Mechanisms of the post-antibiotic effects induced by rifampicin and gentamicin in Escherichia coli

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Objectives: The mechanisms by which antibiotics induce a post-antibiotic effect in susceptible bacteria are poorly understood. To explore the mechanisms more fully we examined the recovery of macromolecular synthesis in Escherichia coli during gentamicin- and rifampicin-induced post-antibiotic effects.

Methods: E. coli ATCC 25922 was exposed to rifampicin and to gentamicin at 5x MIC for 60 min to induce post-antibiotic effects. The antibiotics were then removed from the culture medium by washing the cells. The rates of DNA, RNA and protein synthesis during the post-antibiotic effect and recovery periods were subsequently determined by measuring the incorporation of radiolabelled uridine, thymidine and leucine into trichloroacetic acid precipitable material.

Results: Recovery of E. coli ATCC 25922 from the rifampicin-induced post-antibiotic effect coincided with the recovery of RNA and protein synthesis. Recovery from the gentamicin-induced post-antibiotic effect coincided with the recovery of protein synthesis.

Conclusions: These data support the hypothesis that antibiotic molecules retained in the cell mediate the post-antibiotic effect by suppressing the biochemical activity of their molecular targets.

Keywords: E. coli, PAE, macromolecular synthesis, antibiotic action

Introduction

The continued suppression of bacterial growth following limited exposure to an antimicrobial agent was first noted nearly 60 years ago.1 The term post-antibiotic effect has now become the accepted description of this phenomenon which results from prior exposure of organisms to an antibiotic, rather than persistence of sub-minimal inhibitory concentrations (sub-MICs) of drugs in the medium.1 Determination of the post-antibiotic effect is now an important part of preclinical evaluation of new antibiotics because it is a factor that influences antibiotic dosing intervals.2 However, relatively little is known about the molecular basis of the post-antibiotic effect or the events that lead to restoration of normal growth at the end of the post-antibiotic effect.3

Inhibitors of protein and nucleic acid synthesis including aminoglycosides, tetracyclines, macrolides, fluoroquinolones and rifampicin induce lengthy post-antibiotic effects in Escherichia coli.1 In the few cases examined, recovery from the post-antibiotic effect correlates with re-establishment of the process targeted by the inducing antibiotic. Thus, recovery from the post-antibiotic effect induced by the aminoglycoside tobramycin in E. coli depends upon re-establishment of protein synthesis,3 and recovery from the ciprofloxacin-induced post-antibiotic effect depends upon restoration of DNA synthesis.4 To explore post-antibiotic effect mechanisms more fully we have chosen two further antibiotics, rifampicin and gentamicin, to examine whether recovery from the post-antibiotic effect induced by these agents also depends on recovery of the processes targeted by these antibiotics, namely RNA synthesis for rifampicin and protein synthesis for gentamicin.

The results reported here are consistent with our earlier hypothesis that resumption of growth after the post-antibiotic effect period reflects the time taken for antimicrobial agents to dissociate from their targets and be lost from the cell, releasing sufficient target molecules for growth to resume.5

Materials and methods

Bacterial strain and growth medium

E. coli ATCC 25922 was obtained from the American Type Culture Collection (ATCC) and was grown in M9 minimal salts medium6 supplemented with thiamine (2 mg/L).

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Figure 1. Rates of synthesis (squares) of RNA (uridine) (a), DNA (thymidine) (b) and protein (leucine) (c) in *E. coli* ATCC 25922 during the post-antibiotic effect induced by exposure to 5x MIC of rifampicin. Culture OD (diamonds) is displayed during both the post-antibiotic effect and re-growth periods. Control rates of synthesis (triangles) prior to drug exposure were determined by measuring incorporation of precursors into unexposed control cultures. Values displayed are mean (n = 3) determinations. Error bars represent standard deviations calculated for these readings.
Antibiotics, chemicals and radiochemicals

Antibiotics and chemicals were obtained from standard commercial sources. The following radiochemicals were purchased from Amersham Life Sciences, Little Chalfont, UK: [Me-3H]thymidine (70–95 Ci/mmol), [5,6-3H]uridine (31–56 Ci/mmol) and l-[4,5-3H]leucine (120–190 Ci/mol).

Determination of susceptibility of E. coli ATCC 25922 to antimicrobial agents and induction of the post-antibiotic effect

MICs were determined by broth microdilution in supplemented M9 minimal salts medium using an inoculum of 10^3 cells/mL in a final volume of 70 μL. Microtitre plates (384 wells) containing triplicate
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2-fold dilution series were incubated for 16 h at 37°C in a Spectramax 384 plus microtitre plate reader (Molecular Devices, Abingdon, Oxfordshire, UK), running SOFTmax® PRO 3.1.1 software. Optical density readings (600 nm) were taken at 10 min intervals. Plates were shaken for 30 s before each reading. The MIC was taken as the lowest concentration of antibiotic that prevented growth in the triplicate wells. Susceptibility determinations were performed on three separate occasions and the mean of these values recorded as the MIC.

Post-antibiotic effects were induced by exposing bacteria (2 × 10⁵ cells/mL) in the early logarithmic phase to 5× MIC of each drug for 60 min, followed by washout and re-suspension of bacteria in fresh growth medium at 37°C as described previously.⁷

Incorporation of radiolabelled precursors into macromolecules

E. coli ATCC 25922 was grown to early logarithmic phase (2 × 10⁶ cells/mL) in M9 minimal salts media and then exposed to rifampicin and gentamicin at 5× MIC for 60 min. The cultures were then washed three times in fresh M9 medium (pre-warmed to 37°C) to remove antibiotics. Samples were taken before the addition of antibiotics and periodically during the post-antibiotic effect and recovery periods to establish by pulse-labelling the rates of macromolecular synthesis under the various conditions. For pulse-labelling, samples of culture (450 µL) were added to tubes containing 2 µCi of [Me-³H]thymidine (DNA synthesis), [5,6-³H]uridine (RNA synthesis) or [L-¹⁴C]-leucine (protein synthesis) followed by incubation at 37°C in an orbital waterbath for 5 min at 200 rpm. Ice-cold trichloroacetic acid (TCA) (10% w/v) was then added to the tubes, which were left on ice for 30 min. The TCA-precipitable material was collected on 25 mm GF/C glass microfibre filters which were then washed twice with 10% w/v TCA (5 mL) and 1% v/v acetic acid (5 mL). Filters were dried overnight and then placed in vials containing 5 mL of Optiphase ‘Hisafe’ 2 liquid scintillation cocktail (Perkin Elmer, Beaconsfield, Bucks, UK), before counting in a TRI-CARB-2100TR liquid scintillation counter (Packard Biosciences, Berkshire, Pangbourne, UK). Macromolecular synthesis was expressed as disintegrations per minute (dpm) incorporated per OD₆₀₀ unit.⁴

Results

The MICs of rifampicin and gentamicin for E. coli ATCC 25922 were 4.0 and 0.5 mg/L, respectively. Measurement of culture optical density at 600 nm (OD₆₀₀) following removal of rifampicin and gentamicin revealed that each antibiotic induced a post-antibiotic effect of ~4 h (Figures 1 and 2). Exposure to 5× MIC of rifampicin for 60 min inhibited the rates of macromolecular synthesis in each case by over 80% (Figure 1). RNA synthesis remained at this inhibited level until 5 h, mirroring the stationary state of the cells (Figure 1a). The rate of RNA synthesis returned to control levels at 9 h. Protein synthesis followed a similar pattern of recovery to RNA synthesis since inhibition occurred throughout the post-antibiotic effect period and recovery began between 5 and 6 h after removal of rifampicin (Figure 1c). In contrast, DNA synthesis began to recover immediately after rifampicin was removed from the external environment and before the resumption of growth measured by the OD₆₀₀ (Figure 1b).

Exposure to 5× MIC of gentamicin for 60 min had different effects on the rates of DNA, RNA and protein synthesis. RNA synthesis (Figure 2a) was inhibited by only 60% following exposure to gentamicin. It remained inhibited for 1 h and then began to recover before the onset of growth. DNA synthesis (Figure 2b) was only inhibited by 30% at the beginning of the gentamicin-induced post-antibiotic effect. However, 70–80% inhibition of DNA synthesis was observed in the 1–2 h period following removal of gentamicin (Figure 2b). DNA synthesis began to recover between 2–3 h and, like RNA synthesis, before the onset of growth measured by the OD₆₀₀. Protein synthesis (Figure 2c) was inhibited by >95% at the beginning of the gentamicin-induced post-antibiotic effect but its recovery closely mirrored the resumption of bacterial growth.

Discussion

Data which describe the molecular events that occur in E. coli and other bacteria during the post-antibiotic effect and the immediate recovery period are relatively limited and the effects of rifampicin and gentamicin have not been reported previously.¹ However, in those cases where responses to other antibiotics have been examined, recovery from the post-antibiotic effect corresponds to re-establishment of the process which is the primary target of the inducing antibiotic.³⁻⁴,⁹⁻¹⁰

We found that recovery from the post-antibiotic effect induced by rifampicin in E. coli 25922 correlated with resumption of both RNA and protein synthesis. This is not surprising in view of the connection between transcription and translation. Furthermore, as the maximum half-life of mRNA in E. coli is ~20 min,⁹ mRNA synthesized prior to the addition of rifampicin would have been degraded by the time of antibiotic wash out (60 min). Recovery of protein synthesis would therefore have been dependent upon recovery of RNA synthesis, as observed.

Recovery of E. coli from gentamicin exposure was also consistent with recovery of the process inhibited by the inducing antibiotic. Inhibition of protein synthesis persisted throughout the post-antibiotic effect and recovered rapidly as the cells emerged from the post-antibiotic effect. These data are consistent with the observations of Barmada et al.,³ who showed that recovery of E. coli from the tobramycin-induced post-antibiotic effect depended on the recovery of protein synthesis.

Data presented in the present study are consistent with other published reports that the duration of the post-antibiotic effect is related to the resumption of the biochemical process(es) inhibited by the antibiotic that induced it. Furthermore, the observations reported here support our hypothesis that continued interaction of remaining antibiotic molecules with their intracellular targets is responsible for the post-antibiotic effect, and that resumption of growth after the post-antibiotic effect probably reflects the time taken for antibiotics to dissociate from their targets and be removed from the cell.³

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Transparency declarations

None to declare.
References


