Concentrations in plasma, epithelial lining fluid, alveolar macrophages and bronchial mucosa after a single intravenous dose of 1.6 mg/kg of iclaprim (AR-100) in healthy men

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Objectives: A validated microbiological assay was used to measure concentrations of iclaprim (AR-100) in plasma, bronchial mucosa (BM), alveolar macrophages (AM) and epithelial lining fluid (ELF) after a single 1.6 mg/kg intravenous 60 min iv infusion of iclaprim.

Methods: Male volunteers were randomly allocated to three nominal sampling time intervals 1–2 h (Group A), 3–4 h (Group B) and 5.5–7.0 h (Group C) after the start of the drug infusion.

Results: Mean iclaprim concentrations in plasma, BM, AM and ELF, respectively, were for Group A 0.59 mg/L (SD 0.18), 0.51 mg/kg (SD 0.17), 24.51 mg/L (SD 21.22) and 12.61 mg/L (SD 7.33); Group B 0.24 mg/L (SD 0.05), 0.35 mg/kg (SD 0.17), 7.16 mg/L (SD 1.91) and 6.38 mg/L (SD 5.17); and Group C 0.14 mg/L (SD 0.05), no detectable level in BM, 5.28 mg/L (SD 2.30) and 2.66 mg/L (SD 2.08).

Conclusions: Iclaprim concentrations in ELF and AM exceeded the MIC90 for penicillin-susceptible Streptococcus pneumoniae (MIC90 0.06 mg/L), penicillin-intermediate S. pneumoniae (MIC90 2 mg/L), penicillin-resistant S. pneumoniae (MIC90 4 mg/L) for 7, 7 and 4 h, respectively, and Chlamydia pneumoniae (MIC90 0.5 mg/L) for 7 h. Mean iclaprim concentrations in ELF exceeded the MIC90 for Haemophilus influenzae (MIC90 4 mg/L) and Moraxella catarrhalis (MIC90 8 mg/L) for up to 4 and 2 h, respectively; in AM the MIC90 was exceeded for up to 7 h. Furthermore, the MIC90 for methicillin-resistant Staphylococcus aureus of 0.12 mg/L was exceeded at all sites for up to 7 h. These data suggest that iclaprim reaches lung concentrations that should be effective in the treatment of community-acquired pneumonia.

Keywords: diaminopyrimidine, concentration, respiratory tree

Introduction

Iclaprim (AR-100) is a novel diaminopyrimidine and could be considered as a member of a new generation of dihydrofolate reductase inhibitors, which include trimethoprim. Iclaprim exhibits potent in vivo activity against Gram-positive (including methicillin-resistant Staphylococcus aureus and Streptococcus pneumoniae) and Gram-negative organisms.1 Following single intravenous administration, the $C_{max}$ and AUC of iclaprim increased proportionally with dose, the $t_{1/2}$ (2–4 h) and clearance were independent of dose; plasma protein binding of iclaprim in vitro was 92% to 94% over a wide range of concentrations.2 Moreover, iclaprim has been shown to be active against organisms resistant to trimethoprim. In the treatment of respiratory infection, it is important that adequate concentrations of drug be achieved at potential sites of infection of the respiratory tree.3 The aim of this study was to measure concentrations of iclaprim in plasma and compartments of the respiratory tree at three time intervals after a single 1.6 mg/kg intravenous infusion.
Materials and methods

Subjects

Twenty-four healthy men were enrolled into the study (mean age 25.3 years, range 19–39 years; mean weight 72.8 kg, range 58.8–87.4 kg), at Hammersmith Medicines Research at the Central Middlesex Hospital in London, UK. The Brent Medical Ethics Committee approved the study, and all subjects gave written informed consent. All subjects were screened within 21 days before bronchoscopy: screening included a detailed medical history, physical examination, blood samples for haematological and biochemical analysis, urinalysis and 12-lead ECG.

Dosing with iclaprim

Each subject received a single dose of 1.6 mg/kg iclaprim in 150 mL normal saline, given as an iv infusion over 60 min.

Sampling times

The times (acceptable ranges) between the start of the iclaprim infusion and the start of bronchoalveolar lavage (BAL) in Groups A, B and C were 1.5 (1–2), 3 (3–4), and 6 (5.5–6.5) h, respectively.

Sample collection

Bronchoscopy samples were collected as previously described. Briefly, using standard procedures bronchial mucosa (BM) and BAL were taken during bronchoscopy. For BAL collection, 200 mL of pre-warmed 0.9% saline was divided into four 50 mL aliquots. The aspirate from the first aliquot was discarded to avoid contamination of the sample with proximal airway fluid and cells; the remainder was pooled for analysis.

Microbiological assay

The microbiological assay was a modification of the method described by Allen and Nimmo-Smith. Bacillus pumilis (NCTC 8241) spore suspension (spore count 7 × 10⁷ cfu/mL) 125 µL was added to 150 mL of Iso-Sensitest agar (Oxoid, Basingstoke, UK) and then poured into an assay plate (Mast Diagnostics, Bootle, UK). Antibiotic calibrators were prepared in pooled human serum (range 0.02–0.64 mg/L), pH 7 phosphate buffer (range 0.02–0.32 mg/L), 0.9% NaCl (range 0.02–0.32 mg/L) and 9% NaCl (range 0.02–0.32 mg/L). Internal control samples (0.5, 0.2, 0.06 and 0.03 mg/L for human serum and 0.2 and 0.03 mg/L for pH 7 buffer, 0.9% and 9% NaCl) and quality assurance samples (range 0.048–0.64 mg/L for human serum, 0.024–0.29 mg/L for pH 7 buffer and 0.028–0.27 mg/L for 0.9% and 9% NaCl) were included with every batch of urea estimations were within ±13.4% of the assigned concentration. Ninety-six percent (143/148) of the quality assurance samples were within ±15% of the nominal concentration. Control urea samples included with every batch of urea estimations were within ±15% of the assigned concentration. The mean urea concentration in BAL for 22 subjects was 0.039 mmol/L (range <0.01–0.083 mmol/L), urea not being detected in BAL samples from subjects 24 and 22. In the absence of urea as an endogenous marker, these samples could not be evaluated for iclaprim concentrations (see calculation above). The mean macrophage count and bronchial weight were 1.1 × 10⁵ cells/mL (range 0.5–2.53 × 10⁵ cells/mL) and 9.4 mg (range 3.3–16.7 mg), respectively. Iclaprim was not detected in the 3.3 mg BM sample for subject 8. It is presumed that the small sample weight precluded the measurement of the drug. Unlike other agents that this group has studied, the drug could be measured in all but three of the BAL samples (subjects 20, 1002 and 1004, who underwent BAL at 6.30–6.40 h after start of iclaprim infusion) without the need for concentration by freeze-drying. Mean concentrations of iclaprim in plasma, BM, AM and ELF, respectively, for the three nominal time windows were for 1–2 h (Group A) 0.59 mg/L (SD 0.18), 0.51 mg/kg (SD 0.17), 24.5 mg/L (SD 21.2) and 12.6 mg/L (SD 7.3); 3–4 h (Group B) 0.24 mg/L (SD 0.05), 0.35 mg/kg (SD 0.17), 7.16 mg/L (SD 1.9) and 6.38 mg/L (SD 5.2); 5.5–7.0 h (Group C) 0.14 mg/L (SD 0.05), no detectable level in all samples tested, 5.3 mg/L (SD 2.3) and 2.7 mg/L (SD 0.05). Individual subject’s results are shown in Table 1. Iclaprim infusion was well tolerated, as was the BAL procedure.

Discussion

Published studies that investigate the penetration of trimethoprim into the respiratory tree are few, and those found looked at concentrations in saliva, bronchial secretions and normal and pathological lung tissue. None has investigated concentrations in ELF, BM and AM.

Calculation of iclaprim concentrations in epithelial lining fluid (ELF), alveolar macrophages (AM) and BM

Concentrations of antibiotic were calculated using the following formulae:

**Bronchial mucosa**

\[
AC \times \left(\frac{VB + WS}{WS}\right) = \text{concentration (mg/kg tissue)}
\]

where AC, assayed concentration (mg/L); VB, volume of buffer added to homogenize the sample (μL) and WS, weight of tissue (mg).

**Epithelial lining fluid**

The concentration of urea in BAL was determined using a modified Infinity Kit (Alpha Laboratories, Eastleigh, UK).

\[
ACL \times \frac{(BL/UL)}{\text{ELF concentration (mg/L)}} = \text{ELF concentration (mg/L)}
\]

where ACL, assayed concentration in lavage (mg/L), UL, urea concentration in lavage (mmol/L) and BL, blood urea concentration (mmol/L).

**Alveolar macrophages**

Antibiotic concentration in AMs was determined using a mean cell volume of an alveolar macrophage of 2.48 μL/10⁶ cells.
Co-trimoxazole has in vitro activity against *S. pneumoniae* (MIC distribution for the population lacking a mechanism of resistance 0.12–0.5 mg/L; in-house data), however the British Society for Antimicrobial Chemotherapy (BSAC), following the Committee on the Safety of Medicines recommendations, suggests that the combination should be used only in specific circumstances. In contrast, trimethoprim MICs (MIC distribution for the population lacking a mechanism of resistance 4–16 mg/L; in-house data) exceed the BSAC breakpoint of 0.5 mg/L and *S. pneumoniae* are therefore deemed resistant.

This study has demonstrated that iclaprim concentrations in plasma, ELF and AM exceed the iclaprim MICs for penicillin-susceptible *S. pneumoniae* (MIC₉₀ 0.06 mg/L) for up to 7 h and that mean iclaprim concentrations in ELF exceed the iclaprim MICs observed for *S. pneumoniae* with penicillin-intermediate *S. pneumoniae* MIC₉₀ 2 mg/L and penicillin-resistant *S. pneumoniae* MIC₉₀ 4 mg/L, for up to 7 and 4 h, respectively. For *Haemophilus influenzae* (MIC₉₀ 4 mg/L) and *Moraxella catarrhalis* (MIC₉₀ 8 mg/L), mean iclaprim concentrations exceeded MICs for these organisms for 7 and 4 h after the start of infusion, respectively, in AM. Furthermore, the MIC₉₀ for methicillin-resistant *Staphylococcus aureus* of 0.12 mg/L was exceeded at all sites for up to 7 h. We have shown a high penetration of iclaprim into AM that would suggest that this drug should exhibit efficacy against *Chlamydia pneumoniae* (MIC₉₀ 0.5 mg/L).

These data suggest that iclaprim might be effective for the treatment of respiratory infections, including those caused by pneumococci with low susceptibility to penicillin.

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Transparency declarations

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References