Copper, An Ancient Remedy Returning to Fight Microbial, Fungal and Viral Infections

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Abstract: Copper has potent biocidal properties. Copper ions, either alone or in copper complexes, have been used for centuries to disinfect liquids, solids and human tissue. This manuscript reviews the biocidal mechanisms of copper and the current usages of copper and copper compounds as antibacterial, antifungal and antiviral agents, with emphasis on novel health related applications. These applications include the reduction of transmission of health-associated (nosocomial) pathogens, foodborne diseases, and dust mites loads and treatment of fungal foot infections and wounds. Possible future applications include filtration devices capable of deactivating viruses in solutions, such as contaminated blood products and breastmilk.

Keywords: Copper, biocide, fungicide, antiviral, acaricidal, nosocomial infections, wound healing.

INTRODUCTION

Copper has been used as a biocide for centuries [1]. In ancient Egypt (2000 BC), copper was used to sterilize water and wounds. The ancient Greeks in the time of Hippocrates (400 BC) prescribed copper for pulmonary diseases and for purifying drinking water. Gangajal, "Holy water", given to Hindu devotees to drink as a blessed offering, is stored in copper utensils as it keeps the water sparkling clean. During the Roman Empire, copper cooking utensils were used to prevent the spread of disease. The early Phoenicians nailed copper strips to ships' hulls to inhibit fouling and thus increase speed and maneuverability. The Aztecs used copper oxide and malachite for treating skin conditions. Early American pioneers moving west across the American continent put copper coins in large wooden water casks to provide safe drinking water for their long journey. By the 18th century, copper had come into wide clinical use in the Western world in the treatment of lung and mental disorders. In the Second World War, Japanese soldiers put pieces of copper in their water bottles to help prevent dysentery. Copper sulphate was (and is still) highly prized by some inhabitants of Africa and Asia for healing sores and skin diseases.

Were all these civilizations right in using copper for the above mentioned purposes? Indeed they were! The following sections review scientific studies demonstrating the potent biocidal properties of copper and copper compounds and their current uses in health related applications.

COPPER BIOCIDAL ACTIVITIES

The wide use of copper due to its antifungal properties started in 1761, when it was discovered that seed grains soaked in copper sulphate inhibited seed-borne fungi. Within a few decades, the practice of treating seed grains with copper sulphate had become so general and effective that today seed-borne fungi are no longer of any economic importance. In the 1880s a mixture of copper sulphate, lime and water (called "Bordeaux mixture") and a mixture of copper sulphate and sodium carbonate (called "Burgundy mixture") became the fungicide of choice in the U.S. and France, respectively, for spraying grapes and vines to fight mildew.

The fungicidal properties of copper were demonstrated in controlled laboratory studies starting in the early 1950s (*e.g.* [2-4]) and since then copper and copper compounds have been shown to effectively kill a wide range of yeast and fungi such as *Aspergillus carbonarius* [5]; *Aspergillus fumigatus* [6]; *Aspergillus niger* [6-8]; *Aspergillus oryzae* [7]; *Candida albicans* [6,8-13]; *Cryptococcus neoformans* [6]; *Epidermophyton floccosum* [6]; *Microsporum canis* [6]; *Myrothecium verrucaria* [7]; *Saccharomyces cerevisiae* [14,15]; *Torulopsis pintolopesii* [9]; *Trichoderma viride* [7]; *Trichophyton mentagrophytes* [7,8,13] and *Tricophyton rubrum* [6,13]. Thus, copper fungicides have become indispensable and many thousands of tons are used annually all over the world in agriculture [16,17]. For example, copper sulphate and copper hydroxide are employed for the control of downy mildew on grapes and green slime in farm ponds, rice fields, irrigation and drainage canals, rivers, lakes and swimming pools [17]. However, copper compounds may be very toxic to fish and other organisms. The environmental hazards resulting from copper build-up in sediments and the need for high dosages have led to a constant search for and production of compounds that provide the copper in a chelated form (*e.g.* [18-20]). Chelated copper is non-reactive with other chemical constituents in the water. For example, the copper water-insoluble compound, copper-8-quinolinolate and some of its derivates [7] are used in fruit-handling equipment. This compound is also used to reduce environmental contamination of fungi in hospitals at concentrations above 0.4 µg/ml [16], since infection with fungi, such as *Aspergillus spp*, is a major problem among immunocompromised patients, such as AIDS patients.

Copper sulphate has been in use since 1838 for preserving timber and is today the base for many proprietary wood preservatives [21]. Copper is also used as the active ingredient in products that prevent roof moss formation, such as

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copper granules found in 3M Scotchgard™ Algae Resistant Roofing System [22]. Interestingly, the growth of barnacles, seaweed, tubeworms, and other organisms on boat bottoms produces surface roughness that increases turbulent flow, acoustic noise, drag, and fuel consumption. An average increase of 10 µm in hull roughness can result in a 1% increase in fuel consumption! This has led to the painting of boat bottoms with copper-containing paints that reduce fouling and microbial biofilm formation in ships and save energy [23], and now copper is quite commonly used in antifouling paints [23-25].

Several copper compounds, such as copper sulphate, copper nitrate and cupric chloride-bis-n-dodecylamine, are potent molluscicides. For example, the following snails have been shown to be killed by these compounds: *Biomphalaria glabrata* [26]; *Biomphalaria alexandrina* [27-29] and *Lymnaea natalenesis* [28,29]. Control of snails may be an important strategy in fighting some human diseases, such as bilharziasis. This disease is caused by a trematode parasite, *Schistosoma mansoni*, which uses snails and humans as hosts.

The recognition that copper has potent antibacterial properties followed and was well established in laboratory studies (for a recent review see reference [30]). Some examples include the killing by means of copper or copper compounds of *Acinetobacter calcoaceticus/baumannii* [31,32]; *Bacillus subtilis* [32,33]; *Bacillus macerans* [34]; *Campylobacter jejuni* [35]; *Citrobacter* [32]; *Clostridium difficile* [36,37]; *Enterococci* [11,12]; *Escherichia coli* [10-12,32,33,38-41]; *Legionella pneumophila* [31,42,43]; *Listeria monocytogenes* [12,44-46]; *Klebsiella pneumoniae* [32]; *Mycobacterium tuberculosis* [47]; *Pseudomonas aeruginosa* [48]; *Pseudomonas fluorescens* [45]; *Pseudomonas striata* [34]; *Salmonella sp.* [12,32,35,39]; *Salmonella typhimurium* [45,49]; *Shewanella putrefaciens* [45]; *Shigella flexnerii* [32]; *Staphylococcus epidermidis* [50]; *Staphylococcus aureus* [10-12,31-33,41,45,46,51,52]; and *Streptococcus* [32,53]. Recent studies have demonstrated that non-soluble copper compounds, such as glass coated with thin films of CuO [54], degradable phosphate glass fibres impregnated with CuO [50,53] or metallic and copper alloys [35,36,38,40,44,47,51,55,56] have potent biocidal properties, even against difficult bacterial spores [36]. Interestingly, bacteria exposed to metallic copper surfaces do not enter a viable but nonculturable physiological state, in which they are viable but do not multiply, but are completely inactivated [44]. Importantly, based on the vast amount of antimicrobial efficacy testing (180 tests, utilizing 3235 control and test samples, conducted in independent microbiology laboratories) sponsored by the Copper Development Association (CDA), the U.S. Environmental Protection Agency (EPA) has recently (March 2008) approved the registration of copper alloys as materials with antimicrobial properties, thus allowing the CDA to make public health claims [57]. The following statement is included in the registration: "When cleaned regularly, antimicrobial copper alloys surfaces kill greater than 99.9% of bacteria within two hours, and continue to kill more than 99% of bacteria even after repeated contamination". These public health claims acknowledge that copper, brass and bronze are capable of killing harmful, potentially deadly bacteria, such as Methicillin-resistant *S. aureus* (MRSA). MRSA is one of the most virulent strains of antibiotic-resistant bacteria and a common cause of hospital- and community-acquired infections. Copper is the first solid surface material to receive this type of EPA registration.

Copper demonstrates potent antiviral (virucidal) activity as well. The inactivation of the following enveloped or nonenveloped, single- or double-stranded DNA or RNA viruses by copper and copper compounds, has been reported: bacteriophages [58-62], Infectious Bronchitis Virus [63], Poliovirus [61,64], Junin Virus [59], Herpes Simplex Virus [58,59], Human Immunodeficiency Virus Type 1 (HIV-1) [11,65-67], West Nile Virus [11], Coxsackie Virus Types B2 & B4, Echovirus 4 and Simian Rotavirus SA11 [68]. More recently, the inactivation of Influenza A [55,65], Rhinovirus 2, Yellow Fever, Measles, Respiratory Syncytial Virus, Parainfluenza 3, Punta Toro, Pichinde, Adenovirus Type 1, Cytomegalovirus and Vaccinia [65] has been demonstrated.

BIOCIDAL MECHANISMS OF COPPER

Copper exerts its toxicity to microorganisms through several parallel mechanisms, which eventually may lead to the microorganisms' death even within minutes of their exposure to copper (e.g. [14,38,65]). It is likely that the first site that copper damages is the microorganisms' envelope. Recently, Nan Li and his colleagues [69], by using atomic force microscopy, force-distance curves and inductively coupled plasma mass spectrometer tests, studied the effects of austenitic (a solid solution of ferric carbide or carbon in iron) stainless steel containing or not containing copper on the plasma membrane of *E. coli*. They found that the copper containing steel adhered to the bacteria plasma membrane, via the electrostatic forces exerted by Cu^{2+}, to a significantly greater extent than the austenitic stainless steel not containing copper. They reported that initial damage occurred to the lipopolysaccharide patches on the outer plasma membrane, which collapsed while the inner part of the bacteria remained intact. Already in 1988, Ohsumi and colleagues reported that Cu^{2+} elicits significant permeability changes in intact *Saccharomyces cerevisiae* cells [14], which depending on the plasma membrane fatty acid composition and the membrane permeability, increased markedly in cells enriched with polyunsaturated fatty acids [70]. Extensive copper-induced disruption of membrane integrity inevitably leads to loss of cell viability. However, even relatively small alterations in the physical properties of biological membranes can elicit marked changes in the activities of many essential membrane-dependent functions, including transport protein activity and ion permeability [71].

While copper may interact with many microbial proteins without damaging them, such as with copper chaperones (e.g. [72,73]), copper may damage many proteins, both on the microorganism envelope or within the cell, especially when found in high concentrations, above the threshold by which many microorganisms can cope with excess copper. This may occur via displacement of essential metals from their native binding sites in the proteins, or via direct interactions with the proteins. In both cases, conformational changes in the protein structure or in the protein active site may occur, resulting in the inhibition or neutralization of the protein biological activities. For example, specific oxidation of the cysteine in the active site of vaccinia H1-related protein tyrosine phosphatase by Cu^{2+} results in complete inactivation of the protein activity [74]. Another example is the neutralization of HIV-1 protease, an essential protein for the replication of the virus, by stoichiometric concentrations of copper ions [75]. Direct inhibition by Cu^{2+} required the presence of cysteine residue(s) in the protease [75,76]. Copper also may mediate free radical attack of amino acids, especially of histidine and proline, causing substantial protein alterations and even protein cleavage [77,78].

Copper ions can also damage nucleic acids. By cross linking within and between strands of DNA [79] copper may cause helical structure disorders and DNA denaturation [80]. In single-stranded DNA, such as that found in many DNA viruses, a copper binding site was found on average in every three nucleotides [81]. Guanine-specific covalent binding of Cu^{2+} in double stranded DNA was demonstrated following crystallization (1.2-A resolution) of DNA soaked with cupric chloride [82], explaining the observed specificity of Cu^{2+}-induced oxidative DNA damage that occurs near guanine residues [83]. It has been suggested that subsequent to the specific binding of copper to deoxyribonucleic acids, repeated cyclic redox reactions generate several OH radicals near the binding site causing multiple damage to the nucleic acids [84]. When examining the effects of Cu^{2+} on purified *S. typhimurium* DNA, DNA breakage occurred only when the copper ions and H_2O_2 were present, while no damage to the DNA was detectable after incubation with the metal ions alone or with H_2O_2 alone [49], supporting the notion that copper catalyzes the conversion of H_2O_2 to hydroxyl radicals [85], at least *in vitro*. Although copper binds DNA *in vitro*, stronger competing ligands, such as glutathione and cysteine [86,87], may remove copper away from the DNA *in vivo*. Furthermore, recent studies using *E. coli* lacking copper export genes indicate that copper does not catalyze significant oxidative DNA damage *in vivo* [86]. Electron paramagnetic resonance spin trapping assays showed that the majority of H_2O_2-oxidizable copper was located in the periplasm, away from the DNA. However, it still may be that in some microorganisms, especially in viruses, copper oxidative damage to the genetic material may occur through Fenton mechanisms. Indirect toxic mechanisms have been suggested. For example, exposure to high concentrations of copper may increase the rate of H_2O_2 generation [88], which could have accelerated iron-mediated oxidative DNA damage [86].

In general, the redox cycling between Cu^{2+} and Cu^{1+}, which can catalyze the production of highly hydroxyl radicals, with subsequent damage to lipids, proteins, DNA and other biomolecules [30,89], makes copper reactive and a particularly effective antimicrobial. Other closely related metals, such as zinc and nickel, do not readily undergo redox-cycling reactions and are more stable in their various cationic states. Zinc, which is an essential trace metal like copper and well metabolized by humans, displays antifungal properties [90]. Zinc pyrithione, for example, is widely used as an antifouling agent in paints [91]. However, free zinc ion in solution is highly toxic to plants, invertebrates, and even to vertebrate fish [92] and in high dosage can promote oxidative toxicity in humans [93]. Nickel, which also has potent antimicrobial properties, is a known haematotoxic, immunotoxic, neurotoxic, genotoxic, nephrotoxic, hepatotoxic and carcinogenic agent [94] and is therefore not used.

Many bacteria and fungi have different mechanisms to deal with excess copper (reviewed in [30]). These include exclusion by a permeability barrier, intra- and extra-cellular sequestration by cell envelopes, active transport membrane efflux pumps, reduction in the sensitivity of cellular targets to copper ions, extracellular chelation or precipitation by secreted metabolites including copper, and adaptation and tolerance via upregulation of necessary genes in the presence of copper (e.g. [95,96]). However, above a certain threshold and time of exposure, which differs between the microorganisms, they cannot deal with the copper overload and die. Due to the multisite kill mechanism of copper and mostly non-specific mechanisms of damage exerted by copper (see above and [30]), their tolerance to copper is relatively low, as compared to the resistance to antibiotics demonstrated by some microorganisms (i.e., 10 fold lower sensitivity to copper as opposed to 1000 fold less sensitivity to methicillin, for example by methicillin resistant *S. aureus*). Thus, in contrast to the highly resistant microbes that have evolved to antibiotics in less than 50 years of use, tolerant microbes to copper are extremely rare even though copper has been a part of the earth for millions of years. Viruses do not have tolerance and repair mechanisms, such as DNA repair mechanisms, as bacteria and fungi and thus are highly susceptible to copper induced damage.

COPPER HEALTH RELATED APPLICATIONS

In contrast to the high susceptibility of microorganisms to copper [30], copper is considered safe to humans, as demonstrated by the widespread and prolonged use by women of copper intrauterine devices [97-100]. The risk of adverse reactions due to dermal contact with copper is considered extremely low [101,102]. Copper is an essential trace element involved in numerous human physiological and metabolic processes [103,104], including in wound repair [105], and many over-the-counter treatments for wound healing contain copper [106,107]. The National Academy of Sciences Committee established the U.S. Recommended Daily Allowance of 0.9 mg of copper for normal adults [108].

Copper and copper-based compounds, due to their potent biocidal properties, are now routinely used in several health-related areas. These include 1) control of *Legionella* [42,43,109-111] and other bacteria [112] in hospital water distribution systems. Hospital-acquired Legionnaires' disease has been reported from many hospitals since the first outbreak in 1976. Although cooling towers were linked to the cases of Legionnaires' disease in the years after its discovery, potable water has been the environmental source for almost all reported hospital outbreaks [110,113]. Copper-silver ionisation systems have emerged as the most successful long-term disinfection method for hospital water disinfection systems [42,43,109-111]; 2) prevention of algae and other parasites growth in potable water reservoirs (e.g. [114,115]). The efficacy of silver/copper/chlorine combinations in drinking water for inactivation of protozoa such as *Hartmannella vermiformis* and *Naegleria fowleria* [responsible for primary amoebic meningoencephalitis] amoebas and *Tetrahymena pyriformis*, is being explored [116,117]; 3) reduction of caries in dentistry. Dental cements containing copper have been shown to have antimicrobial and anticariogenic properties [118,119]; 4) reduction of foodborne diseases through the production and use of self-sterilizing metallic copper surfaces [35,40,44,56] or materials containing copper [11,12,53,54,120], in which the food is kept, handled or transported. The addition of copper to drinking glasses has been shown to reduce biofilm formation of *Streptococcus sanguis*, thus reducing the risk of oral infections [53]. The USA Centers for Disease Control (CDC) reported in 1999 that 76 million Americans (~25% of the population) suffer from food poisoning annually. Recently they reported that while several foodborne illnesses - yersinia, shigella, listeria, sampylobacter, and shiga toxin-producing *E. coli* 0157 - have become somewhat less common than they were in 1999, the overall rate of reported foodborne illness hasn't budged much since 2004; and 5) in birth control by using copper intrauterine contraceptive devices [100,121].

Novel uses of copper or copper-based compounds in health-related applications are being explored and/or implemented. One area is the reduction of transmission of health-associated (nosocomial) pathogens in hospitals, clinics and elderly homes, by i) making hospital hard surfaces, like door knobs, bed rails, and intravenous stands, with metallic copper [36,47,51,55] ii) making hospital soft surfaces, like sheets, patient robes, patient pajamas, and nurse clothing, from copper-impregnated biocidal textiles [11,12,122], and iii) disinfecting contaminating cloths with copper-based biocides [31].

Dust mites are considered to be an important source of allergen for perennial rhinitis and asthmatic attacks [123]. Recently it was demonstrated that copper-impregnated fabrics are acaricidal [8,11]. Thus, elimination of house dust mites in mattresses, quilts, carpets and pillows may improve the quality of life of those suffering from dust-mite related allergies.

The use of copper-impregnated socks for the prevention and treatment of fungal foot infections (athlete's foot) has been reported [13,124]. Similarly, the use of copper oxide containing wound dressings for the reduction of dressing and wound contamination has shown excellent results in human and animal studies, not only in reducing the contamination of the wounds, but more importantly in enhancing and allowing wound repair, especially of diabetic ulcers in which conventional treatment modalities failed in closing the wounds (unpublished data).

A possible application of copper due to its potent virucidal properties is its use in filtration devices that can deactivate viruses in contaminated solutions, such as contaminated blood products and breastmilk. Recently the deactivation of HIV-1 and other viruses in suspension by copper-based filters has been reported [65,66].

The capacity to impregnate copper into different textile products, as well as into latex and other polymeric materials [10-12] allows for the production of personal protective equipment (PPE) with antimicrobial and antiviral properties to be used by first responders and laboratory personnel, who may be exposed to pathogens. PPE such as gloves, masks and disposable robes, may increase the safety not only of those using these products but of the immediate environment and assure safer disposal of the used items. Similarly, police or health-workers uniforms that may be exposed to contaminated solutions, such as blood, would reduce the risk of pathogens transmission.

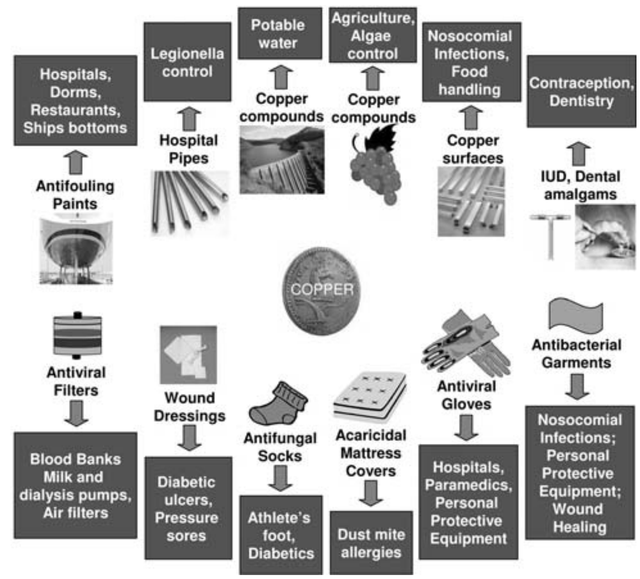

Fig. (1). Current and future potential applications of copper and copper compounds in different areas, which are based on copper's biocidal properties.

In contrast to the above copper health related applications, copper is not appropriate for use for systemic infections, mainly since once copper is ingested it readily interacts with transport proteins as well as small molecular weight ligands [125,126], making it unavailable as an antimicrobial. Furthermore, in cases where no efficacious copper metabolism occurs, the unligated free copper in the body may be involved in disease pathogenesis, such as in Alzheimer's disease [127]. Another limitation of copper may be the price of copper, which has recently escalated. However, this is of special relevance mainly when whole surfaces or products are made with copper or copper alloys. It is significantly less prohibitive when copper compounds, such as copper oxide, are impregnated in low percentages in medical devices or other health related products. In any case, when compared to the alternatives or the consequences of not using copper-containing products, e.g., increased nosocomial infections and food poisoning and the related costs of treatments, the issue of the copper cost is not significant.

In conclusion, the safety of copper to humans and its potent biocidal properties allow the use of copper in many applications (Fig. (**1**)), including several that address medical concerns of the greatest importance. While some of these applications are already being amply used, novel possible applications of copper may have a major effect on our lives.

CONFLICT OF INTEREST

G.B. is the Chief Medical Scientist of Cupron. J.G. is the CEO of Cupron. Cupron is a company that uses copper oxide in its medical and consumer applications.

ABBREVIATIONS

CDA	=	Copper Development Association
EPA	=	U.S. Environmental Protection Agency

MRSA = Methicillin-resistant *Staphylococcus aureus*

HIV-1 = Human Immunodeficiency Virus Type 1

PPE = Personal protective equipment

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