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In vivo immunomodulatory profile of telithromycin in a murine pneumococcal infection model

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Received March 22, 2005, accepted April 28, 2005

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Pharmazie 61: 343–347 (2006)

In addition to bactericidal activity, macrolide antibacterials possess clinically relevant properties such as immunomodulatory activity. Whether such activity extends to novel antibacterials that are structurally related to macrolides, such as the ketolides, remains largely unknown. The objective of this study was to evaluate the *in vivo* immunomodulatory profile of the first ketolide antibacterial – telithromycin – in a murine neutropenic thigh infection model. Specific pathogen-free, female ICR mice were rendered transiently neutropenic with intraperitoneal cyclophosphamide. Thighs were inoculated with 10^6 colony-forming units of a single clinical isolate of *Streptococcus pneumoniae*. Once inoculated, mice ($n = 500$) received single oral doses of telithromycin (10, 25 or 50 mg/kg of body weight) or no treatment (control). Blood was obtained via cardiac puncture prior to and at 2, 4, 8, and 24 h after dose administration for determination of cytokine concentrations. Significant post-inoculation elevations of interleukin (IL)-1β, IL-6, and IL-10 were noted in untreated controls over 24 h. Telithromycin attenuated these increases and the suppression of both IL-6 and IL-10 release was observed to be dose dependent. Systemic concentrations of IL-2 and tumor necrosis factor alpha showed an upward trend over the initial 8-h post-inoculation period in the telithromycin group. These data therefore reveal novel *in vivo* immunomodulatory effects of telithromycin. Further studies are warranted to determine whether such effects contribute to the therapeutic efficacy of the drug in patients with acute respiratory tract infections.

1. Introduction

Macrolide antibacterials appear to exert immunomodulatory properties that are independent of antibacterial activity. Several of these agents have been shown to be active in the carrageenin-induced paw edema model (Culic et al., 2001; Davidson and Peloquin 2002). Macrolides influence several pathways involved in the inflammatory process, including the migration of neutrophils, the oxidative burst in phagocytes and the production of proinflammatory mediators and cytokines (Ianaro et al. 2000). In general, macrolides inhibit the synthesis and/or secretion of proinflammatory cytokines (e.g. tumor necrosis factor alpha [TNF-α], interleukin [IL]-8, IL-6, IL-1β); however, the effects on anti-inflammatory cytokines (IL-10, IL-4) are more variable (Davidson and Peloquin 2002; Ianaro et al. 2000).

The transcription factors activator protein (AP)-1 and nuclear factor-KB (NF-KB) appear to be the most important molecular targets for the inflammatory effects of the macrolides. Both of these factors are key regulators of IL-8 expression. NF-KB regulates the genes encoding various proinflammatory cytokines, chemokines, inflammatory enzymes, acute-phase proteins, and adhesion molecules (Cosentini et al. 2002; Culic et al. 2001).

While the immunomodulatory effects of macrolides are well described (Culic et al. 2001; Davidson and Peloquin 2002; Ianaro et al. 2000), limited data exist for structurally related agents. Telithromycin is the first of a new class of antibacterial agents – the ketolides – that are structurally related to the macrolides, but have structural modifications that confer enhanced antibacterial activity (Ackermann and Rodloff 2003). The major modification is the replacement of an L-cladinose sugar on the erythronolide A ring with a 3-keto functional group that reduces the propensity to induce resistance to macrolide–lincosamide–streptogramin$_B$ antibiotics and confers activity against most Gram-positive cocci that express the *erm* (ribosome methylation) gene (Bonnefoy et al. 1997; Bryskier 2000; Mauvais and Bonnefoy 2000). Telithromycin also contains a substituted side chain at the C11, 12 carbamate residue that results in improved activity against macrolide-resistant strains of *Streptococcus pneumoniae* (Douthwaite et al., 2000; Hansen et al. 1999) and a methyl group at the 6-O position that improves the stability of the molecule (Bryskier, 2000).

Telithromycin possesses an optimally targeted antibacterial spectrum of activity for the treatment of community-ac-

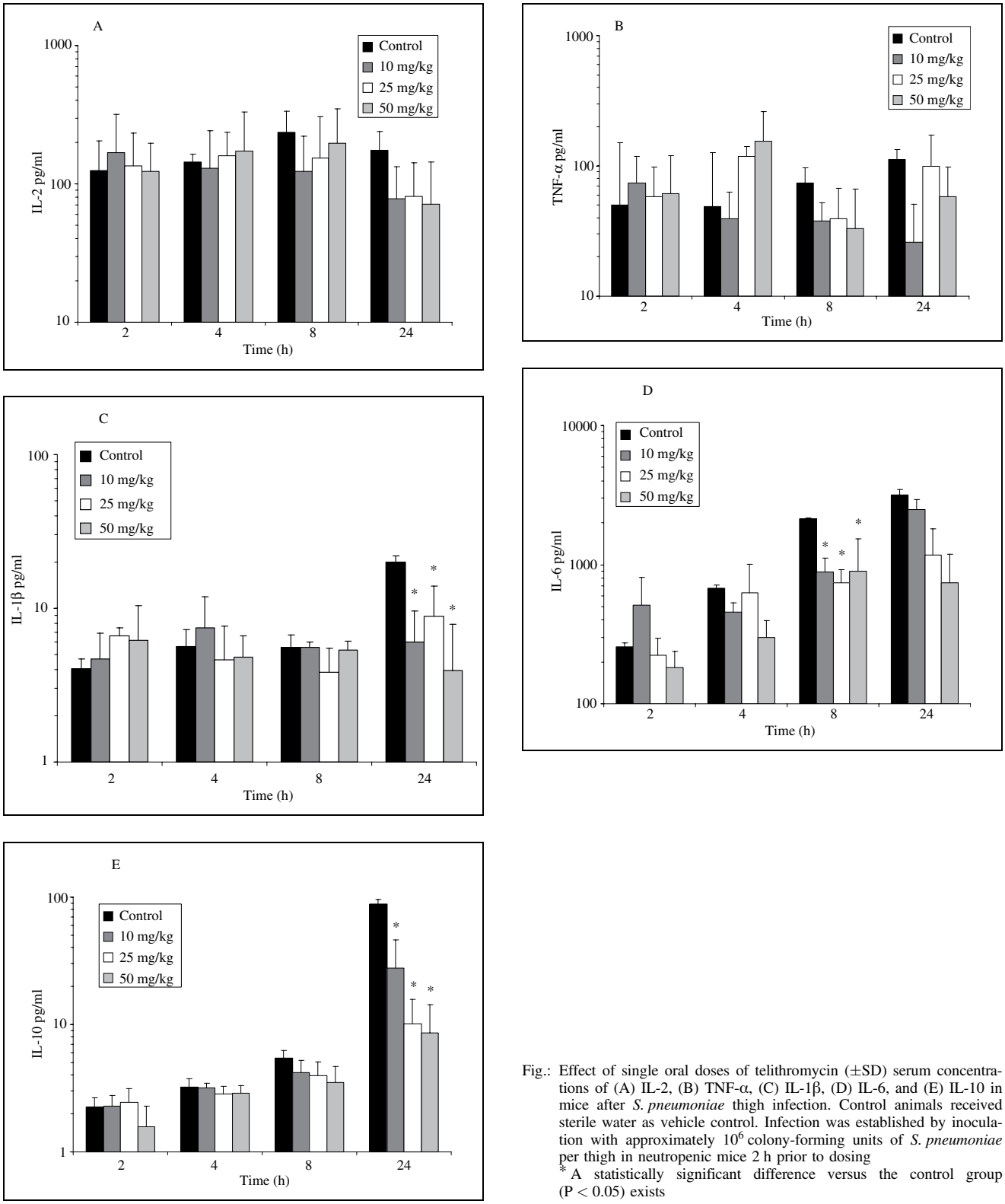

Fig.: Effect of single oral doses of telithromycin (±SD) serum concentrations of (A) IL-2, (B) TNF-α, (C) IL-1β, (D) IL-6, and (E) IL-10 in mice after *S. pneumoniae* thigh infection. Control animals received sterile water as vehicle control. Infection was established by inoculation with approximately 10^6 colony-forming units of *S. pneumoniae* per thigh in neutropenic mice 2 h prior to dosing
* A statistically significant difference versus the control group ($P < 0.05$) exists

quired respiratory tract infections, providing coverage of common causative pathogens, including atypical/intracellular bacteria, and β-lactam- and macrolide-resistant strains of *S. pneumoniae* (Lorenz 2003; Zhanel et al. 2002). The pharmacokinetic profile of telithromycin allows for once-daily oral administration of an 800 mg dose, which achieves respiratory tissue and fluid concentrations exceeding the minimum inhibitory concentrations (MICs) of key respiratory pathogens for up to 24 h (Zhanel et al. 2002). The drug accumulates in a number of cell types, including macrophages, epithelial cells, and neutrophils (Ianaro et al. 2000; Miossec-Bartoli et al. 1999; Pham et al. 1999).

An *in vitro* study using lipopolysaccharide (LPS)-stimulated monocytes noted an immunomodulatory effect of telithromycin, in that secretion of IL-1α and TNF-α was inhibited (Araujo et al. 2002). However, the *in vivo* immunomodulatory profile of this novel ketolide antibac-

terial has not been elucidated. Hence the objective of the present study was to address this issue by evaluating the *in vivo* immunomodulatory effects of telithromycin in the murine thigh infection model.

2. Investigations and results

A total of 87 samples were tested for serum concentrations of all five cytokines. Final replicates (n) at each time point in both control and treatment groups were between 3 and 5. The concentrations of all five cytokines measured in control animals prior to inoculation (−2 h) with *S. pneumoniae* were not significantly different from concentrations in control animals at initiation of dosing (0 h), or at 2, 4, or 8 h.

2.1. Post-inoculation IL-2 and TNF-α concentrations

Serum concentrations of IL-2 in telithromycin-treated mice resembled those in the control animals at 0, 2, 4, and 8 h (Fig. A). At 24 h, however, IL-2 levels in control animals were twice those observed in the telithromycin-treated mice, although statistical significance was not observed. No differences in mean TNF-α concentrations were noted between controls and the telithromycin-treated animals over the 24-h sampling period (Fig. B).

2.2. Post-inoculation IL-1β concentrations

No significant differences in IL-1β serum concentrations were found between the control and telithromycin-treated animals at 2, 4, or 8 h (Fig. C). At 24 h, the mean IL-1β concentration in control animals was statistically higher than the corresponding serum concentration observed in the same group at −2, 0, 2, 4, and 8 h ($P < 0.005$). At this time point, mean IL-1β concentration in telithromycin-treated animals was significantly lower versus controls for all doses ($P = 0.036$, $P = 0.015$, and $P = 0.015$ for the 10, 25, and 50 mg/kg groups, respectively).

2.3. Post-inoculation IL-6 concentrations

In control animals, mean IL-6 serum concentrations showed a progressive increase up to 8 h and remained at this level at 24 h (Fig. D). All telithromycin dosages resulted in significantly lower IL-6 concentrations versus controls at 8 h ($P < 0.003$). While a dose-dependent inhibition of IL-6 was observed among telithromycin-treated animals in relation to controls at 24 h, only the 50 mg/kg dose resulted in significant reductions of this cytokine ($P = 0.05$).

2.4. Post-inoculation IL-10 concentrations

Mean serum concentrations of IL-10 increased over the first 8 h in both the control and telithromycin-treated animals (Fig. E). At 24 h, IL-10 concentrations in control animals had increased 10-fold relative to the 8-h time point, and the mean concentration was significantly different from all previous mean levels ($P < 0.001$). Serum IL-10 concentrations were also elevated at 24 h among telithromycin-treated animals. However, the production of this cytokine appeared to be inhibited in a dose-dependent manner as all regimens resulted in significant decreases in IL-10 concentrations relative to controls ($P < 0.001$).

3. Discussion

In the present study using the mouse pneumococcal thigh infection model, treatment with the ketolide antibacterial telithromycin suppressed the production of the proinflammatory cytokines IL-6 and IL-1β in a dose-dependent fashion, in addition to suppressing IL-10. These findings suggest that telithromycin has an immunomodulatory effect, in accordance with a previous *in vitro* study (Araujo et al. 2002). In the *in vitro* study by Araujo and colleagues, telithromycin significantly inhibited secretion of the proinflammatory cytokines IL-1α and TNF-α by LPS-stimulated human monocytes *in vitro*, without modifying the secretion of IL-1β, IL-6, and IL-10.

The differences between the *in vitro* and *in vivo* studies are probably explained by differences in experimental conditions, such as the induction agent used (i.e. LPS versus pneumococcal infection) and host response mechanisms (i.e. human versus murine). Differences in drug exposure also have to be considered. For example, telithromycin doses in the present *in vivo* study were chosen to span a wide range of drug exposures, with the 50 mg/kg dose producing a free-drug area under the concentration-time curve similar to that observed in human volunteers receiving a therapeutically relevant dose of 800 mg (Perret et al. 2002). In contrast, in the *in vitro* study (Araujo et al. 2002), the greatest immunomodulatory effects were seen at telithromycin concentrations of 5 and 10 mg/l, levels not attainable with conventional dosing in humans (Perret et al. 2002). Another ketolide antibacterial (HMR 3004) has been shown to inhibit release of proinflammatory cytokines (IL-6, IL-1β) and nitric oxide, in addition to down regulating neutrophil accumulation, in a murine model of pulmonary inoculation with heat-killed *S. pneumoniae* (Duong et al. 1998).

Findings from an *in vitro* study using polymorphonuclear neutrophils (PMNs) suggest that telithromycin acts downstream of the priming effects of cytokines on immune cells (Vazifeh et al. 2000). The inhibitory effect of telithromycin on oxidant production by PMNs in response to stimulation by formyl-methionyl-leucyl-phenylalanine or phorbol myristate acetate *in vitro* was abolished by pretreatment of PMNs with TNF-α or granulocyte-macrophage colony-stimulating factor. However, telithromycin did not affect the response of PMNs to stimulation if coincubated with − or added before − the cytokines.

It is well recognized that bacteria release various virulence factors that can cause tissue damage. Moreover, bacterial infection produces a cascade of cytokines that may have beneficial and/or detrimental effects by activating immune cells that are involved in bacterial destruction and inflammation. Indeed, in severe infection, excessive and uncontrolled production of cytokines leads to widespread and potentially fatal tissue injury and organ dysfunction (Bassaris et al. 2003; Coyle 2003). Thus, resolution of bacterial infection can indirectly lead to modulation of the cytokine profile.

The model used in the present study was not able to determine whether the inhibition of cytokines with telithromycin reflected an independent immunomodulatory effect or could be explained solely in terms of its antibacterial action. Indeed, in a recent study in a murine model of *Mycoplasma pneumoniae*-induced pneumonia, clarithromycin demonstrated beneficial microbiologic and immunologic effects in mice infected with live *M. pneumoniae*, but not in animals inoculated with UV light-killed organisms (which also induce significant pulmonary inflammation). The authors sug-

gested that the changes in inflammatory parameters with clarithromycin were a consequence of its antibacterial activity and not an independent immunomodulatory effect. However, these findings may reflect the relatively low immunogenicity of the killed *Mycoplasma* model used in the study, as noted by the authors (Hardy et al. 2003).

The finding that telithromycin was able to induce immunomodulatory effects in a non-infective *in vitro* system (Araujo et al. 2002), and that a related class drug was active in a model of heat-killed pneumococcal infection (Duong et al. 1998), lend support to an independent immunomodulatory effect of telithromycin.

It has been suggested that the clinical benefits of macrolides in the management of such inflammatory diseases as diffuse panbronchiolitis and asthma may reflect their antibacterial activity against an unrecognized chronic infection, in addition to independent anti-inflammatory effects. There is evidence to suggest that the atypical/intracellular respiratory pathogens *Chlamydophila* (*Chlamydia) pneumoniae* and *M. pneumoniae* may play a role in the etiology of asthma, particularly in acute exacerbations and onset of wheezing (Clementsen et al. 2002; Hahn 1999; Kraft et al. 1998). *C. pneumoniae* is known to activate NF-KB, which appears to be important in the pathogenesis of several lung diseases (Culic et al. 2001). There are data to show clinical benefit of macrolides in asthmatic patients with *C. pneumoniae* or *M. pneumoniae* infection (Kraft et al. 1998). Given that telithromycin is bactericidal *in vitro* against these two pathogens (Bebear et al. 2000; Gustafsson et al. 2000; Roblin and Hammerschlag 1998), has proven clinical efficacy in patients with community-acquired pneumonia caused by atypical/intracellular pathogens (Zhanel et al. 2002), and appears to have an additional immunomodulatory potential, it may be found to be beneficial as a supplement to standard-of-care treatment in patients with acute exacerbations of asthma.

In conclusion, telithromycin has *in vivo* immunomodulatory effects, significantly reducing the production of IL-1β, IL-6, and IL-10 in a murine thigh infection model. Additional studies are warranted, not only to determine the underlying mechanism of this immunomodulatory effect, but also to establish whether such effects contribute to the therapeutic efficacy of the drug in patients with acute respiratory tract infections.

4. Experimental

4.1. Test compound

The antibiotic telithromycin was supplied by Aventis (Romainville, France).

4.2. Animals and thigh infection model

Specific pathogen-free, female CD-1/ICR mice (n = 500) weighing approximately 25 g were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, Indiana, USA). All mice were allowed to become acclimatized for a period of one week before use in experimentation. The mice were rendered transiently neutropenic and infected via inoculation with approximately 10^6 colony-forming units of *S. pneumoniae* per thigh as described elsewhere (Tessier et al. 2005). A penicillin-sensitive clinical isolate of *S. pneumoniae* with a telithromycin MIC of 0.008 μg/ml was used. All animal procedures were carried out in accordance with our Institutional Animal Care and Use Committee guidelines which adhere to National Research Council recommendations and were allowed food and water *ad libitum* (National Research Council, 1996).

4.3. Study treatment regimens

Mice were randomized to receive no treatment (control group) or a single oral telithromycin dose of 10, 25, or 50 mg/kg of body weight at 2 h post-inoculation in a volume of 0.2 ml, prepared as described previously (Tessier et al. 2005). In the active treatment groups, a total of 6–8 animals per dose per collection time point were treated. Animals in the control groups (n = 5 per group) were administered sterile water, with the exception of the 0 hour and pre-infection (−2 h) control groups, which received no oral administration.

4.4. Blood collection and sampling scheme

Blood was obtained via cardiac puncture after fatal CO_2 inhalation at the following time points: prior to inoculation (−2 h), prior to dosing (0 h) and at 2, 4, 8, and 24 h after drug administration. This procedure was repeated for a total of 5 runs (i.e. 5 samples per time point per regimen), with the exception of the −2 h group from which 2 samples were taken. After centrifugation, the serum from each animal within the individual treatment groups was pooled and stored at −80 °C pending analysis of cytokine concentrations.

4.5. Cytokine concentration assays

Concentrations of the cytokines Il-1β, IL-2, IL-6, IL-10, and TNF-α in pooled mouse sera were determined in duplicate using commercially available enzyme-linked immunosorbent assay test kits (Biotrak™ Cellular Communication Assay, Amersham Pharmacia Biotech Ltd, Piscataway, New Jersey, USA). The lower and upper limits of detection (assay range) for IL-1β, IL-2, IL-6, IL-10, and TNF-α were 1.14–1000, 21–850, 1.82–2000, 0.17–3000, and 4.44–2450 pg/ml, respectively. Assay variation within runs was ≤5.0%, and was ≤8.0% between runs. Individual cytokine values falling outside ±2 standard deviations of the mean were considered outliers and were excluded from the final data analysis (on this basis, 12, 6, 9, 7, and 11 were excluded from the IL-1β, IL-2, IL-6, IL-10, and TNF-α evaluations, respectively). All analytical assessments were undertaken using a blinded design (i.e. analytical staff were not informed as to whether samples were obtained from telithromycin or control animals).

4.6. Statistical analysis

The mean cytokine concentrations at each time point were tested for statistical differences between control and treatment groups by analysis of variance using Scheffé's test. A P value of ≤0.05 was considered statistically significant.

Acknowledgement: This work was supported by Aventis, Bridgewater, NJ, USA.

This work was presented at the 13th European Congress of Clinical Microbiology and Infectious Diseases, Glasgow, UK, May 2003 (Abstract No. P1620).

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