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Combination therapies for combating antimicrobial resistance

Michael A. Fischbach

Department of Bioengineering and Therapeutic Sciences and the California Institute for Quantitative Biosciences, University of California, San Francisco, San Francisco, CA 94158, USA

Abstract

New drug development strategies are needed to combat antimicrobial resistance. The object of this perspective is to highlight one such strategy: treating infections with sets of drugs rather than individual drugs. We will highlight three categories of combination therapy: those that inhibit targets in different pathways; those that inhibit distinct nodes in the same pathway; and those that inhibit the very same target in different ways. We will then consider examples of naturally occurring combination therapies produced by microorganisms, and conclude by discussing key opportunities and challenges for making more widespread use of drug combinations.

Introduction

Much has been written about the need for new strategies to combat antimicrobial resistance [1–3]. The object of this perspective is to highlight one such strategy: treating infections with sets of drugs rather than individual drugs. The principle of combination therapies as they relate to drug resistance is straightforward: Imagine that the probabilities of spontaneous resistance to drugs A and B are 1×10^{-6} and 1×10^{-7} , respectively. If spontaneous resistance to drugs A and B are independent events, then the probability of spontaneous resistance to the A+B combination will be the product of the two rates, or 1×10^{-13} (for a more in-depth explanation, see [4]).

There are two important provisos: First, the two drugs' mechanisms of action cannot interfere; the drugs cannot have an antagonistic interaction. Second, the target cells are assumed to be in a phenotypically susceptible state. If a subpopulation of cells is insensitive to a single-agent therapy due to persistence or quiescence, they will probably be equally insensitive to a combination therapy, unless one of those drugs has 'sterilizing' activity (see below) [5]. The science of studying how cells and organisms respond to drug combinations is complicated and in its early days, although several recent papers provide a promising start and report some unanticipated phenomena [6–13].

We will highlight three categories of combination therapy: those that inhibit targets in different pathways; those that inhibit distinct nodes in the same pathway; and those that inhibit the very same target in different ways. We will then consider examples of naturally occurring combination therapies from microorganisms, and conclude by discussing key opportunities and challenges for making more widespread use of drug combinations.

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Correspondence: fischbach@fischbachgroup.org.

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Different pathways

The widely used DOTS regimen for treating *Mycobacterium tuberculosis* infections begins with a combination of four drugs: isoniazid, an inhibitor of the enoylreductase subunit of fatty acid synthase; rifampicin, an RNA polymerase inhibitor; ethambutol, an inhibitor of arabinosyl transferases involved in cell wall biosynthesis; and pyrazinamide, whose mechanism of action is not well understood [14,15]. Thus, at least three pathways are inhibited by this combination, so even if the infecting strain of *M. tuberculosis* manages to find a workaround for one of the pathways (e.g., lipids from a host macrophage relieving the need for fatty acid biosynthesis), other key pathways still be blocked.

Pioneering studies performed after the discovery of the first three antibiotics for TB – *p*-aminosalicylic acid (1944), streptomycin (1944), and isoniazid (1951) – showed that combinations of these drugs were more effective at preventing resistance than any of the drugs administered as a single agent [16]. Given TB's infamous ability to persist in the face of drug treatment [17], the effectiveness of the current four-component therapy is a testament to the power of combination therapies. The rising prevalence of MDR- and XDR-TB is a reminder that resistance often behaves as a ratchet [16]. If any of the four drugs are inappropriately administered as monotherapies, TB can develop resistance in short order, and resistance can be maintained indefinitely if the strain periodically encounters the drug – or if the resistant strain does not suffer a selective disadvantage in the absence of drug.

The remarkable gains in survival for HIV-infected patients are also due to combination therapies [18]. Three common combination therapies for HIV are based on a mixture of two nucleoside reverse transcriptase inhibitors, emtricitabine and tenofovir. One of them adds raltegravir, an integrase inhibitor; the second adds the non-nucleoside reverse transcriptase inhibitor efavirenz; and third adds a mixture of ritonavir and darunavir, both protease inhibitors [19]. In this case, the combination therapy does not lead to the eradication of disease, but may enable HIV to become a chronic illness that is not the primary cause of death for those infected. In addition to targeting multiple viral pathways, future HIV combination therapies may inhibit host pathways as well. An interesting twist on HIV combination therapies is the use of ritonavir, originally developed as a protease inhibitor but later found to be a potent inhibitor of the drug-metabolizing P450 CYP3A4, to improve the half-life of other drugs in the regimen, enabling improved dosing schedules [20].

Combination therapies have proven essential for the treatment of the malaria pathogen *Plasmodium falciparum*. While resistance to chloroquine may only have emerged a small number of times during its time in clinical use (perhaps in part because it was being added to table salt in South America) [21], chloroquine-resistant strains have spread widely enough to substantially reduce the efficacy of this drug class. The most effective drug currently used to treat malaria is artemisinin, whose mechanism of action is thought to involve inhibiting the SERCA-family Ca^{2+} -ATPase PfATP6 [22,23]. To prolong the time until it suffers the same fate as chloroquine, an artemisinin derivative (either artesunate or artemether) is generally administered as a combination with amodiaquine, mefloquine, or lumefantrine [24].

Same pathway, different target

The first class of antibiotics was the sulfa drugs, whose founding member sulfanilamide was derived from azo dyes developed at Bayer. Sulfa drugs target dihydropteroate synthase, an enzyme in the biosynthetic pathway for the cofactor folic acid. Starting in the late 1960's another sulfa drug, sulfamethoxazole, was combined with trimethoprim, an inhibitor of dihydrofolate reductase, which regenerates the reduced tetrahydro form of folate from the oxidized dihydro form, allowing it to complete another catalytic cycle [25].

In comparison to inhibiting two different pathways (discussed in the previous section), inhibiting two enzymes in the same pathway is a less diversified bet. If, for example, the target cell has an absolute requirement for folate to synthesize dTMP as a monomer for DNA synthesis, then inhibiting two enzymes in the folate biosynthetic pathway will likely be effective. However, if a subpopulation of target cells can circumvent their need for thymidine (and therefore folate) altogether, then the combination therapy would prove less effective for having placed all its eggs in a single pathway's basket.

One of the most successful subclasses of combination therapy is exemplified by Augmentin, which combines the beta-lactam antibiotic amoxicillin with the beta-lactamase inhibitor clavulanate [26]. The principle of pairing a drug with an inhibitor of its resistance enzyme can be generalized both within and outside the antibacterial therapeutic area. One interesting example can be found among targeted kinase inhibitors for cancer chemotherapy; resistance to these molecules can arise by point mutations in the kinase target, making the mutant form of the kinase a resistance enzyme of sorts. 'Mutation-specific' inhibitors have now been developed, and the possibility of pairing them with the original drug will likely be explored in the coming years [27].

These examples raise several interesting questions: Is it better to wait until the mutant has arisen, or will first-line treatment with a combination of the two drugs be more effective? Would such a first-line drug combination simply select for a different resistance-conferring mutation, or would it prevent resistance-conferring mutations from arising altogether? Are there sets of two drugs that can be administered in a 'toggled' dosing regimen such that treatment with one selects for reversion to a state in which the other is effective, and vice versa?

Same target

The least diversified bet of all is to inhibit not just the same pathway, but exactly the same target with multiple drugs. The first example comes from the grandfather of all targets: the bacterial ribosome. One of the newer antibiotics on the market, synergid, is the latest semisynthetic iteration of a two-drug combination known alternately as the streptogramins, virginamycins, or pristinamycins. These two molecules – one a nonribosomal peptide and the other a polyketide-nonribosomal peptide hybrid – bind in adjacent sites in the 50S subunit, near the peptidyl transferase center [28,29]. They are 10-100-fold more potent as a combination than either molecule is as a single agent [30], raising interesting questions about their coevolution.

Simocyclinone, a DNA gyrase inhibitor, takes this theme to its logical limit [31–33]. A recent crystal structure of the simocyclinone-gyrase complex shows that this natural product is a combination therapy whose components – an anthracycline and an aminocoumarin – bind in distinct sites but are covalently attached by a polyene diacyl linker, presumably providing an entropic benefit for binding [34]. In similar fashion to the streptogramins, variants of simocyclinone lacking either the anthracycline or the aminocoumarin have 100-fold weaker IC₅₀ values for inhibiting gyrase-catalyzed supercoiling [34].

The semisynthetic glycopeptide chlorobiphenyl vancomycin has been proposed to behave similarly; its vancomycin core is known to bind the D-Ala-D-Ala terminus of uncrosslinked peptidoglycan monomers, while its synthetic chlorobiphenyl appendage is thought to inhibit the enzyme peptidoglycan transglycosylase, which is physically collocated with the D-Ala-D-Ala terminus at sites of peptidoglycan synthesis [35]. Another example of a natural product antibiotic that is proposed to have two distinct mechanisms of action is the lipid II-targeting lantibiotic nisin, which is proposed to kill cells not only by forming pores, but also

by depleting lipid II from the septum, thereby blocking the peptidoglycan synthesis required for cell division [36].

Natural combination therapies

Antibiotic resistance is common among bacterial isolates in the 'wild'. Certain soil-derived actinomycetes, for example, are resistant to fifteen different antibiotic classes [37–39]. Continuing with the example of soil actinomycetes, such cosmopolitan resistance raises a critical question: Why do bacteria still produce antibiotics? How are they still effective; still capable of conferring a selective advantage on their host?

One possible reason is that bacteria seldom produce monotherapies [40]. A single strain of *Streptomyces*, for example, can harbor an astonishing ~35 gene clusters for the biosynthesis of natural products [41–43], and a single strain of *Micromonospora* – one of the few strains that has been cultivated in many different growth media to determine its cumulative small molecule production potential – has been shown to produce 50 isolatable natural products [44]. If we assume that less than a quarter of these are antibiotics, then we are still left with an estimate of 8–12 antibiotics from a single strain, which would make for quite a potent combination therapy.

Few examples of natural combination therapies have been characterized, but the ones that have are quite instructive. The first three have already been discussed in previous sections. *Streptomyces clavuligerus* produces a combination of the beta-lactam antibiotic cephamycin and the beta-lactamase inhibitor clavulanate, which are encoded by adjacent biosynthetic gene clusters, probably because they are transmitted horizontally together as part of a large mobile element [45,46]. The same is true for the virginiamycins/pristinamycins/streptogramins, which are produced by physically linked gene clusters in a large (presumably mobile) island in the genome of *Streptomyces pristinaespiralis* [47]. While simocyclinone is a single molecule, the mosaic nature of its gene cluster [32,33] suggests that it arose from the merger of at least three distinct gene clusters: one encoding an anthracycline, the second an aminocoumarin glycoside, and the third a polyene diacid linker [48].

The final example of a natural combination therapy comes from *Micromonospora carbonacea*, also a soil actinomycete. This strain produces three natural products that are completely unrelated in chemical structure, each of which binds the ribosome at a distinct site: Sch 40832, a thiopeptide; everninomicin, an oligosaccharide; and chloramphenicol, a nonribosomal peptide [49,50]. Selective pressure has presumably led to the maintenance of all three gene clusters in *M. carbonacea*, suggesting that each of these (and possibly others?) are required for this strain to compete effectively with other species.

The future of combination therapies

To conclude, we will discuss four key questions that are likely to influence the future of combination therapies:

Can antibiotics that have been shelved for having a high intrinsic resistance rate be resurrected as components of new combination therapies? A number of interesting antibiotic candidates have been shelved due to a high intrinsic resistance rate (e.g., the ADEPs [51,52]). By resurrecting these molecules as components of a combination therapy, a bevy of new antibiotic scaffolds with non-overlapping resistance profiles could be introduced into clinical use. The key challenges are the unpredictable toxicity and PK/PD associated with combination therapies. Given the toxicity-related problems that can bedevil single drugs, pharmaceutical companies will no doubt be skeptical that developing a combination therapy

is worth the resources and risk. Advances in predicting adverse toxicological outcomes – and unexpected amplifications or abatements of toxicity that can arise when drugs are co-administered – would enable the development of more combination therapies, both inside and outside the anti-infective therapeutic area (see below).

Should a systematic effort be made to understand how microorganisms design their combination therapies? Eons of evolution have honed the ability of microbially produced natural products to enter cells, resist degradation, and potentially inhibit a target; through these molecules, microorganisms have taught us myriad lessons about the design principles for individual drugs. Given that the same evolutionary pressures have likely shaped the sets of drugs produced by a microorganism – and that microbes have found a way for their antibiotics to remain effective even in the face of widespread resistance – perhaps we should pay closer attention to the lessons they hold for the design of combination therapies.

Are there clever ways to screen for 'sensitizers' – molecules that do not have killing activity on their own but make co-administered antibiotics more effective or prevent the emergence of resistance? There exist many possible mechanisms by which a molecule could be a sensitizer, including inhibiting a resistance enzyme (e.g., clavulanate), inhibiting an enzyme in a 'workaround' pathway, and inhibiting an enzyme that metabolizes the drug (e.g., ritonavir). These sensitizers, which represent a chemical version of synthetic lethality, would have gone undetected in most academic and industrial screens.

Finally, is there a place for combination therapies outside the anti-infective therapeutic area? Cancer chemotherapeutic regimens have long been combination therapies: most contain at least 2–3 agents, and one regimen for non-Hodgkin lymphoma known as ProMACE-CytaBOM consists of nine different drugs. However, the newer molecularly targeted therapies are, in a sense, much more like antibiotics since they take out single nodes (e.g., the BRAF kinase) rather than causing nonspecific damage (e.g., alkylating DNA). As such, lessons from antibacterial combination therapies may well apply. For example, an infectious disease physician would not be surprised to see a TB patient fail isoniazid monotherapy due to acquired resistance; should the rapid progression observed on kinase inhibitor monotherapies therefore be surprising? Will first-line treatment with combinations of targeted therapies delay (or even prevent) progression?

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Highlights

- New drug development strategies are needed to combat antimicrobial resistance.
- This perspective highlights one such strategy: combination therapies.
- Combination therapies differ in their number of components and pathways targeted.
- Microorganisms produce 'natural' combination therapies.