LEADING ARTICLE

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Aminoglycoside Adaptive Resistance
Importance for Effective Dosage Regimens

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Abstract

There are various pharmacodynamic features of the aminoglycosides that are thought to contribute to the benefits of once-daily administration, of which the ability to induce adaptive resistance is the least understood and discussed. However, this may be the most important characteristic conferring increased efficacy with extended interval dose administration. Adaptive resistance describes a reversible refractoriness to the bactericidal effect of an antibacterial agent. It is well documented for the aminoglycosides but has also been seen with the quinolones. It does not appear to be caused by a genetic mutational change but rather by a protective phenotypic alteration in bacterial characteristics. This includes reversible down-regulation of the active transport of aminoglycosides into Gram-negative bacteria.

In vitro, animal and clinical studies have shown that marked adaptive resistance of Gram-negative bacteria to aminoglycosides occurs within 1-2 hours of the first dose. The duration of adaptive resistance relates directly to the half-life of elimination of the aminoglycoside. With normal human aminoglycoside pharmacokinetics, the resistance may be maximal for up to 16 hours after a single dose of aminoglycoside, followed by partial return of bacterial susceptibility at 24 hours and complete recovery at around 40 hours. With conventional dosage regimens, second and subsequent doses of aminoglycoside are given at the time of maximal resistance and this practice is also likely to reinforce the resistance. Dose administration at 24 hour intervals, or longer, may increase efficacy by allowing time for adaptive resistance to reverse.

The use of larger doses of aminoglycosides administered less frequently has come into favour in the past 10 years in the form of 'once-daily dose administration'. Meta-analyses of controlled clinical trials have shown that this form of administration has resulted in better efficacy and reduced nephrotoxicity when compared with multiple-daily administration.[1] The pharmacodynamic features of the aminoglycosides that have been proposed to explain the improved efficacy with extended interval dose administration include their concentration-dependent killing, long post-antibiotic effect (PAE) and adaptive resistance. Concentration-dependent bacterial killing describes the characteristic of increasing bactericidal effect with increasing peak plasma concentrations. This feature of the aminoglycosides could potentially result in increased efficacy with the use of larger doses given at extended intervals but it is not entirely obvious that 1 high peak concentration per day should be any more effective than 2 or 3 lower peaks, especially when the area under the concentration-time curve is similar for both regimens. The PAE refers to the period during which there is continued bacterial

killing or growth suppression after the antibacterial concentration falls below the minimum inhibitory concentration (MIC). Although PAE may allow extension of administration intervals, PAE also occurs with β-lactam and other antibacterials and seems insufficient to account for the benefits of very long dose intervals. In addition, while PAE may result in sustained efficacy, there is no clear reason why it should result in increased efficacy.

The features of adaptive resistance provide a more plausible explanation for the increased efficacy of extended interval dose administration and it is likely to be more important than the other two pharmacodynamic features, although the three phenomena may act in concert. Adaptive resistance is induced rapidly after a dose of aminoglycoside but is reversible over a number of hours, unlike true resistance. Prolongation of the dose interval may enable time for the return of bacterial susceptibility before the subsequent dose. Adaptive resistance is the focus of this article, from its *in vitro* discovery and characterisation through to clinical evidence of the phenomenon. An attempt is made to put its relative importance into perspective.

1. Definition of Adaptive Resistance

Adaptive resistance is a term that describes a reversible refractoriness to the bactericidal action of an antibacterial agent. It has been characterised best for the aminoglycosides against Gram-negative bacilli, and in particular *Pseudomonas aeruginosa*.[2-4] *P. aeruginosa* is one of the most common organisms in nosocomial infections that are difficult to treat, and has the highest mortality rate of any bacterium in this situation (40 to 93% for *P. aeruginosa* bacteraemia).[5] Aminoglycosides remain a cornerstone of therapy for this pathogen.

Adaptive resistance has also been seen with the quinolone agents against *P. aeruginosa*, Gram-positive cocci and a number of Enterobacteriaceae.[6-8] However, this type of resistance is less reliably produced with the quinolones than with the aminoglycosides, and the quinolones are more likely to induce true resistance early following bacterial exposure.

2. *In Vitro* Studies

In early studies characterising adaptive resistance, a culture of *P. aeruginosa* in the log phase of growth was exposed to a static concentration of an aminoglycoside (usually gentamicin) for between 1 to 2 hours and then the antibacterial was removed rapidly by repeated culture dilutions. The culture was then exposed to the aminoglycoside for a second time to assess susceptibility.[2-4] In these experiments, resistance increased over a 2-hour period following removal of the antibacterial after the first exposure, was maximal between 2 and 4 hours and there was gradual recovery of bacterial susceptibility thereafter. Baseline susceptibility was regained approximately 8 hours after the first drug exposure (fig. 1). The period of adaptive resistance was a little shorter for other Gram-negative bacilli including *Escherichia coli*, *Enterobacter aerogenes* and *Enterobacter cloacae*.[3,7] Bacteria with adaptive resistance induced by one aminoglycoside were shown to have cross-resistance to other aminoglycosides.[3]

Other research showed that when *P. aeruginosa* was exposed to gentamicin continuously, the bacteria with adaptive resistance could survive exposure to increasing concentrations of gentamicin, up to 128 times the MIC.[9] At this concentration, gentamicin would have killed all bacteria that had not had previous exposure to an aminoglycoside. During these continuous exposure experiments, small colony variants were produced on plating the bacteria on agar. However, when the bacteria were cultured in drug-free media the colonies returned to their original morphology and susceptibility.

Combining aminoglycosides with a second antibacterial agent has been shown to be more effective clinically than aminoglycoside monotherapy, especially in neutropenic sepsis.[10,11] This raises the possibility that agents from other classes prevent the induction of aminoglycoside adaptive resistance. *In vitro* experiments showed that adaptive resistance induction in *P. aeruginosa* by netilmicin or amikacin was reduced when rifampicin was added after the aminoglycoside exposure.[12] There are no published studies investigating the effect of

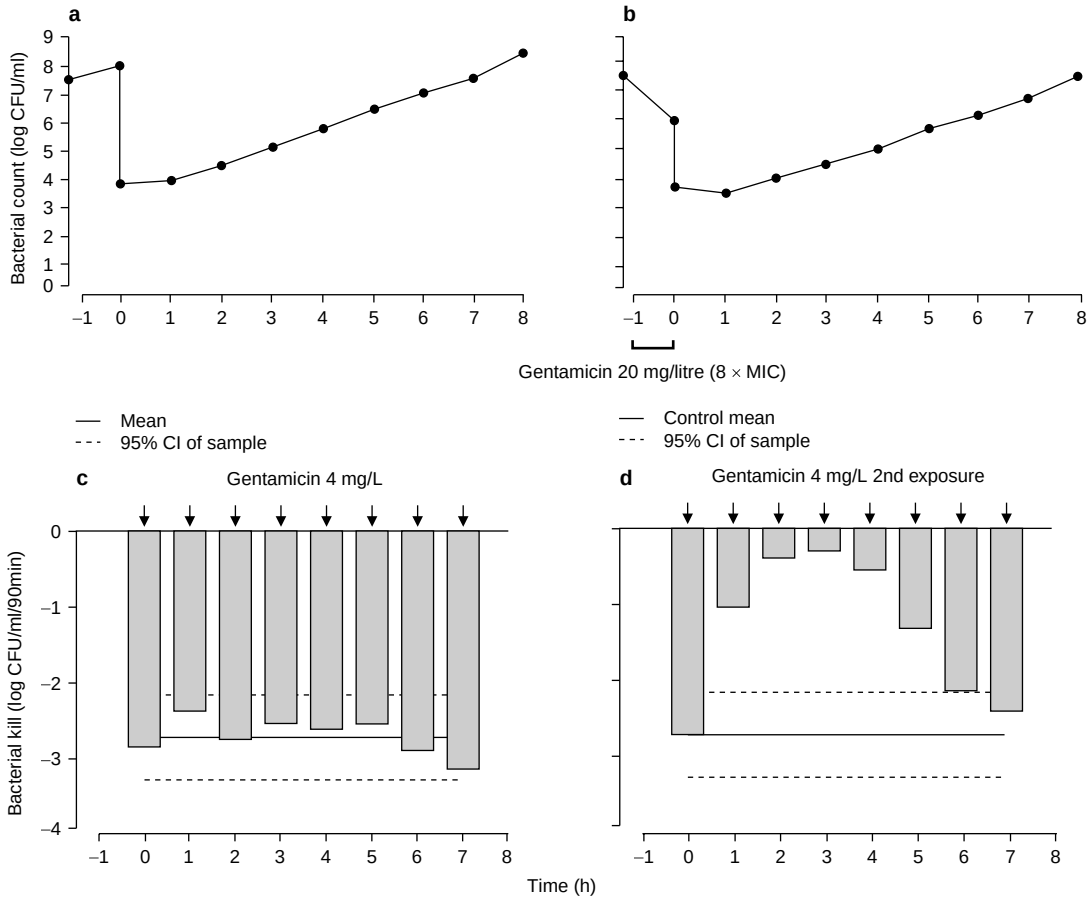


Fig. 1. Antibacterial effects of first and second exposure of *Pseudomonas aeruginosa* to gentamicin. (**a**) Bacterial growth of control culture with no prior gentamicin exposure. (**b**) Bacterial growth with 1 hour of pre-exposure to gentamicin (20 mg/L). Control and experiment cultures were diluted to 10^4 colony forming units (CFU) per ml at time zero. (**c** and **d**) Bacterial killing 90 minutes after addition of gentamicin 4 mg/L to culture samples removed at successive hourly intervals. (**c**) Bactericidal effect of first drug exposure on control culture. (**d**) Bactericidal effect of second drug exposure on experiment culture. Mean bacterial killing and 95% confidence interval (CI) for the control are superimposed on both graphs.[2] **MIC** = minimum inhibitory concentration.

other antibacterials on aminoglycoside-induced adaptive resistance, although we have found *in vitro* that adaptive resistance induced by gentamicin in *P. aeruginosa* was not reduced by concurrent administration of ceftazidime, ciprofloxacin, tetracycline or ticarcillin.[13]

Experiments have been conducted *in vitro* to determine whether the presence of adaptive resistance affects the activity of other antibacterials against *P. aeruginosa*. The antibacterial effects of ceftazidime, piperacillin, imipenem, aztreonam and ciprofloxacin were not altered in the presence of adaptive resistance induced by gentamicin but rifampicin was found to have a more rapid bactericidal effect.[14]

The experiments using static concentrations of aminoglycosides *in vitro* have been valuable in characterising aspects of adaptive resistance, and in particular have produced good evidence of its reversible nature and of the high degree of resistance that could be attained. However, they did not cast light on the time-course of resistance that

might be expected *in vivo*, when drug concentrations decrease with first-order kinetics after a dose of aminoglycoside.

2.1 Dynamic *In Vitro* Studies

A dynamic *in vitro* model of infection that mimics *in vivo* antibacterial pharmacokinetics has been used to investigate the time-course of adaptive resistance. A culture of *P. aeruginosa* in a central chamber was exposed to gentamicin in exponentially decreasing concentrations, with a half-life of elimination of 2.5 hours, the half-life of gentamicin in humans with healthy renal function.[2] The exponential decrease was achieved by the use of a computer-controlled syringe pump that introduced culture medium into the central chamber to dilute the antibacterial. Samples were removed automatically from the central chamber every hour in order to keep the central chamber volume constant and to allow analysis of bacterial susceptibility to gentamicin. The presence of adaptive resistance was determined by the response to a second exposure to gentamicin at 4 mg/L, following doses of gentamicin in the central chamber to achieve initial peak first exposure concentrations of 8 and 25 mg/L. These are the peak concentrations that commonly occur clinically after conventional multiple daily gentamicin administration and once-daily dose administration, respectively.

Adaptive resistance was induced during the first 2 hours after the first dose, as with the experiments using static concentrations of aminoglycoside. However, the resistance remained maximal for up to 12 hours following the peak concentration of 8 mg/L and up to 16 hours after 25 mg/L. The resistance then very gradually subsided so that baseline susceptibility was attained at approximately 40 hours after the dose (fig. 2). It appears that persistence of adaptive resistance prolongs adaptive resistance in direct relation to the concentration-time profile of the aminoglycoside. The duration of adaptive resistance in these experiments was 14 to 18 drug half-lives.

With conventional administration regimens, the second and subsequent doses are usually administered at dose intervals of 8 to 12 hours, at a time when resistance is maximal. It is likely that re-exposure to aminoglycoside at these times will not only result in poor bactericidal effect but will also reinforce the resistance. By comparison, at the 24-hour time-point in these experiments, susceptibility had largely returned, supporting the greater efficacy observed with once-daily administration.

In these dynamic *in vitro* experiments, some samples with adaptively resistant bacteria were exposed to high second dose concentrations of gentamicin, either 25 or 50 mg/L, and it was found that adaptive resistance could not be overcome. Bacterial kill was somewhat higher than with low concentration second exposure, however.

Additional measures were undertaken in these experiments to distinguish between the induction of adaptive resistance and true resistance. The MIC was found to be unchanged for culture samples containing adaptively resistant bacteria when compared with the original culture MIC. The process of MIC measurement involves culture dilution and growth in drug free media over a 16 to 24 hour period, which is long enough to allow reversal of adaptive resistance. The absence of change in the MIC after induction of adaptive resistance suggests that the resistance results from a transient phenotypic change in the bacteria rather than a genetic change.

3. *In Vivo* Animal Studies

Adaptive resistance has been shown to occur *in vivo* in mice with cyclophosphamide-induced neutropenia and *P. aeruginosa* thigh infections treated with netilmicin.[15] A second dose of netilmicin at 2 or 8 hours after the first dose resulted in significant bactericidal effect, but second doses at 4 or 6 hours did not. This was reflected by a 60 to 70% reduction in survival after 12 hours in the mice when second doses were administered at 4 or 6 hours. The duration of adaptive resistance in these experiments was equivalent to 15 to 20 drug half-lives (netilmicin half-life of 15 to 25 minutes in mice) which parallels the results from the dynamic *in vitro* model experiment in which adaptive resis-

tance resolved in 14 to 18 drug half-lives (half-life of 2.5 hours as in humans).

Adaptive resistance has also been documented to occur with amikacin in a rabbit model of *P. aeruginosa* endocarditis.[16] Aminoglycoside half-life in rabbits, at around 50 minutes, is between that in mice and humans. Maximal adaptive resistance in bacteria in excised aortic vegetations occurred between 8 and 16 hours after a dose of amikacin and there was complete refractoriness to amikacin at 12 hours. By 24 hours after the dose, bacteria within vegetations had partially recovered their initial susceptibility to amikacin. In a parallel treatment study, amikacin 80 mg/kg/day administered once-daily was more effective than twice-daily administration (of the same total daily dose) in reducing the density of *P. aeruginosa* in aortic vegetations.[16] The authors noted that the duration of adaptive resistance observed in these experiments was substantially longer than that observed *in vitro* and postulated that the disparity may relate to the persistence of aminoglycoside within cardiac vegetations.

4. Clinical Studies

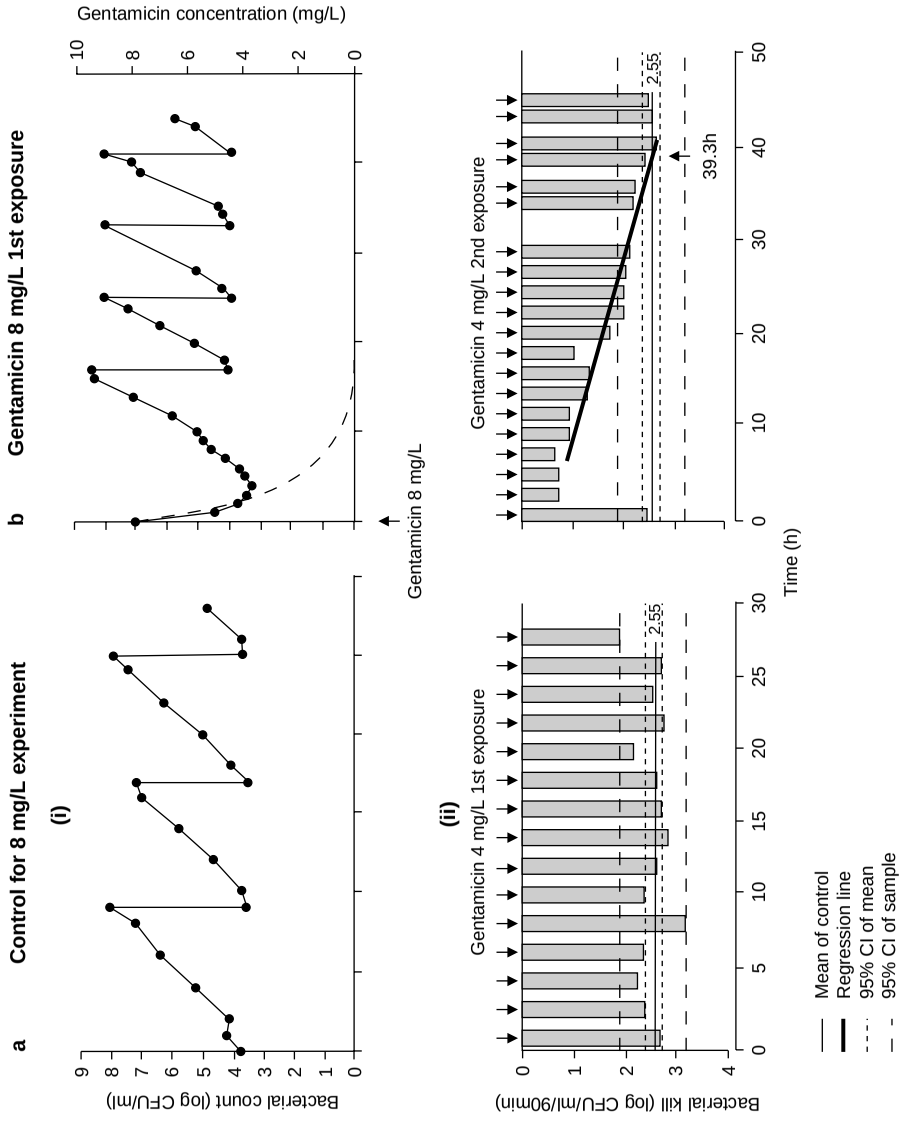
To our knowledge, there is only 1 published clinical study confirming the presence of adaptive resistance. Patients with cystic fibrosis and *P. aeruginosa* lung infection received a single dose of tobramycin by nebuliser. Sputum samples were then collected at predetermined time intervals, homogenised in culture medium and the culture was then exposed to a second dose of tobramycin for 90 minutes *in vitro* to assess bacterial susceptibility.[17] Adaptive resistance was shown to be present in the bacteria in the sputum within 1 hour of the nebulised dose of tobramycin and was still present at 24 hours. Susceptibility then returned at a time point between 24 and 48 hours. The time-course of the adaptive resistance was therefore very similar to that shown in the dynamic *in vitro* model of infection described in section 2.1. Importantly, it was shown that the half-life of elimination of the tobramycin in the sputum was approximately 2.5 hours in these patients, which is similar to the half-life in plasma and the half-life used in the dynamic *in vitro* model study. This seems to confirm that the time-course of adaptive resistance relates closely to the half-life of drug elimination, suggesting that adaptive resistance will persist for as long as bacteria are exposed to the drug above a certain concentration.

5. Mechanism of Adaptive Resistance

The transient and reproducible nature of adaptive resistance suggests that it is not the result of a genetic mutational event, but rather a regulatory event or, less likely, the selection of a subpopulation of bacteria, or both. The exact mechanism of adaptive resistance has not been elucidated but there is circumstantial evidence that the resistance relates to down regulation of active transport of the drug into the bacteria. When Gram-negative bacteria are exposed to an aminoglycoside, a series of events result in the drug being actively transported into the cell, resulting in a greater than 100-fold concentration gradient across the cell wall.[18] The initial step involves concentration-dependent ionic binding of the cationic moieties of the antibacterial to the negatively charged residues in the lipopolysaccharide of the bacterial membrane.[19,20] Calcium and magnesium ions are displaced in this process and binding is reduced in the presence of increased concentrations of these ions.[21,22] Binding of the aminoglycosides to *P. aeruginosa* has been shown to induce a change in the cell wall structure resulting in a change in membrane permeability.[23]

Ionic binding is a passive process, whereas transport of the bound drug into the cell uses at least 2 energy-dependent processes (EDP I and EDP II) which produces the higher internal drug concentration.[24] The early, rapid, concentration-dependent bactericidal effect is thought to relate to the amount of ionically bound drug, whereas a later, slower bactericidal effect is thought to reflect limitations in the active transport capacity.[21]

Adaptive resistance appears to be caused by aminoglycoside-induced reversible down-regulation of the second energy-dependent transport sys-



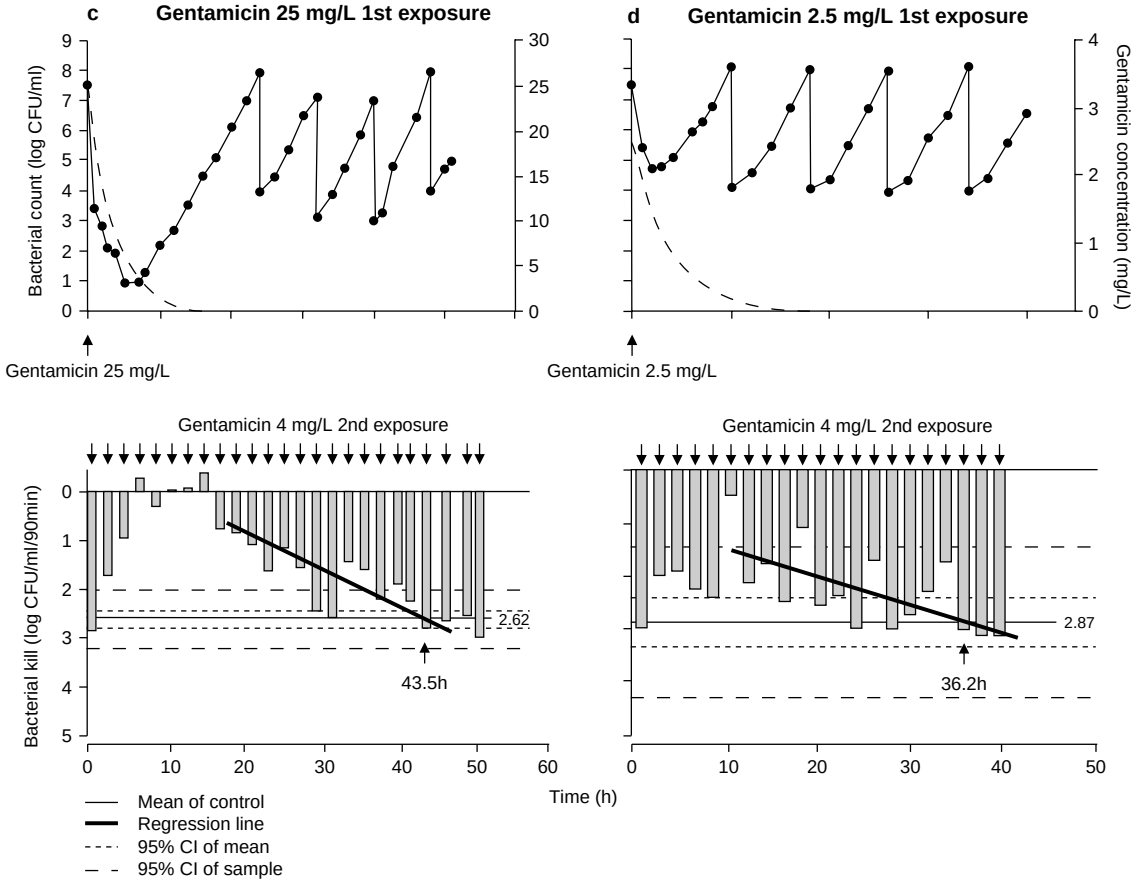


Fig. 2. Adaptive resistance with gentamicin in a dynamic *in vitro* model of infection. **(a)** Control experiment; growth of *Pseudomonas aeruginosa* in the dynamic model and antibacterial effects of exposure of samples to gentamicin. The upper graph (i) shows growth without exposure to gentamicin. Growth is kept in the log phase with 10 000-fold culture dilutions at 8-hour intervals. The lower graph (ii) shows bacterial killing 90 minutes after addition of gentamicin 4 mg/L to culture samples removed at successive 2-hour intervals. The mean and 95% confidence interval (CI) for both the mean and individual samples are shown. (**b**, **c** and **d**) Effects of a second exposure to gentamicin. The upper graphs show growth of *P. aeruginosa* and declining gentamicin concentrations following initial peaks of 8 (**b**), 25 (**c**) and 2.5 (**d**) mg/L of gentamicin. After initial bacterial killing and recovery, growth is kept in a log phase with culture dilutions at 8-hour intervals. The lower graphs show bacterial killing 90 minutes after addition of gentamicin 4 mg/L to culture samples removed at successive 1 to 2 hour intervals. Mean bacterial killing and 95% CI for the control experiments are superimposed. The degree of adaptive resistance is proportional to the first exposure concentration of gentamicin. Regression analysis shows full recovery of susceptibility at 39.3 (**b**), 43.3 (**c**) and 36.2 (**d**) hours.[2] **CFU** = Colony forming units.

tem (EDP II). Studies with ^{14}C-labelled gentamicin have shown that during the period of adaptive resistance the uptake process is turned off.[3] This down-regulation of the uptake can occur during normal bacterial replication after exposure to an aminoglycoside.[3] After bacteria with adaptive resistance are grown in drug-free nutrient media, to allow resolution of the adaptive resistance, the transport system operates normally again.

Adaptive resistance in *P. aeruginosa* coincides with cytoplasmic membrane changes and appears to be independent of changes in either lipopolysaccharide or outer membrane protein.[4] In addition, many characteristics of adaptively resistant *P. aeruginosa* are similar to those of the bacteria when cultured in anaerobic conditions, including reduced ability to accumulate aminoglycoside[25] and changes in intracellular concentrations of some gene products. It has been shown that *P. aeruginosa* cultures adaptively resistant to gentamicin have higher mRNA levels of both *denA* (nitrite reductase), which facilitates terminal electron acceptance in the anaerobic respiratory pathway, and its regulatory protein ANR, in the absence of promoter DNA sequence changes, when compared with controls.[26] These observations suggest that *P. aeruginosa* may regulate the expression of genes in its anaerobic respiratory pathway in response to aminoglycoside insult and may explain, at least partially, the mechanism of *P. aeruginosa* adaptive resistance to aminoglycosides.

As the concentration of aminoglycoside surrounding the bacteria decreases, these processes appear to slowly reverse and bacterial susceptibility gradually returns. More precise knowledge of the mechanism of adaptive resistance may have important clinical implications because it might be possible to block the process of adaptive resistance and, therefore, potentially increase the effectiveness of the aminoglycosides.

6. Conclusion

Induction of adaptive resistance by aminoglycosides provides a satisfactory explanation for the observation that extended dose administration interval regimens seem to improve clinical efficacy. The presence of this resistance may also help explain the failure of aminoglycosides when used as monotherapy. Dose-interval extension allows time for adaptive resistance to resolve, at least partly, before second and subsequent drug exposure. Shorter dose intervals, as with conventional multiple daily administration, are likely to result in reinforcement and persistence of adaptive resistance. The time-course of adaptive resistance appears to relate directly to the concentration-time profile of the aminoglycoside. The concentration-time profile in 'deeper' tissues is likely to be different than in plasma or in the dynamic *in vitro* model. Drug half-lives in these tissues can be quite prolonged and adaptive resistance induced at these sites may last for even longer periods than observed in studies so far. This adds further strength to the argument for dose-interval extension. The optimal dose interval for the aminoglycosides is not known and it may be even longer than 24 hours for some infections, or in renal impairment in which the decline in drug concentration is slower.

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