Intraphagocytic Activity of Erythromycin, Roxithromycin and Azithromycin

D. Milatovic

The intraphagocytic activity of erythromycin, roxithromycin and azithromycin against phagocytosed *Staphylococcus aureus* was compared. Erythromycin and roxithromycin both acted bacteriostatically at concentrations corresponding to 10 × MIC. Azithromycin, however, did not prevent intracellular proliferation of the staphylococci. On comparison of the pH dependency of the antibacterial activity of the three drugs, azithromycin was found to be inactivated earlier in an acidic milieu.

Intraphagocytic activity of an antibiotic is an important property when treating infections due to bacteria which are able to survive intracellularly such as legionella, mycobacteria, listeria, brucella and *Staphylococcus aureus*. Erythromycin, a well established antibiotic, has regained clinical importance as the drug of choice in Legionnaire's disease. Parallel to its clinical efficacy, erythromycin has been shown to accumulate in phagocytic cells (1, 2, 3) and to exhibit intracellular activity against legionella (4, 5, 6). However, data published on the intracellular activity of erythromycin against *Staphylococcus aureus* are contradictory (1, 7, 8). Newly developed macrolide antibiotics such as roxithromycin and azithromycin have been reported to reach even higher intracellular concentrations compared to erythromycin (1, 2, 3, 9). Furthermore, the data obtained on in vitro evaluation of these drugs point towards an expanded spectrum which might include other intracellular microorganisms such as *Mycobacterium avium* complex or *Toxoplasma gondii* (10, 11). The purpose of this study was to compare the intraphagocytic activity of the new macrolides roxithromycin and azithromycin with that of erythromycin.

Materials and Methods. Human polymorphonuclear leukocytes (PML) were isolated from heparinized blood by dextran sedimentation and Ficoll-Hypaque density gradient as previously described (12). *Staphylococcus aureus* strain 502 A opsonized in 10 % pooled human serum was exposed to the PML for 30 min at 37 °C (ratio of bacteria to PML 4:1). Extracellular bacteria were removed by adding 1 U/ml lysostaphin. After incubation for 15 min at 37 °C the PML with the intracellular staphylococci were washed in Hank's Balanced Salt Solution (5 min, 4 °C, 160 × g) and resuspended in Eagles Minimum Essential Medium (MEM) containing 10 % fetal calf serum (FCS) and various concentrations (10 × MIC, 1 × MIC, 1/3 MIC) of erythromycin (Abbott, FRG), roxithromycin (Roussel, France) and azithromycin (Pfizer, UK) respectively. The MIC of the antibiotics for *Staphylococcus aureus* 502 A was 0.5 mg/l, 2 mg/l and 0.5 mg/l respectively. The control PML were suspended in medium without addition of antibiotics. Each experimental set consisted of three identical tubes. The tubes were incubated at 37 °C and rotated end over end. The number of intracellular surviving bacteria was determined at 0, 3 and 18 h. For this purpose at the indicated times one of the three identical tubes was washed twice with ice-cold PBS (5 min, 4 °C, 160 × g) and the pellet resuspended in an equal volume of distilled water to lyse the PML and release intracellular bacteria. From serial tenfold dilutions 100 μl samples were transferred onto blood agar plates. After incubation for 18 h at 37 °C colonies were counted. Furthermore, antibiotic killing of *Staphylococcus aureus* in the absence of PML was evaluated using an antibiotic concentration of 10 × MIC. In these experiments the pH of the medium was modified, ranging from 4 to 8.

Results and Discussion. During incubation for 3 h the number of intracellularly surviving bacteria in the control assay was reduced to 21 % (SD ± 7 %), whereas after 18 h proliferation of the bacteria was observed (Figure 1, closed symbols). Addition of any of the antibiotics tested did not enhance the intracellular killing of the bacteria after 3 h. After incubation for 18 h none of the three macrolides inhibited intraphagocytic multiplication of the bacteria at concentrations equal to the MIC or higher (results with 1/3 MIC not shown in
However, erythromycin and roxithromycin both acted bacteriostatically when the concentration was increased to $10 \times \text{MIC}$. In contrast, azithromycin at the same concentration reduced the growth rate but did not prevent intracellular proliferation of the staphylococci.

Figure 2 shows the killing rate of extracellular staphylococci after incubation for 18 h in Eagles MEM + 10% FCS containing $10 \times \text{MIC}$ of the three antibiotics respectively. The results are broken down by pH modification of the medium. At a pH of 7.4 and 8.0 all three macrolides exhibited moderate bactericidal activity, reducing the initial inoculum by 1–1.5 log. At pH 6 erythromycin and roxithromycin remained active against *Staphylococcus aureus* in contrast to azithromycin which allowed proliferation of the bacteria. None of the antibiotics was effective at pH 4.

These results indicate that roxithromycin is comparable to erythromycin with respect to the bacteriostatic effect on phagocyted *Staphylococcus aureus*. Similar findings were reported by Anderson et al. (1), who used a fluorochrome microassay and a radioassay to test the intracellular activity of erythromycin and roxithromycin. Surprisingly, azithromycin was significantly less active intracellularly, although its activity against extracellular staphylococci was equivalent to that of the other two drugs. However, when comparing the pH dependency of the antibacterial effect, it became evident that azithromycin undergoes earlier inactivation in an acidic milieu than the other two compounds. This could explain why azithromycin lacks intracellular activity at the acidic pH in the phagolysosome. Taking into account the fact that azithromycin is reported to be concentrated intracellularly about 80-fold (3), roxithromycin 15- to 30-fold (1, 2, 9) and erythromycin 10- to 15-fold (1, 2, 3), it becomes clear that the amount of intracellular accumulation of a drug does not necessarily correlate with its intracellular activity.

Clinical studies comparing the efficacy of the new macrolides with that of erythromycin in the treatment of infections due to intracellular organisms are necessary to substantiate these in vitro observations.

### References