Combating antibiotic resistance in bacteria

R. Maheshwari

Abstract Combinations of certain antibiotics select against resistant strains of bacteria. This finding may provide a strategy of combating antibiotic resistant bacteria.

Keywords *E. coli* · Antibiotic · Resistance · Suppression · Selection

Increasingly antibiotics that once cured bacterial infections are no longer effective because of survival of antibiotic-resistant mutants. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to almost all presently known antibiotics. It is now one of the deadliest threats in hospital-acquired infections¹. A recent research finding suggests that a patient with an antibiotic-resistant infection could benefit from treatment of antibiotics in combination². A combined treatment makes the antibiotic effective against its own resistant mutant. The effect of genetic mutation is more than the most apparent.

The effect of drug combinations could be (1) synergistic, when their combined drugs' effect is greater than the sum of their individual effects, (2) additive, when the combined effect is equal to their individual activities, (3) antagonistic, when the effect is smaller than one of the drugs itself, or

Fig. 1 Diagram of types of effects of a combination of two antibiotics on growth rates of bacteria¹.

R. Maheshwari (✉)
Formerly, Department of Biochemistry,
Indian Institute of Science,
Bangalore - 560 012,
India.
e-mail: ramesh.maheshwari@gmail.com

Fig. 2 Chemical structures of doxycycline and ciprofloxacin.

(4) suppressive, when the combined effect is weaker than that of the single drugs (Fig. 1). How the suppressive effect works is not known. Even so, the results of a study carried out by Roy Kishony and his coworkers at Harvard Medical School using the model test bacterium *E. coli* show that antibiotics that are ineffective when used individually may get rid of the resistant bacteria when used in suppressive combination.

The researchers monitored the individual growth rates of strains of sensitive and resistant bacteria in mixed cultures by tagging them with yellow or green fluorescing proteins, respectively, and measuring growth by luminescence using a FACS (fluorescence activated cell sorter). The growth rates of the sensitive and resistant strains in mixed culture in response to combination of two antibiotics (Fig. 2) showed that at certain concentrations of two antibiotics used, there is a region in which the sensitive strain actually outgrows the resistant bacteria, i.e., a region of concentrations in which the combination of drugs that actually selects against the resistant mutant rather than the wild type (Fig. 3).

Fig. 3 Competitive selection of wild type bacteria in combined treatments of doxycycline and ciprofloxacin. Note growth suppression in fig. (d). The growth of sensitive strain (light curve) does not overlap that of resistant mutant strain (dark curve). Figure reproduced (in part) from Nature, 446, p. 669, Nature Publishing Company

The antibiotic doxycycline inhibits protein synthesis. The authors studied the effects of this antibiotic in combination with erythromycin or ciprofloxacin in suppressive interactions on the selection of resistant and sensitive test bacterium *Escherichia coli*, in a growth medium. The results showed synergy with DOX and erythromycin, and suppression with DOX and ciprofloxacin. The DOX-resistant mutant outcompete the wild type under DOX treatment and when DOX was given with erythromycin. However, unexpectedly, in presence of ciprofloxacin, DOX generated selection against its own resistant mutant allele even while maintaining inhibition of the wild type. For example, when $0.1 \mu\text{g ml}^{-1}$ DOX was added to 7.5 ng ml^{-1} ciprofloxacin. The authors stress that ‘our work is limited to sublethal drug concentrations, in a controlled environment *in vitro* and that any possible therapeutic implications from these findings are beyond its scope’. However, these findings may lead to ‘new treatment strategies employing antimicrobial combinations with improved selection against resistance’ bringing hope to languishing patients.

Apparently, when a bacterium becomes resistant to a particular antibiotic, pleiotropic changes occur. Amongst these are alterations in susceptibilities to other drugs. The antibiotic interactions that select against resistance may offer a powerful method to leverage the efficacy of therapeutic agents. Could this finding lead to control of bacterial

infections? The results of the Harvard study suggest that a patient with an antibiotic-resistant infection could benefit from treatment in suppressive drug combination that favours sensitive bacterium and the resistant ones die out through competition.

Basic questions arise: Does the origin of resistance in bacterial cells involve epigenetic changes in addition to primary change in a single gene? How does combination drug therapy tip the balance against resistance, i.e., how does the sensitive bacteria in population outcompete the resistance bacteria that evolved under applied selective pressures? An extension of this finding is in microbial ecology: Are the principles in selection of bacteria also applicable in nature wherein microorganisms secrete toxins or antibiotic substance into their environment? Or in the environment of tumor containing a mixture of cancerous and normal cells?

References

1. Braaten D (2007) Bugs vs. drugs. *Nature Medicine* 13: 522–523
2. Chait R, Craney A & Kishony R (2007) Antibiotic interactions that select against resistance. *Nature* 446:668–671
3. Maheshwari R (2007) Combating antibiotic resistance in bacteria by combination drug therapy *Curr. Sci* 92:1478