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Antiviral Activities of Cu^{2+} Ions in Viral Prevention, Replication, RNA Degradation, and for Antiviral Efficacies of Lytic Virus, ROS-Mediated Virus, Copper Chelation

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ABSTRACT

Copper has been known for decades that marked changes of micronutrient homeostasis in the host are accompanied by infection or inflammation. Copper levels in the serum are significantly elevated in response to inflammation that copper accumulates at sites of inflammation. Easily oxidized copper oxide nanoparticles (CuONPs) are widely used as catalysts that the ability of CuONPs to reduce bacterial population and virus application is enhanced. The mechanism of copper-mediated inactivation of herpes simplex virus (HSV) is by which cupric ions oxidatively damage biomolecules. Virus-mediated subjugation and modulation of host lipids during infection that the life cycle of most viruses proceeds through a series of basic steps: binding and internalization, fusion, uncoating, of the viral genome, its replication, assembly of new particles, and budding or release of the newly made viruses. The HIV-1 protein Vpu is an 81-amino-acid (16-kDa) type I which the presence of Vpu leads to the degradation of BST-2 via an endosome-lysosome degradation pathway. Oxidative degradation by a Cu-metalloenzyme, and ubiquitin-mediated degradation of cellular proteins were exploited. Copper can disrupt the lytic cycle of the Coccolithovirus. Lysins represent a novel class of anti-infectives derived from bacteriophage which lysins are bacterial cell wall hydrolytic enzymes that selectively and rapidly kill specific bacteria. Regarding copper induced cellular toxicity, several mechanisms have been proposed based on the formations of ROS by free Cu ions as cupric and cuprous ions can participate in redox reactions. ROS (O_2^-,・OH, OH^-), Cu^+ and H_2O_2 play the important roles for viral inactivations. Thujaplicin-copper chelates inhibit influenza virus-induced apoptosis. Pyrrolidine dithiocarbamate as a metal ion binding agent inhibits the activity of the viral proteases of polyprotein processing and RNA

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replication of HRV. Chelation enables metals are capable of ligand scavenging via complexation, since reverse transcriptase enzyme inhibits the growth and replication of RNA tumor viruses. Thus, copper complex and copper chelation enhance antiviral efficacy.

***Keywords*:** Copper homeostasis, Cu^{2+} and Cu^{1+} ions, Copper oxide nanoparticles, HSV, DNA/RNA virus, mRNA degradation or decay, Viral replication, Capsid protein, ROS, Copper chelation

Abbreviations: Au/CuSNPs = gold/copper sulfide nanoparticles, CuNPs = copper nanoparticles, CuONPs = copper oxide nanoparticles, DMT = divalent metal ion transporter, DENV = dengue virus, GTPγS = Guanosine 5'-γ-thiotriphosphoric acid, HCMV = human cytomegalovirus, HIV = human immunodeficiency virus, HuNoV = human norovirus, HSV = herpes simplex virus, HRV = human rhinovirus, 8HQ = 8-Hydroquinoline, IC_{50} = half maximal inhibitory concentration, LOX = lysyl oxidase, MBC = Minimum Bactericidal Concentration, MDCK = Madin-Darby canine kidney cell, MIC = Minimum Inhibitory Concentration, MMD = nonsense-mediated mRNA decay, MNV = murine norovirus, oHSV = herpes simplex virus-derived oncolytic viruses, NS3 = non-structural proteins 3, PDTC = Pyrrolidine dithiocarbamate, PGN = peptidoglycan, R = resveratrol, ROS = reactive oxygen species, RT = reverse transcriptase, TG = transglycosylase, TP = transpeptidase, TUFM = Elongation factor Tu, mitochondrial precursor, TUF1, VACV = Vaccina virus, Vhs = virion host shutoff, VNPs = viral nanoparticles, Vpu = HIV-1 acccessory protein

1. INTRODUCTION

Copper is a cofactor of protein and enzymes (cuproenzymes) involved in fundamental mechanisms, such as energy generation, oxygen transcription, hematopoiesis, cellular metabolism and signal transduction. Copper exhibits considerable biochemical action either as an essential trace metal as a constituent of various exogenously administered compounds in humans. The bulk of evidence that copper is involved in the pathogenesis of neurodegenerative disorder is now huge. The average daily intake of copper is between 0.5 and 1.5 mg, coming mainly from seeds, grains, shellfish, nuts, beans and liver [1] that in terms of storage, the total amount of copper in an adult is estimated to be about 90～110 mg which copper is excreted from the body either in a non-absorbed form or via the bile that the estimated amount of copper in feces is 1～1.5 mg/day [1]. In human body, copper Cu is present as Cu^{+} (cuprous) and oxidized Cu^{2+} (cupric) compound that copper is an intermediary for electron transfer in redox reactions. The antimicrobial properties of copper include the ability to accept and donate an electron as it cycles between Cu(I) and Cu(II) oxidation states, such as by that redox property enables to catalyze the production of hydroxyl radicals via the Haber-Weiss and the Fenton reactions:

Reaction 1: $\mathbf{Cu^{2+} + \cdot O_2 \rightarrow Cu^{+} + O_2}$,

and

Reaction 2: $\mathbf{Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{-} + \cdot OH)}$.

The hydrogen peroxide is transformed into the very reactive hydroxyl radical, which combines with nucleic acids, proteins and lipids. Copper proteins have diverse roles in

biological electron transport and oxygen transportation, processes that exploit the easy interconversion of Cu(I) and Cu(II). Copper is found in Cu/Zn superoxide dismutase (SOD), an enzyme that detoxify superoxide, by converting it to oxygen and hydrogen peroxide that copper is a component of lysyl oxidase (LOX), an enzyme that participates in the synthesis of collagen and elastin, two important structural proteins found in bone and connective tissue [2]. Normal copper homeostasis is essential for human growth and development that copper mutations inadequate diet and surgical interventions, may lead to cardiac hypertrophy, poor neuronal myelination, blood vessel abnormalities and impaired immune response [3].

Copper overload is associated with morphological and metabolic changes in tissues which the changes in the expression of human copper transporters alter the sensitivity of cancer cells to major chemotherapeutic drugs [3]. Copper and its complex as medicine have in these agents as potential drugs for therapeutic intervention in various diseases such as Wilson's disease, the Menkes disease, Alzheimer's disease, inflammation, cancer [4].

The other, viruses are made up of nucleic acids, proteins and often lipids, and viruses can emerge because of changes in the host, the environment, or the vector, and new pathogenic viruses can arise in humans from existing human viruses or from animal viruses. Several viral diseases that emerged in the last few decades have now become entrenched human populations worldwide.

Viruses have evolved different strategies for their multiplication and propagation. Enveloped animal viruses use a two-step process to release their genetic material into the cell: first they bind to specific cell-surface receptors anchored to the target cell membrane and then they induce fusion of the viral and cell membranes. In non-enveloped animal viruses, the mechanism for genome delivery have not yet been characterized, although number of steps of the process have been described [5]. The determinations of the structures of virus and viral protein on Cu-enzymes reaction are obtained by X-ray crystallography, so that are of importance in several aspects of the viral life cycle such as cell-receptor recognition, viral entry, nucleic acid transfer, RNA and DNA polymerase, and genome replication of extensively enriched visions of the virus world which many of the structure available correspond to potential targets for antiviral drugs against important human pathogens [5].

Copper toxicity against virus may be considered as regulations of copper metabolism and copper enzyme system that is needed for certain critical enzymes to function in the body which copper is involved in the functioning of the nervous system, maintaining the balance of other useful metals in the body [6]. In this review article, firstly, bacteriolytic mechanisms by Cu^{2+} ion solutions are described against Gram-negative and Gram-positive bacteria. Secondly, copper homeostasis at host-virus interaction during viral infection are taken up. Thirdly, deactivations of virus by copper oxide nanoparticles and on the copper alloy surfaces are treated, and antiviral activities of Cu^{2+} ions indicate to be effective inhibitions for viral entry and replication, mRNA and capsid protein degradations along viral life cycle. Lastly, it may be characterized that copper and copper-complexes have beneficially antiviral efficacies of ROS-mediated virus death, Cu-complex, and Cu-chelation.

2. BACTERIAL KILLING MECHANISM BY Cu^{2+} IONS

Antibacterial susceptibility tests against *E. coli* and *S. aureus* were carried out [7]. The results are shown in **Table 1** that Cu^{2+} ion concentration ranges of 0.10～50 mg/L that the value

of Minimum Inhibitory Concentration, MIC = 50 mg/L above was obtained without killing curves which the commercial antibacterial agent of Cu^{2+} aqueous solution was recognized as appearing of bacteriostatic action, in which serves not bacteriolysis or destruction of bacterial cell wall, but functions maybe as DNA damages. The other, antibacterial susceptibility test of antibacterial agent for nitrate copper reagent $Cu(NO_3)_2{\cdot}3H_2O$ against *S. aureus* were MIC = 625 mg/L, Minimum Bactericidal Concentration, MBC = 1250 mg/L under the Cu^{2+} ion concentration ranging from 78 to 1350 mg/L that these Cu^{2+} ions indicate the appearance of strong killing effect.

Table 1. MIC measurement of Cu^{2+} ion solution commercial agent against *E. coli* and MIC, MBC, and CFU of Cu^{2+} ions in $Cu(NO_3)_2{\cdot}3H_2O$ solution against *S. aureus* by liquid medium method

Antibacterial Commercial Cu^{2+} Ion Agent	**Against *E. coli***										
	Cu^{2+} Ion Concentration (mg/L)										
MIC	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	MIC
	+	+	+	+	+	+	+	+	+	+	50 mg/L above
$Cu(NO_3)_2{\cdot}3(H_2O)$ Solution Agent	**Against *S. aureus***										
	Cu^{2+} Ion Concentration (mg/L)										
	5,000	2500	1250	625	313	156	78	39	20	9.8	MIC· MBC
MIC	–	–	–	–	+	+	+	+	+	+	MIC = 625 mg/L

MBC	−	−	−	+	+	+	+	+	+	+	MBC = 1,250mg /L
CFU (cfu/mL)	<10	<10	<10	1.1×10^{2}	3.1×10^{8}	4.0×10^{8}	4.5×10^{8}	5.1×10^{8}	5.5×10^{8}	5.3×10^{8}	

Bacteriolytic mechanism of Cu^{2+} ions against *E. coli* cell wall is due to the destruction of outer membrane structure by the degradation of outer membrane lipoproteins at N- and C-terminals, and the inhibitions of peptidoglycan (PGN) elongation due to the deletions of PGN-trans- peptidase (TP) enzyme and PGN autolysins. The other, bacteriolytic mechanism of Cu^{2+}ions against *S. aureus* cell wall is composed of the damages of PGN synthesis enzymes transglycosylase (TG)/TP due to the reactions of the polymerization of glycan chains and the cross-linking of peptide chains, and the activations of PGN forth autolysins [8]. A distinction may be made as to whether these bacteriolytic mechanisms could be applicable to virucidal mechanisms for lytic virus in which a further search should be made for a relationship between PGN autolysins and viral lysins.

3. COPPER HOMEOSTASIS AT HOST-PATHOGEN INTERFACE, HOST-VIRUS INTERACTION, Cu-BINDING VIRAL PROTEIN, AND DURING VIRAL INFECTION

Copper is processed by the liver and is transported and sequestered in a safe manner. Inorganic such as that in drinking water and copper supplements, largely bypasses the liver and enters the free copper pool of the blood directly that this copper is potentially toxic because it may penetrate the blood/brain barrier. It has been known for decades that marked changes in micronutrient homeostasis in the host are accompanied by infection or inflammation. Copper levels in the serum are significantly elevated in response to inflammation that copper accumulates at sites of inflammation, within granulomatous lesions of lungs infected with *M. tuberculosis*, and within the exudates of wounds and burns relative to serum [9]. Copper deficiency increases the susceptibility to various pathogens, including coxsackievirus B3 which conversely, copper supplementation is protective against *E. coli*-induced mastitis in dairy cattle and *M. tuberculosis* in mice [9].

Copper plays a role in the effective functions of immune system cells called lymphocytes (T and B types), neutrophils and macrophages that these cells are involved in nonspecific immune functions such as the phagocytosis(eating) and chemical killing of infectious bacteria, and specific immune functions of the production of antibodies and cell-mediated functions of T and B lymphocytes [10]. Cu deficiency is not usually about a lack of Cu but an imbalance of Cu and other minerals in the diet that need to be supplement with minerals and failure to do so may inhibit their potential to produce and can lead to illness [10]. Defects in Cu homeostasis lead to human disease that Wilson's disease results from mutations in ATP7B, leading to

hepatic and neuronal Cu accumulation, Wilson's disease frequently leads to liver malfunction, neurological defects, and Parkinson's disease frequently leads to liver malfunction, neurological defects, including movement disorders and depression that is managed by low Cu diets, chelation therapy or in extreme cases by liver transplant [11]. Whether Cu dysregulation is a cause or a consequence of these neurodegenerative diseases is under considerable study. Since both copper deficiency and copper excess produce adverse health effects, a dose-response modeling strategy for copper toxicity was proposed associated with both deficiency and excess that was applied to multiple studies of copper-induced toxicity, standardized with respect to severity of adverse health outcomes and selected on the basis of criteria reflecting the quality and relevance of individual studies which the existing toxicity data for copper excess or deficiency are effectively utilized in defining the limits of the homeostatic range in humans and other species [12].

Additional data are needed to better define the characteristics of dose-response relationships for copper-induced toxicity in relation to excess or deficiency [12]. The essential toxic nature of copper demands tight regulation of the copper homeostatic machinery to ensure that sufficient copper is present in the cell to drive essential biochemical processes prevent the accumulation to toxic levels which the tandem regulation of the copper uptake and detoxification pathways in response to the chronic presence of elevated concentrations of copper ions in the growth medium [13].

These results were that the nutritional and toxic copper metalloregulatory transcription factors Mac1p and Ace1p must sense and respond to copper ions in a dynamic fashion to appropriately regulate copper ion homeostasis and establish the requirement for a wild-type Mac1p for survival in the presence of toxic copper levels [13]. The intracellular trafficking of Cu to copper-dependent protein is fundamental to normal cellular metabolism. Copper proteins have diverse roles in electron transport and oxygen transportation, processes that exploit the easy interconversion of Cu^{+} and Cu^{2+} [2]. Host-virus interactions show that the mechanisms controlling cellular Cu^{2+} ions trafficking constitute natural antiviral barriers that this, together with the previously gathered broad knowledge of the role Cu^{2+} as a cofactor of viral proteins, suggests that the accessibility of copper ions in infected cells could be a potentially limiting factor in the virus life cycle.

The molecular mechanisms of this enigmatic interplay between viruses and the cellular systems that manage Cu^{2+} or Cu^{1+} flux based on this newly discovered type of host-virus interaction [14]. Further, modulation of host ROS metabolism becomes essential for viral infection that during host-virus interaction, ROS generation was associated with viral infection and specifically with induction of hallmarks of programmed cell death during the lytic phase of infection which inhibition of ROS production by application of a peroxidase inhibitor or an H_2O_2 scavenger inhibited host cell death and reduced viral production [15]. Copper is an essential micronutrient for both pathogens and the animal hosts during viral infection, but copper can be toxic in cells due to its redox reaction and ability to disrupt active sites of metalloproteins. During infection, macrophages can attack invading microbes with high copper that this copper is also elevated at sites of lung infection which copper levels in serum rise during infection with a wide array of pathogens [16]. To defend against this toxic copper, the microbial intruder is equipped with a battery of copper detoxification defenses that promote survival in the host, including copper exporting ATPases and copper binding metallothioneins that the animal host can thwart pathogen growth by limiting their copper nutrients, similarly to the well-documented nutritional immunity effects for starving microbes of essential Zn, Mn,

and Fe micronutrients [16]. Both Cu deficiency and overload can result in abnormal cellular function or damages that given its central role in host-pathogen interaction, subtle alterations of copper homeostasis can occur in the infectious diseases which aim, from the host perspective, either to reduce the availability of respective metals to microbes or to use toxic copper accumulation to eliminate pathogen [17]. Copper(II) chloride dihydrate antiviral compound was tested for inhibitory effect on the replication during infection of dengue virus (DENV-2) in cell culture that the ratio of cytotoxic concentration 50 (CC_{50} = 5.03 μg/ml) to maximal inhibitory concentration 50(IC_{50} = 0.13 μg/ml) was measured [18]. Copper plays the prevention of copper toxicity, as a result increased reactive oxygen species (ROS) and oxidative damage to lipid, DNA, and proteins have been observed in clinical syndromes of severe copper deficiency and inhibition was attributed to released cupric ions which react with cysteine residues on the surface of the protease [18].

4. DEACTIVATION OF VIRUS BY COPPER OXIDE NANOPARTICLES AND INACTIVATION OF VIRUS ON COPPER SURFACE

Copper has potent virucidal properties, and copper's neutralization of infectious bronchitis virus, poliovirus, human immunodeficiency virus type 1(HIV-1), and other enveloped or nonenveloped single- or double-stranded DNA and RNA viruses has been reported that the capacity of copper oxide-containing filters reduced infections titers of a panel of viruses spiked into culture media which enveloped, nonenveloped, RNA, and DNA viruses were affected, suggesting the possibility of using copper oxide-containing devices to deactivate a wide spectrum of infectious viruses found in filterable suspensions [19], and that a novel means to inactivate HIV-1 in medium, may significantly reduce HIV-1 transmission from "mother to child" and /or through blood donations if proven to be effective in breast milk or plasma and safe for use [20]. In addition, the use of protective masks has been shown to reduce the spread of respiratory viruses, especially when used by individuals in enclosed spaces or in close contact with a person with influenza-like symptoms [21]. Thus, impregnation of copper oxide into respiratory protective face masks endows them with potent anti-influenza biocidal properties without altering their physical barrier properties.

The phenomena of contact killing that bacteria, yeasts, and viruses are rapidly killed on metallic copper surfaces, have been known, it is currently received renewed attention. Cupric ions are able to inactivate five enveloped or nonenveloped, single- or double-stranded DNA or RNA viruses that the virucidal effect of this copper is enhanced by the addition of peroxide which mixtures of Cu^{2+} ions and peroxide are more efficient than glutaraldehyde in activating Junin and herpes simplex viruses [22].

Research on antiviral activity of copper nanoparticles (CuNPs) is rarely found, because micrometric metal copper did not cause cell damage as compared with highly biocidal CuNPs at the same mass. However, easily oxidized copper oxide (CuO) nanoparticles (CuONPs) have been focused for viral activity. CuONPs are widely used as catalysts and semiconductors that the ability of CuONPs to reduce bacterial population and virus application is enhanced, releasing Cu ions required for optimum killing [23]. The CuONPs in doses (<400 ppm) are safe for biomedical application and no side-effects, but its high dose (<400 ppm) is toxic [24] and CuO NP inhalation could induce pulmonary fibrosis in C57BL/6 mice that there is an urgent need to prevent the adverse effects of CuONPs in human respiratory system [25]. Nanosized

copper(I) iodide particles show inactivation activity against H1N1 influenza A virus subtitle that Cu(I) may be useful material for protecting against viral attacks and may be suitable for application such as filters, face masks, protective clothing, and kitchen clothes [26]. Further, Gold/Copper Sulfide core shell nanoparticles (Au/CuS NPs) exhibit variable virucidal efficacy against human norovirus (HuNoV) that viral capsid protein degradation and capsid damage appeared to be the mechanisms associated with inactivation [27]. The nanotechnology of nanosized metal preparations is important for medicine, sensor development, catalysis, and biological sciences. The other, viral nanoparticles is an emerging and highly interdisciplinary field in which viral nanoparticles(VNPs) are applied in diverse areas such as electronics, energy and next-generation medical devices [28]. Bioconjugation chemistries, mineralization strategies, and encapsulation techniques are allowed with all these techniques in hand, the potential applications of CuONPs and VNPs are limited only by the imagination.

The mechanism of copper-mediated inactivation of herpes simplex virus(HSV) is by which cupric ions oxidatively damage biomolecules that might be useful in the development of therapeutic antiviral agents [29]. Further, the rate of inactivation of norovirus on dry copper alloy surfaces was initially very rapid and proportional to copper content of alloy tested that the use of chelators and quenchers of reactive oxygen species(ROS) determined that Cu^{2+} and especially Cu^{+} ions are still the primary effectors of toxicity, but quenching superoxide and hydroxyl radicals did not confer protection [30].

Copper surfaces can significantly reduce the number of infectious influenza A virus particles compared to the number found on surfaces made of the widely used material stainless steel that influenza A is a viral pathogen that cause significant mortality and morbidity, which the copper ions have an ability to disorder DNA by binding to and cross-linking between and within strands [31]. If similar mechanisms occurred with the negative-sense RNA genome in influenza A virus, then viral replication could be inhibited by cross-linked RNA damage [31]. Recent work has shed light on mechanistic aspects of contact killing that they are juxtaposed with the toxicity mechanisms of ionic copper and plasmid DNA is completely degraded after cell death by contact killing, preventing the transfer of resistance determinants between organism [32].

The mechanism of copper surface toxicity in virus following wet or dry surface contact could change with contaminated touch surfaces and these results highlight the importance of correct surface cleaning protocols to perpetuate copper ion release and prevent the chelation of ions by contaminants, which could reduce the efficacy of the surface [33].

5. VIRAL PREVENTION

Metal alloys containing copper can destroy human norovirus, according to Applied and Environmental Microbiology, a Journal of the American Society for Microbiology. Specifically, the copper surfaces destroyed both the virus' genome, and its capsid, or protein shell. These copper surfaces can be used on high touch surfaces, like door knobs, hand rails, to prevent environmental transmission of the virus. Application of copper to prevent and control viral infection, thus prevent healthcare-associated infection (HCAI) that installing copper surfaces reduces the incidence of HCAI are required and the cost-effectiveness of such intervention needs to be assessed [34]. Further, 8HQ(8-Hydroquinoline)-based compounds hold medicinal properties such as anticancer, antioxidant, antimicrobial, anti-inflammatory activities

that many diseases arise from the loss of homeostasis including metal overload and deficiency, which are caused by abnormal metal metabolism or metal adsorption [35]. The 8HQ-metal chelation has been possessed for the effective prevention of viral infections.

6. ENTRY/UNCOATING / RELEASE / BUDDING

Virus-mediated subjugation and modulation of host lipids during infection that the life cycle of most viruses proceeds through a series of basic steps: binding and internalization, fusion, uncoating, of the viral genome, its replication, assembly of new particles, and budding or release of the newly made viruses [36].

Inhibition of HIV replication initially targeted viral enzymes, which are exclusively by the virus and not present in the human cell. Antiretroviral drugs are used for the treatment of HIV-infected patients; the reverse transcriptase (RT) inhibitors, nucleoside inhibitors and nonnucleoside inhibitors, the protease inhibitors, the integrase inhibitor raltegravir, the fusion inhibit or enfuvirtide (T-20), and the chemokine receptor 5 antagonist maraviroc [37]. An integrin-binding disintegrin-like domain within human cytomegalovirus (HCMV) envelope glycoprotein B, a protein required for virus entry and fusion throughout the Herpeviridae that accept receptor criteria are met through the use of function-blocking integrin Abs,β1 integrin knockout mouse fibroblasts, and glycoprotein B disintegrin-like peptides, all of which support a critical role for integrins as HCMV entry receptors and signaling mediators acting during the penetration stage of the entry pathway [38].

Dual function of CD81 for influenza virus uncoating and budding has important roles that CD81 belongs to the family of tetraspanins and is expressed on both plasma and endosomal membranes [39].

Lipid rafts play critical roles in many aspects of the influenza A virus cycle that cholesterol is a critical structural component of lipid rafts, and depletion of cholesterol leads to disorganization of lipid raft microdomains whereby the effect of cholesterol depletion by methy-β-cyclodextrin (MβCD) treatment on influenza virus budding was investigated [40]. These results were shown that exogenous cholesterol increased lipid raft integrity, inhibited particle release, and partially restored the infectivity of the released virus particles, and disruption of lipid rafts by cholesterol depletion caused an enhancement of virus particles release from infected cells and a decrease in the infectivity of virus particles [40].

If tetherin is counteracted by small interfering RNA knockdown or expression of the HIV anti-tetherin factor, budding and release capability is bestowed upon an otherwise budding-deficient neuraminidase that budding-competent neuraminidase proteins possess an-yet-unidentified means of counteracting the antiviral restriction factor tetherin and identify a novel way in which the influenza virus neuraminidase can contribute to virus release [41]. Furthermore, the divalent metal ion transporter (DMT1) release from the plasma membrane into extracellular vesicles may represent a novel mechanism which may be important for the regulation of the divalent metal ion transporter [42].

The copper-mediated transport-associated protein Ctr4 can form prion-like epigenetic determinants in *Schizosaccharomyces prombe* [43]. Thus, copper-mediated inhibitions of viral entry, uncoating, release, and budding along viral life cycle are only poorly characterized which appeared to be provided the molecular mechanism of the virus replications.

7. REPLICATION

The first encounter between a virus and a target cell occurs at plasma membrane that viruses first engage binding and entry receptors to initiate the infectious process which during infection, viruses subvert pre-existing cellular lipids and lipid signaling mechanisms for entry and trafficking, and once infection is initiated and viral genes expressed, extensive reprogramming of lipid synthesis and remodeling of lipid distribution serves to promote viral replication, assembly, and egress.

The only inhibitory effect of Cu(II) bound to GI.NOH-Cu(GI.NOH)$_2$ on the infectivity of extracellular virion was found which this is probably due to the observation that Cu(GI.NOH)$_2$ is localized in the extracellular space, a is best compartment specific for Cu(II), and thus, inhibiting the fusion between viral envelope and cell membrane, similarly to the inhibition of cell fusion obtained after action with 2-deoxy-D-glucose [44].

Thujaplicin-copper chelates inhibit influenza virus-induced apoptosis of MDCK cells and inhibit also virus replication and release from the infected cells [45]. Pyrrolidine dithiocarbamate (PDTC) as a metal ion binding agent inhibits the activity of the viral proteases of polyprotein processing and RNA replication of human rhinovirus (HRV) in a metal ion dependent way [46]. Divalent-metal-induced activation of non-structural proteins 3(NS3) helicase domain plays critical roles in NTP-dependent RNA unwinding and translocation during viral replication that a pre-activation state of NS3 helicase in complex with GTPγS, in which the triphosphate adopts a compact conformation in the presence of a divalent metal ions [47]. The effect of cellular copper on influenza A virus replication have been investigated that intracellular copper regulates the influenza virus life cycle, with potentially distinct mechanisms in specific cellular compartments [48]. These observations provide a new avenue for influenza virus pathogenesis.

8. DNA/RNA CLEAVING AND DEGRADING ACTIVITY

The DNA or RNA genome is generally packaged with proteins into a compact structure, the capsid, which may be covered by additional layers of protein or membrane. Resveratrol (R), a plant polyphenol, is known to reduce Cu(II) to Cu(I) generating ROS that can cleave plasmid DNA. A paradoxical synergistic effect between R and Cu(II) whereby plasmid DNA cleaving/degrading activity of R-Cu increased progressively as the ratio of R to Cu was increased i.e., the concentration of Cu was successively reduced with respect to a fixed concentration R, the other, cleavage of plasmid DNA occurred at low molar ratio of R to Cu, at higher ratios, complete degradation of DNA was achieved, and by further increasing the ratio, whereby the concentration of Cu was reduced to very low levels, the DNA degrading activity of R-Cu was lost [49]. This paradoxical synergistic effect is seen with respect to eukaryotic genomic DNA and RNA.

Approximately 0.05 mM concentrations of the metal chelating agents bathocuproine and bathophenanthroline disulphonic acid disodium salts, which form very stable complex with heavy-metal ions inhibited in vitro the RNA-dependent RNA polymerase activity associated with influenza and A/RI-$_5^+$(H_2N_2) viruses in the presence of a large molar excess of Mn(II) and Mg(II) that the chelating agents may inhibit influenza virus-associated RNA polymerase activity by the formation of a ternary complex of enzyme-metal-ligand [50].

9. COPPER IONS INDUCED VIRAL mRNA DEGRADATION OR DECAY, Cu-METALLOENZYME, UBIQUITIN-MEDIATED CELLULAR PROTEINS, AND LYTIC VIRUS

The HIV-1 acccessory protein Vpu is an 81-amino-acid (16-kDa) type I integral membrane phosphoprotein with Rev-dependent (late) expression kinetics that Vpu protein is the degradation of the viral receptor CD4 and enhancement of virion release which the presence of Vpu leads to the degradation of BST-2 via an endosome-lysosome degradation pathway [51]. This understanding the molecular mechanisms of both Vpu-dependent and -independent mediated antagonism of BST-2 will be critical for therapeutic strategies that exploit this novel viral function. In cells, each targets translatable RNAs for cleavage and requires host Xrn1 gene to complete RNA degradation, although the mechanism of targeting and the position of the primary cleavage differ that multiple host shutoff factors have converged upon a common mRNA degradation pathway [52]. Several mammalian viruses encode factors promote mRNA degradation to globally regulate both host and viral gene expression which several antiviral pathways use RNA degradation as a vital restriction mechanism, and these host-encoded ribonucleases target and destroy viral RNA [53]. Human coronavirus 229E was rapidly inactivated on a range of copper alloys and Cu/Zn brasses were very effective at lower copper concentration that Cu(I) and Cu(II) moieties were responsible for the inactivation which was enhanced by ROS generation on alloy surfaces, resulting in even faster inactivation than was seen with nonenveloped viruses on copper [54]. In addition, rapid inactivation, irreversible destruction of viral RNA, and massive structural damage were observed, suggesting that control transmission of respiratory corona viruses [55]. Electron microscopy of purified MNV-1 that had been exposed to copper and stainless steel surfaces suggested that a massive breakdown of the viral capsid had occurred on copper that capsid integrity is compromised upon contact with copper, showing copper ion access to the vital genome [55,56].

During lytic infection, the virion host shutoff (Vhs) protein of HSV accelerates the degradation of both host and viral mRNAs that it helps redirect the cell from host to viral protein synthesis and facilitates the sequential expression of different vital genes which Vhs interacts with the cellular translation initiation factor eIF4H, and several point mutations that abolish its mRNA degradative activity also abrogate its ability to bind eIF4H [57]. Thus, these results suggest a mechanism for linking the degradation of an mRNA to its translation and for targeting Vhs to mRNAs to mRNAs and to regions of translation initiation. The results also indicate that mutations in an mRNA that affect its translation after the sites at which it is cut by Vhs and suggest that Vhs is directed to its preferred cut sites during translation initiation [58]. Nonsense-mediated mRNA decay (NMD) was originally coined to define a quality control that targets mRNAs with truncated open reading frames due to the presence of a premature termination codon which MMD appears to be involved, including homeostatic regulation of gene expression, development and differentiation, as well as viral defense [59]. Individual viral RNAs are often used as substrates for both replication and translation and can contain multiple, sometimes overlapping open reading frames that viral RNAs engage in a wide variety of interactions with both host and viral proteins to modify the activities of important cellular factors and direct their own trafficking, packaging, localization, stability, and translation [59]. Viral RNAs have developed features that mark them as potential targets of host RNA quality control pathways in which viral RNAs run afoul of the cellular mRNA quality control and decay machinery, as well as on strategies developed by viruses to circumvent or exploit cellular

mRNA surveillance [60]. Vaccina virus (VACV) accelerates mRNA decay and limits activation of host defenses that VACV deficient in either decapping enzyme are effective oncolytic viruses which host antiviral enzyme PKR in non-tumorigenic cells compared to wild-type virus [61]. This established a new genetic platform for oncolytic VACV development that is deficient for a major pathogenesis determinant while retaining viral genes that support robust productive replication which VACV mutants unable to execute a fundamental step in virus-induced mRNA decay can be translated into a powerful anti-tumor therapy [61].

The oxidative degradation by a Cu-metalloenzyme [62], and ubiquitin-mediated degradation of cellular proteins in health and diseases [63] were exploited. The former requires the active site of GH61 revealed to contain a type copper and, uniquely a methylated histidine in the copper coordination sphere, thus providing an innovative paradigm in bioinorganic enzymatic catalysis. In the latter, several observations led to the prediction that degradation of intracellular proteins is highly specific process and must be carried out by different mechanism that half live of intracellular proteins vary from a few minutes to several days.

Copper can disrupt the lytic cycle of the Coccolithovirus,EhV86 that the glutathione content is consistent with increase in production of ROS on viral lysis, while increases in phytochelatin, PC content are likely linked to metal homeostasis and indicate that metal toxicity to the host was not affected by viral infection, in which Cu prevents lytic production of EhV86 by interfering with virus DNA synthesis through a transcriptional block, which ultimately suppresses the formation of ROS [64].

Lysins represent a novel class of anti-infectives derived from bacteriophage which lysins are bacterial cell wall hydrolytic enzymes that selectively and rapidly kill specific bacteria [65]. The mechanism of action of lysins is that bacteriophage viruses infected are distributed on the planet, forming part of the normal human microbiome which is estimated that there are phages 10^{31} in the biosphere, representing over 10^{6} distinct species. Whereby, the viral DNA is injected through the tail into the host cell, directing production of progeny phages in a life cycle that may take less than 30 min which these newly produced phages burst from the host bacterial cell and infect other host cells at the production of more phage [65].

Viral metagenomics could represent a potential source of recombinant protein with biotechnology value which in order to identify such proteins, a novel two-step screening technique was devised for cloning phage lytic enzymes from uncultured viral DNA. These two-step screenings result that these proteins include both Gram-positive-like and Gram-negative-like enzymes, as well as several atypical lysins whose predicted structure are less common among known phage which this study represents one of the first functional screens of a viral metagenomic population, and it provides a general approach for characterizing lysins from uncultured phage [66]. This phage lysin LysA2 has been reported as a phage lysin of the lytic profile of a non-tagged recombinant LysA2 against *Lactobacillus* strains [67]. With continued identification and characterization of phage lysins, the use of recombinant phage lysins may offer additional tools to help advance product development of live biotherapeutic products (LBPs) [67]. In addition, autophagy also plays for a virus replication that Tu elongation factor, mitochondrial(LBPs)-dependent autophagy was reduced in TUFM-deficient cells infected with avian influenza A virus, however, that autophagy remained consistent in virus-infected cell [68].

These results suggest that TUFM acts as a host restriction factor that impedes avian-signature influenza A virus replication in human cells in a manner that correlates with autophagy.

10. COPPER INDUCED ROS PRODUCTION IN VIRUS, OXIDATIVE STRESS, AND VIRUS DEATH

Reactive oxygen species(ROS) are well known for being both beneficial and deleterious that cellular metabolisms product different varieties of ROS as byproducts with the role of ROS in ribonucleic acid (RNA) virus pathogenesis which much evidences have accumulated over the past decade, suggesting that patients infected with RNA viruses are under chronic oxidative stress [69]. The Ni/GGH oxidative procedure was used to make covalent attachments to virion by trapping with a functionalized disulfide reagent that crosslinking of and coupling to viral capsid protein occur exclusively at adjacent tyrosine residues oxidations [70]. Regarding copper induced cellular toxicity, several mechanisms have been proposed based on the formations of ROS by free Cu ions as cupric and cuprous ions can participate in redox reactions in the followings:

Super oxide anion generation: $O_2 \rightarrow \cdot O_2^-$

Hydrogen peroxide production: $\cdot O_2^- + 2H^+ + e^- \rightarrow H_2O_2$

Haber-Weiss reaction: $H_2O_2 + O_2^- \rightarrow \cdot OH + OH^- + O_2$

Fenton reaction: $Cu^+ + H_2O_2 \rightarrow Cu^{2+} + OH^- + \cdot OH$

Reduction to Cu(II): $Cu^{2+} + \cdot O_2^- \rightarrow Cu^+ + O_2$

The pathogenesis of influenza virus infection is also related to oxidative stress. However, the role of endogenic genes with antioxidant effect in the control of influenza H5N1 viruses had been studied that the H5N1 infection in lung epithelial cells decreased Cu/Zn superoxide dismutase (SOD1) expression at mRNA and protein levels [71]. Therefore, this study showed the role of SOD1 in the replication of H5N1 replication in epithelial cells that pharmacological modulation or targeting SOD1 may open a new way to fight H5N1 influenza virus.

11. COPPER COMPLEX AND COPPER CHELATION ENHANCE ANTIVIRAL EFFICACY

Chelation enables metals to be transported to or from vulnerable target site, and to hinder or facilitate their carcinogenic potential that in the reverse sense, metals are capable of ligand scavenging via complexation or mixed complex formation. Since reverse transcriptase enzyme inhibits the growth and replication of RNA tumor viruses, zinc metallo-protein nature of reverse transcriptase was reported, using a metal-chelating agent-isoniazid, one of the most potent antitubercular drugs – on which to chelate the zinc ion out of the enzyme to render it inactive, thereby inhibit the viral life cycle [72]. Here, culminated in the use of isoniazid and its copper complex for the specific inhibition of reverse transcriptase as well as the elucidation of the underlying mechanism of this inhibition [72].

Copper chelation is currently being investigated as an antiangiogenic and antineoplastic agent diagnosed with cancer that herpes simplex virus-derived oncolytic viruses (oHSV) are being evaluated for safety and efficacy in patients, but several host barriers limit their efficacy. Copper-chelating agent (ATN-224) can reduce serum-mediated inhibition of oHSV, resulting in improved oHSV's serum stability and therapeutic efficacy [73]. Copper chelates activate the Th1 cellular immune response, enhancing immune processes in the body and protecting it

against infection that the use of copper-chelates increases the response of peripheral blood T lymphocytes to stimulation by mitogens, which include copper [73]. Further, feed supplementation of copper sulphates and copper-glycine chelates intensely absorbed in the intestines in conjunction with phytase may lead to the development of local inflammation in the stomach and intestines, increasing susceptibility to infection [74]. In addition, challenges associated with metal chelation therapy have been carried out attractively for Alzheimer's diseases that the term "metal targeted strategies" brain metal redistribution rather than brain metal scavenging and removal is the major goal of this type of intervention [75].

As mentioned above, antiviral activities of Cu^{2+} ion solutions are summarized in **Table 2** that shows in viral prevention, entry and fusion, uncoating, replication, release and budding along viral life cycle.

Table 2. Antiviral activities of Cu(II) ion solutions in viral prevention, entry and fusion, uncoating, replication, release and budding along viral life cycle

Cu^{2+} ions	Antiviral Activities of Cu^{2+} Ion Solution along Viral Life Cycle						
	Viral Prevention	**Adsorbtion/ Entry/ Fusion**	**Uncoating**	**Replication**	**DNA/RNA**	**mRNA Degradation /Decay**	**Release and Budding**
Cu^{2+} →	→	→ Cu^{2+}, Cu^{+} O_2^-, H_2O_2	→ Cu^{+}, Cu^{2+} O_2^-, ·OH, OH^-, H_2O_2	→ Cu^{2+}, Cu^{+} O_2^-, H_2O_2	→ Cu^{+}, Cu^{2+} O_2^-, ·OH, OH^-, H_2O_2	→ Cu^{2+}, Cu^{+} O_2^-, H_2O_2	→ Cu^{+}, Cu^{2+} O_2^-, ·OH, OH^-, H_2O_2
	• 8HQ-metal chelation possesses the effective prevention of viral infections.	• Integrins as entry receptors (Cu^{2+} ions addition)	• Glycol-proteins inhibition of uncoating • Inhibition of CD81 for uncoating	• Intracellular copper influenza A virus • PDTC and Thujaplicin-Cu chelates replication • Influenza A virus on cellular Cu	• DNA degrading activity of R-Cu • Destruction of Capsid by Cu alloy • PDTC inhibition of RNA	• mRNA degrading on stainless steel and CuZn alloy • Cu-Metallo-enzyme oxidative degradation • Cu(I) generating ROS • Capsid protein degradation by Au/CuSNPs	Thujaplicin-Cu chelates inhibit release • Divalent metal ion transporter inhibit budding • Inhibition of CD81 of budding

12. CONCLUSIONS

Copper has potent virucidal properties, and copper's neutralization of infectious bronchitis virus, poliovirus, human immunodeficiency virus type 1(HIV-1), and other enveloped or nonenveloped single- or double-stranded DNA and RNA viruses.

Easily oxidized copper oxide (CuO) nanoparticles (CuONPs) are widely used as catalysts that the ability of CuONPs to reduce bacterial population and virus application is enhanced. Nanosized copper(I) iodide particles also show inactivation activity against H1N1 influenza virus and Gold/Copper Sulfide core shell nanoparticles (Au/CuS NPs) exhibit variable virucidal efficacy against human norovirus (HuNoV) with inactivation of viral the capsid protein degradation and capsid damage.

Virus-mediated subjugation and modulation of host lipids during infection that the life cycle of most viruses proceeds through a series of basic steps: binding and internalization, fusion, uncoating, of the viral genome, its replication, assembly of new particles, and budding or release of the newly made viruses. Inhibition of HIV replication initially targeted viral enzymes, which are exclusively by the virus and not present in the human cell. Several mammalian viruses encode factors promote mRNA degradation to globally regulate both host and viral gene expression which several antiviral pathways use RNA degradation as a vital restriction mechanism, and these host-encoded ribonucleases target and destroy viral RNA.

The oxidative degradation by a Cu-metalloenzyme and ubiquitin-mediated degradation of cellular proteins had been exploited. Human coronavirus was rapidly inactivated on a range of copper alloys and Cu/Zn brasses were very effective at lower copper concentration that Cu(I) and Cu(II) moieties were responsible for the inactivation which was enhanced by ROS generation on alloy surfaces.

The mechanism of copper-mediated inactivation of herpes simplex virus(HSV) is by which cupric ions oxidatively damage biomolecules.

Lysins exhibit a novel class of anti-infectives derived from bacteriophage which lysins are bacterial cell wall hydrolytic enzymes that selectively and rapidly kill specific bacteria. The mechanism of action of lysins is that bacteriophage viruses infected are distributed on the planet, forming part of the normal human microbiome. Copper can disrupt the lytic cycle of the Coccolithovirus, EhV86 that the glutathione content is consistent with increase in production of ROS on viral lysis.

Regarding copper induced cellular toxicity, several mechanisms have been proposed based on the formations of ROS by free Cu ions as cupric and cuprous ions can participate in redox reactions: Superoxide anion generation**,** hydrogen peroxide production, Haber-Weiss reaction, Fenton reaction, and reduction to Cu(II). ROS (O_2^-, ·OH, OH^-), Cu^+ and H_2O_2 play the important roles for viral inactivations.

Copper-chelating agent (ATN-224) can reduce serum-mediated inhibition of herpes simplex virus-derived oncolytic viruses (oHSV) resulting in improved oHSV's serum stability and therapeutic efficacy.

Thujaplicin-copper chelates inhibit influenza virus-induced apoptosis of MDCK cells and inhibit also virus replication and release from the infected cells. Pyrrolidine dithiocarbamate (PDTC) as a metal ion binding agent inhibits the activity of the viral proteases of polyprotein processing and RNA replication of human rhinovirus (HRV) in a metal ion dependent way. Thus, copper complex and copper chelation enhance antiviral efficacy.

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