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MINIREVIEW

rRNA Binding Sites and the Molecular Mechanism of Action of the Tetracyclines

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The tetracycline antibiotics are known to be effective in the treatment of both infectious and noninfectious disease conditions. The 16S rRNA binding mechanism currently held for the antibacterial action of the tetracyclines does not explain their activity against viruses, protozoa that lack mitochondria, and noninfectious conditions. Also, the mechanism by which the tetracyclines selectively inhibit microbial protein synthesis against host eukaryotic protein synthesis despite conservation of ribosome structure and functions is still questionable. Many studies have investigated the binding of the tetracyclines to the 16S rRNA using the small ribosomal subunit of different bacterial species, but there seems to be no agreement between various reports on the exact binding site on the 16S rRNA. The wide range of activity of the tetracyclines against a broad spectrum of bacterial pathogens, viruses, protozoa, and helminths, as well as noninfectious conditions, indicates a more generalized effect on RNA. In the light of recent evidence that the tetracyclines bind to various synthetic double-stranded RNAs (dsRNAs) of random base sequences, suggesting that the double-stranded structures may play a more important role in the binding of the tetracyclines to RNA than the specific base pairs, as earlier speculated, it is imperative to consider possible alternative binding modes or sites that could help explain the mechanisms of action of the tetracyclines against various pathogens and disease conditions.

The tetracycline group of antibiotics includes naturally occurring polyketide compounds, such as tetracycline, chlortetracycline, and oxytetracycline, as well as semisynthetic compounds, such as methacycline, minocycline, and doxycycline. In addition to their antimicrobial activities, there are also many reports associating them with anti-inflammatory (1), antiapoptotic (2), and neuroprotective activities (1, 3). The mechanism(s) of action in many of these reported activities is still poorly understood (4–6). The ribosomes, which are the sites of protein synthesis in living cells, are composed of proteins and RNA. Many studies have indicated that the tetracyclines bind to the RNA component of bacterial ribosomes. More specifically, they are believed to inhibit translation by binding to the 16S rRNA and inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex (7, 8). A number of binding sites have been identified on the 16S rRNA through photoaffinity labeling and chemical footprinting, for which results indicate certain bases contribute to the binding pocket (9–11). However, these studies do not agree on the exact target/binding site of the tetracyclines on the 16S rRNA (12). In addition, there is increasing evidence of other useful properties of the tetracyclines, such as their antiviral (2, 13, 14), anti-inflammatory (4, 15), antiapoptotic (2), and neuroprotective activities (1), that are not explained by the 16S rRNA binding mechanism currently held for the antibacterial action of the tetracyclines.

Recently, another study showed that the tetracyclines bind to double-stranded RNAs of random base sequence (16), indicating that the double-stranded structures of RNAs may play a more important role in their interaction with the tetracyclines than the specific base sequences. Given that rRNAs and other cellular RNAs form extensive double-stranded structures which are essential for their interaction with other molecules, as well as their functions, the binding of the tetracyclines to cellular RNAs may alter the normal functioning of the various biological processes the RNAs regulate. It is therefore necessary to review the existing information on these studies, with a view to identifying the way forward for research into the mechanism of action of the tetracyclines.

OVERVIEW OF INTERACTIONS OF rRNA WITH ANTIBIOTICS

Ribonucleic acids (RNA) play a very important role in the regulation of gene expression via translation (protein synthesis). Various types of cellular RNAs participate in this process either directly (mRNA, rRNA, tRNA) or indirectly (regulatory RNAs, ribozymes). The interaction of proteins or small molecules with cellular RNAs often affects the functions of the RNAs during gene expression and protein synthesis. Many antibiotics are known to inhibit protein synthesis via their interactions with the ribosomes. These drugs are often able to bind microbial ribosomes and inhibit protein synthesis while leaving those of the host organism relatively unaffected. Although ribosomes are generally composed of RNA and proteins, it is becoming increasingly clear that most of these antibiotics work by binding to rRNAs (17–20). Often, these antibiotics target and inhibit specific functional sites of the translation apparatus. Some are known to bind to the large ribosomal subunit (23S rRNA) and inhibit the peptidyl transferase activity (e.g., chloramphenicol) or interfere with the elongation of the polypeptide chain in various ways (e.g., the macrolides). Some others (e.g., aminoglycosides and tetracyclines) are known to bind the small ribosomal subunit (16S rRNA) and inhibit the initiation of transcription in a variety of ways (17, 18, 21). Of this group of antibiotics that bind to the 16S rRNA, in-depth studies of these interactions have been carried out for some antibiotics of the aminoglycoside group (20, 22, 23). However, there is still controversy

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surrounding the exact target sites and mechanism(s) of action of the tetracycline group of antibiotics (24, 25).

COMPOSITION, STRUCTURE, AND FUNCTION OF rRNA

rRNAs are the RNA components of the ribosome, which is the protein synthesis machinery of the cell. They constitute about 95% of total cellular RNA in *Escherichia coli* (26) and 85 to 90% in eukaryotes (27). They form the active sites of the ribosomes for decoding the message of the mRNA, and they also perform enzymatic functions in the translation process. They also catalyze the formation of peptide linkages in the growing amino acid chain during protein synthesis. In prokaryotes, the small ribosomal subunit (30S subunit) contains 16S rRNA (≈1,542 nucleotides [nt] in *E. coli*), whereas the large ribosomal subunit (50S subunit) contains 23S (≈2,904 nt in *E. coli*) and 5S (≈120 nt in *E. coli*) rRNAs (26). In eukaryotes, the small ribosomal subunit (40S subunit) contains 18S rRNA, whereas the large ribosomal subunit (60S subunit) contains 28S rRNA, 5.8S rRNA, and 5S rRNA. The rRNAs of prokaryotes are cotranscribed from an operon (*E. coli* has 7 copies of rRNA operons in the chromosome), whereas in eukaryotes, the 5S rRNA is transcribed differently from the rest. The ribosomes of eukaryotic mitochondria and chloroplasts (70S) resemble those of bacteria, which is consistent with the idea that mitochondria are derived from bacteria. The processing pathways of prokaryotic and eukaryotic rRNA transcripts also differ greatly. The rRNAs form extensive double-stranded structures which are essential for their interactions with other molecules necessary for their function in protein synthesis (Fig. 1A).

DOUBLE-STRANDED RNAs IN CELLS

Double-stranded RNAs are seen in some viral genomes or are produced as intermediaries of viral and transposon replication. Apart from these viral RNAs, double-stranded RNAs are also formed in cells as a result of RNA:RNA interactions between either two different strands of RNA (sense and antisense strands) or complementary segments of a single strand (folding). Double-stranded RNAs result from base-pairing interactions due to the formation of hydrogen bonds between the nitrogenous bases. Typically, adenine forms a base pair with uracil, while guanine forms a base pair with cytosine. However, guanine-uracil base pairs (known as wobble base pairs) occur in RNA and often lead to secondary structures of functional importance, especially in tRNAs (19, 28–34).

Although RNAs do not typically form long double-helical structures, which are characteristic of DNA, they often contain self-complementary sequences that cause them to fold and form short double helices. This leads to the formation of stem-loop structures within the molecule, and these can be very extensive and complex in the case of long RNA molecules like rRNA. These secondary structures appear as hairpin loops and bulges, with the formation of grooves between the base pairs. Hence, in the base-paired regions, the RNA molecule adopts a helical structure as in DNA, although the helical form adopted has a distinct conformation that differs slightly from that of DNA (Fig. 1B). Double-stranded RNA segments are abundant in living cells, and these secondary and tertiary structures are essential to the function of biologically active RNAs. Since RNA molecules are generally negatively charged (polyanions), metal ions such as Mg^{2+} are often necessary to provide stability within the secondary and tertiary structures (35, 36). Segments of dsRNAs often act as sites of interaction for regulatory proteins and small molecules. These molecules recognize and bind to dsRNA grooves or intercalate between bases (19, 28–34).

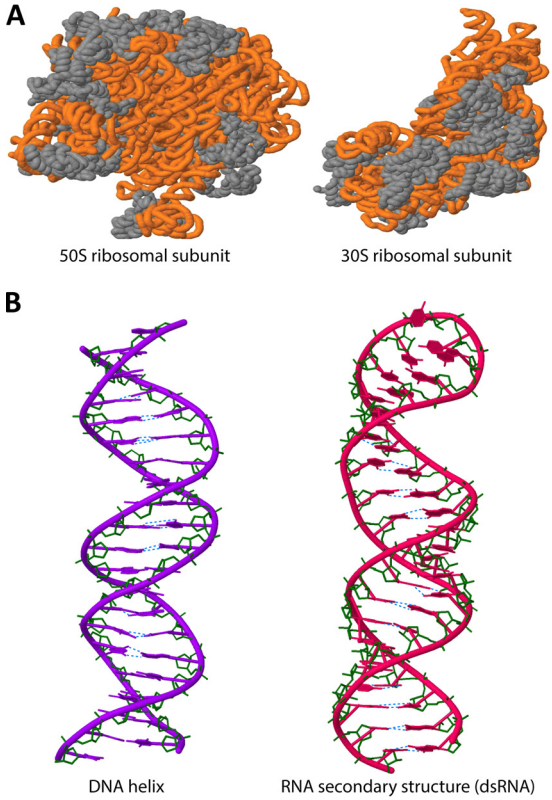

FIG 1 (A) Structures of the 50S and 30S bacterial ribosomal subunits, showing the secondary structures of rRNA (orange) in association with ribosomal proteins (gray). (B) Structures of dsDNA and dsRNA, showing hydrogen bonds between bases (blue) and other intramolecular bonds (green). Structure templates were obtained from the Protein Data Bank (PDB) and drawn using Jmol (an open-source Java viewer for chemical structures in three dimensions).

STRUCTURE AND ANTIMICROBIAL ACTIVITY SPECTRUM OF THE TETRACYCLINES

Tetracycline molecules are characterized by a planar polycyclic structure composed of four ("tetra") hydrocarbon rings, and thus their name, tetracyclines. There are strong indications that the antibacterial activities of members of this group of antibiotics are related to certain aspects of their chemical structure (7, 37, 38). Of particular interest in the context of this study is the planar tetracyclic ring series, which makes them good candidates for intercalation between the base pairs of nucleic acids (Fig. 2). This possibility has been relatively infrequently explored compared to studies of their other possible structure-activity relationships.

The tetracyclines are broad-spectrum bacteriostatic antibiotics which are effective against a wide range of human pathogens and disease conditions (Table 1). Pathogenic microbes against which the tetracyclines are effective include bacteria (39), protozoa (39), viruses (2, 3), and helminths (40). These drugs are quite selective in their action against microbes, with no known major growth-inhibitory activity against larger organisms (7). This has greatly encouraged their use as broad-spectrum antimicrobials, especially in the treatment of chronic infections. They are also currently

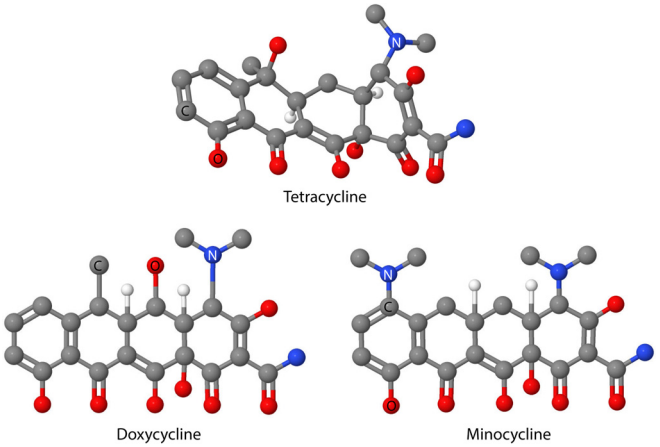

FIG 2 Chemical structures of tetracycline antibiotics in three dimensions. Gray balls represent carbons in the rings, red balls represent oxygens, and blue balls represent nitrogens. Structure templates were obtained from the Protein Data Bank (PDB) and drawn using Jmol.

being used in research to control gene expression in eukaryotes and mammalian cells (41–43).

ROLE OF METAL IONS IN THE PHARMACOKINETICS OF TETRACYCLINES

The tetracyclines are strong chelating agents; hence, their pharmacokinetics and subsequent antibiotic effects are influenced by the presence of metal ions (44, 45). Foods, particularly dairy products, and antacids are generally known to impair the absorption of tetracyclines following oral administration, but minocycline, and to a lesser extent doxycycline, are not affected as strongly as the water-soluble tetracyclines (46, 47). However, there are indications that tetracyclines cross the outer membrane of Gram-negative bacteria as positively charged complexes with cations, most likely magnesium ions (48). This appears to also be the form in which tetracyclines are actively transported out of bacterial cells by the efflux proteins (49, 50). In addition, there is a possibility that the active drug within microbial cells is in a complex with a magnesium ion (39). Mg^{2+} has also been shown to affect the frequency of cross-linking of bases in the 30S ribosomal subunit, as well as the binding of tetracycline to nucleic acids (25). It has also been reported that the interaction of tetracyclines with dsRNAs *in vitro* is enhanced by the presence of magnesium ions in a dose-dependent manner, with little or no measurable interaction in the absence of Mg^{2+} (16).

MECHANISM(S) OF ACTION OF THE TETRACYCLINES: THE JOURNEY SO FAR

Mechanism of antibacterial activity based on interactions of tetracycline antibiotics with bacterial rRNA. The tetracyclines are believed to act by binding to ribosomes and impairing their ability to synthesize proteins necessary for growth and survival of a bacterium (51). They are generally believed to bind to the 30S ribosomal subunit, which is made up of a 1,540-nucleotide RNA (the 16S rRNA) and 21 proteins. It has been established that the tetracyclines inhibit bacterial protein synthesis by inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex (7, 8, 39, 51). However, the exact target site(s) and mechanism(s) of action remain subjects of much debate. It was initially thought that the tetracyclines inhibit protein synthesis mainly by binding the ribosomal proteins of the 30S subunit, as these proteins were photolabeled by tetracycline when photoincorporated into *E. coli* ribosomes (51). Although earlier studies had suggested binding interactions with both 16S and 23S rRNAs (52) and an *in vivo* study from several decades ago indicated an inhibitory effect of tetracycline on rRNA synthesis (53), this early evidence seems to have been largely ignored. The 16S rRNA was subsequently proposed in one of the studies by Oehler et al. in 1997 to be solely responsible for the inhibition of tRNA binding to the A-site of the

TABLE 1 Disease conditions or infections against which tetracyclines are effective, target sites, and mechanisms of action

Infection type (examples) or disease condition	Drug(s) used	Target(s)[a]	Mechanism(s) of action[a]	Reference(s)
Bacterial infection[b] (anthrax, bubonic plague, Rocky Mountain spotted fever [*Rickettsia rickettsii*], borreliosis, psittacosis [*Chlamydia psittaci*], leptospirosis [spirochetes])	All tetracyclines	16S rRNA	Inhibits protein synthesis by inhibiting binding of aminoacyl-tRNA to mRNA-ribosome complex	8, 39
Viral infections (West Nile virus, HIV, Japanese encephalitis virus)	Minocycline	$CD4^+$ T cells	Suppresses activation of $CD4^+$ T cells; anti-inflammatory, antiapoptotic, and neuroprotective	2, 13, 14, 68–70
Protozoan infections (malaria, toxoplasmosis, leishmaniasis, amoebic dysentery, giardiasis, trichomoniasis)	Doxycycline, minocycline	Mitochondria and apicoplasts	Inhibits mitochondrial protein synthesis[c]	5–7, 67, 86
Helminth infections (lymphatic filariasis, onchocerciasis)	Doxycycline	*Wolbachia* endosymbionts	Eliminates bacterial endosymbiont(s) necessary for parasite survival and reproduction	40
Acne and rosacea	All tetracyclines		Anti-inflammatory	15
Elephantiasis	Doxycycline		Reduces plasma VEGF; anti-inflammatory	4
Osteoarthritis	Doxycycline, minocycline	Nitric oxide synthase	Inhibits RNA expression, translation of enzyme	71

[a] Proposed target sites and mechanisms of action.
[b] Includes both Gram-positive and Gram-negative bacteria.
[c] Similar to antibacterial mechanism of action.

protein synthesis machinery (9). In that study, the experiments conducted *in vitro* and designed to correlate the activity of the ribosomal subunits with the binding of tetracyclines to the rRNAs suggested that inhibition of tRNA binding to the A-site is solely due to tetracycline cross-linking to the strong binding site on the 30S subunit (9). Consequently, further studies on the mechanism of action of the tetracyclines have been based on the presumption that the binding of the tetracyclines to the 16S rRNA is strictly responsible for the antibiotic activity of this class of drugs. However, it should be noted that pre-23S rRNA functions well in protein synthesis without maturation, whereas pre-16S rRNA is not functional in protein synthesis (54). This is further complicated by the dependence of the maturation of rRNAs on ribosomal function (55–57), resulting in immature 23S rRNA being functional in the ribosomes while the immature 16S rRNA is unable to function and, furthermore, inhibits 16S rRNA maturation. It is possible that the nonfunctionality of the immature 16S rRNA, in contrast to the functionally active immature 23S rRNA, erroneously led to the assumption that the tetracyclines exert their antibacterial action solely by binding to the 16S rRNA. Hence, most studies on the mechanism of action of the tetracyclines have been concentrated on the 16S component of rRNA instead of rRNA as a whole. Unfortunately, the methods employed in the majority of such studies did not differentiate between the pre-16S and pre-23S rRNA subunits and their mature forms.

A number of these studies explored the binding of the tetracyclines to the 16S rRNA with the objective to identify the exact target site on the rRNA. Photoaffinity labeling, Fenton chemistry, X-ray crystallography, and chemical footprinting studies indicated the involvement of certain bases that contribute to the binding pocket (9–11, 58). However, there have been varied and sometimes conflicting reports with regard to which bases within the 16S rRNA form the core target sites (10). These studies have used the small ribosomal subunit of different bacterial species, and there seems to be no agreement on the binding site of tetracycline on the 16S rRNA (12). In addition, many of these studies were conducted using methods that are prone to introduce errors, such as cross-reactions with products of photolysis during photoaffinity labeling (9, 59). Hence, the proposed binding sites may not represent the actual target sites *in vivo*. Also, the proposed sites do not offer any indication of the mechanism of action. While representing important contributions, the designs of these studies did not take into account possible alternative binding modes or sites that could help explain the interactions between the tetracyclines and ribosomes. Indeed, the studies were limited because they were conducted using specific and isolated ribosomal subunits (especially the 16S rRNA of the 30S subunit) rather than the whole ribosome (9, 10, 12, 60). Also, these reports did not adequately correlate binding with the antibiotic activity of the drugs, because the studies were mostly done *in vitro*. The development of modern sophisticated molecular techniques that are usually applied on specific components of the cell *in vitro* has also encouraged the concentration of subsequent studies on specific components of the ribosome. Nevertheless, these studies established that the tetracyclines bind to bacterial rRNA, although the mechanism of antibacterial action is not clear.

The most common mechanism of resistance to the tetracyclines involves drug efflux proteins, which actively pump the drug out of the cell, but ribosomal protection also plays an important role in tetracycline resistance (7, 8). Ribosomal protection proteins are believed to reversibly distort the structure of ribosomes to prevent the binding of tetracycline, dislodge tetracycline, or still allow tRNA to bind to the ribosomes irrespective of tetracycline binding. Both efflux proteins and ribosomal protection proteins will ultimately reduce the binding of the tetracyclines to rRNA, by reducing the intracellular drug concentration or competitively inhibiting tetracycline binding, respectively. More studies have indicated that base mutations in the 16S rRNA confer tetracycline resistance, especially at positions AGA965 or A965, G966, and A967 in h31 (61), G942 in h29 (58), and G1058 in h34 (62). These mutations have been shown to occur in the tetracycline high-affinity binding sites on the ribosomal 30S subunit (which are often base-paired regions) and are believed to lead to conformational changes that hamper base pairing and affect drug binding (10, 23, 58). They have also been shown to decrease drug binding in direct proportion to the number of base mutations (63, 64). Hence, all the resistance mechanisms against the tetracyclines appear to involve decreased drug binding in some way and indicate the involvement of these binding sites in the mechanism of action of the tetracyclines.

The broad spectrum of antibacterial activity of the tetracyclines can be attributed to the highly conserved nature of ribosomal protein synthesis pathways (and dsRNA structures) among bacterial species. The reversibility of the association of the tetracyclines with the ribosome is believed to account for their bacteriostatic effect (39).

Current theories on the mechanism(s) of action against non-bacterial pathogens and noninfectious disease conditions. The mitochondria are organelles that were derived from free-living proteobacteria acquired by eukaryotic cells via endosymbiosis (65). Hence, they have DNA, and in particular, ribosomes that are similar to those of bacteria. Because of the similarities between bacterial ribosomes and mitochondrial ribosomes, it was believed that the antiprotozoal activities of the tetracyclines were mediated via a similar interaction with the mitochondrial ribosomes of these parasites (66). In addition, some organisms which do not contain mitochondria are known to contain organelles derived from mitochondria, such as mitosomes and hydrogenosomes. Therefore, it remains possible that this theory of tetracycline action via inhibition of mitochondrial protein synthesis is valid, especially for unicellular organisms with very few mitochondria. Similarly, there is increasing evidence that the activity of the tetracyclines against certain parasites is mediated through the inhibition of protein synthesis in other endosymbiotic bacteria necessary for parasite survival and reproduction. For example, the endosymbiotic bacterium *Wolbachia* sp. has been identified as the target for doxycycline in its therapeutic indication against lymphatic filariasis and onchocerciasis (40). Depletion of the *Wolbachia* endosymbionts essential for parasite survival and fecundity by doxycycline leads to sterilization of adult worms in onchocerciasis and death of adult worms in bancroftian filariasis. There are also indications that the activity of the tetracyclines against *Plasmodium* spp. may target the apicoplasts, which are vestiges of nonphotosynthetic plastids derived by secondary endosymbiosis (5, 67). It is possible that these effects are achieved via inhibition of translation in the endosymbionts as in bacteria. However, the susceptibility of protozoa species which lack mitochondria (e.g., *Trichomonas vaginalis*, *Giardia lamblia*, *Entamoeba histolytica*) (7, 66), as well as viral pathogens (Table 1), raises further questions about the exact molecular mechanism(s) of action of the tetracy-

clines. In contrast to the proffered antiprotozoal activity via mitochondrial and endosymbiont rRNA binding, the antiviral activities of the tetracyclines have not been associated with rRNA binding (2, 13, 14, 68–70). Rather, the antiviral activity has been attributed in part to anti-inflammatory (4, 15), antiapoptotic (2), and neuroprotective (1) properties. Just like the antiviral activities, these other useful properties of the tetracyclines (especially the anti-inflammatory activities) are also not explained by the 16S rRNA binding mechanism. There are indications that the anti-inflammatory actions of doxycycline and minocycline in osteoarthritis are mediated via inhibition of nitric acid synthase. The mechanism of action was reported to be, at least in part, at the level of RNA expression and translation of the enzyme (71). Attempts have also been made to elucidate the mechanism by which doxycycline reduces pathology in lymphatic filariasis. Hence, a reduction in plasma levels of lymphangiogenic factors vascular endothelial growth factor-C (VEGF-C) and soluble vascular endothelial growth factor receptor-3 [(s)VEGFR-3] has been indicated (4). However, these proposed mechanisms of action do not offer an in-depth explanation of the activities of the tetracyclines under these conditions.

Consideration of tetracyclines as dsRNA ligands for a potential mechanism of action for their wide range of activities. It is interesting that the viruses against which the tetracyclines have shown some efficacy are RNA viruses, e.g., West Nile virus (WNV) (2), Japanese encephalitis virus (JEV) (70), and HIV (3). Therefore, the action of the tetracyclines against viruses appears to suggest a more generalized interaction with RNAs. This line of thought is supported by the results of another study which indicated that tetracycline induced stabilization of various cellular RNAs (72). Being that most cellular RNAs are partially or fully double stranded, the binding of small molecules such as the tetracyclines to dsRNA is worth considering as a possible explanation for the wide range of effects associated with the tetracyclines. Besides, the 16S rRNAs (like most cellular RNAs) fold into various secondary and tertiary structures, forming collections of short double helices.

A recent study indicated that tetracyclines bind to various synthetic dsRNAs of random base sequences (16). These findings imply that the double-stranded structures may be the target sites of tetracycline-RNA interactions instead of the specific base pairs investigated earlier. This also suggests that the tetracyclines bind to the double-stranded segments of cellular RNAs, especially the rRNAs that have extensive double-stranded structures (Fig. 3). Such binding would be expected to affect not only the stability of the secondary and tertiary structures but also the processing and/or function of the RNAs in some way. Therefore, it is possible that the tetracyclines act by binding to double-stranded RNAs in cells to inhibit their normal processing and function. In bacteria, for instance, this would imply that the tetracyclines bind to the double-stranded portions of the rRNA to inhibit their processing and, consequently, protein synthesis. Indeed, given the diverse functions of RNAs, one can envisage wide-ranging effects commensurate with the wide range of activities of the tetracyclines (which are not limited to antibacterial or even antimicrobial activities). These postulations still need to be adequately investigated.

The differences in the processing pathways could then account for the differences in the relative effects of tetracyclines on the functions of the various cellular RNAs, as well as the differences in their effects on prokaryotic and eukaryotic rRNAs. Endonucleases often generally degraded double-stranded RNAs both *in vitro* and *in vivo*. In cells, they are also known to degrade dsRNAs to process them for the specific functions they subsequently perform. In bacteria, for example, RNase III is essential for the initial processing of precursor rRNA transcripts for further maturation into the functional rRNA necessary for protein synthesis. They cleave the double-stranded secondary structures of the long pre-rRNA to yield the pre-16S and pre-23S rRNA for further maturation (73–75). Hence, the reported inhibition of dsRNA degradation by RNase III in the presence of the tetracyclines *in vitro* (16) may have wide-ranging implications in living cells with respect to RNA processing. This suggests that the binding of the tetracyclines to dsRNA in bacteria could interfere with dsRNA processing pathways, especially those for rRNA processing.

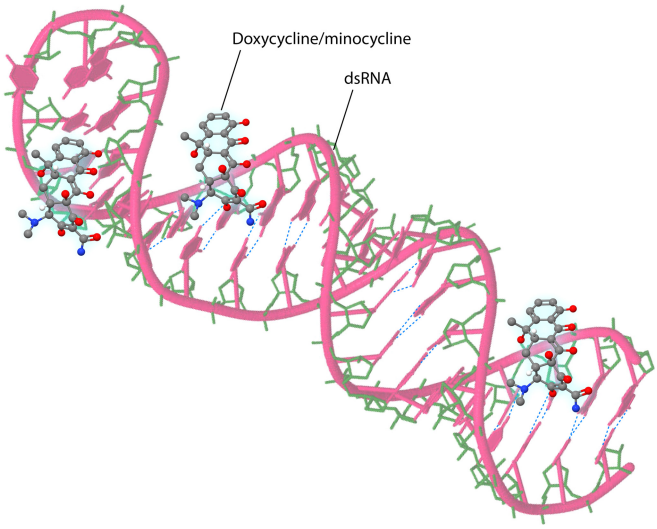

FIG 3 Graphic representation of the binding of tetracyclines to double-stranded segments of cellular RNA.

However, it would be wrong to suppose that the tetracyclines do not affect eukaryotic cells, as there are a range of toxic effects associated with their use, such as photosensitivity and teratogenic effects. The mechanism(s) of action involved in these toxic effects remains unclear but may involve dsRNA binding and processing, at least in part.

The binding of the tetracyclines to dsRNA may also explain the mechanism of their action against viruses. Since the viruses against which they are known to be effective by inhibiting/suppressing viral replication are RNA viruses (14), for example, WNV (2), JEV (70), and HIV (3), the tetracyclines may bind to these viral particles or products to activate or inactivate some other molecular pathways involved in the viral response (76–78). Most viruses produce dsRNA structures during replication. In flaviviruses (WNV and JEV), dsRNAs are often produced by RNA-dependent RNA polymerases during viral replication. In retroviruses (e.g., HIV), dsRNA is produced by the base-pairing of primer tRNA with the genomic RNA, forming a substrate for the reverse transcriptase (79). Also, replication of these viruses, like most RNA viruses, takes place in the cytoplasm of host eukaryotic cells, where ionic conditions (particularly Mg^{2+} concentrations) are favorable for doxycycline/minocycline binding. On the contrary, replication of most DNA viruses takes place in the host

eukaryotic cell's nucleus. In addition, a number of host defense mechanisms and innate immune responses are activated by dsRNA intermediates of viral replication, such as the RNA interference pathway and Toll-like receptor 3 (TLR3) activation of NF-κB and interferon production (78). Some of these immune responses are also indicated in the anti-inflammatory activities of the tetracyclines. Some viruses, particularly HIV, are known to successfully evade these host immune responses. It is possible that minocycline suppresses both viral replication and activation of the inflammatory and cellular responses to these viral infections via an effect on dsRNAs (14). Hence, the antiapoptotic, anti-inflammatory, and antiviral activities of minocycline that have been observed in viral infections (2, 13, 14, 68–70) may also be mediated via interactions with dsRNAs. Further investigations in line with the dsRNA binding perspectives are, therefore, quite imperative.

Proposed mechanism of selective microbial protein synthesis inhibition by the tetracyclines. If the mechanism of action of the tetracyclines involves rRNA binding, two mechanisms could be postulated for their selective inhibition of microbial protein synthesis.

(i) Higher-affinity binding for prokaryotic rRNAs versus eukaryotic rRNAs. Prokaryotic rRNAs are located in the cytoplasm of the cells where ionic conditions (especially Mg/divalent ion concentrations) are ideal for drug binding, as opposed to eukaryotic rRNAs, which are organized in a protected environment (the nuclear compartment). The relative Mg^{2+} concentrations in these cellular compartments may affect the binding of the tetracyclines to the rRNA (16, 46). This mechanism is consistent with the mitochondrial protein synthesis inhibition in eukaryotic cells (in both unicellular organisms like protozoa and higher organisms like animals and humans) at concentrations similar to those required for bacterial protein synthesis inhibition (80, 81). For unicellular organisms (which are mostly intracellular parasites), inhibitory concentrations could easily be achieved in the mitochondria of these parasites (82, 83). However, inhibition of mitochondrial protein synthesis in larger organisms may have additional considerations for tissue penetration and distribution in order for the drugs to access and bind to mitochondrial rRNA in the cytoplasm (80, 81). On the other hand, inhibition of cytosolic protein synthesis in eukaryotes occurs at concentrations that are several orders of magnitude (>10 times) higher than are required for bacterial protein synthesis inhibition (80, 83). These differences are associated with drug binding/affinity (probably due to differences in ionic conditions and drug chemistry) and may account for the mild side effects and toxicities associated with the therapeutic use of the tetracyclines in animals and humans (84).

(ii) Relative effects of drug binding on processing of prokaryotic versus eukaryotic RNAs. Currently, reports have shown that the binding of the tetracyclines inhibits RNase III processing of dsRNA (16). The processing of prokaryotic rRNAs is primarily dependent on RNase III (73); hence, inhibition of RNase III activity/processing would be expected to inhibit mature rRNA formation and function in prokaryotes. On the contrary, the processing of eukaryotic rRNA involves a much more complex pathway that is not dependent on RNase III and occurs in a protected environment (the nucleolus) instead of in the cytoplasm of bacteria (85). The activity of other enzymes involved in dsRNA and rRNA processing may also be differentially affected in prokaryotes and eukaryotes. In addition, tetracycline binding could stimulate conformational changes in the active sites of the rRNAs (where the message of the mRNA is decoded), which could also alter the enzymatic/catalytic functions of the rRNAs themselves. These catalytic functions also differ between prokaryotes and eukaryotes. The differences in the processing pathways could account for the inability of the tetracyclines to generally inhibit protein synthesis in larger organisms (animals and humans), as well as the effects of the drugs in noninfectious conditions (which may be mediated via effects on other dsRNA processing pathways).

CONCLUSION

It is becoming increasingly apparent that the 16S rRNA binding mechanism currently held for the antibacterial action of tetracyclines is not only limited but also does not explain their activities against other, nonbacterial, pathogens and under certain pathological conditions. It has therefore become imperative to consider alternative binding sites/modes that may offer insights into the broad spectrum of activity and antimicrobial selectivity of tetracyclines. The binding of the tetracyclines to cellular dsRNAs and the consequent effects on affected RNA processing and function could be a worthwhile alternative to explore.

REFERENCES

1. **Elewa HF, Hilali H, Hess DC, Machado LS, Fagan SC.** 2006. Minocycline for short-term neuroprotection. Pharmacotherapy **26:**515–521. http://dx.doi.org/10.1592/phco.
2. **Michaelis M, Kleinschmidt MC, Doerr HW, Cinatl J.** 2007. Minocycline inhibits West Nile virus replication and apoptosis in human neuronal cells. J Antimicrob Chemother **60:**981–986. http://dx.doi.org/10.1093/jac/dkm307.
3. **Zink MC, Uhrlaub J, DeWitt J, Voelker T, Bullock B, Mankowski J, Tarwater P, Clements J, Barber S.** 2005. Neuroprotective and anti-human immunodeficiency virus activity of minocycline. JAMA **293:**2003–2011. http://dx.doi.org/10.1001/jama.293.16.2003.
4. **Debrah AY, Mand S, Specht S, Marfo-Debrekyei Y, Batsa L, Pfarr K, Larbi J, Lawson B, Taylor M, Adjei O, Hoerauf A.** 2006. Doxycycline reduces plasma VEGF-C/sVEGFR-3 and improves pathology in lymphatic filariasis. PLoS Pathog **2:**e92. http://dx.doi.org/10.1371/journal.ppat.0020092.
5. **Ralph SA, D'Ombrain MC, McFadden GI.** 2001. The apicoplast as an antimalarial drug target. Drug Resist Updat **4:**145–151. http://dx.doi.org/10.1054/drup.2001.0205.
6. **Dahl EL, Shock JL, Shenai BR, Gut J, DeRisi JL, Rosenthal PJ.** 2006. Tetracyclines specifically target the apicoplast of the malaria parasite Plasmodium falciparum. Antimicrob Agents Chemother **50:**3124–3131. http://dx.doi.org/10.1128/AAC.00394-06.
7. **Chopra I, Roberts M.** 2001. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev **65:**232–260. http://dx.doi.org/10.1128/MMBR.65.2.232-260.2001.
8. **Schnappinger D, Hillen W.** 1996. Tetracyclines: antibiotic action, uptake, and resistance mechanisms. Arch Microbiol **165:**359–369. http://dx.doi.org/10.1007/s002030050339.
9. **Oehler R, Polacek N, Steiner G, Barta A.** 1997. Interaction of tetracycline with RNA: photoincorporation into ribosomal RNA of Escherichia coli. Nucleic Acids Res **25:**1219–1224. http://dx.doi.org/10.1093/nar/25.6.1219.
10. **Pioletti M, Schlunzen F, Harms J, Zarivach R, Gluhmann M, Avila H, Bashan A, Bartels H, Auerbach T, Jacobi C, Hartsch T, Yonath A, Franceschi F.** 2001. Crystal structures of complexes of the small ribosomal subunit with tetracycline, edeine and IF3. EMBO J **20:**1829–1839. http://dx.doi.org/10.1093/emboj/20.8.1829.
11. **Brodersen DE, Clemons WM, Carter AP, Morgan-Warren RJ, Wimberly BT, Ramakrishnan V.** 2000. The structural basis for the action of the antibiotics tetracycline, pactamycin, and hygromycin B on the 30S ribosomal subunit. Cell **103:**1143–1154. http://dx.doi.org/10.1016/S0092-8674(00)00216-6.
12. **Anokhina MM, Barta A, Nierhaus KH, Spiridonova VA, Kopylov AM.** 2004. Mapping of the second tetracycline binding site on the ribosomal

small subunit of E. coli. Nucleic Acids Res **32:**2594–2597. http://dx.doi.org/10.1093/nar/gkh583.
13. **Szeto GL, Brice AK, Yang H-C, Barber SA, Siliciano RF, Clements JE.** 2010. Minocycline attenuates HIV infection and reactivation by suppressing cellular activation in human CD4+ T cells. J Infect Dis **201:**1132–1140. http://dx.doi.org/10.1086/651277.
14. **Dutta K, Basu A.** 2011. Use of minocycline in viral infections. Indian J Med Res **133:**467–470.
15. **Scheinfeld N, Berk T.** 2010. A review of the diagnosis and treatment of rosacea. Postgrad Med **122:**139–143. http://dx.doi.org/10.3810/pgm.2010.01.2107.
16. **Chukwudi CU, Good L.** 20 January 2016. Interaction of the tetracyclines with double-stranded RNAs of random base sequence: new perspectives on the target and mechanism of action. J Antibiot http://dx.doi.org/10.1038/ja.2015.145.
17. **Hermann T.** 2005. Drugs targeting the ribosome. Curr Opin Struct Biol **15:**355–366. http://dx.doi.org/10.1016/j.sbi.2005.05.001.
18. **Spahn CMT, Prescott CD.** 1996. Throwing a spanner in the works: antibiotics and the translation apparatus. J Mol Med **74:**423–439. http://dx.doi.org/10.1007/BF00217518.
19. **Purohit P, Stern S.** 1994. Interactions of a small RNA with antibiotic and RNA ligands of the 30S subunit. Nature **370:**659–662. http://dx.doi.org/10.1038/370659a0.
20. **Vicens Q, Westhof E.** 2003. RNA as a drug target: the case of aminoglycosides. Chembiochem **4:**1018–1023. http://dx.doi.org/10.1002/cbic.200300684.
21. **Hermann T, Tor Y.** 2005. RNA as a target for small-molecule therapeutics. Expert Opin Ther Pat **15:**49–62. http://dx.doi.org/10.1517/13543776.15.1.49.
22. **Tok JBH, Cho JH, Rando RR.** 1999. Aminoglycoside antibiotics are able to specifically bind the 5′-untranslated region of thymidylate synthase messenger RNA. Biochemistry **38:**199–206. http://dx.doi.org/10.1021/bi9819428.
23. **De Stasio EA, Moazed D, Noller HF, Dahlberg AE.** 1989. Mutations in 16S ribosomal RNA disrupt antibiotic-RNA interactions. EMBO J **8:**1213–1216.
24. **Hermann T.** 2003. Chemical and functional diversity of small molecule ligands for RNA. Biopolymers **70:**4–18. http://dx.doi.org/10.1002/bip.10410.
25. **Noah JW, Dolan MA, Babin P, Wollenzien P.** 1999. Effects of tetracycline and spectinomycin on the tertiary structure of ribosomal RNA in the Escherichia coli 30S ribosomal subunit. J Biol Chem **274:**16576–16581. http://dx.doi.org/10.1074/jbc.274.23.16576.
26. **Yi H, Cho Y-J, Won S, Lee J-E, Jin Yu H, Kim S, Schroth GP, Luo S, Chun J.** 2011. Duplex-specific nuclease efficiently removes rRNA for prokaryotic RNA-seq. Nucleic Acids Res **39:**e140. http://dx.doi.org/10.1093/nar/gkr617.
27. **Bustin S.** 2000. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J Mol Endocrinol **25:**169–193. http://dx.doi.org/10.1677/jme.0.0250169.
28. **Jacobs BL, Langland JO.** 1996. When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. Virology **219:**339–349. http://dx.doi.org/10.1006/viro.1996.0259.
29. **Sucheck SJ, Wong C-H.** 2000. RNA as a target for small molecules. Curr Opin Chem Biol **4:**678–686. http://dx.doi.org/10.1016/S1367-5931(00)00142-3.
30. **Neault J-F, Tajmir-Riahi H-A.** 1997. RNA-diethylstilbestrol interaction studied by Fourier transform infrared difference spectroscopy. J Biol Chem **272:**8901–8904. http://dx.doi.org/10.1074/jbc.272.14.8901.
31. **Good L, Nielsen PE.** 1998. Inhibition of translation and bacterial growth by peptide nucleic acid targeted to ribosomal RNA. Proc Natl Acad Sci U S A **95:**2073–2076. http://dx.doi.org/10.1073/pnas.95.5.2073.
32. **Tor Y.** 2009. Exploring RNA-ligand interactions. Pure Appl Chem **81:**263–272.
33. **Xavier KA, Eder PS, Giordano T.** 2000. RNA as a drug target: methods for biophysical characterization and screening. Trends Biotechnol **18:**349–356. http://dx.doi.org/10.1016/S0167-7799(00)01464-5.
34. **Gooch BD, Beal PA.** 2004. Recognition of duplex RNA by helix-threading peptides. J Am Chem Soc **126:**10603–10610. http://dx.doi.org/10.1021/ja047818v.
35. **Tan Z-J, Chen S-J.** 2008. Salt dependence of nucleic acid hairpin stability. Biophys J **95:**738–752. http://dx.doi.org/10.1529/biophysj.108.131524.
36. **Tan Z-J, Chen S-J.** 2007. RNA helix stability in mixed Na+/Mg2+ solution. Biophys J **92:**3615–3632. http://dx.doi.org/10.1529/biophysj.106.100388.
37. **Blackwood RK, English AR.** 1970. Structure-activity relationships in the tetracycline series, p 237–266. *In* Perlman D (ed), Advances in applied microbiology, vol 13. Academic Press, New York, NY.
38. **Dürckheimer W.** 1975. Tetracyclines: chemistry, biochemistry, and structure-activity relations. Angew Chem Int Ed Engl **14:**721–734.
39. **Chopra I, Hawkey PM, Hinton M.** 1992. Tetracyclines, molecular and clinical aspects. J Antimicrob Chemother **29:**245–277. http://dx.doi.org/10.1093/jac/29.3.245.
40. **Hoerauf A.** 2008. Filariasis: new drugs and new opportunities for lymphatic filariasis and onchocerciasis. Curr Opin Infect Dis **21:**673–681. http://dx.doi.org/10.1097/QCO.0b013e328315cde7.
41. **Baron U, Bujard H.** 2000. Tet repressor-based system for regulated gene expression in eukaryotic cells: principles and advances. Methods Enzymol **327:**401–421. http://dx.doi.org/10.1016/S0076-6879(00)27292-3.
42. **Sclimenti CR, Baba EJ, Calos MP.** 2000. An extrachromosomal tetracycline-regulatable system for mammalian cells. Nucleic Acids Res **28:**E80. http://dx.doi.org/10.1093/nar/28.17.e80.
43. **Amar L, Desclaux M, Faucon-Biguet N, Mallet J, Vogel R.** 2006. Control of small inhibitory RNA levels and RNA interference by doxycycline induced activation of a minimal RNA polymerase III promoter. Nucleic Acids Res **34:**e37. http://dx.doi.org/10.1093/nar/gkl034.
44. **Newman EC, Frank CW.** 1976. Circular dichroism spectra of tetracycline complexes with Mg+2 and Ca+2. J Pharm Sci **65:**1728–1732. http://dx.doi.org/10.1002/jps.2600651209.
45. **Avery AM, Goddard HJ, Sumner ER, Avery SV.** 2004. Iron blocks the accumulation and activity of tetracyclines in bacteria. Antimicrob Agents Chemother **48:**1892–1894. http://dx.doi.org/10.1128/AAC.48.5.1892-1894.2004.
46. **Berthon G, Brion M, Lambs L.** 1983. Metal ion-tetracycline interactions in biological fluids. 2. Potentiometric study of magnesium complexes with tetracycline, oxytetracycline, doxycycline, and minocycline, and discussion of their possible influence on the bioavailability of these antibiotics in blood plasma. J Inorg Biochem **19:**1–18. http://dx.doi.org/10.1016/0162-0134(83)85009-0.
47. **Lambs L, Decock-Le Reverend B, Kozlowski H, Berthon G.** 1988. Metal ion-tetracycline interactions in biological fluids. 9. Circular dichroism spectra of calcium and magnesium complexes with tetracycline, oxytetracycline, doxycycline, and chlortetracycline and discussion of their binding modes. Inorg Chem **27:**3001–3012.
48. **McMurry LM, Cullinane JC, Petrucci RE, Levy SB.** 1981. Active uptake of tetracycline by membrane vesicles from susceptible Escherichia coli. Antimicrob Agents Chemother **20:**307–313. http://dx.doi.org/10.1128/AAC.20.3.307.
49. **Yamaguchi A, Udagawa T, Sawai T.** 1990. Transport of divalent cations with tetracycline as mediated by the transposon Tn10-encoded tetracycline resistance protein. J Biol Chem **265:**4809–4813.
50. **Yamaguchi A, Iwasaki-Ohba Y, Ono N, Kaneko-Ohdera M, Sawai T.** 1991. Stoichiometry of metal-tetracycline/H+ antiport mediated by transposon Tn10-encoded tetracycline resistance protein in Escherichia coli. FEBS Lett **282:**415–418. http://dx.doi.org/10.1016/0014-5793(91)80527-A.
51. **Goldman RA, Hasan T, Hall CC, Strycharz WA, Cooperman BS.** 1983. Photoincorporation of tetracycline into Escherichia coli ribosomes: identification of the major proteins photolabeled by native tetracycline and tetracycline photoproducts and implications for the inhibitory action of tetracycline on protein synthesis. Biochemistry **22:**359–368. http://dx.doi.org/10.1021/bi00271a020.
52. **Day LE.** 1966. Tetracycline inhibition of cell-free protein synthesis I. Binding of tetracycline to components of the system. J Bacteriol **91:**1917–1923.
53. **Atherly AG.** 1974. Specific inhibition of ribosomal RNA synthesis in Escherichia coli by tetracycline. Cell **3:**145–151. http://dx.doi.org/10.1016/0092-8674(74)90119-6.
54. **Srivastava AK, Schlessinger D.** 1989. Processing pathway of Escherichia coli 16S precursor rRNA. Nucleic Acids Res **17:**1649–1663. http://dx.doi.org/10.1093/nar/17.4.1649.
55. **Wireman JW, Sypherd PS.** 1974. In vitro assembly of 30S ribosomal particles from precursor 16S RNA of Escherichia coli. Nature **247:**552–554. http://dx.doi.org/10.1038/247552a0.
56. **Ceccarelli A, Dotto GP, Altruda F, Perlo C, Silengo L, Turco E, Mangiarotti G.** 1978. Immature 50S subunits in Escherichia coli polyribo-

somes. FEBS Lett **93:**348–350. http://dx.doi.org/10.1016/0014-5793(78)81137-5.
57. **Srivastava AK, Schlessinger D.** 1988. Coregulation of processing and translation: mature 5′ termini of Escherichia coli 23S ribosomal RNA form in polysomes. Proc Natl Acad Sci U S A **85:**7144–7148. http://dx.doi.org/10.1073/pnas.85.19.7144.
58. **Bauer G, Berens C, Projan SJ, Hillen W.** 2004. Comparison of tetracycline and tigecycline binding to ribosomes mapped by dimethylsulphate and drug-directed Fe2+ cleavage of 16S rRNA. J Antimicrob Chemother **53:**592–599. http://dx.doi.org/10.1093/jac/dkh125.
59. **Ruoho AE, Kiefer H, Roeder PE, Singer SJ.** 1973. The mechanism of photoaffinity labeling. Proc Natl Acad Sci U S A **70:**2567–2571. http://dx.doi.org/10.1073/pnas.70.9.2567.
60. **Moazed D, Noller HF.** 1987. Interaction of antibiotics with functional sites in 16S ribosomal RNA. Nature **327:**389–394. http://dx.doi.org/10.1038/327389a0.
61. **Trieber CA, Taylor DE.** 2002. Mutations in the 16S rRNA genes of Helicobacter pylori mediate resistance to tetracycline. J Bacteriol **184:**2131–2140. http://dx.doi.org/10.1128/JB.184.8.2131-2140.2002.
62. **Ross JI, Eady EA, Cove JH, Cunliffe WJ.** 1998. 16S rRNA mutation associated with tetracycline resistance in a gram-positive bacterium. Antimicrob Agents Chemother **42:**1702–1705.
63. **Nonaka L, Connell SR, Taylor DE.** 2005. 16S rRNA mutations that confer tetracycline resistance in Helicobacter pylori decrease drug binding in Escherichia coli ribosomes. J Bacteriol **187:**3708–3712. http://dx.doi.org/10.1128/JB.187.11.3708-3712.2005.
64. **Dailidiene D, Bertoli MT, Miciuleviciene J, Mukhopadhyay AK, Dailide G, Pascasio MA, Kupcinskas L, Berg DE.** 2002. Emergence of tetracycline resistance in Helicobacter pylori: multiple mutational changes in 16S ribosomal DNA and other genetic loci. Antimicrob Agents Chemother **46:**3940–3946. http://dx.doi.org/10.1128/AAC.46.12.3940-3946.2002.
65. **Edlind TD.** 1991. Protein synthesis as a target for antiprotozoal drugs, p 569–586. *In* Coombs G, North M (ed), Biochemical protozoology. Taylor & Francis Ltd, London, United Kingdom.
66. **Katiyar SK, Edlind TD.** 1991. Enhanced antiparasitic activity of lipophilic tetracyclines: role of uptake. Antimicrob Agents Chemother **35:**2198–2202. http://dx.doi.org/10.1128/AAC.35.11.2198.
67. **Marechal E, Cesbron-Delauw MF.** 2001. The apicoplast: a new member of the plastid family. Trends Plant Sci **6:**200–205. http://dx.doi.org/10.1016/S1360-1385(01)01921-5.
68. **Copeland KFT, Brooks JI.** 2010. A novel use for an old drug: the potential for minocycline as anti-HIV adjuvant therapy. J Infect Dis **201:**1115–1117. http://dx.doi.org/10.1086/651278.
69. **Szeto GL, Pomerantz JL, Graham DRM, Clements JE.** 2011. Minocycline suppresses activation of nuclear factor of activated T cells 1 (NFAT1) in human CD4+ T cells. J Biol Chem **286:**11275–11282. http://dx.doi.org/10.1074/jbc.M110.210518.
70. **Mishra MK, Basu A.** 2008. Minocycline neuroprotects, reduces microglial activation, inhibits caspase 3 induction, and viral replication following Japanese encephalitis. J Neurochem **105:**1582–1595. http://dx.doi.org/10.1111/j.1471-4159.2008.05238.x.
71. **Amin AR, Attur MG, Thakker GD, Patel PD, Vyas PR, Patel RN, Patel IR, Abramson SB.** 1996. A novel mechanism of action of tetracyclines: effects on nitric oxide synthases. Proc Natl Acad Sci U S A **93:**14014–14019. http://dx.doi.org/10.1073/pnas.93.24.14014.
72. **Wei Y, Bechhofer DH.** 2002. Tetracycline induces stabilization of mRNA in Bacillus subtilis. J Bacteriol **184:**889–894. http://dx.doi.org/10.1128/jb.184.4.889-894.2002.
73. **Srivastava AK, Schlessinger D.** 1990. Mechanism and regulation of bacterial ribosomal RNA processing. Annu Rev Microbiol **44:**105–129. http://dx.doi.org/10.1146/annurev.mi.44.100190.000541.
74. **Dunn JJ, Studier FW.** 1973. T7 early RNAs and Escherichia coli ribosomal RNAs are cut from large precursor RNAs in vivo by ribonuclease 3. Proc Natl Acad Sci U S A **70:**3296–3300. http://dx.doi.org/10.1073/pnas.70.12.3296.
75. **Nikolaev N, Schlessinger D, Wellauer PK.** 1974. 30S pre-ribosomal RNA of Escherichia coli and products of cleavage by ribonuclease III: length and molecular weight. J Mol Biol **86:**741–747. http://dx.doi.org/10.1016/0022-2836(74)90350-7.
76. **Vercammen E, Staal J, Beyaert R.** 2008. Sensing of viral infection and activation of innate immunity by toll-like receptor 3. Clin Microbiol Rev **21:**13–25. http://dx.doi.org/10.1128/CMR.00022-07.
77. **Alexopoulou L, Holt AC, Medzhitov R, Flavell RA.** 2001. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. Nature **413:**732–738. http://dx.doi.org/10.1038/35099560.
78. **Ding S-W, Voinnet O.** 2007. Antiviral immunity directed by small RNAs. Cell **130:**413–426. http://dx.doi.org/10.1016/j.cell.2007.07.039.
79. **Dimmock NJ, Easton AJ, Leppard K.** 2007. Introduction to modern virology, 6th ed. Blackwell Publishing, Malden, MA.
80. **van den Bogert C, Kroon AM.** 1981. Tissue distribution and effects on mitochondrial protein synthesis of tetracyclines after prolonged continuous intravenous administration to rats. Biochem Pharmacol **30:**1706–1709. http://dx.doi.org/10.1016/0006-2952(81)90403-2.
81. **McKee EE, Ferguson M, Bentley AT, Marks TA.** 2006. Inhibition of mammalian mitochondrial protein synthesis by oxazolidinones. Antimicrob Agents Chemother **50:**2042–2049. http://dx.doi.org/10.1128/AAC.01411-05.
82. **Beckers CJ, Roos DS, Donald RG, Luft BJ, Schwab JC, Cao Y, Joiner KA.** 1995. Inhibition of cytoplasmic and organellar protein synthesis in Toxoplasma gondii. Implications for the target of macrolide antibiotics. J Clin Invest **95:**367–376.
83. **Chang HR, Comte R, Pechère JC.** 1990. In vitro and in vivo effects of doxycycline on Toxoplasma gondii. Antimicrob Agents Chemother **34:**775–780. http://dx.doi.org/10.1128/AAC.34.5.775.
84. **van den Bogert C, Holtrop M, Melis TE, Roefsema PR, Kroon AM.** 1987. Different effects of oxytetracycline and doxycycline on mitochondrial protein synthesis in rat liver after long-term treatment. Biochem Pharmacol **36:**1555–1559. http://dx.doi.org/10.1016/0006-2952(87)90126-2.
85. **Eichler DC, Craig N.** 1994. Processing of eukaryotic ribosomal RNA. Prog Nucleic Acid Res Mol Biol **49:**197–239. http://dx.doi.org/10.1016/S0079-6603(08)60051-3.
86. **Bunnag D, Karbwang J, Na-Bangchang K, Thanavibul A, Chittamas S, Harinasuta T.** 1996. Quinine-tetracycline for multidrug resistant falciparum malaria. Southeast Asian J Trop Med Public Health **27:**15–18.

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