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Effect of Tilmicosin on Serum Cytokine Levels in the Endotoxemia

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Abstract: The effect of tilmicosin on serum cytokine concentrations were investigated in healthy and lipopolysaccharide-treated mice. The mice were divided into 3 groups. Lipopolysaccharide (250 μg, *Escherichia coli* 0111:B4, intraperitoneally) was injected into the positive control group. The other 2 groups received tilmicosin (20 mg kg^{-1}, subcutaneously) concurrently without or with lipopolysaccharide. After treatment, serum samples were collected at 0, 1.5, 3, 6, 12 and 24 h. Serum tumor necrosis factor, interleukin-1 and interleukin-10 levels were determined by enzyme-linked immunosorbent assay. Lipopolysaccharide increased all cytokine levels in the healthy mice. Tilmicosin slightly induced interleukin-1 production in the healthy mice, while it had no effect on tumor necrosis factor or interleukin-10 productions. However, tilmicosin elevated ($p<0.05$) tumor necrosis factor, interleukin-1 and interleukin-10 levels in lipopolysaccharide-treated mice. In conclusion, these data suggest that tilmicosin stimulates both proinflammatory and antiinflammatory cytokines at the dose recommended for infection.

Key words: Tilmicosin, lipopolysaccharide, TNF, IL-1, IL-10

INTRODUCTION

Tilmicosin (TIL) is a, 16-membered macrolide antibiotic, widely used in veterinary medicine. It is prepared by chemical modification of desmycosin and inhibits protein synthesis by binding to the 50 S subunit of the ribosome. TIL is especially used to the treatment of *Pasteurellae* sp. infections in the cattle and laboratory animals. Macrolide antibiotics may generally cause gastrointestinal side affects, but TIL has also potent cardiotoxic effect and this effect mainly depend on administration route and animal species (Barragry, 1994; Yazar *et al*., 2001, 2002a, 2004; Altunok *et al*., 2002).

Inhibiting the production of inflammatory mediators is current clinical approach to the treatment of inflammation. The activation of the transcription factor nuclear factor κB (NF-κB) by pathogens, including bacterial components such as Lipopolysaccharide (LPS), plays a central role in inflammation through the regulation of genes encoding inducible enzymes and cytokines such as Tumor Necrosis Factor (TNF), Interleukin-1 (IL-1) and IL-10. For this reason, it is believed that activated NF-κB plays a crucial role in the development of inflammation (Hanada and Yoshimura, 2002; Macdonald *et al*., 2003). Cytokines are, primarily produced by macrophages, small and highly active molecules. The circulating concentrations of cytokines are low or undetectable in healthy individuals, but their production is stimulated by microorganisms and/or their products. The main proinflammatory cytokines are TNF and IL-1. Large amounts of proinflammatory cytokines are harmful rather than beneficial. Many experimental studies shown that the overwhelming production of proinflammatory cytokines can lead to vasodilatation, hypotension, multiple organ failure and ultimately shock and death (Netea *et al*., 2003; Goetz *et al*., 2004). IL-10, an antiinflammatory cytokine, possesses potent inhibitory effects on the production of proinflammatory cytokines (Aldridge, 2002).

In recent years, many studies have focused on the antiinflammatory and antioxidant effects of macrolides (Yazar *et al*., 2001, 2002b). Some 14 and 15-membered macrolide antibiotics cause a concentration-dependent reduction in proinflammatory cytokine levels (Labro, 1998a; Tkalcevic *et al*., 2006). However, to the best of our knowledge, there has been no *in vivo* study demonstrating the effect of TIL on cytokine production.

The aim of this study was to determine the effect of TIL on the proinflammatory and antiinflammatory cytokine production in the healthy and endotoxaemic mice.

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MATERIALS AND METHODS

A total of 96 male Balb/C mice (2-3 months old, 25-30 g, Laboratory Animal Unit, Akdeniz University, Antalya, Turkey,) were used and the study protocol was approved by the Ethics Committee of the Veterinary Faculty. The animals were fed a standard pellet diet and tap water *ad libitum*.

The mice were divided into 3 groups (n = 30). LPS was injected (250 μg in 0.5 mL of normal saline solution, intraperitoneally; *Escherichia coli* 0111:B4, Sigma-Aldrich Chemie, Deisenhofen, Germany) into the positive control group. The other 2 groups received TIL (20 mg kg^{-1}, subcutaneously, Micotil® 300 inj, Lilly Ilac Tic. Ltd. Sti. Istanbul, Turkey) without or concurrently with LPS. After treatment, serum samples (n = 6) were collected under thiopental sodium anesthesia (70 mg kg^{-1}, intraperitoneally; Pental® sodium 1 g inj., I. E. Ulagay Ilac Sanayi, Istanbul, Turkey) by cardiac puncture at 0, 1.5, 3, 6, 12 and 24 h. Six mice were used for a 0th sampling point for all groups. Serum TNF (BioSource Mouse TNF kit, Camarillo, CA, USA), IL-1 (BioSource Mouse IL-1 kit) and IL-10 (BioSource Mouse IL-10 kit) levels were determined by Enzyme-Linked Immunosorbent Assay (ELISA) using an ELISA reader (MWGt Lambda Scan 200, Bio-Tek Instruments, VT, USA).

The concentrations of TNF, IL-1 and IL-10 were evaluated with the ANOVA and Tukey test (SPSS 10.0). Data are expressed as means±SE. Significance was accepted at a level of $p<0.05$.

RESULTS AND DISCUSSION

Serum TNF, IL-1 and IL-10 levels are shown in Table 1-3, respectively. TIL did not affect the TNF or IL-10 productions, but it increased the IL-1 levels in healthy mice. TIL increased ($p<0.05$) the elevated TNF (1.5th h), IL-1 (3rd and 24th h) and IL-10 (1.5th h) levels in the LPS-treated animals.

The antiinflammatory effects of the 14 and 15-membered macrolides used in human medicine have previously been demonstrated. However, the effects of 16-membered macrolides on cytokine production in the infection have not been extensively investigated. In this study, LPS increased the serum levels of TNF, IL-1 and IL-10. The stimulatory effects of LPS on cytokine production are well known (Yazar *et al*., 2007). TIL slightly increased IL-1 levels in healthy mice. The stimulatory effect of TIL on IL-1 production in healthy mice in this study is not easily explained. However, it is well known that macrolide antibiotics reach higher concentrations in phagocytes than in the serum and may change their functions (Labro, 1998b). Higher concentrations of TIL within the phagocytes might specifically stimulate only the production of IL-1 via a mechanism that occurs after NF-κB activation. But further studies are required to identify the specific cellular target of TIL. In the current study, TIL increased the elevated antiinflammatory (IL-10) cytokine at 1.5 h in the LPS-treated animals. Similarly, in an *in vitro* study, TIL increased IL-10 expression in LPS-treated macrophages (Cao *et al*., 2006). However, it was reported that TIL reduced proinflammatory cytokine expression in LPS-treated macrophages in same study. This difference may mainly depend on distinctive of experimental study design and/or dose of TIL. *In vivo* and *in vitro* studies may cause different results in order to many factors are involved in *in vitro* studies because of homeostasis. In the present study, TIL increased the elevated proinflammatory (TNF and IL-1) cytokines for 3 h in LPs-treated mice. However, 14 and 15-membered

Table 1: Effects of tilmicosin on serum TNF (pg mL^{-1}) levels in healthy and LPS-treated mice (mean±SE)

Groups	0 h	1.5 h	3 h	6 h	12 h	24 h
LPS	BDL	$771\pm90.9^{a,B}$	$656\pm118^{a,A}$	$123\pm55.2^{b,A}$	17.0 ± 5.79^{b}	BDL
TIL	BDL	BDL	BDL	BDL	BDL	BDL
TIL+LPS	BDL	$1313\pm28.8^{a,A}$	$627\pm57.3^{b,A}$	$64.8\pm26.4^{c,A}$	BDL	BDL

LPS: Lipopolysaccharide (250 μg, intraperitoneally), TIL: Tilmicosin (20 mg kg^{-1}, subcutaneously), TIL+LPS: Tilmicosin (20 mg kg^{-1}, subcutaneously) + (250 μg, intraperitoneally). BDL: Below the Detection Limit. Different letters in the same column (A, B) or line (a, b, c) are statistically significantly different ($p<0.05$)

Table 2: Effects of tilmicosin on serum IL-1 (pg mL^{-1}) levels in healthy and LPS-treated mice (mean±SE)

Groups	0 h	1.5 h	3 h	6 h	12 h	24 h
LPS	BDL	$75.5\pm15.1^{a,A}$	$75.0\pm10.9^{a,B}$	$94.7\pm14.8^{a,A}$	$67.0\pm10.5^{b,A}$	$19.3\pm5.79^{b,B}$
TIL	BDL	$61.3\pm49.9^{a,A}$	$13.2\pm3.99^{a,B}$	$30.5\pm5.63^{a,B}$	BDL	BDL
TIL+LPS	BDL	$149\pm55.9^{a,A}$	$107\pm16.8^{a,A}$	$108\pm15.6^{a,A}$	$48.7\pm14.3^{a,A}$	$62.6\pm16.8^{a,A}$

Different letters in the same column (A, B) or line (a, b) are statistically significantly different ($p<0.05$)

Table 3: Effects of tilmicosin on serum IL-10 (pg mL^{-1}) levels in healthy and LPS-treated mice (mean±SE)

Groups	0 h	1.5 h	3 h	6 h	12 h	24 h
LPS	BDL	$1410\pm263^{a,B}$	$934\pm247^{ab,A}$	$414\pm103^{bc,A}$	$125\pm11.3^{c,A}$	$68.8\pm19.8^{c,A}$
TIL	BDL	BDL	BDL	BDL	BDL	BDL
TIL+LPS	BDL	$2278\pm60.1^{a,A}$	$796\pm154^{b,A}$	$355\pm134^{c,A}$	$121\pm57.6^{c,A}$	$110\pm41.6^{c,A}$

Different letters in the same column (A, B) or line (a, b, c) are statistically significantly different ($p<0.05$)

macrolides (roxithromycin, azithromycin and clarithromycin), except for 16-membered, can decrease the proinflammatory cytokine production (Morikawa *et al*., 1996; Labro, 1998b; Khan *et al*., 1999). Although the 16-membered macrolides (spiramycin and josamycin) have no antiinflammatory effects, some *in vitro* studies have shown that the intracellular accumulation of these drugs may change the functions of immune system cells (Labro, 1998b; Hoyt and Robbins, 2001).

CONCLUSION

In summary, the 16-membered macrolide TIL can increase the cytokine productions at recommended dose in the infection. But this stimulator effect may not negative effect the treatment of infection because of producing the therapeutic levels for 3±4 days after a single subcutaneous injection.

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