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GALLIUM NANOPARTICLES AS DELIVERY SYSTEM AGAINST INFECTIOUS DISEASES AND CANCER

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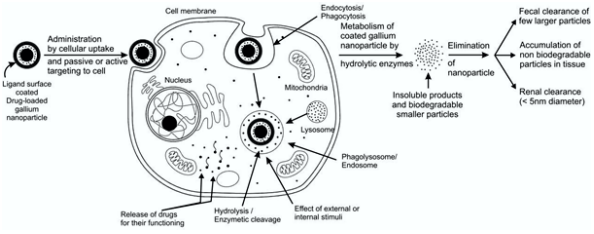
ABSTRACT As viruses, microbes, other pathogenic microorganisms and toxic agents are responsible for global broad spectrum diseases including cancer and malignant hypercalcemia, resulting significant mortality and morbidity, nanobiotechnology-based nanoparticles are being emerged as new nanomedicines for their biological applications owing to their unique shape, size and ease surface functionalizations. To overcome drug resistance and toxicity, gallium (Ga(III)) metal nanoparticles (GaNPs) have attracted attention for their requirements for prolonged treatments, especially, against human immunodeficiency virus, mycobacterium, hypercalcemia and cancer. These nanoparticles remain stable for the longer periods owing to the formation of native and passivating 2-3 nm oxide layer. Therefore, it is needed to encapsulate the NPs with bioactive compounds within vesicular system associated ligand-binding for specific delivery to target-sitesfor getting better efficacies. This review depicts especially the role of GaNPs as delivery system against infectious diseases and cancer.

KEYWORDS : Diseases; Gallium nanoparticles; Mechanism of action; Delivery system



Graphical Abstract

INTRODUCTION

Mycobacterium tuberculosis and type 1 human immunodeficiency virus (HIV-1) are the two crucial bacterial and viral infective agents that cause several million demise of public globally. Their co-infections, development of multidrug resistance due to abuse medications, bacterial biofilm formation and the development of malignant hypercalcemia owing to an enhanced calcium level in the blood for abnormal parathyroid gland activity-related high bone-turnover rates and malignant cancers have emerged a threat to public health to make new drugs with the applications of appropriate technologies [1-5]. The key criterion of the microbial pathogenesis is that the causative organism survives, grows and replicates within the host macrophages by inhibiting the phagosome-maturation for the formation of microbicidal acidic and hydrolytic phagolysosome [6]. Thus, the developing parasitization of the macrophages escapes the immune responses of the host cells [7-13,1,4]. In this context, antioxidant defense system of the body cannot prevent also microbes-induced reactive oxygen species (ROS) generation in the progression of disease-development [4].

Iron (Fe), an essential nutrient for the metabolism and the growth of all living cells including microbes, participates in enzymatic reactions as cofactor with the capability to redox cycle between Fe(III) / Fe(II) enhancing electron transfer [14]. Generally, iron in human becomes bound tightly with transferrin, ferritin, lactoferrin and heme. Pathogenic infective microorganisms usually try to acquire iron chiefly from these complex iron proteins for their survival through highly efficient iron-uptakes mechanisms. Many microbes liberate iron solubilizing compounds such as siderophores to chelate Fe^{3+} intracellularly and extracellularly from host iron-binding molecules for their growth and the maintenance of virulency *in vitro* and *in vivo* [15-19]. Few important microbial enzymes require iron for their survival in human phagocytes. These include superoxide dismutase and catalase for protecting bacteria from phagocyte-derived ROS, ribonucleotide reductase for catalyzing DNA synthesis and iron-possessing enzymes / cytochromes for oxidative phosphorylations. Additionally, many of the bacterial genes become controlled by iron through the iron repressor protein such as IdeR affecting their metabolism for survival [14,20-22]. As host-macrophage-residing microbes become dependent on iron acquisition and utilization for their growth, their targeting of iron acquisition may be a promising strategic treatment for the infective agents [15-19].

Gallium, found generally in the Earth's crest, has been utilized to be effective for the treatments of viral and bacterial infections, malignant hypercalcemia, trypanosomiasis, syphilis and tuberculosis owing to its distinctive physico-chemical characteristics such as Ga-Ga bond formations in molecules, little melting point, rich phase diagram at lower temperature, anomalous volume expansion, chemical stability and anti-multidrug resistant characteristics [23- 27]. Ga, a trivalent cationic metal, is similar to iron. Generally, Ga^{3+} cannot be reduced like Fe^{3+}, and therefore, the concerned enzymes become inactive by their substitute Ga^{3+}-bindings to Fe^{3+}-binding sites as Fe^{3+} binding proteins such as siderophores cannot differentiate Ga^{3+} from Fe^{3+}, and thus, the growth of the pathogens becomes inhibited by Ga through the blockage of the iron dependent pathways.However, free gallium compounds such as gallium- maltolates, nitrates, chlorides, oxides, nitrides and oxynitrides would be more effective approach for the treatment of infections if these particles become nanosized in a regulated synthesized manner and surface-functionalized with bioactive molecules such as sugars, proteins, peptides, genes, ligands and encapsulated within vesicular systems for their targeting to specific site of interest [4,28]. This review illustrates especially the biological efficacies of GaNPs for the treatments of infective organisms and cancer to consider as a probable effective delivery system.

Synthesis Of Gallium Nanoparticles

Biological synthesis

3 mL of flower and root extracts from *Andrographis paniculata* as reducing agent are added separately to 20 mL of gallium nitrate in different flasks. The reaction mixtures are incubated at room temperature for 40 and 50 min respectively, and purified by repeated centrifugations at 10,000 rpm for 10 min. The pellet is stored at 4◦C as nanoparticles, and subjected to sonication before studies.

Colloidal synthesis

At first, 7 mL of 1-octadecene (ODE) is added into a three-neck reflux condenser flask and dried under vacuum for 1 h at 110◦C. After that, the reaction is loaded with inert Ar gas and warmed up to 280◦C following the addition of precursor gallium tris (dimethylamide) dimer ($Ga_2(NMe_2)_6$) solution of 25 mg in dried ODE (4.87 mL) and di-n-octylamine (DOA) (1.13 mL). The heat drops to 230-235◦C, and the reaction flask is chilled to room temperature within 1 min. The unwanted byproducts and non reacted precursors are separated by the addition of 10 mL chloroform, 20 mL ethanol and 1 mL oleic acid (OA) by centrifugations. The pellet is then re-dispersed in chloroform and the precipitation / purification step is repeated 2 to 3 times to get required size of up-scaled Ga NPs by regulating the reaction duration.

Sol-gel synthesis

The precipitations of gallium oxide (Ga_2O_3) precursors are obtained from the hydrolysis of gallium isopropoxide [$Ga(OC_3H_7)_3$] and aqueous gallium chloride ($GaCl_3$) solution by the addition of aqueous tetramethylammonium hydroxide (TMAH). Dominant amorphous and nanosized α-GaOOH particles are obtained by the addition of

TMAH solution and hot water to the solution of $[Ga(OC_3H_7)_3]$ dissolute in 2-propanol. A fully amorphous precipitate is obtained by the hydrolysis of $[Ga(OC_3H_7)_3]$ with pure water at room temperature, while upon heating of the precipitate at 500◦C, nanosized β-Ga_2O_3 particles are formed. On the other way, α-GaOOH particles transform to α-Ga_2O_3 at 500◦C as a single phase, while upon heating at 900◦C for all cases only β-Ga_2O_3 nanoparticles are formed.

For the synthesis of gallium oxynitride nanoparticles (GaON NPs), 0.40 g $Ga(NO_3)_3.8H_2O$ is dissolute in 10 mL distilled water, and 0.14 g hexamethyl enetetramine is adjoined into the solution. Then 2 g acetylene black (AB) is added for forming a viscous mixture and transferred into a Teflon-lined autoclave for hydrothermal treatment for 12 h at 100◦C followed by filtration, cleansing with water and drying. For the removal of AB and the conversion of GaOOH to Ga_2O_3, GaOOH-AB is calcined in air for 8 h at 800◦C to obtain Ga_2O_3-AB followed by nitridation under the NH_3 flow (200 mL/min) at 850, 950 or 1050 ◦C for 3 or 10 h to get GaON NPs, while the ramping rate follows 10◦C/min.

For the synthesis of gallium phosphate nanoparticles ($GaPO_4$ NPs), 0.5114 g of gallium nitrate ($Ga(NO_3)_3$) is dissolute upto 200 mL with deionized water (dIH_2O) by stirring for 10 min in a 1.0L flask. Then 0.25 g of powder polymer vinyl pyrolidone (PVP, 8000 MW) is adjoined into the gallium nitrate solution for providing the reaction to come with nucleation sites for the gallium and stirred for 10 min followed by heating to a rapid boiling. Simultaneously, 1.698 g of 40 mM K_3PO_4 is dissolute upto 200 mL with dIH_2O in another 1.0 L flask by stirring on a hotplate for about 4 min and then is boiled. The $Ga(NO_3)_3$/PVP solution is then adjoined into the K_3PO_4 solution gently and stirred at 700 rpm on the hotplate for 10 min and then heated to a simmering boil and followed at ~200◦C for 30 min. After that, the material solution is kept at room temperature followed by centrifugation at 7000 rpm for 10 min on decanting the solution or removing the top 2/3 rd of the solution or throwing out the bottom material to achieve appropriate smaller nanoparticles with their simulataneous checking by dynamic light scattering (DLS) spectroscopy. The collected $GaPO_4$ NPs are then dialyzed in a T1 dialysis bag for purification and lyophilized for storing or dissolved in distilled water or PBS with sonication before any study.

Preparations of gallium nanoformulations

5 mg dry glucan particles (GPs) synthesized from Fleishmann's Baker's yeast [29] are swelled in a sub-hydrodynamic volume (5 µL / mg GP) of an aqueous gallium chloride ($GaCl_3$) for loading GPs by diffusion of the gallium solution into the GPs through their porous surface structure. The particles are then lyophilized and the loading cycle is repeated to achieve maximum concentrations of $GaCl_3$ inside GPs. After that, the dry GP-$GaCl_3$ particles are swollen in a sub-hydrodynamic volume of a suitable reagent solution for a fast displacement reaction to generate insoluble gallium carbonate ($Ga_2(CO_3)_3$), gallium phosphate ($GaPO_4$) or gallium oxide (Ga_2O_3) nanoparticles inside GPs. The excess reagents and untrapped insoluble gallium salts (<30 nm) are cleansed from GP samples in 0.9% saline aseptically and sterilized in 70% ethanol.In this aspect, GPs-trapped water-insoluble gallium materials refers to the mixtures of compounds such as Ga_2O_3, amorphous $Ga(OH)_3$ and crystalline α-GaOOH, by the reactions of $GaCl_3$ with sodium hydroxide, sodium triphosphate and sodium bicarbonate [30]. The sample concentrations are then adjusted to $1x10^8$ nanoparticles / mL in 0.9% sterile saline and stored frozen for future use.

For the preparation of polymeric formulations, 100 mg pluronic p407 polymer, 200 mg gallium(III)meso tetraphenylporphyrine chloride (Ga TP) and 100 mg sucrose are adjoined to 20 mL of 1% HEPES solution and dispersed by stirring overnight in dark conditions. The mix is then homogenized until polydispersity and consistent size are acquired. To prepare homogenized suspension, the mixture is transferred to an Avestin C5 high-pressure homogenizer and extruded at 20,000 pounds / sq inch. The obtained suspension is cleansed 2 times with 0.5% p407 polymer-containing HEPES solution by spinning at 10,000 rpm for 30 min at 4◦C. The consequence pellet is resuspended in 1% HEPES and homogenized for getting consistent size and polydispersity of the NPs.

Gallium-containing PLGA nanoparticles may be prepared by double emulsification sonication method. In brief, PLGA is dissolute in HPLC-grade dichloromethane (DCM). Then the drug is adjoined to the PLGA/DCM solution and assorted properly by stirring. The dissolute solution is then adjoined into cooled 1% polyvinyl alcohol (PVA) and sonicated using a Cole Parmer Ultrasonic processor for 10 min at 20% amplitude. The suspension is assorted overnight at room temperature for evaporating DCM and gathered or rotovaporised gently and spun at 8000xg for 20 min at 5◦C. After that, the supernatant is decanted, and the pellet is cleansed twice in 25 mL dI H_2O by spinning at 8000xg for 20 min. The pellet is suspended in dI H_2O or PBS for further use.

Characterizations Of Gallium Nanocomposites

The characterization of the gallium nanocomposites regarding shapes, sizes, quantities, chemical stabilities on fine structural transitions are analyzed by utilizing several techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), UV-visible spectroscopy, Fourier transmission infrared spectroscopy (FT-IR), X-ray diffraction (XRD), dynamic light scattering (DLS) and surface plasmon resonance (SPR) spectroscopy.

Mechanism Of Actions Of Gallium Nanoparticles

Though group IIIA metallic galliums have similar characteristics like irons, they cannot be reduced to Ga(II), but can interact with important biological proteins involved in iron metabolism of the microorganisms interfering with both of the iron acquiring mechanisms and the functions of iron-reliant enzymes, incorporating Fe superoxide dismutase and catalase for superoxide and hydroperoxide - neutralizations, ribonucleotide reductase for conversion of ribonucleotides into deoxyribonucleotides (Fig.1) and the tyrosine radical formation, implying their antimicrobial activities, inhibitions of biofilm formations and hypercalcemia associated cancer [18,19,31-34]. The insertions of Ga(III) into the active sites of the iron-containing enzymes render them inactive, as Ga(III), compared to Fe, cannot be reduced to Ga(II) in biological system providing less redox toxicity [35]. After administration of Ga NPs in the biological system, they can bind incomplete assembled apo-transferrins for forming the Ga^{3+}-transferrin complexes for uptaking into the cells via transferrin receptor (TfR)-mediated endocytosis to promote antineoplastic activity of cancer cells (Fig.2, Fig.3). Some iron-deprived cancer cells upregulate TfR expressions onto their surfaces to allow the cells to take up higher levels of available Ga^{3+}-transferrin complexes to gather more irons for their required growth, and thus become susceptible to shelve-destructions by the usages of proper concentrations of gallium [36]. In this context, Ga^{3+} ions can directly induce Bax protein complex to enhance ROS production and down-regulate cyclin D1 for inhibiting the cell cycle progress from the G1 to S phase, and also block the proteasome complex to produce apoptosis in some diseased cells.

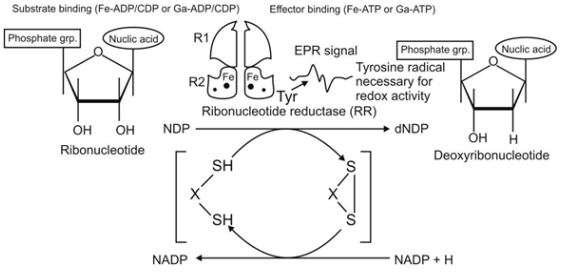


Figure 1. Ribonucleotide Redustase (rr) Needed To Convert Ribonucleotides Into Deoxyribonucleotides Where Iron Is Required For The Redox Chemistry. Gallium Ion Or Its Complex Can Interfere With The Tyrosine Radical And Deoxy-ribonucleotide Formations.

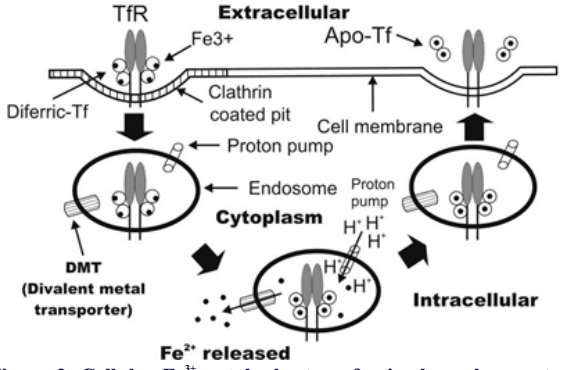


Figure 2. Cellular Fe^{3+}-uptake by transferrin through receptor-

mediated endocytosis. Iron-loaded transferrin / transferrin receptor (Tf / TfR) complex is delivered into endosomes where the pH - decline triggers the iron-release from the Tf / TfR complex. Then, iron is taken out into the intracellular matrix by the divalent metal transporter, and the Tf / TfR complex is recycled to cell surface.

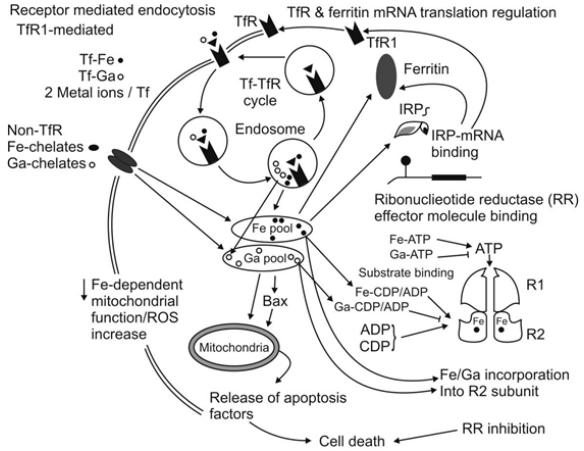


Figure 3. Transferrin-uptake of Gallium ions and / or iron. The transferrin / transferrin receptor (Tf / TfR) cycle is exhibited along with TfR and Ferritin mRNA regulation.

Gallium Nanoparticles As Delivery System

It has been demonstrated that 1 mol% GaNPs can provide significant antibacterial efficacy as potential therapeutic agent for pathogenic microbes such as methicilin-resistant *Clostridium difficile* and *Staphylococcus aureus* [37]. It is reported that GaNPs diminished the *Mycobacterium*-growth for 15 days after their single loading [38], while they showed their good bactericidal activities against *Pseudomonas aeruginosa* [39]. Some researchers prepared GaNPs to target *Mycobacterium tuberculosis* -infected macrophages, while the release of GaNPs inhibited the bacterial growth with iron acquisition significantly by promoting the phagosome- maturation as therapeutic anti-tuberculous drug [40]. Few other researchers elucidated that GaNPs disrupt *Francisella tularensis* -iron metabolism for inhibiting their infections at low drug concentrations also [41]. The potentiality of GaNPs has also been reported in suppressing co- infections of tuberculosis and HIV by the NPs' interactions with the CD4 membrane following endocytosis and pH-dependent endosomal escapes of the GaNPs into the cellular cytoplasm leading to inhibitions of viral proteases [42]. Few investigators prepared GaNPs and GaNPs- loaded glucan particles for the delivery of gallium and inhibitions of hypercalcemic malignancy and HIV-infected macrophages [25,43]. As GaNPs lack specificity to the cells, their loading or encapsulation with carriers may result in efficient targeted delivery to macrophages by the receptor-mediated active or passive uptakes mechanisms [43,4,28].

Immune Responses

When a host is exposed to pathogenic microorganisms, the host immune-defense mechanism becomes functional to produce a variety of molecules including chemokines, cytokines and T- cell-mediated immunity [44]. The T-cells, inturn, generate interferon gamma (IFN-γ) to activate macrophages for producing nitric oxide and reactive oxygen species, and key cytokines such as tumor necrosis factor alpha (TNF-α) and IL-12 to control host-infections [45-47]. Co-infections of monocyte-derived macrophages (MDMs) with HIV and *Mycobacterium tuberculosis* produce two significant interleukins e.g. IL-6 and IL-8 in huge amounts and insignificant amounts of TNF-α, IFN-γ, macrophage colony-stimulating factor (M-CSF), IL-1β and IL-4 [48-50]. Generally, the productions of IL-6 and IL-8 exhibit a negative effect on the host defense mechanism against infection, while IL-6 promotes the growth of the microbes by inhibiting the production of IL-1β and TNF-α for the maximal microbial killing, and IL-8 enhances the levels of inflammations and granuloma formations [51-53]. *In vitro* GaNPs-treated MDMs exhibited diminished levels of IL-8 and IL-6-productions through the inhibitions of microbial infections and / or GaNPs-mediated interfering of macrophages signaling IKK-β/NF-κB pathway/s.

Elimination

Chemically, the Ga^{3+} ions become hard Lewis acids, favoring ligands with the oxygens as the donor atoms for forming stable coordinated compounds lacking Ligand Field Stabilization Energy. Thus, kinetically labile Ga^{3+}-complexes become susceptible to the trans-metalations, de- complexations and hydrolysis reactions *in vivo* [54,55]. In the human body, under physiological conditions, the Ga^{3+} ions exist mostly as the hexa-aqua species $[Ga(H_2O)_6]^{3+}$ following their subsequent hydrolysis to give the insoluble gallium hydroxide $(Ga(OH)_3)$ [56,54] .

$$[Ga(H_2O)_6^{3+}(aq) + H_2O(I) \rightarrow [Ga(H_2O)_5(OH)]^{2+}(aq) + H_3O^+(I)$$

$$[Ga(H_2O)_5(OH)]^{2+}(aq) + H_2O(I) \rightarrow [Ga(H_2O)_4(OH)_2]^+(aq) + H_3O^+(I)$$

$$[Ga(H_2O)_4(OH)_2]^+(aq) + H_2O(I) \rightarrow [Ga(OH)_3](S) + H_3O^+(aq) + 3H_2O(I)$$

Such hydrolysis reactions may limit the extracellular availability of Ga^{3+} ions for binding transferrin after the oral or intravenous administration of soluble GaNPs. Moreover, the deposition of insoluble- gallium hydroxide and gallium oxhydroxide (GaO(OH)) in the kidneys may cause renal toxicity in human beings, though few of the larger particles may excreted through fecal clearances, while the others having sizes <5 nm can pass through renal glomerular filtrations [57].

CONCLUSIONS AND FUTURE PERSPECTIVES

Most of the drugs utilized to treat infections, bear drug-resistance, toxicity and non-selectivity. Metal-based nanoparticles have attracted attention as potential therapeutic to overcome these barriers, while nanoparticles can show exemplary cellular interactions with biomolecules within the cells and on the cell surfaces, and can be surface-functionalized by instigating selective biological moieties with particular binding activity to targeted cells for enhancing their therapeutic efficiency at the diseased site/s. The nanoparticles are characterized by increased cellular uptakes, good interactions with cells and good selectivities to the targeted cells with their proper surface-functionalization-modifications. The antibacterial activities are attributed generally to their capabilities to produce ROS that damage the microbial cell walls and to bind to microbial DNA or RNA, hindering their replication process, disrupting microbial enzymes and mitochondrial activities with the hindrance of electron transmembrane transport and inhibition of ATP production, which are reliant on some factors such as the shape, size, concentration, and methods of preparation of NPs, while antiviral activities are performed by their interactions with the viral surface of virions by the inhibition of the receptor binding sites on the virus to inhibit viral replications.

To get maximum therapeutic efficiency, GaNPs should be controlled in size (<5 nm) for glomerular clearance and surface-functionalized with ligands, sugars, proteins, peptides, drugs, genes and vesicles to overcome all the biological barriers and to deliver cargos to the targeted site of interest [28,4]. However, there is a need for a thorough and systemic *in vivo* study on oral and intravenous administrations of GaNPs with biodistribution, pharmacokinetics, toxicity, immune responses and biological efficacies to consider GaNPs as potential delivery system before going to a translational clinic.

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Disclosure Statement

No potential conflict of interest was reported by the author.

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