Interaction of macrolides with adornase during DNA hydrolysis

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Since patients with cystic fibrosis are often treated with adornase to reduce sputum viscosity, and because of preliminary reports of efficacy of long-term low-dose erythromycin therapy in chronic airway diseases, it is likely that adornase and macrolides might be given together in such patients. A possible interaction between these drugs was therefore investigated. Using hyperchromic effect to quantify adornase activity, a time- and dose-dependent inhibitory effect on human DNA hydrolysis has been observed for erythromycin, roxithromycin and azithromycin. Inhibitory doses 50% for adornase were graphically determined. Azithromycin exhibited the strongest inhibitory effect.

Introduction

Cystic fibrosis (CF) is a recessive autosomal disease complicated by persistent respiratory tract infections with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Haemophilus influenzae*.

After colonizing the respiratory tract, *P. aeruginosa* secretes alginate and exoenzymes which contribute to the persistence of infection, parenchymal lesions and destruction of host cells. In patients with CF, airway secretions are viscous and hinder clearance of infection. Sputum of patients infected with *P. aeruginosa* contains proteins such as immune complexes which participate in inflammatory damage to the lung, as well as high concentrations of DNA derived from inflammatory cells. This latter constitutes the main component of the typical viscous, purulent lung secretion which reduces bacteriological clearance and diffusion of antibiotics.

Since 1958, purified bovine pancreatic DNase I, and later recombinant human DNase I (adornase), have been used successfully in CF patients to decrease viscosity of lung secretions by degrading leucocyte-derived DNA (Shak et al., 1995). In several clinical studies, aerosolized adornase has been shown to improve pulmonary function and reduce the risk of respiratory tract infections in such patients (Hubbard et al., 1992; Ramsey et al., 1993; Ranasinha et al., 1993).

On the other hand, in several Japanese clinical trials of chronic airway diseases including diffuse panbronchiolitis, low-dose long-term erythromycin therapy has been shown to be effective in reducing neutrophil accumulation, elastase-like activity and *P. aeruginosa* colonization (Oishi et al., 1994; Kobayashi, 1995). This clinical efficacy
was inferred from a reduction of purulent secretion and an increase in vital capacity. In some cases *P. aeruginosa* has been eradicated. Although the mechanisms of this clinical improvement remain unclear, experiments *in vitro* have pointed out the ability of macrolides to interfere with inflammatory phagocytic cells and with bacterial physiology. Evidence of reduction of IL8 production by stimulated macrophages (Oishi et al., 1994) and suppression of secretion of *P. aeruginosa* virulence factors were obtained when subinhibitory concentrations of macrolides were used. Such concentrations did not affect the growth rate of these microorganisms (Molinari et al., 1993). The beneficial effects of macrolides involved decrease of expression and/or activity of *P. aeruginosa* exoenzymes such as exotoxin A, elastase, proteases, lecithinase, phospholipase C and DNase. Moreover, *P. aeruginosa* DNase activity was completely blocked by azithromycin, erythromycin or clarithromycin in six, four and three out of seven tested strains, respectively.

Such clinical and experimental results and the potential mechanisms of action of low-dose long-term macrolide therapy could open new prophylactic approaches in CF patients chronically infected with *P. aeruginosa*. In such patients adornase and macrolides might therefore be given together.

The aim of this study was to investigate a possible interaction of two macrolides, erythromycin and roxithromycin, and one azalide, azithromycin, on adornase hydrolytic activity against human purified DNA.

**Materials and methods**

Quantitative analysis of adornase activity was carried out using the hyperchromic effect of DNase during DNA hydrolysis (Adams, Knowler & Leader, 1986). DNase activity was measured by an increase of optical density at 260 nm (OD 260 nm) due to double-strain or single-strain DNA break-down corresponding to the release of ultraviolet-absorbing acid-soluble products. It allowed the determination of (i) the minimum inhibitory concentration for each macrolide as the lowest concentration able to inhibit all the hydrolytic activity of adornase and (ii) for each antibiotic the inhibitory dose 50% (ID₅₀) at each recorded time.

**Purified DNA**

Human genomic DNA was isolated from peripheral blood leucocytes of healthy volunteers, extracted with phenol/chloroform and precipitated with ethanol. After resolubilization in 10 mM Tris/1 mM EDTA pH 8, DNA concentration was estimated at 0.5 g/L (OD 260 nm).

Adornase (Roche, France) was used at a concentration of 1000 U/mL.

**Antibiotics**

Erythromycin (Abbott, France), roxithromycin (Roussel, France) and azithromycin (Pfizer, France) were dissolved in 50% aqueous methanol to obtain an initial concentration of 5.12 g/L.
Measurement of adornase activity

Kinetic determination of adornase activity was carried out by recording spectrophotometric OD 260 nm values each minute for ten minutes at room temperature. Twenty units of adornase were tested with a constant amount of human DNA (5 μg) and six different concentrations of antibiotics (4, 6.5, 13, 26, 51 and 77 mg/L) in a total volume of 1 mL. Reaction components were mixed in a 10 mm spectrophotometric quartz cell (Hellman, Germany) by inverting the cell ten times. Zero OD 260 nm was read as the cell was inserted in the spectrophotometer (Ultraspec II, LKB Biochrom, England) and the chronometer was started at the same time.

Results

Recorded OD 260 nm values corresponding to DNA alone were stable during 10 min. Fifty per cent aqueous methanol solution alone did not exhibit any effect on DNA hydrolysis. Also, in the absence of adornase, the highest concentrations of antibiotic used had no detectable spectrophotometric effect on purified human DNA (data not shown). Without antibiotics, adornase-related hydrolysis was responsible for an increase of 37 units of OD 260 nm and the maximum of OD value was reached in 7 min, corresponding to the complete hydrolysis of DNA (Figure).

![Figure. Kinetics of human DNA hydrolysis activity of adornase without antibiotics (—, control) or when macrolides ((a) azithromycin; (b) erythromycin; (c) roxithromycin) were added at varying concentrations (▲, 4 mg/L; △, 6.5 mg/L; ●, 13 mg/L; ○, 26 mg/L; ■, 51 mg/L; □, 77 mg/L).](https://academic.oup.com/jac/article-abstract/37/5/987/749832)
Each antibiotic used had an inhibitory effect on adornase activity. The lowest concentration of azithromycin showing inhibition was 4 mg/L (Figure (a)) while for erythromycin and roxithromycin inhibition was detected at a concentration of 6.5 mg/L and above (Figure (b), (c)). Complete inhibition of DNA hydrolysis was obtained using 77, 77 and 51 mg/L of erythromycin, roxithromycin and azithromycin respectively.

From each reaction, the inhibition rate was calculated and the ID$_{50}$ at each recorded time was deduced (Table). The ID$_{50}$ of azithromycin was always lower than that of erythromycin or roxithromycin.

**Discussion**

The DNase hyperchromic effect is used commercially to define the activity unit of bovine DNase I. Adornase activity has been determined in the same way and two 14-membered ring macrolides, erythromycin and roxithromycin, and a 15-membered ring macrolide, azithromycin, were shown to have a strong inhibitory effect on hydrolytic activity against human DNA *in vitro*.

Azithromycin was the most inhibitory, being effective at a concentration as low as 4 mg/L. After a single 500 mg oral dose, azithromycin sputum concentrations range from 0.12 to 9.5 mg/L. It can therefore be assumed that inhibition may occur during long-term combination treatment with macrolides and adornase because alveolar macrophages concentrate azithromycin and may release it gradually (Baldwin *et al.*, 1990). In contrast, lower levels of erythromycin and roxithromycin, from 0.17 to 1.86 mg/L are present in sputum of infected patients (Oishi *et al.*, 1994) and these drugs would not interact with adornase *in vivo*.

The biochemical mechanisms of the inhibitory effect described in this study have not yet been identified. Though the modes of action of macrolides are still unclear, possible mechanisms can be speculated upon. In prokaryotes, macrolides block protein synthesis by binding to the 50S ribosomal subunit involving both 23S rRNA and ribosomal proteins such as L22 protein which has been shown to be located near the active centre of peptidyl transferase (Arévalo, 1988). Macrolides are thus able to create specific links.
Macrolides and adornase inhibition.

not only with proteins such as peptidyl transferase, which controls peptidyl-tRNA during translocation, but also with nucleosidic components (23S rRNA).

Macrolides could therefore interact with and bind to adornase as a protein constituent and/or to purified human DNA as a nucleoside constituent, possibly creating steric hindrance between catalytic domain of adornase and DNA.

Studies are currently being undertaken in this laboratory to determine the biochemical mechanism involved in the inhibitory effect of macrolides on adornase activity. Therapeutic trials are also needed to determine (i) if this effect can be detected in vivo during macrolide-adornase treatment and (ii) if so, the posological conditions which allow both molecules to exert their beneficial effects.

References


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