The Implications of Ferroptosis in Antibiotic Resistance

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Abstract

Bacterial infections in the United States are becoming increasingly resistant to existing antibiotic treatments. Due to projected increases in resistance and the recent decrease in novel antibacterials, experts have determined that the United States is in the "post-antibiotic era." The scientific community has failed to resolve resistance despite the continual discovery of new antibiotic compounds. In the past decade, a novel form of cell death called ferroptosis has been implicated in antibiotic treatment by employing the use of nanotechnology. This literature review will describe the problem of bacterial resistance and demonstrate how current research is pioneering a new age of medicine using ferroptosis and nanotechnology.

Ferroptosis: A Potential New Target for the Treatment of Resistant Bacterial Infection

The Cost of Antibiotic Resistance

Bacterial infections have antagonized human health for thousands of years. While most Americans are acquainted with a treatable form of bacterial infection, 2.8 million Americans each year suffer from a more serious disease: infection with a multidrug-resistant bacterial strain (Centers for Disease Control and Prevention [CDC], 2021). A stronger combination of drugs is mostly effective against these strains of bacteria, but pre-existing health conditions can make serious infections deadly for 35,000 people in the U.S. each year. These additional healthcare expenses cost an estimated $20 billion each year (Ballal, 2016). Even with some treatment available, the death toll from resistance continues to rise. Ten million deaths are expected worldwide in 2050 because of antibiotic resistant infections (Park et al., 2022). Resistance is an immediate threat to the health of the United States, and infections from resistant strains will persist unless measures to prevent and treat resistance in bacteria are put into practice.

Emergence of the Antibiotic Era

Investigating the origin of antibiotics gives a clue into the motivations and thoughts behind modern systems of prescribing and manufacturing antimicrobial medicine. Antibiotic resistance was a foreign concept to continental Europe at the end of the 19th century when Louis Pasteur and Robert Koch identified *Bacillus anthracis* as the causative agent of the endemic disease: anthrax (Mock & Fouet, 2001). Scientists raced against death to find a cure. An initial treatment involved the use of common, non-lethal bacteria, like *Streptococcus* and *Staphylococcus aureus,* both of which conferred a protective effect to cattle (Sams et al., 2014). Investigations into how these common bacteria antagonized the growth of pathogenic bacteria led to the isolation of antimicrobial chemicals such as pyocyanin from *Pseudomonas aeruginosa*.

These studies inspired the vision of Paul Ehrlich to create a “magic bullet” which could treat bacterial infections without harming host cells (Aminov, 2010, p. 2). In 1909, Ehrlich discovered Salvarsan, a derivative of Atoxyl, as a treatment for syphilis caused by *Treponema pallidum* (De Sousa Oliveira et al., 2016). Ehrlich utilized systematic testing to discover sulfa drugs. His work inspired other scientists to uncover thousands of antibiotic targets (Aminov, 2010). These antimicrobials were shown to be bacteriostatic and even bactericidal, and when used synergistically, the outcomes were even more effective.

Antibiotics would rise to become a predominant pharmaceutical in the drug industry. Following his discovery of penicillin in 1929, Alexander Fleming persistently pushed for the implementation of penicillin in regular treatment for bacterial infections (Aminov, 2010). By 1945, penicillin was distributed in excess to millions of Americans, with some patients even self-prescribing for illnesses unrelated to bacteria. This period from the 1950s to the 1970s is often considered the “antibiotic era,” characterized by the treatment of many bacterial infections with thousands of new antibiotics (Martens & Demain, 2017, p. 520). Within the last decade of this era came a surge of antibiotics including cephalosporins, tetracyclines, aminoglycosides, chloramphenicol, macrolides and more. These treatments revolutionized healthcare for everyone, and as a result, the life expectancy in the United States increased by over 20 years. To America, this was a sign of man overcoming another boundary to life. To scientists such as Fleming, the abuse of this drug was a warning of what was yet to come (Aminov, 2010). While pharmaceutical industrialization flourished, resistance in bacteria only worsened.

Antibiotic Resistance in Bacteria

Although antibiotic resistance was largely unknown for a long time, signs of resistant bacteria have existed for decades. Antibiotic resistance is a natural evolutionary phenomenon

during which bacteria that survive antimicrobial treatment persist. The surviving strains give rise to new, drug-resistant strains. New antibiotics introduced at a nationwide level place a large pressure on bacteria to retain random, drug-resistant mutations. Antimicrobials target an essential structure, protein, or molecule of a particular strain of bacteria, resulting in the elimination of all bacteria with those features. As a result, an estimated 70% of pathogenic bacteria are resistant to at least one antibiotic (Uddin et al., 2021). During the "golden era" of antibiotics, resistance was avoided by the continual discovery of new antibiotic groups (Uddin et al., 2021, p. 1753). For the past 50 years, no new antibiotic classes have been discovered, contributing to persistent infections. To mount a defense against bacterial resistance, it is essential to have knowledge of the various kinds of resistance that may arise from antimicrobial treatments.

Types of Antibiotic Resistance

To treat an infection with antibiotics, the antibiotic must detect and destroy a bacterial target. Antibiotic resistance arises when the antibiotic is rendered ineffective or the desired target is modified to avoid antibiotic action. For instance, penicillin, an antibiotic, targets and destroys components of the bacterial cell wall. Acquiring the ability to produce penicillinase results in the destruction of the antibiotic. This is classified as a form of resistance distinct from modifications of the bacterial cell wall that prevent penicillin binding (Gumustas et al., 2017). Distinguishing between modifications of the antibiotic or the antibiotic target is a tool used to categorize different types of antibiotic resistance.

Target modification refers to the alteration of the protein or structure the antibiotic means to affect. Protein structures are particularly vulnerable to inactivation due to their reliance on an exact DNA sequence. A simple point mutation may turn an antibiotic binding site inside out. Numerous other factors also contribute to disabled targets, including mutation, enzymatic

methylation, protection by other proteins, or protection by cellular barriers (Aminov, 2010). One study found that protection can be conferred to targets on cell surfaces (Kobras et al., 2020). ATP-binding cassette (ABC) transporters remove antimicrobial peptides (AMPs) from their site of action. BceAB-type transporters are ABC transporters composed of one permease (BceB) and two ATPases (BceA). They have been found to free lipid II cycle intermediates from inhibitory AMPs in *Bacillus subtilis*. New categories of target modification are continuously being discovered, posing an additional challenge to therapies directed at these specific changes.

Another resistance mechanism is the mutation of porins, which prevents antibiotic entry into the cell. The impairment of these passages confers resistance to drugs such as imipenem and meropenem in *Pseudomonas aeruginosa* (De Sousa Oliveira et al., 2016). Other types of resistance destroy the antibiotic. *Enterobacteriaceae* have evolved to combat carbapenem antibiotics by producing carbapenemases (Eichenberger & Thaden, 2019). Extended-spectrum beta-lactamase (ESBL) genes in *Enterobacteriaceae* plasmids encode enzymes which can hydrolyze numerous types of antibiotics (Kammili et al., 2020). Some types of resistance utilize efflux pumps to reduce cellular concentrations of antibiotics. *Acinetobacter baumannii* possess numerous efflux systems that combat a wide range of antibiotics (Coyne et al., 2011). According to Coyne et al., there are five major families of efflux systems which differ in their target substances, structures, and mechanisms of action (2011). The large swath of resistance mechanisms is a testament to the power and versatility of bacterial habituation.

The various forms of antibiotic resistance may be transmitted from one strain to another. Gene transfer offers the receiving strain the ability to generate novel proteins and survive antibiotic treatment. Disseminated vertically during binary fission or horizontally to surrounding bacteria, this mechanism increases the evolutionary fitness of infectious bacteria. Through

conjugation involving plasmids, transformation using environmental DNA, or transduction involving bacteriophages, genetic resistance may spread rapidly to surrounding bacteria (Munir et al., 2020; Liu et al., 2022). Additionally, bacteria may acquire resistance to numerous antibiotics, giving rise to multidrug-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA). Infection with a multidrug resistant strain is more dangerous and more difficult to treat compared to regular strains (Peterson & Kaur, 2018). Bacteria alone sustain and prolong their existence, but there are numerous environmental factors contributing to their success as well.

Factors Amplifying Bacterial Resistance

Environmental factors such as overmedication and a lack of new antibiotics intensify the evolution of multidrug-resistant bacteria. Overmedication is the process of prescribing unnecessary medication. This may occur with an improper diagnosis or with self-medication practices and has led 70% of infection-causing bacteria to acquire resistance to at least one prevalent drug (Turkel et al., 2017). In an assessment of prescription effectiveness of gram-negative antibiotics, researchers found that 26% of antibiotic prescriptions were unsuited to treat the desired infection (Hsieh & Amin, 2016). Proper medication practices are one practical way to suppress antibiotic resistance.

Antibiotics are overused in the animal industry as well. Resistant *Streptomycin* was first discovered in turkeys around the 1950s, right around the start of increasing antibiotic use (Dibner & Richards, 2005). Further studies confirmed antibiotic-resistant strains of bacteria in poultry could be transferred to human microbiota. As a result, multidrug-resistant extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* was created, with the ability to survive through dormancy at 55 °C (Wang et al., 2023). Neglected contributors to antibiotic resistance have been

ignored for too long, leading to the potential for small infections to become lethal due to the ineffectiveness of the most potent drugs available.

Antibiotic resistance is a concern unless new methods are available to combat developing resistant strains. The lack of novel antibiotics is currently a major problem. Recent trends demonstrate a rise in the number of resistant strains and a decrease in the amount of approved antibiotics (Preußke et al., 2023). Antibiotics are one of the easiest drugs to approve in the market; however, certain FDA expectations are abstruse. Researchers are often at a loss when they have developed an antibiotic only to realize later that it failed to meet an FDA standard (Kesselheim & Outterson, 2010). Clarity of these expectations as well as increased funding for antibiotic research would benefit the drug market greatly.

An Evaluation of Antibiotic Prescription Practices and Costs

The first question to ask to evaluate antibiotics is the following: what do antibiotics target? Most antibiotic targets are protein-based, DNA-based, and ribosome-based, meaning antibiotics are highly prone to resistance due to simple genetic modifications (De Sousa Oliveira et al., 2016). The numerous antibiotics and their targets have always given rise to resistant strains. Table 1 compares the different antibiotics, their mechanisms of action, and associated antibiotic-resistant strains.

Secondary impacts on bacterial metabolism are another way to evaluate antibiotics in place. Some bacteriostatic antibiotics inhibit growth by suppressing cellular respiration while other bactericidal drugs promote cell death by accelerating respiration (Lobritz et al., 2015). Most antibiotics that target translation are bacteriostatic and result in the loss of energy consumption due to the inability to produce proteins. Bactericidal drugs typically do the

Table 1

Comparison of Antibiotic Class, Antibiotic Targets, and Presence of Resistance Strains

Antibiotic Class	Examples	Target	Strains Resistant to Antibiotic
Beta lactams	amoxillin, penicillins	Cell wall synthesis	*Acinetobacter*, *Clostridioides difficile*, *Enterobacteriaceae*
Macrolides	azithromycin, erythromycin	Protein synthesis	*Clostridioides difficile*, *Neisseria gonorrhoeae*, *Shigella*
Fluoroquinolones	ciprofloxacin, levofloxacin	DNA synthesis	*Clostridioides difficile*, *Neisseria gonorrhoeae*, *Campylobacter*
Tetracyclines	tetracycline, doxycycline	Protein synthesis	*Clostridioides difficile*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*
Trimethoprim-sulfamethoxazole	trimethoprim-sulfamethoxazole	Folic acid synthesis	*Neisseria gonorrhoeae*, *Salmonella* serotype Typhi, *Shigella*
Urinary anti-infectives	nitrofurantoin	DNA, protein, cell wall synthesis	*Neisseria gonorrhoeae*
Lincosamides	clindamycin	Protein synthesis	*Clostridioides difficile*, Group A *Streptococcus*, Group B *Streptococcus*

Note. The data for types of antibiotic classes, examples, and targets are adapted from *Antibiotic Resistance & Patient Safety Portal: Outpatient Antibiotic Prescription Data,* by the Centers for Disease Control and Prevention, n.d. (https://arpsp.cdc.gov/resources/OAU-Antibiotic-Class-Definitions.pdf). In the public domain. Data for resistant strains are from *Chapter 5 – Effect of polymer-based nanoparticles on the assay of antimicrobial drug delivery systems*, by Gumustas et al., 2017, Elsevier (https://doi.org/10.1016/B978-0-323-52725-5.00005-8). Copyright 2017 by Elsevier.

opposite, resulting in the upregulation of cell metabolism and respiration. Antibiotics that work against metabolism are always limited in scope. Penicillin targets cell wall biosynthesis but only impacts one molecule in the process (Aminov, 2010). Table 1 demonstrates that this is a trend for multiple antibiotics. Elucidating the mechanics of host-infection interplay could pave the way for an antimicrobial with a robust impact on infections.

Prescription Practices and Patient Compliance

The effective treatment of infection is also dependent on proper prescription practices and patient compliance. Qualitative assessments of the use of antibiotics in the United States, Europe, Australia, and Korea reveal that anywhere from 20-55% of antibiotic prescriptions are inappropriate (Park et al., 2022). Decisions to prescribe may be complicated by comorbidities, allergies, side effects, and the interactions with other drugs, making it difficult to design a universal assessment system. The attempt at an antimicrobial quality assessment, called AQUA, was made in 2015 to retrospectively analyze antibiotic treatment in the United States (Magill et al., 2021). Various details that might impact prescription such as illness severity, infections during hospitalization, and culture tests were investigated. The study found that 71.3% of patients with community-acquired pneumonia received treatment longer than the recommended duration. Under 40% of patients who received antibiotics did not demonstrate the symptoms of infection required for prescription. This system along with the opinions of many health professionals emphasize the urgency to adopt better prescription practices in healthcare settings.

On top of the improper prescription of medication is the poor compliance from patients. An analysis of 87 studies from 33 different countries found that treatment cost, treatment frequency, and poor knowledge of treatments were barriers to appropriate antibiotic use

(Zanichelli et al., 2018). This emphasizes the importance of informing patients about their treatment and addressing their concerns and beliefs about antibiotics.

Production Costs and Procedures

An important factor to consider in the analysis of current antibiotics is the methods used to discover and produce them. The use of bacteria in production is a surprisingly effective method of antibiotic manufacturing, accounting for the creation of over 60 types of drugs (Martens & Demain, 2017). Bacteria are maintained under controlled oxygen, pH, carbon dioxide, temperature, and feeding conditions, and while these demands seem arduous, the process is very inexpensive. For instance, the cost of penicillin production is only $10-$20 per kilogram (Elander, 2003). Coupled with a recovery yield of over 90%, antibiotic production is more effective than any existing drug in the market. That leaves the following question: where are the new antibiotics?

Manufacturing drugs becomes more complicated considering the cost of experimental trials, poor returns on investments, and lack of government funding. Ten dollars is a meager fee compared to the millions it costs to approve new drugs (DiMasi et al., 2016). Alongside these huge expenses are often painful losses, rendering decades of research and design useless. Even if some drugs pass FDA regulations, proper prescription practices and the low cost of production result in little return for shareholders. Institutions may help mitigate these terrible expenses; however, the National Institute of Health only spends $200 million per year on antimicrobial related research and government funding is similarly insufficient (Kesselheim & Outterson, 2010). The problem of resistance is clearly a financial issue as well as a scientific one.

The antibiotic era, as some experts claim, is coming to an end, ushering in what is called the “post-antibiotic era” (Hansson & Brenthel, 2022, p. 385). Future projections indicate that by

2050, 10 million people will die from resistant bacteria due to ineffective antibiotics. Although new DNA, RNA, and protein targets can be developed, the promise of continual evolution remains, as more antibiotics will be phased out due to resistance (Pulido et al., 2016). For some, this era is a dystopian crisis during which antibiotics are defeated by increasing amounts of resistant strains. Others continue to believe in the dying antibiotic market. Regardless of competing perspectives on this era, the fact remains that antibiotic resistance is an enduring dilemma. Investigations into alternative forms of treatment could introduce treatments less prone to resistance. On the frontier of uncovering such a treatment are investigations into methods of targeted drug delivery and novel forms of cell death.

Nanotechnology in Drug Development

Science is only beginning to uncover the many applications of nanotechnology. Decades ago, manipulating atoms and molecules was unimagined, but in this post-antibiotic era, nanotechnology holds the key to multiple kinds of versatile treatments (Sharon, 2019). Nanotechnology works on the scale of the nanometer, which is 1 billionth of a meter. As a result, nanotechnology can confer specificity on the molecular level. Research began predominantly in 1950 with the introduction of the first controlled drug delivery system (Yun et al., 2014). Since then, numerous nanomaterials have been developed for imaging, diagnostic, and therapeutic functions, including structures such as quantum dots, nano micelles, liposomes, and carbon nanotubes (Singh et al., 2023). Among the first of these inventions is fullerene: a spherical nanocarbon structure made from chemical vapor deposition. By nature of the high surface area to volume ratio of these nanomaterials, these nanocarriers have the potential to significantly improve drug delivery systems in medicine. The shapes of these complexes variably contribute to their solubility, biocompatibility, and bioconjugation (Grace & Pandian, 2007). With

increased potential to internalize drugs in targeted areas, nanotechnology holds significant promise for the future of pharmaceuticals.

Recent applications of nanotechnology in the realm of microbiology promise a future of more effective, targeted delivery to specific cells in the body. Utilization of biodegradable polymeric nanoparticles, polymeric micelles, polymeric type dendrimers, and other types of technology have demonstrated an increase in drug accumulation and retention compared to current antimicrobials (Gumustas et al., 2017). Aerosol inhalation of liposome-encapsulated ciprofloxacin demonstrated a 90% entrapment rate in the lung tissue of mice infected with *Francisella tularensis* (Wong et al., 2003). Nanotechnology surpasses the drug delivery abilities of current pharmaceuticals.

A variety of environmentally conscious and inexpensive processes exist to produce nanotechnology. Plant extracts often serve as the basis for gold and silver nanoparticles (Sharon, 2019). Camphor is a plant-derived precursor used to synthesize fullerene. Although it may be impossible to predict the waste regulation needed upon implementation, nanoparticles are expected to be produced in a controlled manner to protect workers and the environment. More research is needed to uncover more efficient manufacturing procedures before bulk production is possible.

Overview of Ferroptosis

Alongside the recent applications of nanotechnology is a novel form of cell death implicated in many human physiological processes and a variety of diseases, including bacterial infections. This is an iron-dependent form of cell death unique from apoptosis termed ferroptosis. The name was first coined in 2012 by Brent Stockwell, though related work began as early as the 1950s (Tang & Kroemer, 2020). Ferroptosis is a type of necrosis triggered by the

influx of radical oxygen species (ROS), produced through the iron-dependent Fenton reaction, mitochondria, or the NADPH oxidase (NOX) family. The ferroptosis-promoting free iron is controlled by processes such as iron transport and the autophagy-dependent degradation of ferritin, the iron storage protein. Metabolic stress triggered by an influx of fatty acids initiates ferroptosis in some situations as well. Numerous studies have revealed a strong relationship between ferroptosis and the pathogenesis of lower respiratory tract infections, tuberculosis, and more (Dar et al., 2018; Amaral et al., 2019). An investigation of ferroptosis will reveal the potential targets of ferroptosis-based antibiotics.

Ferroptosis was discovered in studies searching for drugs targeting mutated RAS in cancer cells (Tang & Kroemer, 2020). Often mutated in human cancers, RAS proteins are tightly regulated GTPases maintaining cell proliferation and survival (Prior et al., 2012). RAS-selective lethal compounds and various anticancer drugs were found to initiate cell death in ways distinct from apoptosis (Kang & Tang, 2017). Supplemental studies discovered the iron chelator deferoxamine was able to prevent cell death and transmission electron microscopy (TEM) confirmed this mechanism of death was distinct from known forms. Marked by a decrease in mitochondrial size, reduced mitochondrial cristae density, and increased mitochondrial membrane potential, ferroptosis was found to be distinct from other forms of cell death. Embedded in a network of related cellular processes, scientists are only beginning to understand the mechanisms and applications of this unique class of necrosis.

Activation of Ferroptosis

Ferroptosis is intricately tied with metabolism and other cellular processes. Activation of ferroptosis can occur by targeting iron metabolism, glutathione (GSH) biosynthesis, and glutathione peroxide 4 (GPX4) (Chen et al., 2023). Sensitization to ferroptosis can be achieved

using lipophilic radical-trapping antioxidants, coenzyme Q, and tetrahydrobiopterin (BH_4). Ferroptosis may be initiated intrinsically or extrinsically. The extrinsic pathway involves the regulation of transporters such as the inhibition of the amino acid antiporter system x_c^- or the activation of transferrin and lactotransferrin–both of which are iron transporters (Chen et al., 2021). The intrinsic pathway induces ferroptosis by preventing the expression of intracellular antioxidant enzymes, notably glutathione peroxidase 4 (GPX4). Other stresses may trigger ferroptosis, these may include drugs, extreme temperatures, hypoxia, and radiation. The addition of zinc will trigger ferroptosis, regardless of the presence of chelation inhibitors. Abnormal triggers of ferroptosis mainly involve the protein-degradation pathways: autophagy and the ubiquitin-proteasome systems (Tang & Kroemer, 2020). Pathologies associated with these pathways such as acute tissue damage, infection, cancer, and neurodegeneration can alter normal patterns of ferroptosis. These pathways are balanced by counter systems to prevent the destruction of an entity due to ferroptosis.

Inhibition of Ferroptosis

Like activators, inhibitors of ferroptosis exhibit crosstalk with numerous cellular processes. Antioxidants, radical trapping agents, and iron chelators are three categories of ferroptosis inhibitors (Chen et al., 2023). Inhibitory mechanisms exist to prevent ferroptosis, one notably being the glutathione system. GSH is an antioxidant and a component of system x_c^- –an amino acid transporter that imports cystine into the cell and exports glutamate out of the cell. Intracellular cystine is easily reduced to cysteine–the limiting precursor for GSH synthesis. Cysteine is also obtained from the metabolism of sulfur-containing amino acids in the trans-sulfuration pathway. The presence of GSH allows for the activity of the ferroptosis inhibitor: Glutathione peroxidase 4 (GPX4) (Kang & Tang, 2017). A member of an antioxidant enzyme

family including 1-8, GPX4 is unique in that it regulates ferroptosis. *In vivo* studies determined the effect of GPX4 depletion to be increased ferroptosis in mice. GPX4 is a selenoenzyme which reduces phospholipid hydroperoxides (PLOOH) to non-toxic alcohols (PLOH), preventing the build-up of antioxidants to the threshold which triggers ferroptosis (Tang & Kroemer, 2020).

Iron and zinc chelation is another method in which ferroptosis is inhibited. ZIP7 is a zinc transporter which regulates ferroptosis by monitoring zinc efflux between the cytosol and other cellular components (Chen et al., 2021). When this transporter is inhibited, it leads to the ER stress response, inducing ferroptosis.

Coenzyme Q (CoQ10, or ubiquinone) refers to an ubiquitously expressed family of lipophilic metabolites (Jiang et al., 2021; Tang & Kroemer, 2020). The mitochondrial CoQ10 system contributes to the inhibition of apoptosis while the non-mitochondrial forms prevent ferroptosis (Tang & Kroemer, 2020). The enzymatic activity of this family is regulated by numerous transcription factors. The activity of CoQ10 runs parallel to the GPX4 pathway to reduce lipid peroxides. The apoptosis-inducing factor mitochondria-associated 2 (AIFM2) transcription factor works to regulate the expression of the enzyme and has implications in active membrane repair to prevent ferroptosis.

Antioxidants work to inhibit ferroptosis as well. Tetrahydrobiopterin (BH_4) is an example of such, and while it mainly serves as the precursor for certain neurotransmitters like dopamine, serotonin, and nitric oxide, it can also tip the balance of radical oxygen species to favor ferroptosis inhibition (Tang & Kroemer, 2020). Inhibition pathways are carefully calibrated to reduce extraneous instances of ferroptosis.

Crosstalk with Autophagy and Other Degradation Pathways

Degradation pathways have been heavily implicated in the regulation of ferroptosis, as studies commonly find autophagy upregulated in ferroptosis. The main form of autophagy that contributes to ferroptosis is ferritinophagy, which mediates the degradation of ferritin (Kang & Tang, 2017). The light (FTL) and heavy chains (FHL) of ferritin work to influence iron metabolism by oxidizing and storing iron in bioavailable forms to prevent oxidative reactions. Ferritin degradation by ferritinophagy relies on nuclear receptor coactivator 4 (NCOA4), which subsequently leads to iron release, inducing oxidative stress characteristic of ferroptosis.

The degradation of anti-ferroptotic regulators commonly occurs during autophagy (Tang & Kroemer, 2020). Studies of the role of system x_c^- in autophagy regulation demonstrated that GSH deficiency triggered the AMPK-independent induction of autophagy in spermatogonia-type germ cells (Mancilla et al., 2015). Other studies discovered that acyl-CoA synthetase long-chain family member 4 (ACSL4) improves cell sensitivity to erastin-induced ferroptosis by mediating the production of 5-hydroxyeicosatetraenoic acid (5-HETE) (Yuan at el., 2016). ACSL4 also regulates the activity of mechanistic target of rapamycin (mTOR) complex I and mTOR complex II in autophagy, suggesting a further connection between ferroptosis and autophagy in need of further research (Kang & Tang, 2017). Heat shock 70 kDA protein 5 promotes cell survival during endoplasmic reticulum stress-induced autophagy, but in pancreatic cancer cells, it limits lipid peroxidation to defend against oxidative stress. Every new pathway that intersects with ferroptosis is another step towards illuminating its implications in the treatment of bacterial infections.

Interplay between Bacterial and Host Survival involving Ferroptosis

Bacterial infections often trigger ferroptosis in host cells to promote disease development. Fer-1, an inhibitor of ferroptosis, reduced the amount of necrotic cell death in macrophages infected by *Mycobacterium tuberculosis* (Li et al., 2022, Amaral et al., 2019). Increased iron levels during an infection increase the amounts of reactive oxygen species, causing ferroptotic lipid peroxidation in host macrophages (Li et al., 2022). This cell necrosis allows *Mycobacterium tuberculosis* and other pathogenic bacteria to invade the body. *Pseudomonas aeruginosa* expresses lipoxygenase to oxidize host arachidonic acid–phosphatidylethanolamines to trigger ferroptosis in host bronchial epithelium (Dar et al., 2018). Normal microbiota have been found to preserve the host by inhibiting ferroptosis. Studies found that capsiate, a metabolite produced by gut microbiota, inhibits host cell ferroptosis-dependent ischemia/reperfusion injury by promoting GPX4 expression (Deng et al., 2021). In general, targeting mechanisms of inhibition or activation in ferroptosis are reliable targets for infection treatment.

Nanotechnology and Ferroptosis-Based Treatments

An important element to the usage of ferroptosis as an antibacterial treatment is the use of nanotechnology to deliver drugs to a site of infection. Many current studies are underway to uncover new ferroptosis targets for treatment. Ferroptosis has been induced in both humans and bacteria using targeted iron delivery, system x_c^- suppression, GSH depletion, and GPX4 inhibition, and there are numerous ways research has uncovered to selectively trigger these processes (Sun et al., 2023). The following is a list of numerous nanotechnologies used to target ferroptosis.

Gold Nanoparticles

Gold nanoparticles are nanocarriers at the crossroad between antibiotic development and nanotechnology. Gold nanoparticles are particularly versatile for this area, as they can be shaped into spherical, rod-like, and core-shell formations (Vigderman & Zubarev, 2013). Their size can be as small as 1 nm and reach over 100 nm, allowing for a variety of different optical and functional qualities. These types of nanomaterials can absorb light in the near infrared (NIR) region, to which bodily tissues are transparent. Drugs are easily attached to smaller gold nanoparticles, allowing for the enhanced antibacterial effect of aminoglycosides on gram-positive and gram-negative strains of bacteria.

Although nanotechnology is not yet predominant in the pharmaceutical setting, scientists are investigating the possibility of nanoparticle-delivered drugs in tumor treatment (Yun et al., 2014). The selective activation of ferroptosis results in the physical disruption of cancerous cellular membranes. Provided the target could alter NADPH production or redox balance when applied to bacterial infection, ferroptosis treatment could avoid exacting selective pressures leading to antibiotic resistance.

Single-Atom Catalysts

Single-atom catalysts (SACs), alternatively referred to as single-atom nanozymes (SAzymes), are bio-inspired nanozymes which mimic natural enzyme structures, catalytic ability, and potential to generate excessive ROS for bacterial inhibition in host tissues (Sun et al., 2023). Active sites on SACs can be precisely identified, and they have great catalytic ability and stability. In addition, transition metal-based SACs have exhibited pro-ferroptosis effects in cancerous cells. A palladium-based SAC has been able to simulate the activity of double peroxidase and glutathione oxidase to initiate ferroptosis in tumors (Cao et al., 2022). A study

conducted in May 2023 discovered that bacterial nonferrous ferroptosis could be induced in bacteria by anchoring iridium and ruthenium metal sites on sp^2c-linked nanoscale covalent organic frameworks (COF). Light irradiation or hydrogen peroxide was effective to activate the single-atom catalysts (SACs) to cause an influx in reactive oxygen species, deactivate GPX4, and alter nitrogen and respiratory metabolisms, hence proving that ferroptosis could be induced in gram-negative and gram-positive strains (Sun et al., 2023).

Iron Sulfides

Metastable iron sulfides have been found to suppress *Gardnerella vaginalis*, a type of bacteria that causes bacterial vaginosis (Fang et al., 2022). Greigite (Fe_3S_4) permeates thin bacterial cell walls and inhibits glucokinase. Iron sulfides overcome metronidazole-resistance in *G. vaginalis* and induce ferroptosis-like death *in vivo.* Iron sulfides release iron ions and polysulfides in water, suggesting their potential effectiveness for human antibiotic treatment (Cao et al., 2023). Ferrous iron can eradicate a broad spectrum of bacteria, allowing it to obliterate biofilms and reduce persistent intracellular bacteria. Otitis media (OM), a common ear infection caused by *Staphylococcus aureus*, has been successfully treated using a thermosensitive hydrogel to deliver metastable cystine-denoted iron sulfide molecules to the site of infection (Li et al., 2023). This treatment performed well *in vivo* and *in vitro*, with low cytotoxicity and anti-inflammatory effects in the host. Additionally, nanotechnology has been employed using iron sulfides to develop antiviral treatment targeting extracellular viral particles without impacting host cells (Miao et al., 2023). Iron sulfides show significant promise for biocompatible treatments.

Vesicle and Membrane-Related Proposed Therapies

Vesicle therapy involves using cellular membranes to package and deliver various kinds of therapeutic substances such as proteins and RNAs (Cecchin et al., 2023). This is an alternative delivery mechanism for promoters or inhibitors of ferroptosis and has demonstrated the ability to target and treat infections in various vesicle studies. In response to high levels of lipid peroxidation, macrophages directed ferrous iron importation away from host cells and into bacterial vacuoles, inducing ferroptosis in the infectious bacteria (Ma et al., 2022). Sodium alginate has been used to wrap iron oxide (Fe_3O_4), a known ferroptosis inducer in MRSA, and cinnamaldehyde, a GSH consumer, to selectively deliver ferroptosis into the sites of infection (Hu et al., 2023). Using ultrasonic stimulation, the antibacterial particles released the intravesicular contents, promoting ferroptosis in mice cells infected with MRSA. No toxicity was reported in this procedure, and the outcomes of the treatment resulted in decreased bacterial load and increased survival of the mice, suggesting these therapies could perform well clinically.

"Nanoswords"

In the last year, scientists have manufactured what is called a "nanosword" for the purpose of precisely targeting and treating post-operative infections following implants (Xue et al., 2023). By increasing the pH in a particular region, this specific mechanism can inhibit ATP synthesis by weakening the proton motive force. Nanoswords cause breaks in the bacterial membrane, increasing intracellular iron ion concentrations, resulting in ferroptosis. The low cytotoxicity of this type of therapy holds promise for infection control.

Cultivating a Better Future for Bacterial Infections

Resistant bacterial infections will continue to emerge over the next few years, to a dangerously high extreme. In this "post-antibiotic era," it is more important than ever to stay on

top of this issue. Developing new antibiotics to target different proteins is a salve on the growing issue of resistance. Ferroptosis has showed significant prowess in the targeting of bacterial infection. To mount a better defense against resistance, some light must be shed on the limits of ferroptosis-based treatments and the intersections between ferroptosis and other fields of study.

Limitations of Ferroptosis-Based Treatments

Both bacteria and humans use similar mechanisms to promote ferroptosis, posing a challenge for the specificity of ferroptosis-based therapies. Ferroptosis is heavily involved in the pathogenesis of diseases with high polyunsaturated lipid states including antioxidant-mediated immune responses, cancer, and neurodegenerative disorders (Zou & Schreiber, 2020; Han et al., 2020). The exact mechanism of ferroptosis in these diseases remains mostly unelucidated. In addition, ferroptosis inducers without nanotechnology demonstrate poor water solubility and specificity (Liu et al., 2023). While nanotechnology seeks to resolve these therapeutic conflicts, without an understanding of how host and bacterial ferroptosis interact, ferroptosis-based treatments will remain impossible. Studying the implications of ferroptosis in other domains of science would elucidate its full therapeutic potential.

Intersections in Other Fields of Study

Good science involves the integration of details in a larger picture that can be mobilized for effective applications. Ferroptosis is an isolated topic in a sea of possibility, and to build confidence in its effectiveness in the realm of science, its important connections must be illuminated.

Cell Metabolism. The efficacy of antibiotics is in part based on their metabolism. Bactericidal antibiotics can be potentiated with decreased carbon flux through the TCA cycle as well as other factors linked to the availability of oxygen. Fumarate was found to increase

antibiotic sensitivity of *Escherichia coli* microcolonies with extracellular matrix-induced resistance by enhancing TCA metabolism (Han et al., 2023).

Iron metabolism is an important regulator of ferroptosis and essential for most organisms. Because both humans and bacteria require iron to thrive, it is imperative to uncover the nature of iron competition between the host and bacterial infection. Five strategies are employed in bacteria to manage iron: an iron transport system to scavenge various forms of iron, use of intracellular iron stores for controlled uptake, redox stress resistance systems to control iron-induced ROS and damage, controlled iron consumption, and an overarching iron-responsive regulatory system (Andrews et al., 2003). Studies have demonstrated that symbiotic gut microbiota control iron uptake in human intestinal epithelium (Das et al., 2020). Through the downregulation of host hypoxia-inducible factor (HIF-2α), a promoter upregulating host iron importers, iron overload is resisted due to the increased production of 1,3-diaminopropane, butyrate, and propionate by symbiotic *Lactobacillus* species. This host-bacteria iron interaction potentially mirrors that of a host infected with resistant bacterial strains. Further studies should illuminate the impacts of iron regulation on ferroptosis induction.

Processes involved in oxidative phosphorylation have been connected to antibiotic resistance as well. Scientists have restored susceptibility of *Staphylococcus aureus* towards polymyxin antibiotics by the inhibition of ATP synthase (Vestergaard et al., 2017).

Reactive oxygen species play a role in activating ferroptosis, and studies prove they can be targeted. The formation of reactive oxygen species in response to antibiotics is one additional cause of death for bacteria, making it an important molecule to regulate (Lobritz et al., 2015). Additionally, ROS are continuously produced as by-products or signaling molecules for other reactions (Apel & Hirt, 2004). Elevated ROS has been used to signal the release of ferroptosis-

inducing drug combinations in experimental tumor treatment (Zhang et al., 2021). Such experiments demonstrate the specificity and safety of a potential treatment for bacterial infection.

Ferroptosis regulators are also involved in the trans-sulfuration pathway. This is a key metabolic system with numerous layers of regulation, involving the transfer of sulfur from homocysteine to cysteine (Sbodio et al., 2019). This pathway oversees the regulation of critical metabolites such as cysteine, GSH, and hydrogen sulfide. Activation of the reverse system of metabolism through the antioxidant transcriptional factor NRF-E2-related factor and cystathionine β-synthase has enabled ovarian cancer cells to develop resistance to erastin-induced ferroptosis (Liu et al., 2020). This pathway can be considered for potential side effects or further infection targets.

Glutathione metabolism involves the production and degradation of glutathione. GSH is produced by the ATP-dependent reaction converting glutamate and cysteine and an additional reaction with the addition of glycine (Bachawat & Yadav, 2018). Amino acid availability plays a role in the regulation of this cycle. Specific enzymes manage the degradation of glutathione back into glutamate, cysteine, and glycine. Some bacteria lack glutathione, but several gram-negative strains have the machinery to produce it. Nucleotide metabolism is also interconnected with this process. One study discovered that the suppression of ribonucleotide reductase (RNR), an enzyme producing dNTPs necessary for DNA replication, inhibited ferroptosis by maintaining baseline levels of GSH (Tarangelo et al., 2022). Clearly, ferroptosis is a highly regulated system of cell death with multiple potential application points in the realm of antibiotics.

Alternative Forms of Cell Death. Scientists have been speculating that the different types of cell death should no longer be viewed in isolation. Everything from autophagy to other

forms of degradation have continually demonstrated a deeper connection with all forms of cell death.

Autophagy is a highly selective degradative process involving hydrolytic enzymes to dispose of dangerous or unnecessary cell products, hallmarked by the formation of double-membrane-limited vesicles (Wollert, 2019). In response to cytotoxic stress, such as a highly oxidative state produced during ferroptosis, autophagosomes are produced to randomly capture large amounts of cytoplasm without discretion. Along with this, the ubiquitin-proteasome system (UPS) is another prominent degradation pathway. Both autophagy and the UPS have been used to respond to microbial infections. Bacteria have evolved the ability to remove host proteasomes by autophagy using the T3E Hrp outer protein M1 protein (Üstün et al., 2018). Further research is needed to illuminate the intersections between bacteria and degradative pathways.

Apoptosis is a type of programmed cell death with potential clinical connections to bacterial infections such as tuberculosis. Macrophage apoptosis is triggered by the addition of TNF-α synthesis as well as the infection with *Mycobacterium tuberculosis* (Duan et al., 2001). Concomitant to this process is the release of arachidonic acid, which is amplified by transcription factor 6 α (ATF6α) to promote the ferroptosis of tumor cells (Zhao et al., 2022).

Necrosis is irreversible cell injury characterized by swelling, plasma membrane rupture, cell lysis, and spillage of intracellular materials (Khalid & Asimpouran, 2023). While ferroptosis is a form of necrosis, it may still interact with alternative subcategories of necrosis. Phospholipase A2 (PLA2) is important in the signaling of many forms of cell death, including necrosis, and has been implicated in the signaling of ischemia/reperfusion-induced cellular injury, which as previously mentioned, has been linked to the ferroptosis-inducing metabolite: capsiate (Arcila et al., 2007, Deng et al., 2021). Necrosis promotes anaerobic bacteria growth in

wounds with poor circulation, causing infections which are difficult to treat (Nayeri, 2016). The intersections between ferroptosis and other modes of study continue to be unveiled.

Phage Therapy. Phage therapy involves the use of bacterial viruses to treat bacterial infections (Lin et al., 2017). Research suggests that bacteriophages could be used to replace or supplement antibiotics to treat multi-drug resistant bacteria. Various types of phages exist, inducing different effects on different strains of bacteria. New forms of gene editing have been used with phage therapy to manipulate the expression of antibiotic-resistant bacterial genes. With diverse functions and the ability to target specific bacteria, phage treatment has found significant experimental success in the treatment of cholera and various antibiotic-resistant bacterial infections. Like nanotechnology, phages are another vehicle that can confer specificity to ferroptosis treatments for antibiotics, yet there is no current research on the use of bioengineered bacteriophages on bacterial infections. More studies are also necessary to detail the interactions between the host, phage, and bacterial infections before this treatment can be implemented in clinical settings.

The Call to Participate

The implementation of a new frontier for medicine is a complicated process requiring communication between financial, political, and scientific bodies of thought. More research must be done to reveal different processes and complexities involved in bacterial infections and ferroptosis. In addition, regulations for drug processing must be clearly communicated with drug developers. Incentives for research into antibiotic treatment would likely increase research outcomes. Encouraging investors to fund the use of nanotechnology in medicine would likely contribute immensely. Additionally, the participation of the local government in antibiotic-prescription control and regulation would reduce the rate at which bacteria are developing

resistance to antibiotics. It is the responsibility of everyone to use the skills and knowledge they have to protect the community from antibiotic resistance. Provided everyone does their part, nanotechnology-based treatments targeting ferroptosis in bacteria could mean that antibiotic resistance fades into a distant memory.

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