min prior to lipopolysaccharide injection (0.5 mg/kg, i.p.). Amounts of interleukin-10 and TNF-α in the serum were measured 1.5 h after lipopolysaccharide injection. Results are expressed as the mean ± S.E.M. $^{**}P < 0.01$ significantly different from control (Dunnett method).

laboratories. Lipopolysaccharide from *Escherichia coli* (serotype 0111:B4 or 055:B5) was purchased from Difco Laboratories (Detroit, USA). Y-39041 was suspended in 0.5% hydroxypropylmethylcellulose solution. Lipopolysaccharide was dissolved in 0.9% saline.

Y-39041 was orally administered to mice 30 min prior to i.p. injection of lipopolysaccharide (0111:B4, 0.5 mg/kg). Blood samples were obtained at 1.5 h after lipopolysaccharide injection. The samples were centrifuged and the serum was collected and stored at −30°C until use for cytokine determination. Amounts of interleukin-10 and TNF-α were measured using enzyme-linked immunosorbent assay (ELISA) kits. The specific ELISA for murine interleukin-10 and TNF-α were purchased from Genzyme (MA, USA) and BioSource (CA, USA), respectively. Assays were performed as indicated by the manufacturer's instructions. The lower detection limit is 15 pg/ml for interleukin-10 and TNF-α.

Endotoxin shock was induced by i.p. injection of lipopolysaccharide (055:B5, 7.5 mg/kg). The compound was orally administered 30 min prior to or after lipopolysaccharide injection. Survival was monitored 3 days after lipopolysaccharide injection.

Significant differences in cytokine production were determined by the one-way analysis of Dunnett method. Significant differences in survival rate were calculated using Wilcoxon method. *P* values less than 0.05 were considered statistically significant.

3. Results

The i.p. injection of lipopolysaccharide (0.5 mg/kg) to mice caused the elevation of interleukin-10 and TNF-α serum concentration at 1.5 h (Fig. 1). The oral treatment with Y-39041 (30, 100 mg/kg) significantly augmented lipopolysaccharide-induced interleukin-10 production. Especially, in Y-39041 (30 mg/kg)-treated group, an approximately 10-fold increase in interleukin-10 production over the untreated control was observed. In addition, lipopolysaccharide-induced TNF-α production was significantly decreased by Y-39041 treatment at doses of 10–100 mg/kg, p.o. (about 80% suppression).

Eight of ten mice died within 1–2 days after i.p. injection of lipopolysaccharide (7.5 mg/kg). In the group

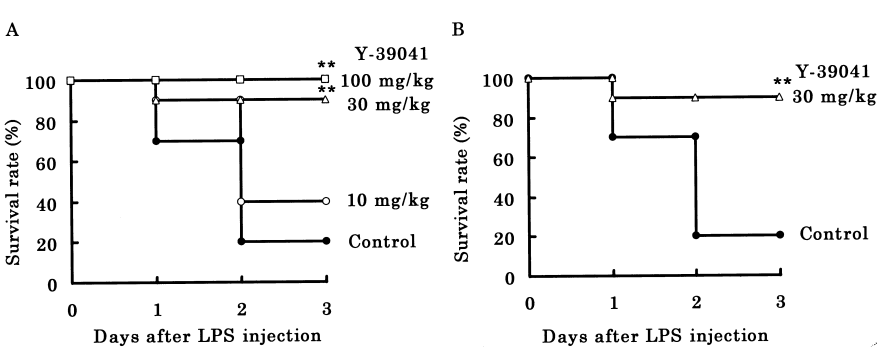

Fig. 2. Y-39041 protects mice against lipopolysaccharide lethality. Groups of 10 mice were orally administered with Y-39041 30 min before (A) or after (B) lipopolysaccharide injection (7.5 mg/kg). Survival was monitored 3 days after lipopolysaccharide injection. $^{**}P < 0.01$ significantly different from control (Wilcoxon method).

administered with 30 and 100 mg/kg of the compound 30 min prior to lipopolysaccharide injection, 90% and 100% of lipopolysaccharide-injected mice survived, respectively (Fig. 2). In the group administered with 30 mg/kg of the compound 30 min after lipopolysaccharide injection, 90% of lipopolysaccharide-injected mice survived.

4. Discussion

In patients with septic shock, a blood TNF-α level is detected up to 10 days after the onset of shock. TNF-α also plays a major role in systemic toxicity associated with sepsis (Deitch 1998). An anti-TNF-α chimera antibody improved survival and organ function in animal endotoxemia models (Beutler et al., 1985). Interleukin-10 has already been shown to protect mice against lethal endotoxemia (Howard et al., 1993). It has been reported that the combined treatment with anti-TNF-α antibody and interleukin-10 produces an additive therapeutic effect on development of arthritis in collagen-induced arthritis in mice (Walmsley et al., 1996). Therefore, elimination of TNF-α with anti-TNF-α antibody and administration of interleukin-10 are expected to be therapeutic for diseases associated with TNF-α, suggesting that agents having the dual regulatory activities, namely interleukin-10-enhancing and TNF-α-inhibiting activities, should be used clinically in the near future.

Y-39041 is a dual cytokine regulator suppressing TNF-α production and augmenting interleukin-10 production at the same time and at almost the same dose. In in vivo preliminary experiments, the pretreatment of mice with a murine anti-interleukin-10 antibody augmented lipopolysaccharide-induced TNF-α production, and Y-39041 inhibited the overproduction of TNF-α. The above findings suggest that the compound independently regulates the production of TNF-α and interleukin-10. The mechanism of its dual regulatory effects is unclear. The compound showed no inhibitory activity on lipopolysaccharide-induced TNF-α production from human peripheral blood mononuclear cells at 10^{-5} M in vitro. However, lipopolysaccharide-induced TNF-α production was inhibited by addition of serum in mice given orally with 30 mg/kg Y-39041. Therefore, it is considered that Y-39041 decreases amounts of TNF-α via an induction of TNF-α inhibitory factors in serum. This compound showed no inhibitory activity for phosphodiesterase-IV (−2%), cyclooxygenase-I (−5%), or cyclooxygenase-II (−17%) and no binding affinity for adenosine A_{2A} receptor (7%) at 10^{-5} M in vitro. Therefore, the pharmacological profile of the compound is different from that of phosphodiesterase-IV inhibitors and non-steroidal anti-inflammatory agents. The compound had no binding affinity for TNF-α in binding assay (22% inhibition at 10^{-5} M), suggesting that it has no antagonistic effect on TNF-α.

We observed that oral administration of Y-39041 prior to lipopolysaccharide injection protected mice from lethal endotoxic shock. As this administration schedule of the compound in lipopolysaccharide-induced lethality of mice was almost the same as the lipopolysaccharide-induced cytokine production, the compound may protect mice from lipopolysaccharide-induced shock via a drastic increase of interleukin-10 production and at the same time suppression of TNF-α production. Interestingly, Y-39041 also protected mice from lipopolysaccharide-induced shock by the administration after lipopolysaccharide injection. In a cecal ligation puncture model, interleukin-10 decreased mortality when given after induction of sepsis, but anti-TNF-α antibody had no effect on the survival rate (Kato et al., 1995). Therefore, it is considered that the inhibitor of TNF-α production may not be effective in septic shock in humans. The compound that augments interleukin-10 production and inhibits TNF-α production at the same time may be effective in septic shock patients. These results and findings suggest that the therapeutic effect of Y-39041 on lipopolysaccharide-induced shock model may occur through up-regulation of endogenous interleukin-10.

Nitric oxide (NO) is thought to play a major role in lipopolysaccharide-induced lethality. In patients with rheumatoid arthritis, the treatment of anti-TNF-α antibody reduced NO overexpression (Perkins et al., 1998). Though Y-39041 has no inhibitory activity on inducible NO synthase at 10^{-5} M in vitro, it cannot be ruled out that it indirectly inhibits NO production in vivo. Our results show that Y-39041 augments the production of interleukin-10 and suppresses the production of TNF-α at the same time. TNF-α is known to be involved in the pathogenesis of rheumatoid arthritis and Crohn's disease. Y-39041 would be a therapeutic agent in patients with not only septic shock but also rheumatoid arthritis and Crohn's disease.

In conclusion, we found a novel synthetic compound, Y-39041, as a dual cytokine regulator suppressing TNF-α production and augmenting interleukin-10 production at the same time. In addition, Y-39041 completely protected mice from lipopolysaccharide-induced death. These findings suggest that Y-39041 would be a useful therapeutic drug for the treatment of TNF-α-associated diseases such as septic shock, rheumatoid arthritis, and Crohn's diseases.

Acknowledgements

We thank Dr. M. Terasawa for helpful discussion.

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