Cephalosporin clinical concentration–time profile modelling and in-vitro bactericidal effects on *Escherichia coli*

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We assessed the cephalosporin concentration–time curve area (AUC), peak concentration, maintained concentration and duration of exposure on in-vitro bactericidal effects on *Escherichia coli* NCTC 10418, using exposures modelling cephazolin clinical profiles after 1 g and 2 g im injection, equal AUC exposures (288 mg·h/L, 576 mg·h/L; 48 h) and constant exposures to 6, 12 and 24 mg/L. Cephalosporin dosage exposures based on maintenance of concentrations at multiples (6–24 times) of the MIC were not as effective in early or sustained (24 h) bactericidal effect as exposures modelling im injection profiles with equal or lower AUC \((P < 0.05, \text{ANOVA})\). Similar results applied to im comparisons with equal AUC exposures modelling extremes of concentration and time exposures. These results indicate a need for intermittent dosage to produce optimally effective profiles, and raise the possibility that these optimum dosing profiles may allow an extension of minimum interdose intervals beyond 8 h.

Introduction

Dependence of the bactericidal effect of \(\beta\)-lactam antibiotics in general, and cephalosporins in particular, on antibiotic concentration, time of exposure or area under the serum concentration–time curve (AUC) is important for dosage design.\(^1,2\) Reported results are variable and contradictory. Some workers have reported that in-vitro Gram-negative bactericidal activity increased with cephalosporin concentration\(^3–7\) and similar results have been reported in animal studies where bactericidal effect correlated with the extent that minimum bactericidal concentrations were exceeded.\(^8\) In contrast, others have reported that cephalosporins exhibited largely time-dependent bactericidal effects which were independent of concentration except for early bactericidal action.\(^9–11\) Similar contradictions apply to the issue of the dependence of cephalosporin efficacy on AUC. Vogelman and coworkers\(^12\) reported strong time dependence and weak AUC dependence, while other investigators have found continuous administration to be more effective than higher doses at intermittent intervals.\(^13\) In contrast, Lavoie and Bergerson found the opposite results for efficacy between continuous and intermittent dosage regimens, suggesting dependence on AUC rather than the pharmacokinetic profile of exposure.\(^14\) Inevitably such conflicting evidence has led to a variety of views as to the most efficacious method for clinical delivery of cephalosporins. These range from the view that antibiotic concentration should be maintained above the MIC via constant infusion or frequent intermittent dosing\(^15–17\) through the use of high peak concentrations on an intermittent basis, to advocacy of large doses as an initial therapy.\(^14,18\)

The aim of this study was to model clinical cephazolin concentration–time profiles in vitro as a benchmark of exposure, then to systematically re-examine the individual influence of AUC, peak and maintained antibiotic concentration, ratio of concentration(s) to MIC, and duration of exposure on the action of cephalosporins.

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**Materials and methods**

**In-vitro bacterial culture**

Escherichia coli NCTC 10418, with an MIC of cephazolin of 1 mg/L, were cultured under standard conditions in the presence and absence of standardized concentration-time profiles of cephazolin (Eli Lilly, West Ryde, NSW, Australia). An overnight culture of E. coli in Brain-Heart Infusion Broth (BHIB) (Oxoid, Basingstoke, UK) was diluted to 10^7 cfu/mL in 0.1% peptone water (Difco Laboratories, Detroit, MI, USA) and a 1 mL sample of the 10^7 cfu/mL culture was added to the experimental culture broth resulting in an initial density of 10^6 cfu/mL. Viable counts were determined from colony formation on the surface of nutrient plates (Oxoid). The lower limit of detection was 20 cfu/mL.

**Modelling of 1 g and 2 g im cephazolin post-injection profiles**

In-vitro modelling of clinical exposures following 1 g im dosing involved determination of the AUC from published data using the trapezoidal rule, followed by design of an equal AUC profile approximating the clinical profile (Figure 1 (i)a). Cephazolin in BHIB was added in four increments over 30 min to produce a peak of 60 mg/L (Figure 1 (i)a). After 2 h, a 1:1.5 dilution was made to reduce the concentration to 40 mg/L, then cephazolin concentrations were halved every 2 h until 10.5 h. The concentrations were halved again at 24 h (Figure 1 (i)a). Samples for the determination of viable cell counts were taken at 0 and 0.5 h then two hourly for 10.5 h and at 24 and 48 h. For the 2 g im dose profile, all drug concentrations were doubled assuming linear extrapolation of data (Figure 1 (i)b).

**Bacterial exposure to constant AUCs equivalent to 1 g and 2 g profile exposures**

In studies of time and concentration dependence, the cephazolin AUC was kept constant while time and concentration were varied. Maintaining a constant AUC of 288 mg·h/L (the AUC associated with a 1g im injection) cephazolin concentrations of 288, 48, 24, 12 and 6 mg/L were kept for 1, 6, 12, 24 and 48 h, respectively, after which time the drug was washed out by centrifuging the culture twice for 12 min at 3.2 × 10^3 rpm. The E. coli pellet was resuspended in drug-free BHIB after each centrifugation and incubated at 37°C for the remainder of the experiment. To determine viable cell counts, samples were taken at 0, 1, 2, 4, 6, 8, 10, 12, 24 and 48 h post-dose. The AUC studies related to the 2 g im profile (576 mg·h/L) involved double the concentrations of the above profiles.

**Cephazolin stability studies**

The stability of cephazolin in the culture medium was studied by addition of cephazolin as cefazolin sodium powder (Lot No. A 7080A; Eli Lilly, Indianapolis, IN, USA) to broth at concentrations across the range 6–24 mg/L with incubation at 37°C for 72 h. Samples were taken at 0, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0, 24.0, 48.0 and 72.0 h. Samples were immediately frozen at -20°C until assay, and assays were performed within 20 min of thawing each sample.

Cephazolin determinations were performed using an HPLC system (Waters Associates, Milford, MA, USA), consisting of a Model 501 Pump and a Model 440 UV absorbance detector at 254 nm with an Omniscribe chart recorder (Houston Instruments, TX, USA). Cephazolin samples (20 μL) were injected directly on to the column and chromatographed on a Waters C-18 10 μm radial-pak column with a mobile phase of 18% methanol, 82% 0.03 M phosphate buffer at a flow rate of 2.0 mL/min. The assay was linear over the range 2–25 mg/L with an intra-assay CV of 10.78 % at 2 mg/L and 2.1% at 24 mg/L.

The stability of the cephalosporin was high with no significant between-sample change in concentration detectable over a 72 h period at concentrations of 6 and 12 mg/L. Among the 24 mg/L replicates there was a small (13%) but significant difference between the 24 h and the 48 h and 72 h samples; however, the 48 and 72 h samples did not differ from each other.

**Determination of pharmacokinetic parameters and statistical analysis**

The AUC was calculated using the trapezoidal rule and a one-way Tukey A NOVA (Minitab Release 8, Minitab Inc, PA, USA) was used for statistical analysis using a significance level of 0.05.

**Results**

E. coli growth after exposure to concentration-time profiles modelling 1 g and 2 g im doses

Control studies of E. coli growth from seeding concentrations of approximately 10^6 cfu/mL showed a plateau at or
near $10^9$ cfu/mL (Figure 1 (ii)a,b). Exposure to concentration modelling 1 g and 2 g im dosing with cephalozin caused initial bactericidal effects from 0.5 h. Live colony counts fell from $10^6$ to $10^3$ within 30 min, and continued to decline until 10.5 h (Figure 1). Early (2.5 h) bactericidal action was concentration-dependent (Table). There were no detectable live colonies from plated subcultures at 8.5 h for the 2 g profile and at 10.5 h with both 1 g and 2 g exposure modelling. This was followed by a variable recovery process measured at 24 h and 48 h (Figure 1 (ii)a,b). There were individual between-dose differences in subculture colony count means at 24 and 48 h. However, there was considerable variation in response and no systematic differences between the sustained effects of 1 g and 2 g profiles (Table).

E. coli growth after exposure to constant AUC profiles equivalent to 1 g and 2 g dose modelling

The dependence of cephalosporin effect on concentration and time was investigated by exposures designed to reflect the opposite extremes of maximum concentration exposure and maximum time exposure. Exposure to cephalozin for 1 h caused a concentration-dependent effect ($P < 0.05$; Figure 2a,b). With maintained concentrations of cephalozin, bacterial populations continued to decline steadily to levels at or near $10^2$ cfu by 8 h. After 12 h variable regrowth resumed (Figure 2). Continuing 24 h exposure to 6, 12 and 24 mg/L cephalozin failed to prevent resumption of growth (Figure 2c).

There were systematic differences in the time–kill responses to the 1 g and 2 g clinical profiles at 2.5 h and 24 h compared with the corresponding responses to equal AUC exposures (Table).

E. coli growth after maintained concentration exposure

Exposure to cephalozin, maintained at concentrations of 6, 12 and 24 mg/L, caused a sustained decrease in cfu over the first 6 h (Figure 2c). Cultures exposed to 12 and 24 mg/L declined until 8 and 10 h, respectively, with variable recovery to 48 h (Figure 2c).

Figure 1. (i) Cephalozin concentration-time profiles under in-vitro conditions and (ii) kill kinetics and regrowth patterns of E. coli following (a) a cephalozin 1 g im profile (□) and (b) a cephalozin 2 g im profile (○). A control profile (antibiotic free; ○) was also run in parallel. For all studies, $n = 6$. 

473
There was clear-cut concentration dependence of initial (1 h) exposure over these exposure ranges (P < 0.05). However, the trend to systematic continuing differences did not reach statistical significance.

**Discussion**

The major outcome of this study was the finding of a quantitative difference in the time–kill responses to im injection profiles compared with all combinations of maintained equivalent AUC profiles or maintained exposures to constant supra-MIC concentrations over the range 6–24 mg/L for 24 h. Early (2.5 h) bactericidal activity was found to be concentration-dependent.

Upon exposure to constant AUC profiles, an initial (1 h) concentration-dependent bactericidal effect was observed with all cephazolin profiles. Such concentration dependence of the initial antibacterial activity of cephalosporins is in agreement with the conclusions of several studies.\(^3,4,11,22\) Maintained concentrations were most efficacious for continued suppression of bacterial growth over 24 h in these AUC profile studies.

Where cephazolin concentrations of 6, 12 and 24 mg/L were maintained for 48 h, there were initial (1 h) concentration-dependent differences between the results of these three concentrations. While there was a trend to earlier recovery of bacterial growth in cultures exposed to lower concentrations, these differences were not statistically significant.

The most effective cephazolin exposure profile was that based on the im injection profile, hence, further analysis of the exposure parameters of the im injection profile is needed in order to identify the major determinants of...
bactericidal efficacy of cephalosporins beyond concentrations, A U C and time of exposure.

Our results showing regrowth of bacteria despite concentrations maintained well above M I C indicate that the design of cephalosporin dosage schedules based principally upon the maintenance of concentrations above the M I C may not be adequate. The current general clinical practice of intermittent dosing should be maintained in the interim. If the responses to the im dose profiles presented here represent empirical evidence of an additive effect between bactericidal responses to dynamically variable initial concentrations and maintained concentrations, then this principle may represent a basis for improving the efficacy of cephalosporins. Equally the results from the im profiles could provide a basis for allowing the extension of interdose intervals with cephalozolin and other cephalosporins beyond 8 h to 12 h.

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References


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