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Commentary

Phagomimetic Action of Antimicrobial Agents

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A wide variety of extracted and synthesised drug molecules have electron transfer capabilities which allow them to generate reactive oxygen species (ROS). In particular, many antibiotics that kill or inhibit bacteria, yeasts and cancer cells readily transfer electrons to oxygen making superoxide and hydrogen peroxide in the process. When suitable redox active forms of iron are available, Fenton chemistry occurs generating the highly damaging hydroxyl radical. This type of chemistry is very similar to that which evolved within phagocytic cells as part of their microbial killing armoury. Many antibiotics, when used in model systems, have well defined pharmacological actions against key cellular functions, but their clinical usefulness is also often demonstrable at concentrations in vivo well below their in vitro minimum inhibitory concentrations. These observations have led us to propose that a common mechanism exists whereby phagocytic cells and antibiotics exploit the use of ROS for microbial killing.

Keywords: Reactive oxygen species, transition metal ions, phagocytosis, antibiotics, electron transfer, DNA damage

ELECTRON TRANSFER AND DRUG ACTIONS

A large number of biologically active compounds, or their metabolites have clearly recognisable electron transfer (ET) functionalities and these include, quinones (or phenolic precursors), metal complexes and complexors, conjugated iminium species (Figure 1), and aromatic nitro compounds (or their reduced derivatives). It is the recently recognised ability[1,2] of a large number of antibacterial agents to undergo redox cycling, and to transfer electrons to oxygen to produce free radicals and other reactive oxygen species (ROS), that is the subject of this brief review. In many cases, the production of ROS by redox cycling drugs will be part of drug efficacy, whilst for others it may only contribute to unwanted toxicity.

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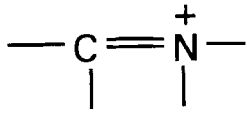


FIGURE 1 (iminium species)

ANTIBIOTICS: A GENERAL CONSIDERATION

Antibiotics can be defined as chemicals produced by one organism that are inhibitory to the growth of other organisms. Most antibiotics have been isolated from soil actinomycetes, although marine micro-organisms remain a vast potential resource. Antibiotics of current therapeutic value are used to treat bacterial, fungal and cancerous growths, and to a lesser extent protozoal and viral infections. Some antibiotics, such as the tetracyclines, are widely used in animal feeds to promote growth, and there is concern that they, or their metabolites, might enter food chains and cause serious problems.

Of the thousands of antibiotics isolated from micro-organisms, relatively few have proved suitable for human, clinical, and veterinary use. Problems of toxicity and solubility have been major restrictions. Many of the antibiotics presently used, such as the penicillins, are now synthesised or chemically modified.

The Target-Specific Nature of Antibiotics and Bactericides

The bactericidal and bacteriostatic actions of antibiotics are thought to arise primarily through their ability to inhibit key microbial metabolic processes or key enzymes (reviewed in 3–7).

Since bacterial cells have a cell wall, this can be targeted by chemicals such as bacitracin (polypeptide antibiotic) that binds to polyprenyl pyrophosphate, an essential precursor in cell wall synthesis. The penicillins and cephalosporins (β lactam antibiotics) are the most widely used group of cell wall active drugs, that prevent transpeptidation between peptidoglycan chains by inhibiting key enzymes. In addition, ET-ROS processes may also contribute to their biological effects.

The cell membrane can also be targeted, and in this case antibiotics that bind to sterols can be exploited to inhibit the growth of yeast and fungi that have sterol-containing membranes. The polyene antifungal drugs include candicidin, amphotericin B, and nystatin, and the polypeptide drugs include gramicidin and polymyxin. By binding to cell membranes these antibiotics disrupt normal cell function, often by causing the formation of permeable pores through which ions and other low-molar-mass solutes can pass.

Protein synthesis takes place on ribosomes, and here another class of antibiotics bind to ribosomes and interfere with normal bacterial protein synthesis. This group includes streptomycin, chloramphenicol, erythromycin and the tetracyclines.

The most toxic group of antibiotics are those which target cellular DNA. They are mutagenic and probably carcinogenic as well, and should be handled with great care. Antitumour antibiotics are used clinically to treat malignant growths, although their success against solid tumours is often poor, probably due to restricted blood flow to the tumour centre. Many other factors contribute to cell resistance, such as levels of GSH and topoisomerases.[8] In spite of their poor penetration and toxicity, they form an essential part of modern cancer treatment regimens. The anthracylines and aureolic acid antibiotics are sugar-containing drugs which intercalate the DNA molecule (insert between complementary DNA strands within the double helical structure). Intercalation prevents further DNA replication and topoisomerase is provoked to cleave DNA. In addition redox cycling of certain drug moieties can transfer electrons to oxygen to generate reactive and damaging drug and oxygen radicals. Antibiotics of this group include bleomycin, mitomycin C, doxorubicin and actinomycin D. RNA can also be targeted by the rifamycin antibiotics which inhibit bacterial RNA

synthesis by binding to a DNA-dependent RNA polymerase (for reviews see 3–7).

Metal Ion-Sequestration by Antibiotics

Many antibiotics are metal chelators and metal ions frequently play key roles in their biological actions. Indeed, many of our most useful iron-chelators are produced from microbial siderophores, which, under our definition can also be classified as antibiotics. Almost all aerobic life forms have an essential requirement for iron in order to facilitate oxygen metabolism. Unfortunately for bacteria most environmental iron is present in the highly insoluble ferric state [Fe (III)] at neutral pH values, and not readily available to them. To overcome restricted iron availability, micro-organisms produce high affinity iron-binding chemicals (siderophores) which capture, solubilise, and internalise iron by using special receptors. Animals have evolved to capture and transport iron by using high affinity iron-binding proteins, such as transferrin and lactoferrin. These ligands facilitate iron transport and metabolism, and help us to survive in competition with micro-organisms.[9] Desferrioxamine is the most widely used microbial siderophore in clinical medicine, and is used to treat patients with iron-overload conditions.

Metal-Binding by Antimicrobial Agents

The tetracycline families of antibiotics have been in clinical use for nearly 40 years, and are avid metal ion binding agents.[10] It is thought likely that a magnesium complex participates in the inhibition of bacterial protein synthesis at the ribosome.[3] Patients treated with tetracyclines show the presence of calcium complexes in their plasma, and calcium complexation may account for reports of inhibited bone development[11] in some patients.

The aminoglycoside family of antibiotics is known to inhibit bacterial protein synthesis. They are most effective in the treatment of gram negative infections but their clinical use has been limited by reports of their oto- and nephro-toxicities.

Metal ions are known to bind to aminoglycosides such as streptomycin with evidence of copper, calcium and nickel complexes being formed.[12] Evidence suggests that certain transition metal ions may enhance some of the toxicities associated with these antibiotics. For example iron supplementation has been shown to increase gentamycin nephrotoxicity in rats,[13,14] and iron chelators have been shown to reduce ototoxicity in Kanamycin treated guinea pigs.[15] Recently, an iron gentamycin complex was shown to promote peroxidation of arachidonic acid in vitro.[16] These findings together with decreased side effects observed when free radical scavengers were administered together with the antibiotics,[17] suggest transition metal ion-catalysed production of ROS by aminoglycosides is important in their toxicity.

The beta-lactam antibiotics most widely used in clinical and veterinary practice are the penicillins and cephalosporins. In vitro experimental evidence shows that these antibiotics readily form complexes with a range of metal ions including zinc, copper and iron (reviewed in 18). Indeed the iron-binding properties of penicillin are such that iron deficiency anaemia can result from penicillin therapy.[18]

Bacitracin antibiotics bind metal ions, and are often stabilised as a zinc complex (reviewed in 19). Since EDTA suppresses the antibacterial activity of bacitracin, it has been concluded that metal ions are involved in its biological activity.[20]

Metal-Binding by Antitumour Antibiotics

Several of the quinone-group containing antitumour antibiotics bind metal ions. The aminoquinone, streptonigrin, is a highly substituted derivative of the iron chelator picolinic acid. The ability of streptonigrin to damage DNA has been shown to depend upon the binding of transition metal ions such as iron or copper (reviewed in 21,22), which also enhance its antibacterial activ-

ity. Evidence for the formation of a ternary complex between antibiotic, metal ion, and DNA has been established.[23] The clinically used anthracycline antibiotics doxorubicin (Adriamycin) and daunorubicin (Daunomycin) bind a variety of metals such as iron and copper ions which can facilitate DNA damage (in model systems), (reviewed in 24). When ferric ions bind to doxorubicin, the drug can transfer an electron to the ferric ion reducing it to the ferrous state.[25,26] This type of electron transfer from ligand to the bound metal ion, paticularly iron ions, is not uncommon and is seen to occur when ferric ions are bound to modified desferrioxamine,[27] citrate,[28] ethyleneglycol-bis (β-aminoethyl ether)N,N,N′,N′-tetraacetic acid (EGTA) and nitrilotriacetate (NTA).[29]

The bleomycins are a unique group of antitumour antibiotics that bind a variety of transition metal ions. When ferrous ions are bound to bleomycin, the drug acts as a ferrous-ion oxidising catalyst (ferroxidase), catalysing oxidation to the ferric state.[30] When DNA is bound to the bleomycin-ferrous complex, substantial base and deoxyribose sugar damage occur to the DNA molecule[31,32] via reactive oxo-iron intermediates. Bleomycin can undergo several DNA-damaging cycles before being degraded itself.

PHAGOMIMETIC ACTIONS OF ANTIBIOTICS

The term 'phagomimetic' was introduced by the authors in 1989[33] to describe the ROS producing properties of antibiotics that may contribute to microbial cell killing, in the same way that ROS produced by phagocytes are known to do.

Production of ROS by Phagocytic Cells

Human blood contains large numbers of neutrophils (about 2.5–7.5 million per ml in healthy subjects), whose function is to recognise, engulf, and destroy foreign organisms, such as bacteria and viruses. This process of engulfment is called phagocytosis, and cells which can do it are called phagocytes.

Recognition of foreign organism by a neutrophil usually occurs because the foreign organism has been coated by an antibody protein produced by the immune system of the body. As soon as an organism, recognisable as foreign, touches the surface of the neutrophil, an enzyme in the membrane of this cell is activated. This enzyme removes electrons from NADPH inside the cell, oxidising it to $NADP^+$. The electrons are passed across the membrane and used to reduce O_2 to $O_2^{\bullet-}$ on the outer surface of the neutrophil. Hence when the foreign organism is engulfed by the neutrophil, it is wrapped up in a piece of membrane generating $O_2^{\bullet-}$. This $O_2^{\bullet-}$ production is one of the mechanisms by which engulfed foreign organisms are killed by phagocytes (reviewed in 34). Another killing mechanism sometimes used is the production of toxic amounts of nitric oxide $NO^{\bullet}$. The microbial killing process ultimately leads to the death of the phagocytic cells from oxidative damage.

The importance of $O_2^{\bullet-}$ production by neutrophils to human health is simply illustrated by examining patients whose neutrophils lack the ability to do it such as those from patients with chronic granulomatous disease. Patients with this disease suffer persistent infections with certain bacteria, presumably those whose killing by neutrophils is largely superoxide dependent. Although the symptoms of chronic granulomatous disease tell us that $O_2^{\bullet-}$ is important in the killing of bacteria by phagocytes, the mechanism by which killing occurs is uncertain. Superoxide is not very toxic and it cannot get inside bacteria. It can form H_2O_2.[1]

$$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2 \qquad (1)$$

which is toxic to many bacterial strains. The H_2O_2 easily enters the bacterium (since it resembles water) and reacts with iron or copper inside the bacteria to produce damaging $^{\bullet}OH$. Activated phagocytes utilise myleoperoxidase to produce

hypochlorous acid (HOCl) from H_2O_2 and chloride ions. HOCl can react with $O_2^{\cdot-}$ or with ferrous ions to make $^{\cdot}OH$.[35] Superoxide could also react with $NO^{\cdot}$ (if both were generated at the same time) to form peroxynitrite.[2]

$$O_2^{\cdot-} + NO^{\cdot} \rightarrow ONOO^{\cdot} \quad (2)$$

Peroxynitrite is itself likely to be damaging to bacteria, and it might also break down to give a species with reactivity similar to that of the $^{\cdot}OH$ radical.[3]

$$ONOO^{\cdot} + H^+ \rightarrow NO_2^{\cdot} + {}^{\cdot}OH\text{-like species.} \quad (3)$$

Thus, activated phagocytes can kill bacteria by imposing severe oxidative stress upon them. How might antibiotics do the same?

Generation of ROS by Antibiotics

Quinone-containing molecules are ubiquitous in nature, particularly in plants. In many cases they serve as 'toxins' to deter predators and so protect the genetic material of the plant. One of the best examples of quinones contributing to the defence of an insect is seen in the bombardier beetle. When disturbed the beetle sprays a pulsed jet (500 times per second) of hot solution containing hydroquinones and hydrogen peroxide—the perfect 'anti-mugging' spray. Many drugs contain quinone or hydroquinone rings, particularly the antitumour antibiotics. Hence, a mechanistic discussion of anticancer action in the ET-ROS context is appropriate.

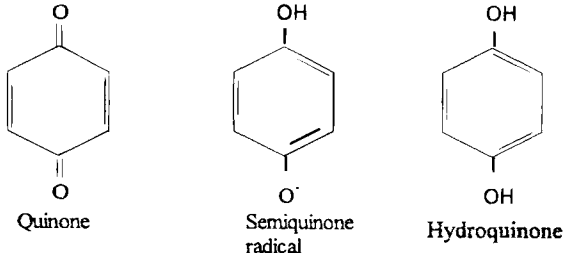


FIGURE 2

In 1959, one of the authors (PK) proposed the oxo-radical hypothesis of anticancer action of drugs stating that many anticancer drugs are also carcinogens, and vice versa.[36] In a wider context this has become known as 'Haddows paradox'. With considerable experimental evidence to support it, the thesis was elaborated upon in greater detail to include metal complexes, quinones, iminoquinones, iminium salts, and radiation in 1986.[37] Since antitumour agents are one of the most extensively investigated areas of drug research, large numbers of additional agents, or their metabolites, have appeared that incorporate ET functionalities. Representative examples are metal complexes (Cu, Fe, Ni, Ru, Rh),[38–41] quinones (benzo,[40,42] naptho,[43] dipheno,[44] streptonigrin complexor,[45] imine or iminium types (methotrexate,[46] α-difluoromrthylornithine,[46] rhodamine 123,[39] quinoline salts,[44,47] acridine salts,[39,40,48] and neocarzinostatin.[49,50]

There is general consensus that the mode of action of cis-Pt entails adduct formation with DNA, which shields the strands from excision repair.[51] However, it appears that binding alone is not sufficient, and that some mechanism which occurs after the Pt species attaches itself to DNA must account for the antitumour activity.[38,53] For transition metal ions, as we discussed in section 2.2.2, associated with antitumour antibiotics electron transfer reactions can result in the generation of ROS.

Several features common to most antitumour agents can be identified.[37] These are.

1. Binding to DNA by alkylation, complexation, or intercalation.
2. Presence of an ET entity.
3. ET with disruption of vital host processes, usually by oxidative stress.
4. DNA strand cleavage.

It has been shown that intercalation alone is not sufficient[53] and that many intercalators display ET functionalities e.g. quinone, iminium (aromatic N-heterocycles), and nitro aromatic compounds,[54,55] and are pro-oxidants.[56] DNA

strand cleavage can occur either by generation of ROS or by the p-phenylene diradical formed from enediynes[57] (core depiction in figure 3).

As early as 1964 ET processes were being examined in DNA[58] with extension to anticancer agents[38] and carcinogens.[58,59] Experiments involving donor and acceptor entities in DNA or oligomers demonstrated that ET could occur over appreciable distance.[60] When applied to carcinogens, anticancer agents, and other drugs, the intercalator functions as the acceptor coupled with the DNA base functioning as donor. For alkylating agents, the resulting conjugated iminium species derived from a DNA base would operate as the electron attractor.[59] DNA bases have the ability to act as both donors and acceptors.[61] Over the years an appreciable number of reports deal with the ability of alkylating species to induce oxidative stress.[59,62,63] ROS and lipoperoxidation are common features assioiated with the functioning of anticancer agents.[64–66] Let us discuss some of these with special reference to antitumour antibiotics.

ROS from Antitumour Antibiotics

From the original pioneering work[67,68,69] we know that most of the antitumour antibiotics with redox cycling moieties and or metal-binding properties can be made to generate free radicals in model systems. The possibility therefore exists that free radicals might participate in cancer cell killing by damaging DNA. The quinone group-containing drugs (Q) (Figure 2) can be reduced by the microsomal electron transport chain, and by the electron transport chain located on the nuclear envelope, to reactive semiquinones ($SQ^{\bullet}$) that can react with cellular components, or combine with oxygen to form superoxide (Equations 4–5)

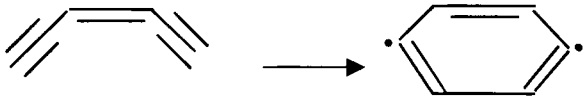


FIGURE 3

$$Q + e \rightarrow SQ^{\bullet} \quad (4)$$

$$SQ^{\bullet} + O_2 \rightarrow O_2^{\bullet -} + Q \quad (5)$$

Superoxide will dismute to hydrogen peroxide (H_2O_2) and oxygen (Equation 6), and hydrogen peroxide in the presence of a suitable reduced transition metal ion will form the highly reactive and damaging hydroxyl radical ($^{\bullet}OH$) (Equations 6–7)

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (6)$$

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \quad (7)$$

As previously mentioned, contaminating iron ions from the environment are usually present in the oxidised ferric state Fe (III). Reduction of Fe (III) to Fe (II), which facilitates $^{\bullet}OH$ generation, can be achieved by two pathways: (A) involving superoxide, (B) involving semiquinones (Equations 8–9).

$$\text{A} \quad O_2^{\bullet -} + \text{Fe(III)-complex} \rightarrow O_2 + \text{Fe(II)-complex} \quad (8)$$

$$\text{B} \quad SQ^{\bullet} + \text{Fe(III)-complex} \rightarrow Q + \text{Fe(II)-complex} \quad (9)$$

In general, route B is not favoured until O_2 concentrations are low. Reduction of the quinone group-containing drugs can be catalysed in vitro by enzymes such as xanthine oxidase or ferredoxin reductase. Provided that $^{\bullet}OH$ radicals are formed very close to DNA, they could participate in DNA damage. However, intercalated anthracyclines are no longer accessible to enzymatic reduction.[70,71,72] A mechanism for $^{\bullet}OH$ generation which bypasses enzymatic reduction has been proposed for doxorubicin, whereby autoreduction of an intercalated iron complex generates $^{\bullet}OH$ (for a general review see 72).

Recent evidence has shown that transgenic mice constitutively over expressing cardiac catalase, are largely protected from lipid peroxida-

tion and other cardiotoxic side effects associated with the adminsitration of doxorubicin.[73] Additionally, transgenic mice which express increased levels of manganese superoxide dismutase (mitochondrial SOD) are protected from the cardiotoxic effects of doxorubicin, thereby implicating oxidative damage to the mitochondria as a key adverse event.[74]

ROS from Antifungal Antibiotics

Several of the antifungal antibiotics (AFH) such as candicidin, nystatin and amphotericin B are lipophilic molecules that contain conjugated double bonds, and are known as 'polyenes'. Conjugated double bonds make these molecules readily oxidisable,[75] resulting in a loss of antifungal activity.[76] Indeed it has been shown that antioxidants can enhance the antifungal action of amphotericin B.[77] Autooxidation of polyenes, resulting in the formation of peroxyl radicals and aldehydic products similar to those formed from peroxidised unsaturated fatty acids (Equations 10–12), has been demonstrated, and suggested to contribute to antifungal activity.[78,79] Indeed oxidative damage has been implicated in the red blood cell lysis induced by amphotericin B in vitro.[81]

$$AFH + OH^{\bullet} \rightarrow AF^{\bullet} + H_2O \qquad (10)$$

$$AF^{\bullet} + O_2 \rightarrow AFO_2^{\bullet}\ \text{(peroxyl radical)} \qquad (11)$$

$$AFO_2^{\bullet} + AFH \rightarrow AFOOH \qquad (12)$$

(hydroperoxide) + $AF^{\bullet}$

↓

Fragments to aldehydes

$AF^{\bullet}$ Continues the chain reaction as above.

ROS and ET from Antibacterial Antibiotics

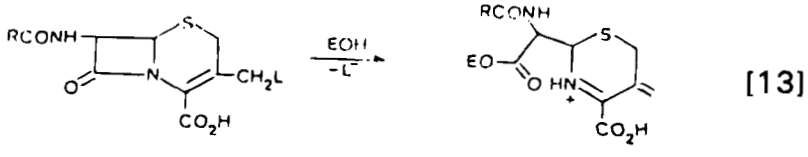
Here we consider some diverse, structurally unrelated antibiotics that can participate in electron transfer reactions resulting in the generation of ROS in some cases.

β-Lactam Antibiotics There is widespread acceptance that the major antibacterial action of β-lactams is dependent upon inactivation of cell wall enzymes. A questioning of this oversimplified view can, however, now be found in the literature.[80,81] For example, loss of bacterial viability is more complicated than simple inactivation of enzymes, and lysis and death are secondary, indirect responses to enzyme binding. Interaction with enzyme protein (EOH) does not automatically result in lysis. Hence, the mechanism of the irreversible effects must involve events in addition to enzyme binding.

Recently, the iminium hypothesis of β-lactam action was proposed entailing ET reactions, which occur after site binding.[82] As applied to bicyclic types, usually lactam ring opening generates a basic imine that can form iminium (Equation 13) on protonation by nearby acidic hydrogen.

Examples are cephalosporins, penicillin, bisnorisopenicillin, thienamycins, and olivanic acid. In most cases, the imine arises in conjugation with the carboxyl group. Isomerisation of the nonconjugated imine in the case of penicillin was addressed in a computational approach.[83] In electrochemical studies, iminium salts of Δ^1-pyrroline-2-carboxylic acid (Figure 4) and Δ^3-thiazoline-4-carboxylic acid (Figure 5) were used as models.[82]

Reduction potentials fell in the range of –0.18 to –0.37 V which should be capable of inducing ET transformations Thus, after binding there



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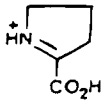


FIGURE 4

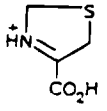
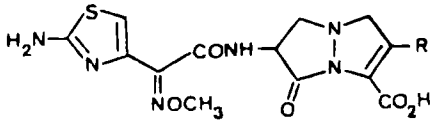


FIGURE 5



a) R = CN
b) R = SO_2CH_3

FIGURE 7

may be electrochemical interference with normal bacterial ET processes culminating in death.

In contrast to the fused ring types, the monocyclic β-lactams cannot generate iminium on ring opening. It is quite suggestive that the monocyclic category is usually characterised by a conjugated imine, e.g., oxime derivative, in the amide chain as shown for aztreonam (Figure 6).

Reduction potentials of the protonated (iminium) forms were in the range of –0.47 to –0.64 V.[83] Various antibiotics with modified ring structures, e.g., pyrazolidinones (Figure 7), lactivicin (Figure 8), and β-lactones, can also be readily incorporated into the theoretical framework.

Hence, essentially all of the lactam antibiotics and related types can be mechanistically accommodated within the dual concept of enzyme acylation and generation of a conjugated iminium moiety.[85] In an electrochemical classification of antibacterial effects, a systematic survey was made of the potentiometric responses induced by β-lactams.[86]

If the ET hypothesis is correct, placement of a second electrochemically active site might be beneficial. This has proved the case in a number of more recent investigations. Incorporation of the methoxyimine group (iminium precursor) into the acyl moiety at the 7-position of cephalosporins significantly increased the drug activity.[87] Ester derivatives of carboxyl were synthesised that contained $ArNo_2$[88] and metal-complexing quinolone.[89] A review was devoted to β-lactams with amide side chains linked to iron-sequestering siderophores.[90] Obafluorin, a β-lactone antibiotic, contains side chains with $ArNo_2$ and a catechol group (metal coordinator and o-quinone precursor).[91] A number of investigators have examined the relationship between β-lactams and metals (see 2.2.1) in living systems.

In the presence of ferric and cupric salts, several of the penicillins are able to generate ROS.[92] Methicillin, penicillin G, and carbenicillin produce damage to carbohydrate and DNA characteristic of the 'OH radical. To explain this damage, the following mechanisms have been proposed (Equations 14–15).

$$\beta\text{-lactam (reduced)} + \text{Fe(III) or Cu(II)} + O_2 + 2H^+ \rightarrow \beta\text{-lactam (oxidised)} + \text{Fe(II) or Cu(I)} + H_2O_2 \quad (14)$$

$$H_2O_2 \xrightarrow{\text{Fe(II), Cu(I)}} OH^- + {}^{\cdot}OH \quad (15)$$

Further evidence for the formation of ROS by β-lactams comes from the finding that the neph-

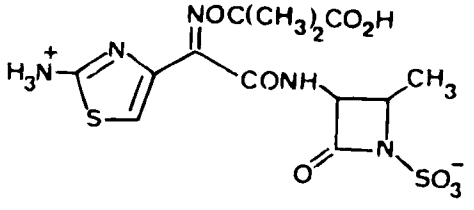


FIGURE 6

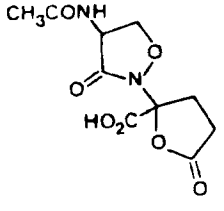


FIGURE 8

rotoxicity associated with large dosage administration of certain cephalosporins, is probably mediated by ROS production resulting in molecular damage.[93]

Tetracycline Antibiotics In the presence of iron and copper salts, tetracycline, oxytetracycline, chlortetracycline and doxycycline generate hydroxyl radicals which damage carbohydrate and DNA.[94,95] Membrane lipid peroxidation is also stimulated by drug activation in the presence of metal ions.[94,96] The pathway to $^{\bullet}OH$ radical formation is similar to that proposed for the β-lactams above, that is the antibiotic may transfer electrons to oxygen or transition metal ions (or both) forming superoxide, hydrogen peroxide and reduced metal ions (Equations 16–18).

$$\text{Tetracycline (reduced)} + O_2 \rightarrow \text{Tetracycline (oxidised)} + O_2^{\bullet -} \quad (16)$$

$$\text{Tetracycline (reduced)} + \text{Fe(III) or Cu(II)} \rightarrow \text{Tetracycline (oxidised)} + \text{Fe(II) or Cu(I)} \quad (17)$$

$$H_2O_2 \xrightarrow{\text{Fe(II), Cu(I)}} OH^- + {}^{\bullet}OH \quad (18)$$

The tetracyclines are also good scavengers of HOCl and ONOO and can depress phagocyte killing.[97]

Bacitracin Antibiotics The bacitracins are a complex mixture of related neutral polypeptide antibiotics. Unstable bacitracin A converts to bacitracin F. In the presence of ferric and cupric salts, bacitracin A generates $^{\bullet}OH$ radicals which damage DNA and carbohydrate[33] and convert the antibiotic to its oxidised "F" form (equations 19–20).

$$\text{Bacitracin 'A'} + O_2 + \text{Fe(III) or Cu(II)} + 2H^+ \rightarrow \text{Bacitracin 'F'} + \text{Fe(II) or Cu(I)} + H_2O_2 \quad (19)$$

$$H_2O_2 \xrightarrow{\text{Fe(II), Cu(I)}} OH^- + {}^{\bullet}OH \quad (20)$$

Rifamycin Antibiotics Rifamycin 'SV' has a hydroquinone structure (QH_2) which, in the presence of oxygen and divalent metal ions, oxidizes to the quinone or 'S' form,[98] Mn^{2+} ions being most effective at pH 9.0[99] and Cu^{2+} ions at pH 7.4.[100] When ferric or cupric salts are present, hydroxyl radicals are generated that degrade carbohydrate and DNA.[100,96] Additionally, NADH-dependent microsomal oxidation of the antibiotic has been shown to cause DNA damage in the presence of iron.[101] A proposed mechanism for hydroxyl radical generation is shown below (Equations 21–25).

$$QH_2 + O_2 \rightarrow SQ^{\bullet} + O_2^{\bullet -} + H^+ \quad (21)$$

$$SQ^{\bullet} + O_2 \rightarrow Q + O_2^{\bullet -} \quad (22)$$

$$2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (23)$$

$$O_2^{\bullet -} \text{ or } SQ^{\bullet} \quad \text{reduced Fe(III) or Cu(II)} \quad (24)$$

$$H_2O_2 \xrightarrow{\text{Fe(II), Cu(I)}} OH^- + {}^{\bullet}OH \quad (25)$$

Stimulation of Phagocytosis by Antibiotics As we have already discussed, antibiotics are believed to act as pharmacological agents blocking key enzymes and metabolic functions in bacteria. However, we believe that the ability of antibiotics to generate ROS may also contribute to their antimicrobial armamentarium. For example, it is well known that antibiotics can exert measurable effects on the growth of bacteria when present in vivo at concentrations well below their in vitro minimum inhibitory concentrations (MIC). In addition to their ability to undergo electron transfer reactions and generate ROS, antibiotics also have the potential to pharmacologically stimulate phagocytic cells to generate ROS. Arbekacin (ABK), a newly developed aminoglycoside antibiotic, has been shown to stimulate mouse phagocytic cells against *S. aureus* infection. This protective effect was accompanied by increased ROS and interleukin 1α production by the phagocytes. ABK was also shown to act as an adjuvant in mice by stimulating antibody production against various challenges.[102] These

results therefore suggest that ABK may have immuno-modulating activity which enhances its antibacterial activity in addition to its direct antibiotic action. In fact it is well established that a whole array of antibacterial, antitumour, and antifungal antibiotics exhibit immuno-modulating activities which can result in either pro or anti-inflammatory actions. Such immuno-modulation results primarily from the ability of such drugs to influence the production of inflammatory mediators by neutrophils and macrophages. For instance the tetracylines have been shown to decrease TNFα and IL-1β levels released from LPS challenged human monocytes in vitro.[103] Whereas β lactams, lincosamides, and teicoplanin all increase TNFα, IL-6 and IL-4 production by monocytes.[104] Macrolide antibiotics such as erythromycin have also been shown to induce IL-6 production.[105] The mechanisms by which these events occur are at present unclear, but it is interesting to speculate that some of the immuno-modulating effects noted may be mediated by second messenger effects of ROS, generated by antibiotics, including inhibition of enzymes and metabolic transformations.

The authors believe that the phagomimetic hypothesis of antimicrobial drug actions involving ROS and ET, helps answer many of the unsolved observations concerning tissue concentrations and antibiotic behaviour. This unifying theme is in keeping with the multiple actions of a variety of drugs and the toxicity of many antibacterial agents. Both features may be a reflection of ET and ROS actions.

Synthetic Antibacterial Agents: ET functionalities and electrochemistry

In the 1980's a broad based approach to drug ET and ROS was begun.[106,107] Thus investigations[107,108,109] showed that both major and minor classes of synthetic agents, such as metal derivatives of mercury (merbromin, merthiolate), metal chelators (nalidixic acid and oxine), nitroheterocycles (nitrofurazone, nitrofurantoin), nitro-aliphatics (chloropicrin, bronopol), quinones (halogentated 1,2-napthoquinones), azo dyes (scarlet red), and iminium species (triaryl- methane dyes, N-heterocyclic salts, and heterocyclic di-N-oxides), could be accommodated in the hypothesis. Electrochemical reductions were, for the most part, reversible with potentials generally in the favourable range of –0.20 to 0.58 V. Similarly, as already discussed, large numbers of naturally occurring antibiotics readily fit into the same theoretical framework, which ultimately leads to the generation of ROS and DNA damage. These drugs represent one of the largest groups of pharmacological agents, and an increasing number appear to fit the unifying hypothesis. In summary we can say that, metal complexors,[110–115] quinones[110,113,116] and quinone precursors,[116,119] conjugated iminium ions,[110,118,119] $ArNO_2$[120] pheothiazines[121] and xanthones,[115] are examples of pharmacologically active agents that depend upon electron transfer for their biological activities.

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