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REVIEW

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Are antibiotics naturally antibiotics?

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Abstract Antibiotics have been used for more than 50 years and are the cornerstone of infectious disease treatment; in addition, these low-molecular-weight bioactive compounds have been applied to many other therapeutic purposes. However, there is almost no information on the evolutionary biology or ecology of naturally occurring low-molecular-weight compounds. The large number of different structural types and the extremely broad range of biological activities of organic molecules produced by microbes raise many questions concerning their roles in nature. Recent evidence for the enormous complexity of microbial populations in the environment favors the notion that the principal roles of small molecules in microbial ecology are cell–cell communication and not antibiosis.

Keywords Hormesis · Natural products · Sociomicrobiology · Transcription modulation

Introduction

Let us begin with a definition. What is an antibiotic? In 1945 Selman Waksman proposed that the word be defined as ''a chemical substance of microbial origin that possesses antibiotic powers'' [21]. What, then, are these powers? When growth inhibition results from the interactions of two microbes, it is considered to be the manifestation of antibiosis.

Webster's Third International Dictionary (1981) defines an antibiotic as ''a substance produced by a microorganism (as a bacterium or a fungus) and in dilute solution having the capacity to inhibit the growth of or kill another microorganism (such as a disease germ)''. Brock's well-known textbook of microbiology [11] defines an antibiotic as ''a chemical agent produced by one organism that is harmful to other organisms''. Other texts produce variations of this theme but often ignore the original requirement for the active compound to be made by a microorganism. It should be emphasized that all definitions are based on sparse knowledge of the ecology and biology of naturally occurring low-molecular-weight organic compounds.

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What antibiotics do?

It has been shown that all so-called antibiotics at very low dilution (as low as 1/100 of inhibitory concentrations) do not exhibit antibiosis but have major effects on metabolism; global transcription patterns are altered significantly (Fig. 1). The identification of the latter activity can be achieved with simple assays by using a selection of bacterial promoter–reporter constructs [10, 18]. Some 5–10% of cell transcripts are modulated, approximately half up-regulated and half down-regulated. How does one describe compounds that exert antibiosis at high concentration but have a different, stimulatory effect at low concentration? The phenomenon of distinct concentration-dependent activities has been described as hormesis [5]. There is considerable evidence that both the target pathogen and the host exhibit transcriptional changes; it is probable that all types of living cells have specific responses to small molecules. The duality of small molecule activity is universal. A couple of glasses of wine with dinner are pleasant and even beneficial, but the consumption of a jeroboam could have serious side effects. Thus, microbially produced small molecules should not be inferred to be antibiotics indiscriminately, since this represents only a partial description of their bioactivity. In fact, antibiosis has proven to be an insensitive and inaccurate method to identify or describe the bioactivity of a given compound and may not even detect its presence. The requirement of high concentrations for antibiosis leads

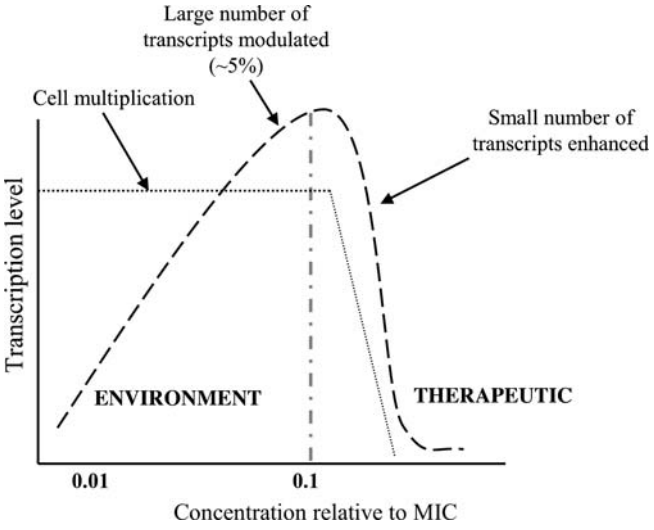

Fig. 1 An idealized diagram showing the relationship between small molecule (antibiotic) concentration, transcription modulation (low), and growth inhibition (high). *MIC* refers to the minimal inhibitory concentration

one to argue that, under true physiological (environmental) conditions such as in soil and marine habitats, inhibitory concentrations of small molecules are rarely achieved. If such is indeed the case, modulation of cellular transcription patterns represents the 'normal' function of the vast majority of low-molecular-weight natural products. Perhaps it is best said that "an antibiotic is a therapeutic agent produced by a pharmaceutical company", this definition being useful only in the context of a property of small molecules outside of their normal environment. Waksman himself concluded that antibiotic activity itself was not important in natural microbial populations [21].

The isolation and identification of antibiotics in the laboratory often requires the identification of a pitifully small amount of compound that is made only because the producing microorganism is placed in a foreign environment. This is generally an extremely rich monoculture in liquid medium with unlimited nutrients and oxygen and at a constant temperature (except for equatorial denizens) [8]. Under such conditions cell metabolism becomes disturbed with the result that normal functions may be over- or under-expressed, permitting production at measurable amounts. Clearly the organism is placed under strong physiological stress, although the roles of stress responses in the overproduction of small molecule secondary metabolites under laboratory conditions (relative to levels synthesized in the wild) have not been analyzed. It should be remembered that in nutritionally depleted soil environments most of the resident microbes grow at generation times that may be 50–100 times lesser than in the three-star-restaurant environment of a bacteriology laboratory [1].

There has been considerable anthropocentric discussion on the roles of small molecules in nature, most of which is based on the pharmaceutical definition given above [6, 12]. Until recently their significant activities at subinhibitory concentrations were unknown, and it can be argued that inhibitory concentrations are rarely produced in the environment. In any event, the accurate determination of the concentration of an antibiotic activity in soil is difficult, to say the least, especially when the active compounds are components of complex mixtures of small molecules with different chemical properties and biochemical activities. The detection of a defined antibiotic activity in soil has not been reported. The premise of a wide range of antibiosis necessarily assumes that microbial small molecules in nature exert inhibitory activity against bacteria, fungi, plants, insects, and even animals. Such antagonistic roles have rarely been demonstrated. On the other hand, a good case for universal small molecule signaling can be made.

Consideration of evolutionary biology leads to the conclusion that the enormous number of small molecules made naturally by bacteria, fungi, plants, and animals have important functions in the environment. They are produced because cells evolved the complex genetic and biochemical machinery to make them. Having said this, how can the ecology and evolutionary biology of small molecules be studied in a logical and meaningful manner? The processes are finely regulated and it is apparent that not all of the biosynthetic pathways are expressed under laboratory conditions; the signals required for activation are known in a few cases only. Are the chemicals produced by any given microbe in the environment identical to those made by the same microbe in the laboratory, and do they have the same biological activity?

Most studies to date suggest that antibiotics and other bioactive small molecules are not required for growth under laboratory conditions. However, it has to be admitted that only limited studies have probed this question, and of course, laboratory conditions hardly represent the environment. It is common knowledge that bacterial mutants dependent on streptomycin for growth can be isolated in the laboratory. Are bacterial strains dependent for growth or function on the presence of streptomycin (or any such small molecule) present in nature? Small molecules are known to play hormone-like roles in the interactions of microbes, plants, and animals. This is consistent with the notion that the main environmental role of microbial small molecules is cooperative inter-species interactions within complex microbial and other communities. In defense of antibiosis-minded microbiologists, one cannot exclude the possibility that some compounds may play various competitive roles as antagonists and to suppress plant pathogens; numerous examples of these types of activity have been demonstrated [15]. That being said, positive interactions are vital functions that were established during primordial development, and cells (organisms) capable of community relationships possessed an evolutionary advantage (the family that preys together stays together). All cells, be they prokaryote or eukaryote, exist and function cooperatively in their particular environments, in some form of organized structure [13]. The co-existence of

microbes in biofilms, in lichens, and other symbiotic relationships are particularly well characterized and ancient examples. Can it be assumed that small molecules are the keys to all such associations?

The evidence for a universe of small molecules in nature is compelling; for example, most streptomycetes have the theoretical capacity to produce as many as 20–30 different molecules, each with defined and regulated biosynthesis pathways [3]; many of these pathways are of unidentified function. To increasing extents, small molecule biosynthetic capacity is being identified in many other bacterial genera; there is nothing unique about streptomycetes or actinomycetes (except perhaps for the number and chemical diversity of compounds produced). Other members of the Actinobacteria [7], the Pseudomonads [4], and the Bacilli [9] are all rich sources of small molecules that have been inadequately investigated. There is no shortage of bioactive small molecules in nature. This is especially true when we consider that 99% of microbial strains cannot be grown under laboratory conditions—so much for the dearth of potential pharmaceutical agents! The exciting studies of quorum-sensing mechanisms in Gram− and Gram+ bacteria, the expanding catalog of auto-inducing molecules and their roles in the regulation of development, antibiotic production, and pathogenicity continue to provide information on the ubiquitous production and biological importance of low-molecular-weight compounds in microbial ecology [19]. Studies of the roles of acylhomoserine lactones and other compounds in interkingdom communication are a growing field of interest [2, 17] and multiple biological responses in a variety of eukaryotic hosts have been shown. This is but the tip of the iceberg.

The study of sociomicrobiology [16] is an expanding discipline; increasingly, bacterial families are found to use chemical signals to establish networks of communication. The time has come to expand these concepts more broadly (all microbes in all environments?). Interesting evolutionary relationships are becoming apparent, for example, between acylhomoserine-lactone signaling in Gram-negative bacteria and the γ-butyrolactone signaling system in actinomycetes. Cationic peptides, originally identified as antimicrobials, are common components of inter- and intra-cellular signaling in many complex cellular systems, from the microcins of intestinal bacteria, to antibiotics, and to innate immunity in everything from flies to humans [14]. This has opened up an entirely new aspect of small molecule biology. Until now, these wide-ranging studies have largely focused on cationic peptides and their derivatives, but one can predict with confidence that future work will implicate other structural types of small molecule.

Conclusion

The presence of an enormous structural diversity of small molecules in nature implies the generality, variety, and specificity of cell–cell interactions. How can we find out what these millions of small molecules are doing? Are they all components of cell–cell communication systems? How can the chemical languages be deciphered? Microbes are talking to each other, but we do not understand what they are saying; we do not know what wavelength to tune-in to listen [20]. More importantly, they also communicate with other creatures and vice versa. How do we study the potential roles of small molecules in complex environments?

Parenthetically, if antibiosis is not a major role of small molecules in complex microbial environments such as soils, one has to question the function of antibiotic resistance—resistant organisms are prevalent. This may be a means of modulating small molecule signaling or changing its specificity. The topic clearly needs additional investigation.

In recent years there has been increasing interest in the field of chemical biology; this is not a new discipline; nature invented it and chemical biology has been a vital element of cellular function for aeons. The biology of small molecules is an important and largely unexplored field that deserves more attention, especially to provide a better understanding of the inter- and intra-generic interactions responsible for maintaining homeostasis in the environment. Complex microbial populations exist in nature under conditions of biological solidarity and interdependence linked by networks of chemical signals, using many types of molecule. Better understanding of these many processes will be of enormous value to science and medicine. Traditional approaches to the discovery and application of natural products for antibiotic use, albeit successful, have obscured the fact that small molecules are as critical to cell function and survival as DNA, RNA, proteins, carbohydrates, and lipids. New and valuable applications will be revealed only if discovery and bioactivity screening of natural small molecules is carried out with due consideration of their evolution, biology, and chemistry. More significant and targeted collaboration between academia, industry, and government is required. Could we perhaps learn some principles for effective interactions from the microbial world?

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