Immunomodulatory Properties of Antibiotics

S.C. Tauber and R. Nau*

Department of Neurology, Georg-August-University, Göttingen, Germany

Abstract: There is growing evidence that certain antibiotics exert their beneficial effects not only by killing or inhibiting the growth of bacterial pathogens but also indirectly by immunomodulation. This review aims at giving an overview of the immunomodulatory properties of antibiotics in different diseases: The antiinflammatory properties of macrolides in chronic inflammatory pulmonary disorders were recognized more than 15 years ago and have been well documented in the last decade. Recent data suggest that several antibiotics such as tetracyclines and cephalosporins may have a beneficial immunomodulatory or neuroprotective effect on neuroimmunological and neurodegenerative diseases including multiple sclerosis and amyotrophic lateral sclerosis. Moreover, the non-bacteriolytic but bactericidal antibiotics rifampicin, clindamycin and aminoglycosides kill bacteria without releasing high quantities of proinflammtory cell wall components. The use of bactericidal, non-bacteriolytic protein synthesis inhibitors reduces mortality and long-term sequelae in experimental bacterial sepsis, plague and meningitis. Clinically, macrolides have been well established as an adjunctive treatment to β-lactam antibiotics in pulmonary diseases. For other indications, appropriate clinical trials are necessary before using the immunomodulatory properties of antibiotics in clinical practice.

Keywords: Immunomodulation, bacterial infection, inflammation, macrolide, fluoroquinolone, tetracycline, β-lactam antibiotic, central nervous system, neurodegenerative disease.

INTRODUCTION

For more than 15 years, it has been noted that certain antibiotics – in addition to their antiinfectious qualities – have immunomodulatory properties that improve the long-term outcome of patients with chronic inflammatory pulmonary diseases. Although the best investigated family of antibiotics during the last decades is the group of macrolides, there also is a growing body of data about the immunomodulatory actions of other antibiotics. Here, recently published studies on the immunomodulatory properties of antibiotics and their impact on pulmonary and neurological diseases are reviewed.

MACROLIDES

Macrolides belong to the family of 14- or 15-membered lactone ring antibiotics. These antibiotics achieve high intracellular concentrations and have good activity against Gram-positive bacteria such as *S. pneumoniae* and *S. pyogenes*. Macrolides distinctly influence the release of cytokines such as interleukin-8 (IL-8) and tumor necrosis factor α (TNFα) as well as mediators of inflammation such as nitric oxide (NO). They are able to inhibit leukocyte chemotaxis by suppressing synthesis of endogenous chemotactic factors [1, 2]. Furthermore, the inhibition of bacteria-epithelial cell interaction and modulation of signaling pathways involved in the inflammatory response and direct effects on neutrophils have been observed [3]. The molecular mechanisms responsible for the effects of macrolides on neutrophil cell function in pulmonary diseases are not completely understood; however it has been postulated that inhibition of protein kinase A leads to reduced oxidant production [4] or that neutrophil

activity is altered by changes in the phospholipase D-phosphatidate phosphohydrolase transduction pathway, which is responsible for cell degranulation [5].

Erythromycin can attenuate the detrimental effects of cerebral ischemia when applied before the hypoxic event. Pretreatment with the macrolide (25 mg/kg i.m.) improved postischemic survival of hippocampal CA1 and CA3 neurons, reduced functional deficits, and upregulated anti-apoptotic bcl-2 mRNA in the hippocampus [6]. Furthermore, the gene expression profiles of preconditioned (25 mg/kg i.m. erythromycin) and non-preconditioned rats were assessed by complementary DNA expression array and RT-PCR. Erythromycin-treated animals exposed to 15 minutes of cerebral ischemia revealed a distinct suppression of mRNA expression of pro-inflammatory genes. In contrast to the previous study, there was little effect on the expression pattern of genes involved in apoptosis [7]. These results confirm the immunomodulatory properties of macrolides and also give insight into changes in gene expression. Taken together, these studies suggest that these antibiotics apparently influence both gene expression and protein synthesis and thereby exert neuroprotective properties.

Telithromycin belongs to the group of ketolide antibiotics and is a derivative of the 14-membered ring macrolides. This drug also appears to exert immunomodulatory effects. In lipopolysaccharide (LPS)-induced systemic inflammation in mice, a single dose of 150 mg/kg telithromycin decreased the LPS-induced mRNA expression and protein synthesis of pro-inflammatory cytokines such as TNFα, IL-1β and interferon γ (IFNγ) [8].

TETRACYCLINES

Tetracyclines display several properties in addition to their antimicrobial activity. Doxycycline, for example, is able to inhibit collagenase activity: a 50% reduction of human neutrophil collagenase activity can be achieved by 7 –

*Address correspondence to this author at the Dept. of Neurology, Georg-August-University Göttingen, Robert-Koch-Straße 40, D-37075 Göttingen, Germany; Tel: +49-551-39-8455; Fax: +49-551-39-8405;
E-mail: rnau@gwdg.de

15 mg/l doxycycline (a concentration temporarily achievable *in vivo* after intravenous infusion of 200 mg doxycycline) [9]. Tetracyclines have also been shown to inhibit matrix metalloproteinases (MMPs), as the activity of these enzymes is zinc-dependent and the antibiotic is able to chelate zinc from their active site [10]. Moreover, doxycycline has the ability to inhibit leukocyte function *in vitro* via divalent cation binding [11] and may reduce leukocyte adhesion.

The immunomodulatory and neuroprotective effects of tetracyclines – especially minocycline – in neurological diseases are considered to be due in part to the suppression of microglia activation. To further illuminate the molecular signal transduction pattern resulting in an inhibition of microglia activation *in vitro*, rat microglial and neuronal cells were pre-treated with 10 microM minocycline or doxycycline and then exposed to hypoxia. Both antibiotics suppressed microglia activation as assessed by Iba1 (ionized calcium-binding adaptor molecule 1) staining and activation of ED1, a membrane-bound lysosomal glycoprotein, both markers for microglial activation. This effect was accompanied by down-regulation of pro-inflammatory molecules such as NO, IL-1β and TNF-α. In contrast, tetracycline treatment did not increase the concentrations of neuroprotective proteins such as brain-derived neurotrophic factor (BDNF) or glial cell line-derived neurotrophic factor (GDNF). From these results the authors concluded that neuroprotection presumably is achieved by the regulation of microglial activity and not by changes in microglia proliferation or viability [12].

FLUOROQUINOLONES

Fluoroquinolones have also been studied for their modulatory activity on the immune response to bacterial infections. The best investigated agent seems to be moxifloxacin and was shown to have immunomodulatory actions both *in vitro* and *in vivo*.

To illuminate the molecular pathways involved in the immunomodulatory effects of moxifloxacin, the release of pro-inflammatory cytokines by LPS-activated human monocytes was measured. An inhibition of inflammatory mediators and of three major signal transduction pathways involved in inflammatory responses was observed: NF-kappaB, mitogen-activated protein kinase ERK and c-Jun N-terminal kinase (JNK) [13]. Analysis of clinically relevant doses of moxifloxacin (2.5-10 mg/L) in the A549 lung epithelial cell line stimulated with both IL-1β and IFNγ showed an inhibition of cytokine-induced NO secretion and of intracellular signaling pathways involving ERK1/2, p-JNK as well as NF-kappaB [14]. Similar results were recently obtained in an *in vitro* study with IB3 cells, a cystic fibrosis bronchial cell line, that were activated by either TNFα, IL-1 or LPS with or without 5-50 microg/ml moxifloxacin, ciprofloxacin or azithromycin. In agreement with former results, moxifloxacin as well as high concentrations of ciprofloxacin were shown to inhibit the major signaling pathways NF-kappaB, ERK and JNK, while no effect of azithromycin was observed [15].

In addition to investigations of the effects of fluoroquinolones in bacterial infections, infections with other pathogens such as opportunistic fungi were also performed.

Changes in cytokine expression pattern and inhibition of pro-inflammatory signal transduction pathways by fluoroquinolones seem to be quite similar in opportunistic infections of different origin and therefore relatively independent of the infectious pathogen. Activation of human peripheral blood monocytes and the human monocytic cell line THP-1 by *Aspergillus fumigatus* and subsequent treatment with 5-20 mg/L moxifloxacin led to an inhibition of the Aspergillus-induced increase in IL-8, IL-1β and TNF-α as well as mediators such as ERK1/2, p38 and p65-NFkappaB involved in pro-inflammatory signal transduction pathways [16]. Similar results were obtained *in vivo* in immunocompromised mice (by injection of cyclosphosphamide) infected intratracheally with *Candida albicans* and treated with 22.5 mg/kg/day moxifloxacin. Mice treated with the antibiotic displayed lower levels of IL-8, TNFα and INFγ in the lung. Furthermore, clinical signs of pneumonia, weight loss and mortality were significantly decreased by moxifloxacin despite the lack of antifungal activity. This led to the hypothesis that the immunomodulatory effects of the antibiotic are responsible for the beneficial outcome even in non-bacterial infections [17].

The molecular mechanisms causing the immunomodulatory effects of fluoroquinolones, however, are still under investigation. Very recently, activation of the p38 mitogen-activated protein kinase (MAPK) pathway was proposed as the main effect of levofloxacin. Mouse macrophage-like cells (RAW264.7) stimulated by LPS and treated with levofloxacin released more pre-synthesized IL-1β in part *via* the MAPK pathway. In contrast, the production of newly synthesized IL-1β was inhibited [18].

Another member of the fluoroquinolone family, alatrofloxacin, appears to have a short-term immunostimulatory effect. Within the first 60 minutes after phagocytosis of bacteria by human THP-1 cells, the agent induced a pro-inflammatory response involving the release of TNFα, IL-1, IL-6, NO, activation of cAMP and lysosomal hydrolytic enzymes. This effect was time-limited since the inflammatory response returned to normal values within 2 – 4 hours. The authors interpreted this observation as suggesting a mechanism inhibiting the spread of infection and thereby reducing tissue damage [19].

β-LACTAM ANTIBIOTICS

Relatively few data exist on the immunomodulatory effects of β-lactam antibiotics in comparison to macrolides, tetracyclines and fluoroquinolones. One of the major differences between β-lactam antibiotics and the other groups seems to be the lack of anti-inflammatory properties of β-lactams. The most thoroughly investigated agent is cefaclor: it was shown to promote phagocytosis by enhanced chemotaxis and potentiated bactericidal activity by inducing a shift towards a type 1 pro-inflammatory response [20]. There is contradictory *in vitro* data on the actions of cefaclor and cefpodoxime in human leukocytes: while the histamine release induced by *E. coli* and *S. aureus* from human basophils was increased by the cephalosporins, the synthesis of leukotrienes from neutrophils was decreased. Furthermore, phagocytosis of *E. coli* by granulocytes and bactericidal activity of these cells were both increased. In contrast, synthesis of IL-6

and of the pro-inflammatory TNFα was decreased [21]. After short (3 or 6 days) treatment with 10, 50 or 100 mg/kg cefaclor, *ex vivo* stimulation of rat spleen cells with the polyclonal mitogen PHA produced increased lymphoproliferative activity and increased levels of IFNγ, IL-2 and IL-10 as well as decreased levels of IL-4 und IL-6 in comparison to control rat spleen cells [22]. These changes in cytokine synthesis indicate immunostimulatory changes towards a type 1 pro-inflammatory response, possibly enhancing antibacterial activity.

RIFAMPICIN

Suppression of T-cell activity by rifampicin has long been noticed in both patients suffering from tuberculosis and healthy controls [23]. The activation of human glucocorticoid receptors by this antibiotic was postulated to be a possible mechanism of its immunosuppressive effect [24, 25]. However, in human neuroblastoma and alveolar cells as well as mouse hippocampal cells, no activation of glucocorticoid receptors by rifampicin was detected [26, 27].

MACROLIDES IN INFLAMMATORY PULMONARY DISORDERS

The favorable immunomodulatory actions of macrolides have been known for 15 years. In the meantime several clinical trials on the effects of therapy with these drugs in patients with chronic inflammatory pulmonary diseases – mainly diffuse panbronchiolitis (DPB) and cystic fibrosis (CF) – have been carried out and have confirmed the beneficial effects of long-term treatment. For the sake of completeness, a brief summary of previous reviews is provided [3, 28-31].

The reduction of morbidity and the dramatically reduced mortality of DPB patients following long-term treatment with macrolides has been well documented. DPB is a pulmonary disease of unknown etiology that is found nearly exclusively in Japan. Several clinical trials from 1987 until the beginning of the 21st century confirmed the attenuation of DPB by macrolide treatment. Most patients were treated with 400 – 600 mg/d erythromycin or 200 mg/d clarithromycin for several months to years and displayed improved pulmonary function, decreased mucus hypersecretion, reduced neutrophil numbers and lower concentration of cytokines in bronchoalveolar lavage (BAL) and serum [31].

CF shares many similarities with DPB. Antiinfectious treatment is absolutely needed in this disease since lung infections with *S. aureus* and *P. aeruginosa* are very common and worsen the prognosis. Antibiotic treatment with macrolides alleviates the course of disease even if the drugs lack direct bactericidal activity against these pathogens. Several clinical trials from 2002 and 2003 confirmed improved quality of life, better lung function and less antimicrobial colonization in children and adults with CF after long-term treatment with 250-500 mg/d or three times a week azithromycin [32, 33]. Improvement of CF by long-term treatment with macrolides is believed to be the result of the macrolide´s intrinsic immunomodulatory actions, increase of mucus clearance and reduction of biofilm formation (biofilms pro-

tect the mucoid *P. aeruginosa*) that reduce exacerbations und improve lung function. Fortunately, long-term treatment with macrolides turned out to be safe and caused only a few side effects, mostly nausea and headache. However, some studies did not reveal beneficial effects of macrolides in CF: treatment with 250-500 mg azithromycin daily for 6 months did not significantly change exercise tolerance, subjective well-being, sputum bacterial densities or inflammatory markers in a placebo-controlled trial with 41 children suffering from CF [34]. Similarly, 6 weeks of 500 mg clarithromycin daily did not improve forced expiratory volume in 1 s (FEV$_1$) or any inflammatory indices in BAL in a trial with 10 young adult patients with CF [35].

Immunomodulatory effects of macrolides were also observed in other pulmonary diseases. By the end of three months, 17 children with brochiectasis who had received 15 mg/kg clarithromycin displayed a significant decrease in total cell count, IL-8 synthesis and neutrophil ratio in BAL fluid. Additionally, sputum production was distinctly reduced by the end of the third month [36]. Recent analysis of antibiotic therapy in a large group of patients admitted to US hospitals because of community-acquired pneumonia revealed that the initial use of an antibiotic agent active against atypical organisms was independently associated with lower 30-day mortality and re-admission to hospital within 30 days of discharge. Moreover, the beneficial effects of agents active against atypical bacteria were associated only with macrolides but not with fluoroquinolones or tetracyclines [37]. Comparison of combination antibiotic therapy and monotherapy in severe bacteremic pneumococcal pneumonia revealed an association of lower 14-d mortality among critically ill patients with combination therapy, independent of the class of antibiotic [38]. Moreover, combination of a β-lactam antibiotic with a macrolide appeared to be advantageous in bacteremic pneumococcal pneumonia in comparison to treatment with a β-lactam only which was an independent predictor of in-hospital mortality [39]. Table **1** gives an overview of human studies investigating antibiotic regimes and their effect on outcome in different bacterial and autoimmunological disorders.

ANTIBIOTICS IN NEUROIMMUNOLOGICAL AND NEURODEGENERATIVE DISEASES

Multiple Sclerosis (MS)

A growing body of research postulates a neuroprotective role of minocycline in MS. In 2002, the first major studies of the beneficial immunomodulatory effects of minocycline in experimental autoimmune encephalomyelitis (EAE), an animal model of MS, were published. Minocycline (45 mg/kg for 20 or 42 days) was able to attenuate disease activity, delay disease onset, and reduce overall severity of EAE in rats (Fig. **1**). This was accompanied by reduced inflammation and demyelination in the spinal cord. These observations are believed to be the result of decreased microglial activation in the central nervous system (CNS) as well as diminished T-cell infiltration and MMP-2 expression in the spinal cord [40]. Similar results reporting beneficial effects of minocycline were observed in mild EAE: mice treated

Table 1. **Human Studies Investigating Immunomodulatory Properties of Different Antibiotics and their Effects on Disease Activity and Outcome**

Disease	Number of Patients/Type of Study	Therapy	Molecular Effects	Disease Activity/Outcome	Reference
Community-acquired pneumonia	2,209 between 1998 – 2001 (retrospective analysis)	Antibiotics active against atypical organisms (macrolides, fluoroquinolones, tetracyclines)		Use of macrolides associated with lower in-hospital mortality, 30-d mortality and readmission within 30 d of discharge	37 [Metersky *et al.,* 2007]
Severe bacteremic pneumococcal pneumonia	844 (prospective observational study)	Combination antibiotic therapy or monotherapy		Combination antibiotic therapy associated with lower 14-d mortality among critically ill patients, independent of the class of antibiotic	38 [Baddour *et al.,* 2004]
Bacteremic pneumococcal pneumonia	409 between 1991 – 2000 (retrospective analysis)	β-lactam antibiotic with or without macrolide		β-lactam antibiotic only was an independent predictor of in-hospital mortality	39 [Martinez *et al.,* 2003]
Multiple sclerosis	10 (open-label single center study)	Minocycline (100 mg twice daily for 6 months)	Increase of p40 subunit of IL-12 and sVCAM-1 and decrease in MMP-9 activity in serum	No relapses between 6 and 24 months; one patient with gadolinium-enhancing lesion in MRI	44 [Zabad *et al.,* 2007]
Huntington´s disease	11 (pilot study with an open-label design)	Minocycline (100 mg/d for 2 years)		Reduction of psychiatric symptoms and stabilization of motor and neuropsychological functions after 24 months of treatment	63 [Bonelli *et al.,* 2004]
Rheumatoid arthritis	535 (metaanalysis of 10 trials)	Tetracyclines (minocycline, doxycycline)	Reduction of eosinophil sedimentation rate when antibiotic administered for 3 or more months	Reduction in disease activity and in tender joint count when antibiotic administered for 3 or more months, no change on radiological progression of disease Minocycline had greater effect on reduction of disease activity than other tetracyclines	68 [Stone *et al.,* 2003]
Gram-negative urosepsis	30 (prospective observational study)	Imipenem or ceftazidime (500 mg imipenem or 1000 mg ceftazidime every 8 hours for 72 h)	4 h after onset of treatment, blood endotoxin levels had decreased in all imipenem patients, but in only half of the ceftazidime patients		82 [Prins *et al.,* 1995]
Children with *S. pyogenes*-caused deep tissue infections	56 children between 1983 – 1997 (retrospective analysis)	Protein synthesis inhibitors (mainly clindamycin) and β-lactam antibiotics (mainly nafcillin)		Favorable outcome with protein synthesis inhibitors in comparison to β-lactam antibiotics	83 [Zimbelman *et al.,* 1999]

IL-12 – interleukin-12; MMP-9 – matrix metalloproteinase-9; sVCAM-1 – soluble vascular adhesion molecule-1.

with 25 and 50 mg/kg minocycline prior to immunization exhibited a delayed onset and milder course of the disease. Inhibition of MMP activity as the probable beneficial mechanism was also proposed in this study because MMPs are known to promote transmigration of T-lymphocytes across a fibronectin matrix barrier [41]. Additionally, intraperitoneal long-term treatment with 45 mg/kg minocycline for 30 days also resulted in a distinct suppression of EAE activity in mice [42].

Beneficial effects of this tetracycline have just recently been confirmed in a rat model of optic neuritis. Administration of 50 mg/kg minocycline i.p. for 8 days starting from the day of immunization delayed disease onset and reduced

the severity of symptoms. Furthermore, it improved electrophysiological function of the optic system and activated anti-apoptotic pathways (phosphorylation of MAPK and Akt, a downstream component of phosphtidylinositol-3 kinase signaling; decrease of the pro-apoptotic protein Bax associated with simultaneous increase of the anti-apoptotic protein bcl-2). Finally, glutamate levels in the retina were decreased, suggesting a neuroprotective effect of minocycline [43].

Recent data from a small pilot study with 10 patients with relapsing-remitting MS who received 100 mg minocycline twice daily for 6 months also showed encouraging results. No relapses occurred during follow-up between 6 and 24 months and there was only one patient with a gado-
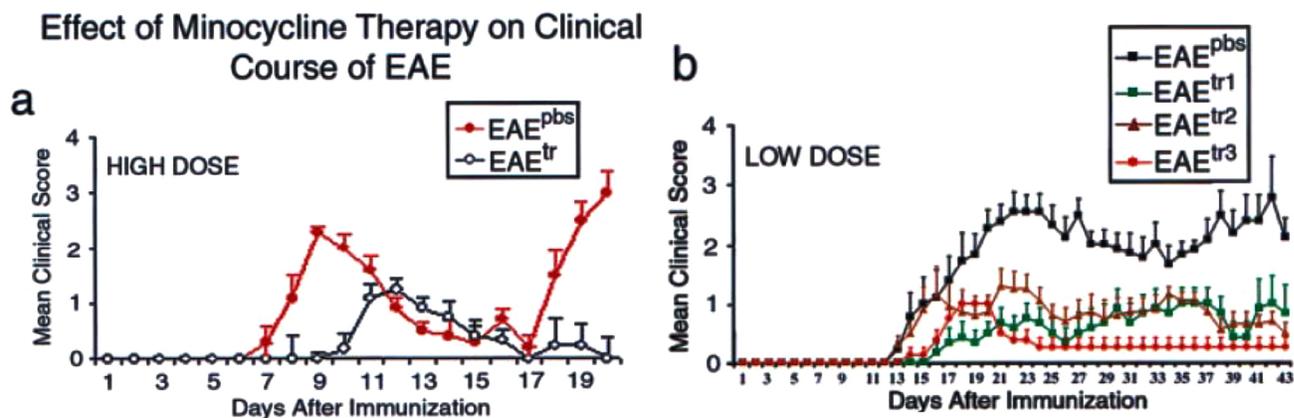
Fig. (1). Minocycline treatment delayed the onset and attenuated the clinical course of experimental autoimmune encephalomyelitis (EAE). (**a**) In the high-dose myelin oligodendrocyte glycoprotein (MOG) immunization paradigm, minocycline attenuated the onset and severity of EAE (EAEtr) in comparison to phosphate-buffered saline-treated controls (EAEpbs). (**b**) In the low-dose MOG immunization paradigm, severity of EAE was significantly reduced when minocycline treatment started before (EAEtr1) or at onset of disease, with once-daily (EAEtr2) or twice daily (EAEtr3) injections for the first 2 days compared to controls (EAEpbs). Reprinted from reference [40] with permission of the publisher.

linium-enhancing lesion in the MRI. On the molecular level, the p40 subunit of IL-12 and soluble vascular cell adhesion molecule-1 (sVCAM-1) were increased in serum while the activity of MMP-9 was decreased (pooled observation 3, 6, 12 and 18 months after treatment). The drug was relatively well tolerated except in two patients in which the dose had to be reduced because of headache and nausea [44].

STROKE

The neuroprotective effects of the tetracyclines doxycycline and minocycline were first described in 1998 in a model of global cerebral ischemia in gerbils. Since inflammation plays a role in the course of neuronal death due to ischemia, anti-inflammatory agents as adjuvant therapies may be beneficial. Treatment with high doses of both doxycycline and minocycline (either 45 mg/kg 12 hours before ischemia followed by twice daily injections at a dose of 90 mg/kg during the first day after ischemia and 45 mg/kg starting 36 h after stroke or the same regime without application before ischemia but starting 30 min after ischemia) increased the survival of CA1 pyramidal neurons and reduced microglial activation even when administered 30 minutes after the ischemic event. The reduced activity of microglia was accompanied by a reduction in gene expression of ICE (interleukin-1β-converting enzyme), an apoptosis-promoting gene that is induced in microglia after ischemia, and iNOS (inducible nitric oxide synthase), an enzyme which may produce toxic amounts of NO in non-neuronal cells [45]. Moreover, 10 mg/kg doxycycline reduced the infarct volume and improved functional efficacy in a rat model of focal CNS reperfusion injury when applied before the ischemic event [46]. The mechanism responsible for this effect was not further investigated in this study. However, doxycycline has the ability to inhibit leukocyte function *in vitro* via divalent cation binding [11] and may have influenced post-ischemic events through reduced leukocyte adhesion.

Minocycline was also studied in combination with other potentially neuroprotective agents. In stroke, a drug cocktail

including minocycline as an immunomodulatory drug, riluzole as a glutamate antagonist and nimodipin as a voltage-gated calcium channel blocker was tested in mouse models of transient and permanent ischemia. Infarct size and clinical recovery were improved in both experimental models, and a more efficient neuroprotective effect of the drug combination vis-à-vis any individual component was observed. The beneficial effect is believed to be the result of less microglial and caspase-3 activation and preserved astrocyte structure and function as documented by unaltered GFAP (glial fibrillary acidic protein) immunoreactivity after transient ischemia. However, no immunohistochemical differences in microglia and caspase-3 activation were observed after 72 h of permanent ischemia. In single drug experiments, minocycline was protective in both experimental models while riluzole had a protective effect after transient ischemia only [47].

Favorable effects of another antiinfectious agent, rifampicin (20 mg/kg single dose), were described in a mouse model of focal brain ischemia: application of the antibiotic before or within 30 minutes after ischemia reduced the number of apoptotic cells in the striatum [48].

AMYOTROPHIC LATERAL SCLEROSIS (ALS)

A study that received much attention explored the effect of ceftriaxone on the expression of the glutamate transporter GLT1. An association of GLT1 dysfunction and several neurological diseases such as ALS [49], stroke [50] and epilepsy [51] was postulated. GLT1 is believed to be involved in preventing glutamate toxicity by rapid removal of glutamate from the synaptic cleft [52]. β-lactam antibiotics increased GLT1 expression and reduced neuronal cell death in embryonic cortical cultures exposed to oxygen-glucose deprivation (OGD). Treatment of transgenic mice expressing a mutation of superoxide dismutase occurring in familial ALS (G93A-SOD1) with 200 mg/kg/d ceftriaxone i.p. for 5 weeks led to a delayed loss of neurons and muscle strength and hence increased survival in comparison to saline-treated animals [53].

Hippocampal slices obtained from P21-28 rats treated with 200 mg/kg i.p. ceftriaxone for 5 days were also exposed to OGD. Patch-clamp techniques indicated increased glutamate transporter activity after ceftriaxone treatment, and the delay to OGD-induced hypoxic spreading depression was longer in slices from ceftriaxone-treated animals, both observations suggesting a neuroprotective effect. However, this effect could not be repeated in organotypic hippocampal slices obtained from P7-9 rats where similar damage was observed after pre-treatment with the antibiotic and in controls. In contrast to the study of Rothstein *et al*. [53], no increase of GLT1 protein synthesis was detected in these experiments [54]. The authors concluded that the β-lactam antibiotic worked through changes in transporter activity rather than in transporter protein synthesis. However, these results were highly dependent on the experimental model used.

There are also data on the impact of minocycline on the course of experimental ALS. Antibiotic treatment (10 mg/kg/d) delayed the disease onset and extended the survival of G93A-SOD1 mice. This effect was thought to be due to the inhibition of mitochondrial permeability-transition-mediated cytochrome c release that was demonstrated *in vivo* and *in vitro* in cells and isolated mitochondria [55].

HUNTINGTON´S DISEASE (HD)

Studies on the effect of minocycline therapy in experimental HD produced inconsistent data, in part depending on the animal model used. In a phenotypic toxic 3-nitropropionic acid (3-NP) mouse model of HD, application of 45 mg/kg i.p. minocycline 30 minutes before injection of 3-NP led to deterioration of motor function. Histopathological analysis revealed more neuronal cell loss in the dorsal striatum in mice treated with minocycline and 3-NP than in mice receiving 3-NP only [56]. Similarly, in a 3-NP rat model and *in vitro* using primary striatal cells, minocycline was not protective although attenuation of inflammation was observed. The authors additionally used quinolinic acid (QA) to induce a phenotypic model of HD. In contrast to the model induced by 3-NP, minocycline reduced striatum lesions and inflammation induced by injections of QA. Since intoxication with 3-NP – in contrast to QA – leads to calpain-dependent neuronal death, minocycline might not be effective in calpain-dependent cell death [57]. As a mechanism responsible for the effects of minocycline in HD, inhibition of both caspase-dependent (Smac/Diablo) and caspase-independent (apoptosis-inducing factor) signal pathways were postulated [58].

In addition to phenotypic models of HD, minocycline was also tested in a transgenic mouse model of HD (R6/2) in which the beneficial effects were more pronounced than in the phenotypic models. R6/2 mice express exon-1 of huntingtin with an expanded polyglutamine repeat under the control of its native reporter and develop a progressive neurological phenotype with features of HD [59]. Treatment of R6/2 mice with 5 mg/kg body weight i.p. minocycline daily for seven weeks delayed disease progression, inhibited caspase-1 and caspase-3 mRNA upregulation, and decreased iNOS activity [60].

High dose coenzyme Q10 (CoQ10) was also shown to be effective in R6/2 mice leading to extended survival and im-

proved motor function [61]. Since both CoQ10 and minocycline improved behavioral and neuropathological deficits in R6/2 mice, experiments using a combined therapy were carried out. All beneficial effects such as amelioration of behavioral and neuropathological alterations, improved motor performance assessed by rotarod test and extended survival were observed to increase in the combination therapy group in comparison to each individual treatment group [62].

Clinical trials with minocycline in patients suffering from confirmed HD are still being evaluated (e.g. DOMINO study, www.clinicaltrials/gov). In a small trial consisting of 11 patients with genetically confirmed HD and 100 mg/d minocycline treatment, a stabilization of general motor and neuropsychological functions was found after 24 months of treatment. Moreover, a significant reduction of psychiatric symptoms was observed after 24 months of minocycline which was not apparent after the first 6 months of treatment [63].

OTHER DISEASES

Both cerebral inflammation and apoptosis are thought to be involved in the tissue damage occurring after traumatic brain injury. Thirty minutes after induction of a closed head injury mice were treated with 45 mg/kg minocycline followed by 90 mg/kg daily. The animals displayed a decreased lesion volume and improved outcome one day after injury. Unfortunately, no substantial clinical differences between minocycline and control animals were noted four days after the injury. Minocycline-treated animals showed less microglial activation and IL-1β expression while neutrophil infiltration, cytokine expression and density of apoptotic cells were unaltered [64]. Further studies are required to evaluate a potential therapeutic option of minocycline for patients with traumatic brain injury.

Administration of ciprofloxacin was investigated in a mouse model of experimental antiphospholipid syndrome. Treatment with 30 mg/kg ciprofloxacin three times daily for 5 days starting on day 1 of pregnancy led to a decrease in the incidence of pregnancy loss and reduction of clinical symptoms. These effects were associated with increased levels of IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) [65].

Adjuvant therapy with 30 mg/kg doxycycline given 18 hours after infection in addition to the bacteriolytic antibiotic ceftriaxone in a rat model of pneumococcal meningitis reduced the mortality, blood-brain barrier disruption and the extent of cortical brain injury. Adjuvant therapy at the same daily dose for 4 days attenuated hearing loss, one of the major long-term sequelae after pneumococcal meningitis (Fig. **2**) [66]. It is unclear whether this beneficial effect is caused by the immunomodulatory action of doxycycline or by an inhibition of the release of proinflammatory bacterial products (see below).

Whether there is a role for tetracyclines in adjuvant treatment of rheumatoid arthritis is still a matter of debate. Data from animal models were contradictory. Administration of minocycline and tetracycline for seven days starting directly or within one week of induction of rheumatoid arthritis by sensitization led to a decrease in paw volume in the
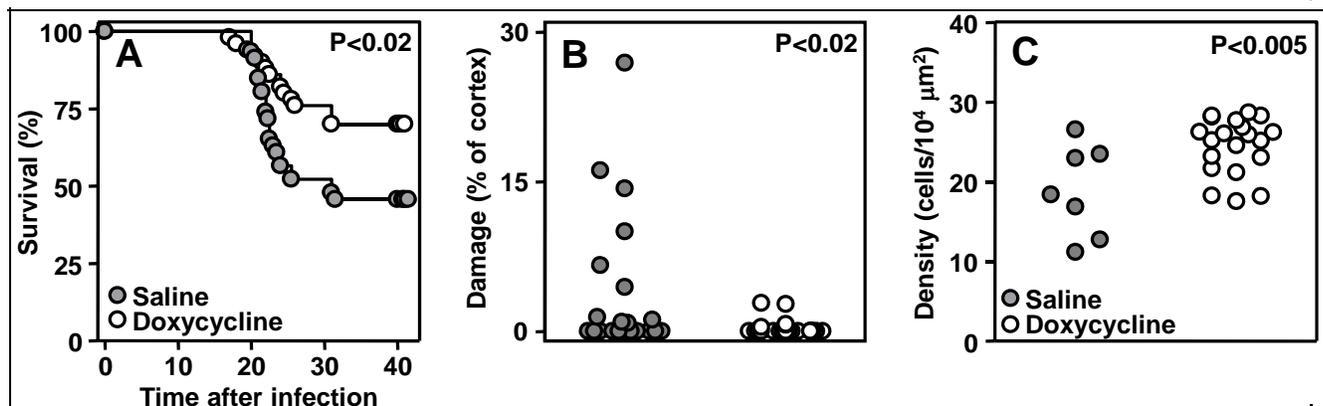
Fig. (2). Adjuvant application of doxycycline (30 mg/kg/d for 4 days, open circles) in addition to ceftriaxone in comparison to ceftriaxone only (gray circles) in a rat model of pneumococcal meningitis. Doxycycline significantly improved survival (**A**), reduced the extent of cortical neuronal injury (**B**) and reduced neuronal loss in the cochlear spiral ganglion (density of type 1 neurons in the Rosenthal´s canal 3 weeks after infection) (**C**). Reprinted from reference [66] with permission of the publisher.

footpad-thickness test in both treatment regimes. According to the authors, these changes indirectly indicated a decrease in lymphokine production or release induced by the antibiotic treatment. However, leukocyte migration used as a measure for the cell-mediated immune response was influenced differently depending on the time of administration: treatment directly after sensitization from day 1-7 led to the desired inhibition of leukocyte migration while administration from day 7-13 after sensitization resulted in an undesired increase of leukocyte migration [67]. Analysis of ten randomized controlled trials focusing on the role of tetracyclines, especially minocycline, in rheumatoid arthritis revealed that antibiotic treatment for at least 3 months was associated with a significant reduction in disease activity. This effect was more pronounced in seropositive patients with duration of the disease for less than one year. Unfortunately, only three out of the ten chosen trials were considered to be of high quality so that further studies are needed to answer this question conclusively [68].

THERAPY FOR INFECTIONS USING PROTEIN SYNTHESIS-INHIBITING ANTIBIOTICS

One therapeutic approach to attenuating the hosts´ inflammatory response is to take advantage of the immuno-modulatory properties of the antibiotics described above. Another therapeutic approach is to reduce the release of bacterial components by using protein synthesis-inhibiting antibiotics.

Bateriolytic antibiotics such as β-lactams work by inhibiting bacterial cell wall synthesis. This leads to lysis of the pathogen and consequently to the release of pro-inflammatory bacterial components. Especially in bacterial meningitis and systemic infections with a high bacterial load, the inflammatory burst upon initiation of treatment with β-lactam antibiotics increases mortality and sequelae [69-71]. In contrast, non-bacteriolytic but bactericidal antibiotics such as rifampicin and clindamycin inhibit bacterial protein synthesis and prevent the initial inflammatory burst (Fig. **3**).

In vitro data from our laboratory showed that killing the *S. pneumoniae* type 3 strain by β-lactam antibiotics (ceftriaxone and meropenem) significantly increased the release of lipoteichoic acid (LTA) and teichoic acid (TA) in comparison to cultures exposed to protein synthesis inhibitors (rifampicin, rifabutin, quinupristin-dalfopristin) or trovafloxacin [72]. Similar results were obtained in cultures of *S. aureus* that were incubated for 4 hours in the presence of β-lactams (imipenem, flucloxacillin or cefamandole) or protein synthesis inhibiting antibiotics (erythromycin, clindamycin or gentamicin). The levels of LTA and peptidoglycan in the supernatants of the bacterial cultures were increased by β-lactams. In contrast, protein synthesis inhibitors did not increase peptidoglycan release and even decreased LTA levels in comparison with control cultures without antibiotics. The capacity of supernatants from *S. aureus* cultures exposed to β-lactam antibiotics to stimulate the release of TNFα and IL-10 in human whole blood was higher than that of cultures exposed to protein synthesis inhibitors [73].

These *in vitro* data support the idea that therapy with non-bacteriolytic antibiotics causes less inflammation and could possibly improve the outcome of severe infections. This led to investigations in different animal models of bacterial meningitis and sepsis in our laboratory. In a mouse model of *S. pneumoniae* meningitis treatment with rifampicin in comparison to ceftriaxone (both twice daily for 3 days) resulted in a reduced mortality during the first 24 h and reduced overall mortality in animals receiving rifampicin. Eight hours after a single 2 mg-dose of rifampicin or ceftriaxone, rifampicin-treated mice had lower concentrations of LTA and TA in serum and cerebrospinal fluid (CSF) [74]. Comparison of trovafloxacin and ceftriaxone in a rabbit model of pneumococcal meningitis showed that treatment with the fluoroquinolone delayed but did not inhibit the release of the pro-inflammatory cytokines TNFα and IL-1β into the CSF, presumably because of the delayed liberation of bacterial cell wall components [75]. In the same experimental model, application of clindamycin resulted in reduced release of LTA into the CSF (Fig. **4**), lower CSF leukocyte count and lower extracellular concentrations of hydroxyl radicals and glutamate in the hippocampal formation in comparison to treatment with ceftriaxone. Moreover, there was a lower incidence of neuronal apoptosis in the dentate gyrus in clindamycin-treated animals [76]. Further data supporting advantageous effects of protein synthesis inhibiting antibiotics come from a murine model of *S. aureus* sepsis. Antibiotic treatment with clindamycin 5 hours after intrape-
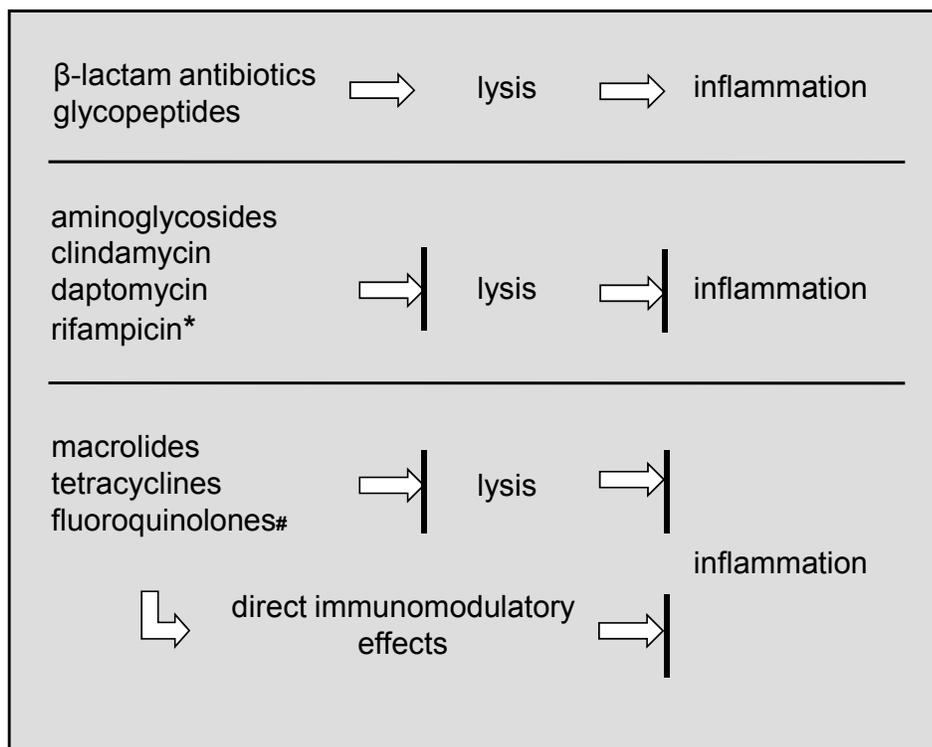
Fig. (3). Illustration of the anti-inflammatory and immunomodulating effects of different classes of antibiotics.

ritoneal inoculation decreased the morbidity and mortality of mice compared to mice receiving bacteriolytic therapy with ceftriaxone [77].

Comparison of therapy with ceftriaxone to therapy with the non-bacteriolytic polypeptide antibiotic daptomycin in rat pneumococcal meningitis revealed less CNS inflammation (assessed by MMP-9 concentration in CSF) and cortical injury in daptomycin-treated animals. The authors concluded that the positive effect of daptomycin was due to its reduction of the release of pro-inflammatory bacterial products [78].

Analysis of combined antibiotic therapy in a rabbit meningitis model with initial application of rifampicin followed by ceftriaxone 6 hours after the start of antibiotic treatment showed that pretreatment with rifampicin prevented the release of pneumolysin, an important virulence factor in *S. pneumoniae* [79]. Pretreatment with rifampicin only one hour before application of ceftriaxone reduced the release of proinflammatory bacterial products *in vitro* and attenuated inflammation and neuronal damage *in vivo* in rabbits with bacterial meningitis [80].

Minimizing the release of pro-inflammatory compounds by the use of protein synthesis-inhibiting antibiotics could also be useful in infections complicating other, in particular autoimmunological, diseases. Our laboratory therefore investigated the effect of treatment of *S. pneumoniae* infections with non-bacteriolytic antibiotics (100 mg/kg minocycline or rifampicin) on the course of EAE induced 7 days prior to intraperitoneal *S. pneumoniae* infection. In the minocycline-treated group, a delay of disease onset by approximately one day was observed, while rifampicin treatment had no effect on the course of disease [81]. This delay of one day is presumably not clinically relevant in most conditions, but may still represent a subjective benefit for the individual patient.

The clinical significance of the differential release of bacterial components following treatment with individual classes of antibiotics is still a matter of debate. Large scale prospective studies of infections to investigate whether antibiotics inhibiting protein synthesis can decrease mortality have not been carried out. In a study of Gram-negative urosepsis, 30 patients were randomized to therapy regimes with either imipenem or ceftazidime. Four hours after the onset of treatment, blood endotoxin levels had decreased in
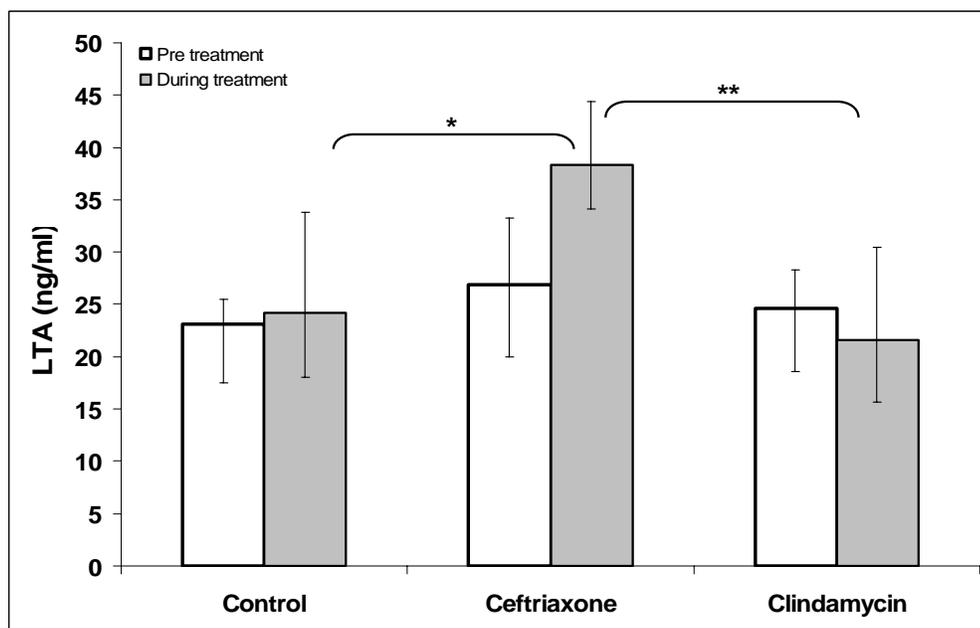
Fig. (4). Lipoteichoic acid (LTA) concentrations in cerebrospinal fluid (CSF) of rabbits with pneumococcal meningitis before and during treatment with either saline, ceftriaxone or clindamycin. Ceftriaxone-treated rabbits had significantly higher concentrations of LTA compared to clindamycin-treated animals and controls. Reprinted from reference [76] with permission of the publisher.

all patients receiving imipenem whereas endotoxin levels had decreased only in half of patients with ceftazidime. The study was not suitable for measuring outcome differences because mortality in both groups was 0% [82]. Children with deep tissue infections caused by *S. pyogenes* were more likely to have a favorable outcome if initial treatment included a protein synthesis inhibitor (mainly clindamycin) as compared to therapy with cell wall-inhibiting antibiotics only [83]. The concentration of lipoteichoic acid (LTA) and teichoic acid (TA) in CSF upon admission and the outcome – assessed by the Glasgow Coma Scale – upon discharge were correlated in patients with *S. pneumoniae* meningitis. LTA and TA concentrations in CSF were significantly associated with neurologic sequelae and mortality [84].

Taken together, these data support the concept of a beneficial effect of bactericidal protein synthesis-inhibiting antibiotics in comparison with β-lactams in infections with high bacterial load such as meningitis and sepsis. Randomized clinical studies, however, are necessary before applying this concept in clinical practice.

CONCLUSIONS

The immunomodulatory properties of macrolides were discovered over 15 years ago. Convincing *in vitro* studies as well as *in vivo* data gained from animal models of chronic pulmonary diseases led to clinical trials that confirmed the beneficial immunomodulatory effects of macrolides in chronic inflammatory pulmonary disorders such as DPB and CF. This improved the quality of life and long-term outcome for many patients. The concept of using the immunomodulatory properties of antibiotics to reduce the severity of pulmonary diseases has been so convincing that investigations were expanded to other classes of antibiotics and different diseases, particularly to tetracyclines in infectious and degenerative diseases. Initial promising observations were

made on the use of minocycline and evidence for immunomodulatory actions of other antibiotics was found. Appropriate clinical trials are necessary to demonstrate whether adjunctive therapy with antibiotics other than macrolides is beneficial in clinical practice.

In experimental bacterial meningitis and sepsis, bactericidal protein synthesis-inhibiting antibiotics are able to reduce mortality and sequelae compared to conventional treatment with β-lactam antibiotics. The probable mechanism is not a direct immunomodulatory effect of these antibiotics but an inhibition of the release of pro-inflammatory bacterial products compared to β-lactams.

ABBREVIATIONS

3-NP	=	3-Nitropropionic acid
ALS	=	Amyotrophic lateral sclerosis
BAL	=	Brochoalveolar lavage
BDNF	=	Brain-derived neurotrophic factor
CF	=	Cystic fibrosis
CNS	=	Central nervous system
CoQ10	=	Coenzyme Q10
CSF	=	Cerebrospinal fluid
DPB	=	Diffuse panbrochiolitis
EAE	=	Experimental autoimmune encephalitis

GLT1	=	Glutamate transporter 1
GFAP	=	Glial fibrillary acidic protein
GDNF	=	Glia cell line-derived neurotrophic factor
GM-CSF	=	Granulocyte-macrophage colony stimulating factor
HD	=	Huntington´s disease
ICE	=	IL-1β converting enzyme
IFN	=	Interferon
IL	=	Interleukin
iNOS	=	Inducible nitric oxide synthase
JNK	=	c-Jun N-terminal kinase
LTA	=	Lipoteichoic acid
LPS	=	Lipopolysaccharide
MAPK	=	Mitogen-activated protein kinase
MMP	=	Matrix metalloproteinase
MS	=	Multiple sclerosis
NO	=	Nitric oxide
OGD	=	Oxygen-glucose deprivation
QA	=	Quinolinic acid
SVCAM-1	=	Soluble vascular adhesion molecule-1
TA	=	Teichoic acid
TNF	=	Tumor necrosis factor

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