Use of a clinical *Escherichia coli* isolate expressing lux genes to study the antimicrobial pharmacodynamics of moxifloxacin

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**Introduction**

The pharmacodynamics of antimicrobial agents are usually studied by observing changes in viable counts of bacteria over time for a range of drug concentrations, demonstrating the ability of the agent to prevent bacterial cell replication. However, it is useful to monitor effects on metabolic activity, rather than replication, and these have been observed indirectly by adding luciferase and luciferin externally to monitor intracellular ATP of bacterial cells after treatment with antibiotics. Bacterial *lux* genes of different origins have been expressed successfully in a number of Gram-negative bacteria and provide a simple, direct means of monitoring metabolic activity. We describe transfer of *lux* genes into a clinical isolate of *Escherichia coli*, using the recombinant plasmid pLITE27. This plasmid carries a 7 kb *EcoRI* fragment, containing the *luxCDABE* operon of *Xenorhabdus luminescens* cloned into pUC18, allowing transcription of the *lux* genes from the *lac* promoter of pUC18.

Our aim was to observe the effects of moxifloxacin on *E. coli* metabolic activity, measured by bioluminescence, as compared with its effects on cell replication, measured by viable counts. We also wished to evaluate the use of bioluminescent bacteria to determine the post-antibiotic effect (PAE) and control-related effective regrowth time (CERT) of moxifloxacin.

**Materials and methods**

**Bacteria and plasmids**

*E. coli* 16906 was a clinical isolate from Southmead Hospital (Bristol, UK), maintained on nutrient agar. *E. coli* DH5(pLITE27) was a gift from F. Marincs (Grasslands Research Centre, Palmerston North, New Zealand) and was maintained on nutrient agar containing ampicillin 50 mg/L.

**Antibiotics and media**

Moxifloxacin (BAY 12-8039) was from Bayer AG (Wuppertal, Germany); ampicillin was from Sigma (St Louis, MO, USA). Nutrient agar (NA) and iso-sensitest broth (ISB) were from Oxoid (Basingstoke, UK). Luria–Bertani (LB) broth and LB agar were supplemented throughout with ampicillin at 50 mg/L and 100 mg/L, respectively.

**Electroporation of *E. coli* 16906**

A single colony of *E. coli* DH5 (pLITE27) was transferred to 10 mL LB broth and incubated at 37°C overnight with shaking. Rapid preparation of pLITE27 plasmid DNA was carried out.

The recipient *E. coli* 16906 was grown overnight in 2 mL LB broth without ampicillin and washed cells were trans-
formed with pLITE27, using a Bio-Rad Gene Pulser (Bio-Rad, Richmond, CA, USA).

Concentration-dependent killing

The MIC of moxifloxacin for *E. coli* 16906 (pLITE27) was determined by broth macrodilution. Ten millilitres ISB plus ampicillin at 50 mg/L were inoculated with a single colony of *E. coli* 16906 (pLITE27) and incubated at 37°C overnight with shaking; 0.5 mL of the overnight culture was added to each of five bottles of 50 mL of pre-warmed ISB (without ampicillin). Bioluminescence was measured by removing 1 mL samples to a luminometer (BioOrbit 1250; BioOrbit, Turku, Finland); samples were held at 37°C and mixed (LKB Wallace 1250-105 Mixer; Milton Keynes, UK) during measurement. Viable counts were carried out using a spiral plater (Autoplate Model 3000; Spiral Biotech, Bethesda, MD, USA) and plated onto NA plus ampicillin 50 mg/L plus 1% MgCl₂.

At time zero, $1 \times $MIC, $4 \times $MIC, $10 \times $MIC or $16 \times $MIC of moxifloxacin were added to each of four bottles, leaving the fifth as a control. The bottles were incubated at 37°C with shaking for 54 h, sampling for bioluminescence and viable counts at hourly intervals for 9 h and then at 24, 48 and 54 h.

Measurement of bacterial regrowth

Ten millilitres ISB plus ampicillin 50 mg/L were inoculated with a single colony of *E. coli* 16906 (pLITE27) and incubated at 37°C overnight with shaking; 0.1 mL of overnight culture was added to three bottles of 10 mL of ISB (without ampicillin). Bioluminescence and viable counts were measured and, at time zero, $4 \times $MIC and $10 \times $MIC of moxifloxacin were then added to each of two bottles, leaving the third as a control. After incubation at 37°C with shaking for 30 min, 1 mL samples from each bottle were microfuged for 1 min. The bacterial pellet was washed in 1 mL pre-warmed ISB, microfuged again and finally re-suspended in 1 mL fresh pre-warmed ISB; 0.5 mL of this suspension was added to 50 mL of pre-warmed ISB (without ampicillin) and incubated at 37°C with shaking. Samples were immediately removed for bioluminescence determination and viable counting, and thereafter these measurements were taken at half-hour intervals for 12 h.

PAE¹ and mCERT² were calculated from the regrowth curves, by calculating the difference in time taken by treated and untreated cultures to increase one log above the resuspended concentration (PAE) or the initial concentration (mCERT).

Results

Electroporation

Approximately 120 colonies grew on LB agar + ampicillin 100 mg/L; all emitted visible light; single purified colonies showed identical API profiles to *E. coli* 16906. Transformants showed strong constitutive light emission when growing exponentially in ISB at 37°C. Viable counts and bioluminescence readings over 24 h showed no significant difference with and without ampicillin selection, indicating that the plasmid was stable in the absence of ampicillin. Addition of hypochlorite to bioluminescent cultures or removal of cells by filtering gave luminometer readings of 0 mV, indicating that bioluminescence readings were from metabolizing cells.

Concentration-dependent killing

The MIC of moxifloxacin for *E. coli* 16906 (pLITE27) was 0.06 mg/L. Viable counts (Figure 1a) indicated concentration-dependent killing. However, bioluminescence measurements (Figure 1b) showed more inhibition with $1 \times $MIC moxifloxacin, compared with four-, 10- and 16-fold higher concentrations. The viable counts in Figure 1 also indicated that no replicating cells remained after 7 h for

![Figure 1. Activity of moxifloxacin against E. coli 16906 (pLITE27). Antibiotic concentrations used were 1 × MIC (⧫), 4 × MIC (⧫), 10 × MIC (⧫), 16 × MIC (⧫) and antibiotic-free control (⧫). (a) Measured by viable counts; (b) measured by bioluminescence.](image-url)
Bioluminescent *E. coli* to study moxifloxacin effects

16 × MIC and 24 h for 4 × MIC and 10 × MIC. In direct contrast to this, bioluminescence measurements showed evidence of persistent metabolic activity after 54 h for all concentrations of moxifloxacin.

**Bacterial regrowth after exposure to moxifloxacin**

Regrowth occurred after 30 min exposure to 4 × MIC and 10 × MIC of moxifloxacin followed by washing and diluting 1:100 in fresh broth (Figure 2). For 10 × MIC the viable counts dropped between 1 and 2.5 h, which was not seen in bioluminescence measurements. The PAE was method dependent for 4 × MIC (95 min with viable counts, 150 min with bioluminescence) and for 10 × MIC (100 min with viable counts, 390 min with bioluminescence).

However, the CERT values for both methods of measurement were similar (Figure 2). The CERT with 4 × MIC was 160 min with viable counts and 150 min with bioluminescence. At 10 × MIC it was 380 min measured by bioluminescence and 370 min measured by viable counts.

**Discussion**

Although bioluminescent bacteria have been used to monitor antimicrobials, they have not previously been used to determine PAE or CERT. Our results suggest that clinical isolates expressing *lux* genes have potential use for rapid, real-time evaluation of drug dosage regimens and that CERT determined by this method is an estimate of recovery of both bacterial metabolism and replication. Moxifloxacin (BAY 12-8039) is an 8-methoxyquinolone that showed concentration-dependent killing on colony counts of *E. coli* 16906 (pLITE27). In contrast, its effect on the bioluminescence of this isolate was not concentration dependent: 1 × MIC resulted in a much greater inhibition of metabolic activity than higher concentrations. For clinical isolates, producing virulence factors, this has relevance to treatment of established infections. A low concentration of moxifloxacin which inhibits metabolism and therefore virulence factor production may be more satisfactory than higher dosage regimens. This type of ‘Eagle effect’ has been previously noted with ciprofloxacin. It may be caused by uncoupling of metabolism from transcriptional control at high concentrations.

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**References**


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