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Anti-inflammatory activity of azithromycin attenuates the effects of lipopolysaccharide administration in mice

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Abstract

Macrolide antibacterials inhibit the production of various cytokines and the migration of inflammatory cells. These anti-inflammatory actions of macrolides may be beneficial in attenuating inflammatory processes involved in bacterial sepsis. Therefore, we investigated the ability of azithromycin to attenuate the deleterious effects of lipopolysaccharide (LPS), in three different LPS-induced inflammatory models. Our results show that azithromycin (10 and 100 mg/kg) significantly attenuated the intraperitoneal LPS-induced increase in plasma TNF-α concentration. It also increased survival rate in a septic shock model in mice challenged with intravenous LPS. Oral treatment with azithromycin (up to 300 mg/kg) was less effective in suppressing neutrophil infiltration into the lungs 24 h after intranasal LPS challenge, possibly because of a slower onset of action or inadequate dosing. In the same model, azithromycin given intraperitoneally significantly improved inflammatory markers (total cell number, neutrophil percentage and MIP-2 concentration) in bronchoalveolar lavage fluid. In conclusion, azithromycin exhibits significant anti-inflammatory properties but the potency of such effects varies depending on the experimental model and route of administration.

Keywords: Azithromycin; Lipopolysaccharide; Pulmonary neutrophilia; Septic shock; TNF-α

1. Introduction

Macrolides, a family of antibiotics isolated from streptomycetes, are widely used for the treatment of moderate to severe bacterial infections (Zhanel et al., 2001). A growing number of reports indicate that, in addition to anti-microbial properties, macrolides possess a broad spectrum of anti-inflammatory and immunomodulatory effects (reviewed in Culic et al., 2001; Labro, 2004; Tsai and Standiford, 2004). Macrolides accumulate in inflammatory cells, especially neutrophils and macrophages (Gladue et al., 1989; Wildfeuer et al., 1989, 1996), inhibiting the synthesis of reactive oxygen species and/or secretion of pro-inflammatory cytokines (Culic et al., 2002; Ianaro et al., 2000; Khan et al., 1999; Suzaki et al., 1999; Tamaoki et al., 1999; Terao et al., 2003). Additionally, macrolides are reported to inhibit adhesion molecule expression on epithelial cells, suppressing leukocyte adhesion and infiltration into tissues (Anderson et al., 1996; Culic et al., 2002; Kadota et al., 1993; Sanz et al., 2004; Tamaoki et al., 1995). The most probable mechanism by which macrolides express their anti-inflammatory properties is inhibition of two of the most important transcription factors for the inflammatory response, nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1) (Kikuchi et al., 2002; Okamoto et al., 1994).

As a consequence, anti-inflammatory macrolides are being used in the treatment of various chronic inflammatory diseases, including diffuse panbronchiolitis, cystic fibrosis and chronic sinusitis. In order to achieve a therapeutic effect, they are usually administered for long time periods, several months or even years (Azuma and Kudoh, 2005; Baumann et al., 2004; Pirzada et al., 2003). However, it was reported recently that short-term treatment with azithromycin modulates neutrophil

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and inflammation markers in chronic obstructive pulmonary disease (Parnham et al., 2005). Therefore, it is possible that even short-term treatment with macrolides could efficiently ameliorate acute inflammatory diseases such as sepsis.

The pathogenesis of sepsis involves a progressive and dynamic expansion of the systemic inflammatory response to bacterial infection (Glauser, 2000). A major factor contributing to sepsis is the shedding of lipopolysaccharide (LPS) from the cell wall of Gram-negative bacteria into the circulation. This pro-inflammatory molecule interacts with a variety of cell types and induces hyperproduction of various cytokines. One of the central cytokines involved in sepsis is tumour necrosis factor alpha (TNF-α), systemic release of which induces increased vascular permeability and disseminated intravascular coagulation, which often leads to a state of shock (Annane et al., 2005). Furthermore, sepsis is associated with the activation and migration of leukocytes into various organs (Annane et al., 2005). The most common event is infiltration of activated neutrophils into lung tissue, inducing acute lung injury (Welbourn and Young, 1992).

As the time course of sepsis is extremely short, especially once the systemic inflammatory response has been initiated, we were interested in whether single oral azithromycin administration to mice could attenuate the deleterious effects of LPS from Gram-negative bacteria. We studied the ability of azithromycin to ameliorate the effects of LPS in three different murine experimental models, mimicking various stages of the pathological cascade in sepsis: LPS-induced plasma TNF-α production, septic shock and pulmonary neutrophilia.

2. Materials and methods

2.1. Animals

All studies were performed on male BALB/cJ mice weighing 25–30 g obtained from IFFA CREDO Laboratories, Lyon, France. Mice were kept on wire mesh floors with irradiated maize granulate bedding (Scobis Due, Mucedola, Italy) and maintained under standard laboratory conditions (temperature 22±2 °C, relative humidity 55±10%, approx. 20 air changes per hour, filtered on HEPA 99.97%, artificial lighting with circadian cycle of 12 h). Food (Mucedola, Italy) and tap water were provided ad libitum. Mice were allowed to acclimatize for 10 days before the beginning of each experiment.

All procedures on animals were performed in accordance with (a) the EEC Council Directive 86/609 of 24th November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes; and (b) Statute of Republic Croatia, Animal welfare law, NN 081-99-266/1 of 9th February 1999.

2.2. Materials: chemicals, antibodies and drugs

LPS from *Escherichia coli* serotype 0111:B4 and *Serratia marcescens* were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Mouse TNF-α antibodies were purchased from R&D Systems (McKinley Place, MN, USA). Azithromycin used in all experiments was obtained from Rockville (USP, MD, USA). Diff-Quick staining set was purchased from Dade Behring Inc. (Newark, DE, USA). All antibodies for enzyme-linked immunosorbent assay (ELISA) were purchased from R&D Systems (MN, USA). All other reagents, if not indicated otherwise, were from Sigma Chemical Co. (St Louis, MO, USA).

2.3. Preparation of azithromycin for in vivo administration

In all experiments, azithromycin was first dissolved in dimethylsulfoxide (DMSO) and then diluted with 0.5% methylcellulose (final concentration of DMSO up to 5%). In each experimental model, the azithromycin and vehicle were administered orally by gavage (10 ml/kg), at the doses indicated in the experimental procedures. In an additional comparative experiment on lung neutrophilia, azithromycin was also administered intraperitoneally in the same vehicle.

2.4. In vivo experimental procedures

2.4.1. LPS-induced plasma TNF-α production

LPS induction of plasma TNF-α production was performed according to Badger et al. (1996). In order to determine the time course of TNF-α production, a preliminary experiment was performed. Animals were divided into a single group of six animals (healthy control) and four groups of 10 animals that were challenged intraperitoneally with 25 μg of LPS from *E. coli*/0.2 ml sterile saline. Animals were anaesthetised with Thiopental (25 mg/ml saline; Nycomed, Germany), injected intraperitoneally and exsanguinated by puncturing the *v. jugularis* and *a. carotis communis* 60 min, 90 min, 120 min and 180 min after the LPS injection. Blood was collected in heparinized tubes (BD, Franklin Lakes, NJ, USA) and centrifuged (2000×*g*) for 15 min at room temperature. The resulting plasma was transferred into new tubes and frozen at −20 °C for subsequent determination of TNF-α concentration by ELISA.

In a subsequent experiment, in which the dose-dependency of the effect of azithromycin on plasma TNF-α was assessed, animals were divided into four groups of eight animals (healthy control and azithromycin-treated groups) and one group of 10 animals (vehicle-treated group). To induce TNF-α production, 25 μg of LPS from *E. coli*/0.2 ml sterile saline were administered intraperitoneally to each animal, except those in the healthy control group. Azithromycin (1, 10 and 100 mg/kg) and vehicle were administered orally 30 min before injection of LPS. The 30-min pretreatment regimen was used to allow for an approximate coincidence of peak tissue concentrations of the drug and peak plasma cytokine concentrations. Ninety minutes after the LPS injection, animals were anaesthetised and exsanguinated as previously described. Blood was collected in heparinized tubes and centrifuged (room temperature, 2000×*g*, 15 min). Plasma was transferred into new tubes and frozen at −20 °C for TNF-α concentration analysis by ELISA.

2.4.2. LPS-induced septic shock

Mice were divided into 3 groups of 10 animals (azithromycin-treated groups) and 1 group of 15 animals (vehicle-treated group). All animals received an intraplantar injection of 4 μg LPS from *S. marcenes*/0.05 ml sterile saline. Approximately 19 h later, a second dose of LPS from *S. marcenes* (90 μg of LPS/0.2 ml sterile saline per animal) was administered intravenously. Azithromycin (1, 10 and 100 mg/kg) and vehicle were administered orally, twice, 30 min before each LPS application. Survival of animals was recorded 0.5 h, 5 h and 24 h after the second intravenous LPS application.

2.4.3. LPS-induced pulmonary neutrophilia

Experimental pulmonary neutrophilia, a model that mimics the acute respiratory distress syndrome (ARDS), was induced according to Szarka et al. (1997), with minor modifications. All mice were anaesthetised with a mixture of ketamine hydrochloride (2 mg/mouse; Narketan Vetoquinol, Bern, Switzerland) and xylazine hydrochloride (0.07 mg/mouse; Rompun, Bayer, Leverkusen, Germany) injected intraperitoneally. While still anaesthetized, healthy control mice received 60 μl of PBS intranasally and all other animals received 2 μg LPS from *E. coli*/60 μl PBS intranasally, to induce pulmonary neutrophilia. Vehicle and azithromycin at doses of 1, 10, 100 and 300 mg/kg were administered orally 30 min before intranasal challenge with LPS. In one experiment, azithromycin (150 mg/kg) and vehicle were administered intraperitoneally 2 h before LPS for comparison with the effect of azithromycin (300 mg/kg) given orally. This treatment regiment was chosen according to Kadota et al. (1993). Approximately 24 h after LPS administration, mice were killed by an intraperitoneal overdose of Thiopental. Tracheostomy was performed and a tracheal catheter was clamped into the trachea. The lungs were washed three times with PBS in a total volume of 1 ml (0.4, 0.3 and 0.3 ml). After bronchoalveolar lavage, lungs were excised and fixed in 10% buffered neutral formalin fixative.

The bronchoalveolar lavage samples were centrifuged for 5 min (100×*g*) at 4 °C to collect whole cells in the pellet. The supernatant was carefully removed and stored at −20 °C for analysis of the cytokines, TNF-α and interleukin-6 (IL-6) and the chemokine, macrophage inflammatory protein 2 (MIP-2), the mouse homologue of human interleukin-8 (IL-8). The pellet of cells remaining after centrifugation of the bronchoalveolar lavage samples was resuspended in an equal volume of fresh PBS and used for total and differential cell counts. The total number of cells in bronchoalveolar lavage fluid was counted in a hemacytometer (Sysmex SF 3000). The percentage of neutrophils was determined by morphological examination of at least 200 cells in cytocentrifuged preparations (Cytospin-3, Shandon Instruments, UK), stained with the Diff-Quik staining set.

2.5. Measurement of cytokines by ELISA

TNF-α concentration in plasma was determined by sandwich ELISA, using capture and detection antibodies according to the manufacturer's recommendations. Sensitivity of the assay was 2.8 pg/ml for TNF-α. Prior to analysis, plasma samples were diluted four-fold.

Concentrations of TNF-α, IL-6 and MIP-2 in bronchoalveolar lavage fluid were determined by sandwich ELISA, using capture and detection antibodies according to the manufacturer's recommendations. Sensitivity of the assay was 2.8 pg/ml for TNF-α, 2 pg/ml for IL-6 and 1.5 pg/ml for MIP-2. Bronchoalveolar lavage fluid samples were used undiluted for cytokine concentration analysis.

2.6. Histopathological examination of lungs

Lungs were formalin-fixed, embedded in toto, cut into 3-μm sections and stained routinely with hematoxylin and eosin. In four sections (containing main stem bronchi) from each lung specimen, granulocyte infiltration into peribronchial areas was investigated observer-blind and graded according to the following criteria:

0	No granulocytes
1	Few scattered granulocytes
2	Larger aggregates of granulocytes
3	Marked accumulation of granulocytes

In borderline cases, an intermediate grade was used (0–1, 1–2, 2–3), extending the scoring to a total of seven grades.

2.7. Data analysis and statistical evaluation

Differences in plasma TNF-α concentrations between vehicle and azithromycin-treated groups were analysed statistically using the unpaired *t*-test. Differences in cytokine concentrations, number or percentage of cells in bronchoalveolar lavage fluid between groups were examined statistically using one-way analysis of variance (ANOVA) or the Kruskal–Wallis test, followed by the Tukey–Kramer or Dunn's multiple comparison test, respectively. For histopathological scoring of neutrophil infiltration into lung tissue, the χ^2-test for trend was used for comparison of single pairs of groups. For survival of mice in LPS-induced septic shock, the two-sided Fisher exact test was used. All statistical analyses were done using the program GraphPad InStat v. 3.06 (GraphPad Software Inc., San Diego, CA, USA). The level of significance was set at $P<0.05$ in all cases.

3. Results

3.1. Effect of azithromycin on LPS-induced plasma TNF-α production

We tested the ability of azithromycin to suppress the LPS-induced increase in plasma TNF-α, an important proximal mediator of sepsis syndromes. Prior to administration of LPS to healthy mice, TNF-α was not detectable in plasma. As expected, LPS administration markedly increased plasma concentrations of TNF-α (Figs. 1 and 2). As determined in a

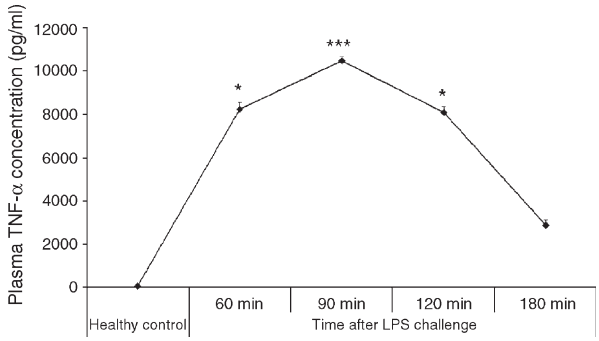

Fig. 1. Time-course of tumour necrosis factor alpha (TNF-α) concentrations in mouse plasma after intraperitoneal LPS challenge (25 μg/animal). Data are presented as group means±S.E.M. Signs indicate significant difference from LPS-challenged mice (*$P<0.05$, ***$P<0.001$).

preliminary experiment, the peak plasma TNF-α was recorded 90 min after intraperitoneal LPS administration (Fig. 1). Therefore, we limited investigation of the effect of azithromycin on plasma TNF-α only to this time point. In comparison to the vehicle-treated group, azithromycin (10 and 100 mg/kg) significantly decreased the concentration of TNF-α in mouse plasma (Fig. 2).

3.2. Effect of azithromycin on LPS-induced septic shock

As azithromycin markedly and dose-dependently inhibited LPS-induced production of plasma TNF-α, we tested whether azithromycin treatment could increase survival of animals in a mouse model of septic shock. Mice were first sensitised to LPS by intraplantar administration, in order to make them highly susceptible to the lethal effects of LPS. A second, intravenous injection of LPS rapidly increased mortality in vehicle-treated mice and none of them (0/15) survived for 24 h (Fig. 3). In contrast to the vehicle-treated group, survival rates in the azithromycin-treated groups (10 and 100 mg/kg) were significantly higher, 60% (6/10) and 70% (7/10) of animals surviving to the end of the experiment, respectively (Fig. 3).

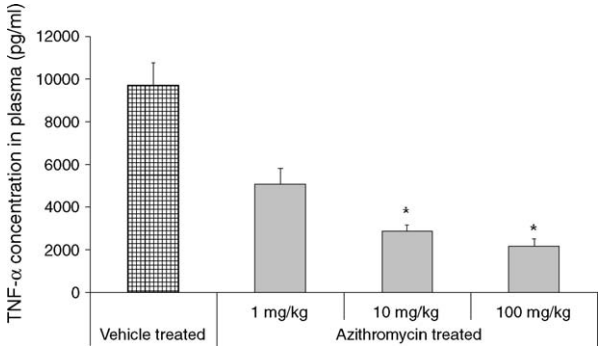

Fig. 2. Effect of oral azithromycin treatment on tumour necrosis factor alpha (TNF-α) concentrations in mouse plasma after intraperitoneal LPS challenge (25 μg/animal). Data are presented as group means±S.E.M. (*) indicates significant difference from vehicle-treated and LPS-challenged mice ($P<0.05$).

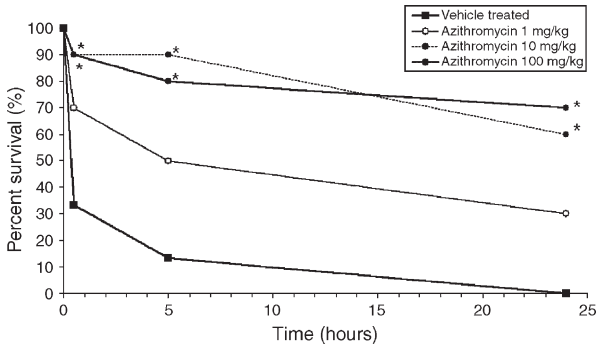

Fig. 3. Oral azithromycin treatment improves survival of mice in a model of LPS-induced septic shock. Survival (=10 mice per group) was monitored for 24 h after intravenous injection of LPS (90 μg/animal). (*) indicates significant difference from vehicle-treated group ($P<0.05$).

3.3. Effect of azithromycin on LPS-induced pulmonary neutrophilia

Progression of sepsis is associated with dysfunction of various organs, especially lungs, due to leukocyte infiltration and edema. In order to mimic the acute respiratory distress syndrome, LPS was administered intranasally into the lungs. LPS in vehicle-treated mice significantly increased total cell number as well as neutrophil percentage in bronchoalveolar lavage fluid in comparison to healthy controls (Fig. 4A,B). Furthermore, the concentrations of pro-inflammatory cytokines, TNF-α, IL-6 and MIP-2, were also significantly increased (Fig.

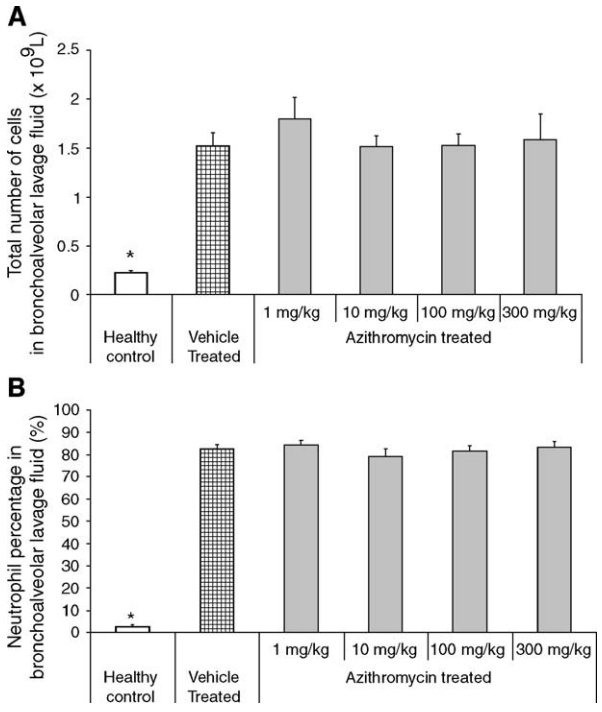

Fig. 4. Effect of oral azithromycin treatment on (A) total cell number and (B) percentage of neutrophils in bronchoalveolar lavage fluid after intranasal LPS challenge (2 μg/animal). Data are presented as group means±S.E.M. (*) indicates significant difference from vehicle-treated and LPS-challenged mice ($P<0.05$).

5A–C). Histopathological analysis showed that intranasal challenge with LPS induced significant granulocyte infiltration into lung tissue (Figs. 6 and 7).

Treatment with azithromycin at doses up to 300 mg/kg significantly changed neither total cell number nor neutrophil percentage in bronchoalveolar lavage fluid (Fig. 4A,B), nor the infiltration of granulocytes into lung tissue, in comparison to the vehicle-treated and LPS-challenged group (Figs. 6 and 7). There was, however, a tendency for neutrophil infiltration to decrease at the highest dose (Fig. 6). In mice treated with this high dose (300 mg/kg), the concentrations of TNF-α, IL-6 and MIP-2 in bronchoalveolar lavage fluid also were not significantly changed in comparison to the vehicle-treated and LPS-challenged group, MIP-2 tending to be increased (Fig. 5A–C).

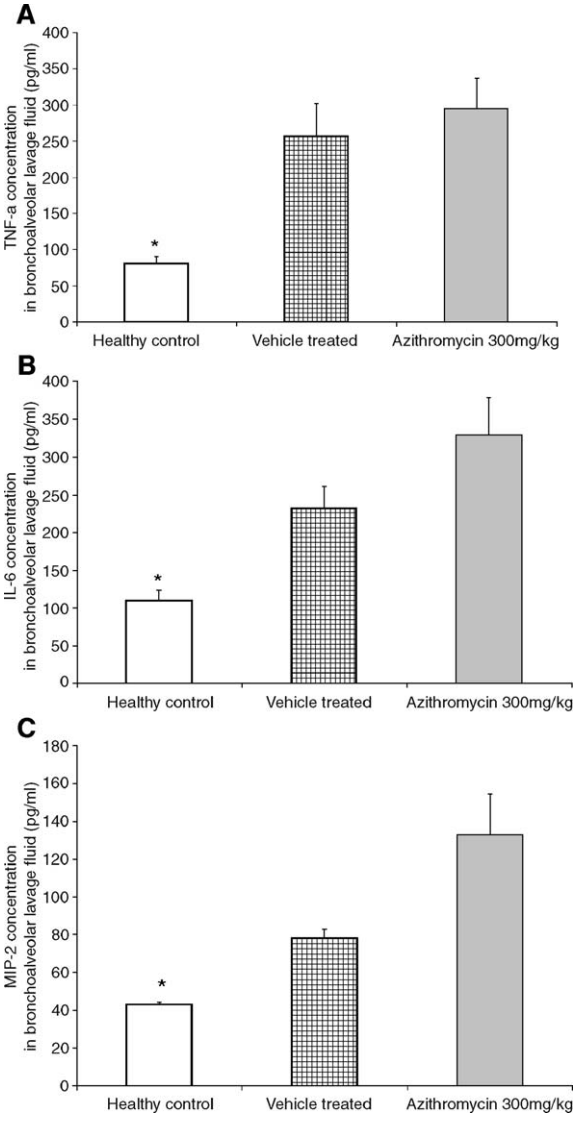

Fig. 5. Effect of oral azithromycin treatment on (A) tumor necrosis factor alpha (TNF-α), (B) interleukin-6 (IL-6) and (C) macrophage inflammatory protein 2 (MIP-2) concentrations in bronchoalveolar lavage fluid after intranasal LPS challenge (2 μg/animal). Data are presented as group means ± S.E.M. (*) indicates significant difference from vehicle-treated and LPS-challenged mice ($P < 0.05$).

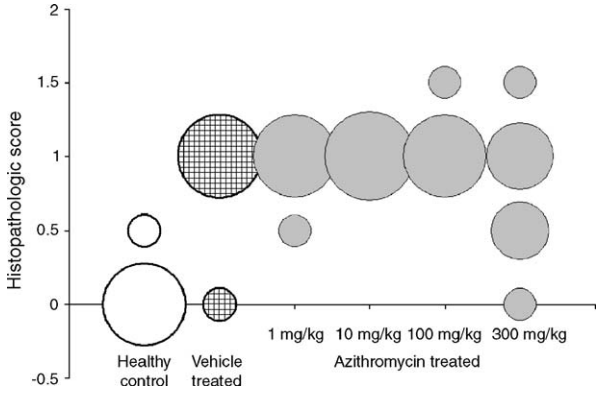

Fig. 6. Effect of oral azithromycin treatment on granulocyte infiltration into peribronchial lung tissue areas after intranasal challenge with LPS. Sample distribution is shown according to histological scores of granulocyte infiltration into peribronchial lung tissue. The area of each circle corresponds to the number of samples with the same histological score. All mice, except those in the healthy control group, were treated as indicated and after 30 min challenged intranasally with LPS (2 μg/mouse).

To test whether the lack of effect of oral azithromycin (300 mg/kg) on lung neutrophilia at 24 h was due to lack of efficacy or to potential pharmacokinetic reasons, in one experiment, a single 150 mg/kg dose given i.p. 2 h before LPS was also included. As shown in Table 1, azithromycin given i.p. at 150 mg/kg tended to inhibit cytokines-achieving statistical significance for IL-6- and also significantly inhibited neutrophil infiltration. (As I have mentioned before, I do not believe that this 200 mg p.o. and 2 h experiments tells anything; therefore, I would not mention it at all. Namely, only at least 450–500 mg/kg p.o. where azi does not work would support this line of thinking.)

4. Discussion

Earlier investigations in an animal model of sepsis indicated the usefulness of combined drug therapy directed against the bacteria, the circulating endotoxin and the induced cytokines (Cross et al., 1993a). As macrolide antibiotics have a large volume of distribution and achieve relatively low concentrations in sera (Nilsen, 1987), they are not considered to be primary antibacterials for the treatment of infections that are accompanied by a severe sepsis syndrome. Therefore, very few reports have considered the activity of macrolides in animal models of severe sepsis and septic shock. Recently, Giamarellos-Bourboulis et al. (2004) investigated the effect of clarithromycin in experimental sepsis induced by multidrug-resistant *Pseudomonas aeruginosa*. The investigation showed that increased survival of experimental animals was achieved after co-administration of clarithromycin and amikacin, and was probably attributable to the immunomodulatory properties of clarithromycin. However, the study also detected a significant decrease in the number of bacterial colonies in several organs after clarithromycin administration, indicating a possible dual, antibacterial and anti-inflammatory effect of clarithromycin (Giamarellos-Bourboulis et al., 2004).

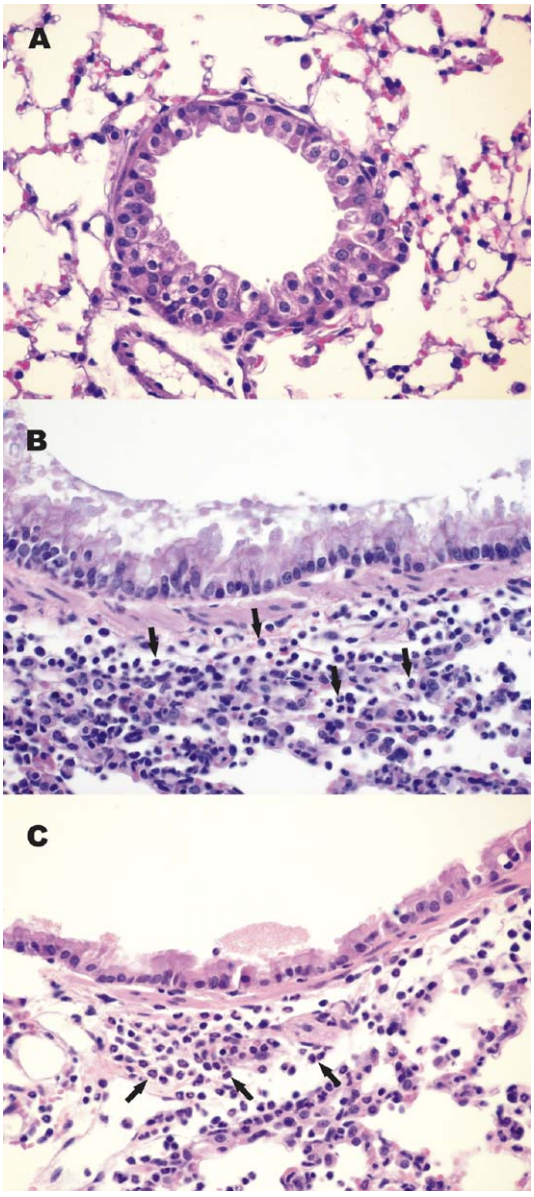

Fig. 7. Representative photomicrographs of lung tissue (hematoxylin and eosin, ×400) from mice after intranasal treatment with LPS. Arrows indicate granulocyte infiltration. The PBS-challenged group showed normal lung histology (A). In the LPS-challenged (2 μg/animal intranasally), vehicle-treated group, infiltration of granulocytes into peribronchial lung tissue areas was observed (B). Oral azithromycin treatment (300 mg/kg) did not significantly decrease infiltration of neutrophils into lung tissue in comparison to the LPS-challenged group (C).

Earlier investigations showed that macrolide antibiotics accumulate in inflammatory cells, especially neutrophils and macrophages (Gladue et al., 1989; Wildfeuer et al., 1989, 1996), inhibiting their migration, generation of reactive oxygen species and/or secretion of pro-inflammatory cytokines (Culic et al., 2002; Ianaro et al., 2000; Khan et al., 1999; Suzaki et al., 1999; Tamaoki et al., 1999; Terao et al., 2003). Macrolides have also been reported to inhibit leukocyte adhesion and infiltration into tissues (Anderson et al., 1996; Culic et al., 2002; Kadota et al., 1993; Sanz et al., 2004; Tamaoki et al., 1995). Therefore, we hypothesized that macrolides, due to their anti-inflammatory or immunomodulatory properties, could be considered as possible agents for the therapeutic control of the inflammatory component of the sepsis syndrome. To test this hypothesis and in order to avoid confounding effects due to concomitant antibacterial activity, we investigated the ability of azithromycin to attenuate the effects of LPS administration to mice. This approach was chosen because putative therapeutic modulators of the sepsis syndrome are frequently evaluated first in models involving LPS administration (Beutler et al., 1985; Cross et al., 1993b; Hinshaw et al., 1977).

In our study, single oral azithromycin pre-treatment significantly increased the survival of experimental animals in the model of LPS-induced septic shock. It is well known that, in blood, LPS binds to a lipopolysaccharide binding protein (LBP) (Shapiro and Gelfand, 1993). The LPS-LBP complex is a ligand for the CD14 receptor at the surface of monocytes and macrophages. The interaction of LPS-LBP with its receptor triggers the monocytic secretion of several pro-inflammatory cytokines, among which the early synthetized TNF-α represents the archetype. TNF-α orchestrates the major alterations observed during septic shock, such as vasodilatation, impaired coagulation and fibrinolysis and elevation of white blood cells. Several earlier studies showed that azithromycin decreases TNF-α production, both in vitro (Khan et al., 1999) and when used in vivo in models such as rat carrageenin pleurisy and a mouse model of mucoid *P. aeruginosa* endobronchial infection (Ianaro et al., 2000; Tsai et al., 2004). Since our results also clearly indicate that single oral administration of azithromycin inhibited intraperitoneal LPS-induced plasma TNF-α production in a dose-dependent manner, increased survival rate observed with the macrolide under the LPS-sensitization schedule could be attributed to inhibition by azithromycin of LPS-induced TNF-α production. Moreover, the inhibition of the lethal effects of LPS could explain the common clinical finding that treatment of community-acquired respiratory tract infections with azithromycin and other macrolides is associated with decreased mortality (Amsden, 2005).

Table 1
Effect of azithromycin given intraperitoneally (150 mg/kg) 2 h before intranasal LPS on cytokines and neutrophil counts in bronchoalveolar lavage fluid after 24 h

	Healthy control	LPS-challenged	
		Vehicle-treated	Azithromycin
Total number of cells ($\times 10^9$/l)	0.198±0.017*	1.397±0.016	0.277±0.019 [93]*
Neutrophil percentage (%)	3.0±1.6*	83.0±1.7	23.9±3.1 [71]*
Cytokine concentration (pg/ml)			
TNF-α	81.3±9.6*	256.4±45.1	126.7±21.4 [74]
IL-6	109.8±12.6*	231.4±29.2	110.4±10.9 [99]*
MIP-2	43.0±1.0*	78.2±4.7	54.8±1.9 [66]

Data are means±S.E.M. (n=9 in each group). Numbers in brackets represent percent inhibition in comparison to the appropriate positive control (%).
* Indicates significant differences (p<0.05) versus appropriate vehicle-treated group.

In addition to shock, systemic overproduction and release of TNF-α in sepsis often induces the organ specific production and secretion of many other pro-inflammatory cytokines (Shapiro and Gelfand, 1993). Among these cytokines, IL-8 is responsible for the recruitment and activation of polymorphonuclear cells in various organs. Activated polymorphonuclear cells induce tissue damage due to excessive production of oxygen free radicals and degradative enzymes, leading to the organ dysfunction. Irrespective of the aetiology of the sepsis, the lungs were found to be the most endangered organ (Welbourn and Young, 1992). Earlier investigations showed that intranasal administration of LPS into the lungs mimics the pulmonary neutrophilia that is characteristic for the adult respiratory distress syndrome (Szarka et al., 1997).

Various macrolides, including azithromycin, were shown to effectively suppress pulmonary neutrophilia in animals when applied either intraperitoneally or orally in a "low-dose, long-term" treatment regimen (Pinto et al., 2004; Sanz et al., 2004; Suzaki et al., 1999; Tamaoki et al., 1995; Terao et al., 2003). However, this treatment regiment does not seem to be practical for clinical use in sepsis. Therefore, we investigated the efficacy of single oral azithromycin treatment in the mouse model of LPS-induced pulmonary neutrophilia. Our results showed that, at oral doses up to 300 mg/kg, 30 min prior to the intranasal LPS challenge, azithromycin did not impair the accumulation of TNF-α, IL-6 and MIP-2 nor the neutrophil recruitment into the lungs 24 h later. In agreement with our findings, a previous study showed that 4 days daily pre-treatment with oral azithromycin (500 mg loading dose, then three doses of 250 mg) did not inhibit ozone-induced airway neutrophilia and cytokine release in humans (Criqui et al., 2000). A similar 3-day oral pretreatment (500 mg/day) with azithromycin was found to enhance peripheral blood neutrophil activity in healthy volunteers over the first 24 h after treatment, causing inhibition of neutrophil function in the succeeding period (Culic et al., 2002). It seems likely that, by evaluating lung inflammation at 24 h, we may have been too early to detect a later inhibitory effect of oral azithromycin. In contrast, Kadota et al. (1993) showed that single intraperitoneal erythromycin administration at a dose of 5 mg/animal 2 h before LPS, significantly inhibited intrapulmonary influx of neutrophils in a mouse model of LPS-induced lung neutrophilia. Using the same dose of azithromycin (≅150 mg/kg) as Kadota et al. (1993) had used of erythromycin and a pretreatment period of 2 h as applied in the earlier study, we also found that azithromycin given intraperitoneally was an inhibitor of cytokines and lung neutrophilia 24 h after LPS administration. Consequently, differences in the pharmacokinetics of azithromycin, depending on the route and time of administration, may account for these contrasting results. Additional investigations are needed to clarify this issue.

In conclusion, the present study has revealed that azithromycin and possibly other macrolides with similar anti-inflammatory properties could be considered as potential adjuvant anti-inflammatory therapeutic agents in the treatment of sepsis by inhibiting the initial TNF-α response, and thereby preventing septic shock. However, the findings also emphasise the need for further research on the potential for azithromycin as an inhibitor of the endotoxin-induced organ dysfunction by inhibiting the recruitment and stimulation of neutrophils.

Acknowledgments

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