Macedonian Journal of Chemistry and Chemical Engineering, Vol. **33**, No. 1, pp. 39–52 (2014)

MJCCA9 – 635 ISSN 1857-5552
Received: November 12, 2012 UDC: 615.31:546.681
Accepted: February 5, 2014 *Review*

METALLOMICS RELATED TO GALLIUM COMPOUNDS: BIOCHEMICAL AND XENOBIOCHEMICAL ASPECTS

Zeno Gârban[1*], Radu Silaghi-Dumitrescu[2], Gabriela Gârban[3], Adina Avacovici[1], Nicoleta Hădărugă[4], C. Baltă[1], G.-D. Ghibu[1], Cristina Bischin[2], Olga-Alina Rada[1]

[1]*Department of Biochemistry and Molecular Biology, University of Agricultural Sciences and Veterinary Medicine, Calea Aradului Nr. 119, RO-300 645 Timişoara, Romania*
[2]*Department of Chemistry and Institute for Interdisciplinary Research on Bio-Nano-Sciences, "Babeş-Bolyai" University, Str. Arany Janos Nr. 11, RO-400 028 Cluj-Napoca, Romania*
[3]*Department of Environment and Nutrition, National Institute of Public Health – Branch Timişoara, Blvd. Dr. V. Babeş Nr. 16, RO-300 226 Timişoara, Romania*
[4]*Department of Food Quality, University of Agricultural Sciences and Veterinary Medicine, Calea Aradului Nr. 119,RO-300 645 Timişoara, Romania*

Issues concerning chemical compounds studied in the framework of metallomics are of interest both for the domain of biochemistry dealing with the metabolization of nutrients and for xenobiochemistry – a different domain of biochemistry dealing with the biodegradation of xenobiotics. In this context, in vivo and in vitro data are reviewed here on inorganic and organo-metallic compounds of gallium (Ga), ranging from cellular and molecular biology to biochemistry and pathobiochemistry and on to pharmacology and its relations to homeostasis and nutrient degradation.

Keywords: gallium compounds – biochemistry; xenobiochemistry; biomedical effects; in vivo; in vitro

МЕТАЛОМИКА НА ГАЛИУМОВИ СОЕДИНЕНИЈА: БИОХЕМИСКИ И КСЕНОБИОХЕМИСКИ АСПЕКТИ

Проблемите поврзани со хемиските соединенија од областа на металомиката се од интерес не само за биохемијата – домен што се занимава со метаболизмот на хранливите состојки, туку и за ксенобиохемијата – дел од биохемијата што се занимава со биодеградацијата на ксенобиотиците. Во овој труд е направен преглед на податоците *in vitro* и *in vivo* за неоргански и органски соединенија на галиум, од аспект на клеточна и молекуларна биологија до биохемија и патобиохемија и фармакологија во однос на хомеостазата и разложувањето на хранливите состојки.

Клучни зборови: биохемија; ксенобиохимија; биомедицинско дејство; соединенија на галиум *in vivo*; *in vitro*

1. INTRODUCTION

Problems related to biomineral compounds that, as constituents of the human organism, have as their precursors metals and metalloids present in food products of vegetal and animal origin as well as pharmaceutical products (either extracted or synthesized), attract continuous interest for their biochemistry, xenobiochemistry, physiology, pathophysiology, pharmacology etc. Toxicological aspects regarding inorganic or organo-metallic compounds of biological or economic importance are also a key issue. The interplay between these aspects across several metal/metalloid compounds

has led to the emergence of the new field of metallomics [1–4].

Obvious connections may be noted to proteomics – dealing with metalloproteins (e.g. metalloenzymes); to genomics – due to the interaction of metal ions with nucleic acids (DNA, RNA) in the genetic structure (starting from viruses to humans); and as a whole to metabolomics – dealing with the interaction of metal ions with various bioconstituents (e.g. lipoproteins, glycoproteins etc.) as well as to the speciation of metallic elements in biological systems [4, 5].

Gallium (Ga) is a metallic element belonging to group III A and has atomic number 31. It was discovered by Paul-Emile Lecoq de Boisbaurdan in 1875. In the earth's crust Ga is found at concentrations of 5–15 mg/kg. Its melting point is at 28.76 °C.

From a biochemical point of view, there are no known data regarding Ga as a biogenic element. Also, there are no physiological data suggesting a clear role or clear metabolic pathways concerning Ga in the human organism.

From a xenobiochemical point of view, some gallium compounds can be considered as xenobiotics of pharmaceutical interest, e.g. Ga trinitrate, tris(3-hydroxy-2-methyl-4*H*-pyran-4-one)gallium (Ga maltolate). The use of gallium in therapy was initiated by Levaditi in 1931, as cited by Bernstein (2005) [6]. In this context the use of Ga tartrate is mentioned, with a single dose (30–45 mg/kg) administered intramuscularly in the case of testing the eradication of experimental syphilis induced in rabbits. Experiments performed with Ga citrate (injection) revealed that it concentrates in bones, liver and kidneys [7].

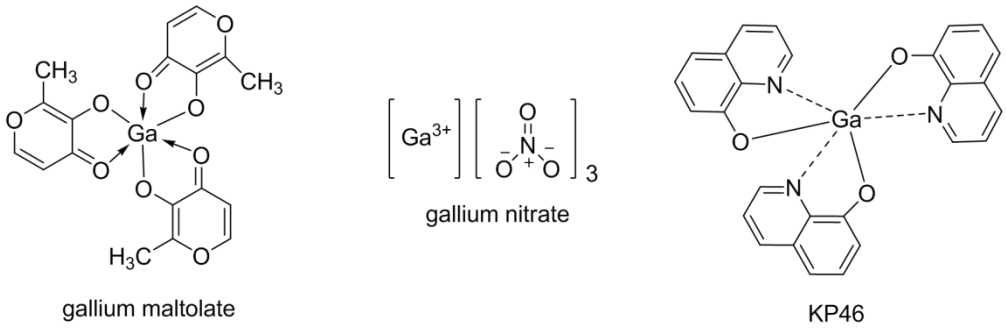


Fig. 1. Schematic structure of some gallium compounds of pharmaceutical interest

Also, there are gallium compounds such as Ga arsenide, which is used in the electrotechnical industry and can be considered xenobiotics of toxicological interest.

General data regarding the effects of non-radioactive gallium make reference to studies carried on in the period 1949–1952 [6]. The first studies highlighted poor absorption following oral administration of non-radioactive Ga compounds as well the accumulation of injected Ga citrate in bones, liver and kidneys. Studies undertaken after early 1970 demonstrated that non-radioactive gallium has many therapeutic effects, including: decrease in bone mineral resorption and decrease in high plasmatic calcium levels; inhibition of neoplastic proliferation; and treatment of some infectious diseases. Accordingly, one can conclude that various compounds of Ga may be useful in the diagnosis of certain cancers (radioactive gallium), in the treatment of various carcinomas, in cancer related-hypercalcemia and in infectious diseases.

2. CHARACTERISTIC REACTIONS OF Ga COMPOUNDS

2.1. *Reactions of gallium in aqueous media*

Gallium shows an amphoteric character in aqueous medium. Thus, at 25 °C and pH 7.4 in aqueous solution one can find $[Ga(OH)_4]^-$ at 98.4% and $Ga(OH)_3$ at 1.6% (in equilibrium with solid GaO(OH) [8]. The amphoteric character is evidenced by the presence of Ga hydroxyde $Ga(OH)_3$ and GaO(OH), the solubility of which increase at either higher or lower values of pH. Thus, at pH = 2 the $Ga(OH)_3$ form appears in an amorphous phase as a precipitate. In time this may be converted (transformed) in a quasicrystalline phase to GaO(OH).

In the amorphous phase present in alkaline medium, $Ga(OH)_3$ may form:

$$Ga(OH)_3 \rightleftharpoons Ga^{3+} + 3OH^-$$

In the crystalline phase in neutral medium, GaO(OH) may interact as follows:

$$GaO(OH) + H_2O \rightleftharpoons Ga^{3+} + 3OH^-$$

If the medium is alkaline the following reaction can occur:

$$GaO(OH) + H_2O + OH^- \rightleftharpoons [Ga(OH)_4]^-$$

Additionally, in acidic media the Ga^{3+} ion may be found in various other compounds revealing a metallic character, such as Ga nitrate – $Ga(NO_3)_3$, Ga sulfate – $Ga_2(SO_4)_3$, and Ga phosphate – $GaPO_4$ a.o.

2.2. *Reactions of gallium in biological systems*

In biological systems Ga^{3+} ions display effects similar to those of Al^{3+}, In^{3+} (being in the same group in the periodic system of elements) and especially to Fe^{3+}. The similarity between the biochemical interactions of Ga^{3+} and Fe^{3+} ions can be explained by the binding of these ions to proteins in a context where the two ions have radii of comparable values. Thus, in octahedral structures, one finds 0.620 Å for Ga^{3+} and 0.645 Å for Fe^{3+} but 0.350 Å for Al^{3+} [9]; furthermore, like iron, Ga^{3+} accommodates not only octahedral but also tetrahedral coordination spheres [10].

The ionization potentials also follow similar trends: 64 eV in the case of Ga^{3+}, 54.8 eV for Fe^{3+} and 119.99 eV for Al^{3+} [9, 11]. Nevertheless, there are also significant differences between Ga^{3+} and Fe^{3+} ions. The Fromm biochemical point of view these differences are:

1) Ga^{3+} has a constant valence while Fe^{3+} may be reduced to ferrous ion (Fe^{2+}) or oxidized to ferryl (Fe^{4+}), a highly-oxidizing toxic state (found in haeme enzymes and proteins) that has been implicated in oxidative stress, apoptotic cell death, lipid oxidation, degradation of carbohydrates and protein cross-link formation [12–18]. In biological molecules (e.g. heme, enzymes), ferrous ion (Fe^{2+}) may block the substitution with Ga^{3+}.

2) Fe^{3+} is largely insoluble at a pH of 7.4 (specifically in biological media). In contrast, Ga^{3+} may be present as gallate $Ga(OH)^{4-}$, a soluble form which can thus easily reach potential biochemical targets such as proteins or other chelatable compounds [10, 19]. The respective solubilities of the two +3 ions are 10^{-18} M for Fe and 10^{-6} M for Ga [20].

2.3. *Effects of gallium compounds in the environment*

Gallium can be found in bauxite, germanite and coal. Also, Ga can be a by-product of zinc and copper refining. In this context, the specific industrial profile regarding the extraction of ores, the processing and purification technologies may be topics of xenobiochemistry.

Certain compounds of Ga (e.g. gallium arsenide) are used in the electronic industry as semiconductor materials useful in integrated circuits [21]. The use of gallium in optoelectronic devices (photodetectors, LEDs, laser diodes, and solar cells), in smartphones, wireless communication, defence, and aerospace may carry an implicit risk of exposure and toxicity for the environment [22]. Very little information has been found regarding the toxicity of gallium in humans. In our knowledge, there are only two cases of severe toxicity reported in the medical literature, one studying the development of neurological sequelae after exposure to fumes from gallium fluoride crystals [23] and another revealing an acute gallium poisoning as a result of accidental exposure to gallium halide complexes. After the incident, the symptoms rapidly progressed from an apparent relatively non life-threathening dermatitis to a dangerous condition of vertigo, tachycardia, dyspnoea and unexpected black-outs [24].

Studies made in the optoelectronic industry in Taiwan have shown that exposure to gallium arsenide was reflected in significant increases in gallium and arsenide in the urine of workers. The use of masks and gloves had a protective effect against gallium arsenide exposure [25]. Another study showed elevated blood and urine levels of malondyaldehyde (a product of lipid peroxidation), in electronic industry workers exposed to aluminium, gallium, indium and arsenic [26].

Gallium alloys were considered to be less toxic dental materials than mercury, which possesses a toxic effect by affecting ion channels and transporters in the brain and kidney. Gallium does not have a significant effect on the channels, but still exerts a cytotoxic effect [27].

Various studies on the toxicology of Ga have been performed on experimental animals, e.g. mice, rats and hamsters. For example, a chronic experiment with gallium arsenide inhaled in doses of 0.1, 0.5 or 1.0 mg/m^3 for 6 h per day, 5 days per week for 105 and 106 weeks, on mice, evidenced no carcinogen activity [28]. Studies on rats showed that gallium arsenide induces pneumocyte hyperplasia and, over time, lung lesions that can lead to fibrosis [29]. This aspect suggests that Ga is a xenobiotic of toxicological interest.

Toxicity tests have also been developed with a new material with potential use in orthopaedics, obtained by introducing gallium ions into the crystalline lattice of hydroxyapatite [30].

3. STUDIES OF BIOLOGICAL INTEREST

3.1. *In vitro studies*

Experiments regarding the effects of Ga compounds have been the object of cell biology studies, but also of work in biochemistry and molecular biology [31–33]. Thus, in vitro studies evidenced modifications in protein synthesis, inhibition of the action of certain enzymes (ATP-ase, DNA-polymerase, ribonucleotide reductase etc.), disturbance in the polymerization of tubulines followed by destabilization of the assembly of microtubules in cells [34]. This effect on ribonucleotide reductase is also related to the similarity of Ga with Fe, as gallium binds at the diiron site of the enzyme, thereby blocking the tyrosyl-generating function of this site and hence blocking DNA synthesis [20].

The major effects of metal complexes in chemotherapy occur as a result of the interaction of metal ions with nucleoproteins and especially with deoxyribonuleotide acid [35]. In vitro studies performed on cell cultures revealed that certain Ga complexes induce effects similar to apoptosis in which the genomic DNA is fragmented. The resulting genomic DNA fragments with lengths of 180 bp (base pairs), or multiples of this, are considered characteristic of the dimension of the nucleosome in the DNA-histone complex [36]. At low ionic concentrations Ga^{3+} can bind to phosphate groups in the DNA macromolecule forming stable complexes, with no binding to nucleobases.

Changes were also observed in the tridimensional structure of DNA induced by its interactions with metals, followed by disturbances in the biosynthesis of the macromolecule [37]. All these can be explained by the fact that Ga intervenes in the inhibition of DNA biosynthesis in the replication phase [36, 38]. Generally, metals present in the environment, in food or water, and in chemotherapeutic drugs may induce toxic effects, depending on their concentration, which can be evidenced by homeostatic changes [39, 40].

Cell biologists followed the transit of Ga and its penetration into cells, determining that the process is due to Ga^{3+} binding to transferrin. Although its affinity is reduced compared to that of Fe^{3+}, when in excess, Ga^{3+} replace Fe^{2+} from this protein [41]. Nevertheless, using gel electrophoresis autoradiography experiments with ^{67}Ga and ^{59}Fe, it was shown that the two ions in fact entail slightly different pathways of entry and efflux into cells. Additionally, and perhaps not unexpectedly due to its increased solubility, Ga, unlike Fe^{3+}, is not sequestered inside cells by ferritin [42]. Gallium was also shown to affect iron uptake indirectly, by inhibiting the acidification of endosomes [20]. Also in relation to iron metabolism, the presence of gallium in transferrin inhibits haemoglobin formation [43].

It was further observed that malignant cells have a higher number of transferrin receptors on their surfaces [41] which can facilitate the penetration of Ga compounds into the cells. The process is also influenced by the antagonistic effects of Ga^{3+} toward the divalent metal ions Mg^{2+}, Fe^{2+}, Zn^{2+} and Ca^{2+}.

Cell biologists also observed that Ga compounds cause a calcium efflux at the mitchochondrial level. The process is more intense in cases where this is more calcium in the mitochondria.

Additionally, Ga nitrate also induces cellular oxidative stress via a series of events that leads to overexpression of haeme oxygenase-1 and metallothionein 2A [44]; the metal-responsive transcription factor-1 and zinc transporter-1 were also found to be affected [45]. Also, gallium nitrate is a potent inhibitor of protein tyrosine phosphatases (PTPases), a group of enzymes which play important roles in cell grow regulation and transformation. These enzymes are not affected by other Group IIIa metal salts (aluminum and indium) or Group IIIb metals (zinc) [46]. The ability of gallium to trigger an oxidative environment was revealed by other studies that demonstrated an overexpression of glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase-NADPH, two NADPH producing enzymes. Also, catalase activity was shown to be diminished, Fe metabolism being severely braked by Ga toxicity [47]. A series of $[Ga(LX)_2]ClO_4$ compounds ($(LX)^-$ = deprotonated form of ligands containing pyridine and 4,6-substituted phenol moieties with X = methoxy, nitro, chloro, bromo, and iodo) displayed growth-inhibition activity on cisplatin-resistant human neuroblastoma cells [48, 49]. This class of compounds was also shown to target the proteasome [50].

The gallium adduct of a paullone derivative, 9-bromo-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5*H*)-one (denoted kenpaullone), displayed a 1.5–18-fold increase in cytotoxicity compared to the free ligand on a range of human tumour cell lines [51].

Gallium(III) complexes of the naphtol-Schiff base ligand are recognized as substrates for P-glycoprotein (Pgp), one of the most characterized barriers to cytostatic treatment in cancer. Radiolabeled analogues of this complex could be useful in noninvasive imaging of Pgp-mediated transport *in vivo* [52].

Derivatives of (ethylenediamine)-*N*,*N*′-bis-[propyl[(2-hydroxy-3-methoxybenzyl)imino]] and (ethylenediamine)-*N*,*N*′-bis[propyl[(2-hydroxy-4,6-dimethoxybenzyl)-imino]] ligands with trivalent ions including gallium were shown to be cytotoxic against human epidermal carcinoma KB-3-1 cells. However, in colchicine-selected KB-8-5 multidrug resistant cells, the MDR1 P-glycoprotein apparently acted as an efflux transporter to induce resistance in the case of gallium but not in the case of indium [53].

Two novel organogallium(III) complexes containing *N*-phtaloylglycine and *N*-phtaloyl-DL-alanine ($Me_2Ga(\mu\text{-}O_2\text{-}CCH_2N(CO)_2C_6H_4)]_2$ and RS-$[Me_2Ga(\mu\text{-}O_2CCHMeN(CO)_2C_6H_4)]_2$) were evaluated regarding their antitumoral activity and the mechanism of cell death induction. The results showed that these compounds present an antiproliferative effect higher than that of gallium(III) nitrate, causing cell death by the induction of apoptosis [36].

Studies regarding the cytotoxic activities of some new gallium(III), titanium(IV) and tin(IV) containing 2,6-dimethoxypyry 3-carboxylato ligands showed a higher dose dependent cytotoxic effect of gallium(III) and tin(IV) compared with those of titanium complexes. Furthermore, electrostatic interactions of all these complexes with DNA were observed [54].

New bi- and tetranuclear gallium(III) complexes ($[Me_2\text{-}Ga(S\text{-}imi)]_2$, $Me_2\text{-}Ga(S\text{-}oxa)]_4$, respectively) with heterocyclic thiolato ligands demonstrated an apoptotic activity against cancer cell lines, most probably due to upregulation of caspase, sometimes faster than occurs with cisplatin [55].

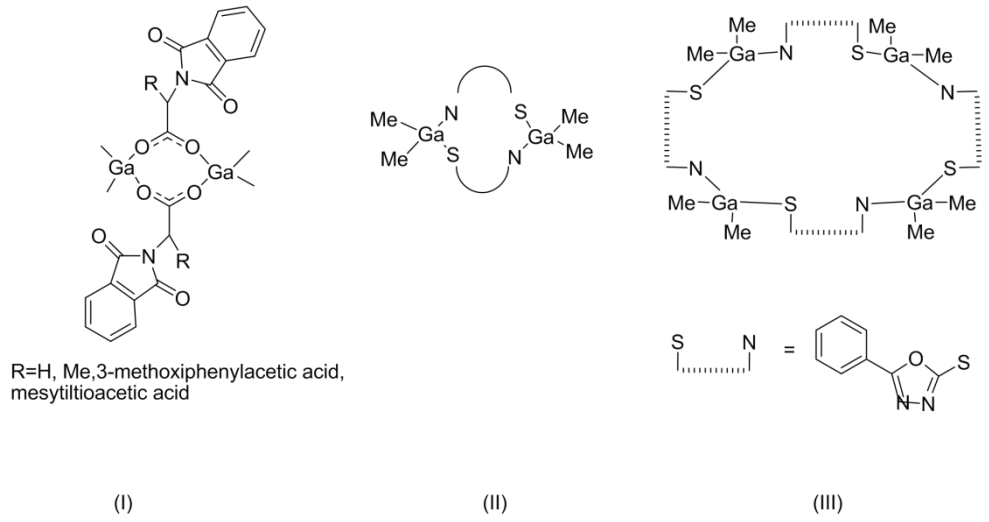


Fig. 2. Schematic structure of a carboxylate complex of gallium(I) and two other potential antitumoral bi- and tetranuclear gallium complexes (I, respectively II)

Other studies showed that gallium(III) complexes containing carboxylate or heterocyclic ligands revealed similar effects as cisplatin when tested on some types of human cell carcinoma, the mechanism of action of these complexes consisting of the induction of apoptosis through a caspase-independent pathway [56].

The use of gallium in low power laser therapy was also investigated. Thus, a Ga-Al-As laser produced an anti-inflammatory effect by stimulation of adrenal corticosteroid hormone release [57].

The ICP-MS method was used to quantify the in vitro binding of gallium quinolinolate (KP46) to transferrin and serum albumin. This method efficiently provides valuable information about binding parameters, including changes in the content of gallium-protein adducts and equilibrium binding constants [58]. Other studies used CE-MS to evaluate the binding of gallium nitrate and gallium quinolate to transferrin and human serum albumin [59]. Having similar coordination geometries and strong affinity for groups that contain oxygen, Ga^{3+} is used as a substitute for Fe^{3+} in some applications regarding iron-binding proteins and binding of phosphopeptides. Thus, a new method of immobilized metal affinity chromatography using Ga as the metal was developed for the purification of phosphopeptides [60]. A metal tag, gallium *N*,*N*-biscarboxymethyl lysine (Ga-LysNTA), was recently used by Camp et al. in phosphoproteomics, a new and more selective and precise method for the characterization of protein phosphorylation.

Modern techniques are applied for detection (liquid chromatography coupled to inductively coupled plasma mass spectrometry (ESI-MA)) and characterization of the resulting complex (ion trap electrospray ionization mass spectrometry (ESI-MS), Fourier transform mass spectrometry (FT-MS) and molecular modelling data) [61].

In Table 1 literature data are presented concerning the half maximal inhibitory concentration (IC50) obtained for different cell lines treated with some of the gallium compounds discussed here.

T a b l e 1

IC50 (μM) of some gallium(III) compounds in different cell lines

Compound	**Types of cell line**	**IC50 (μM)**	**Reference**
Gallium maltolate	Human HCC cell lines (Hep3B, HepG2, SNU475)	25–35 (144 h)	[62]
Gallium nitrate	Human HCC cell lines (Hep3B, HepG2, SNU475)	60–250 (144 h)	[63]
KP46	Variety of melanoma cell lines (ex. VM1, VM8) and human tumor cell line (breast, lung, colon, liver, cervix, bone, leukaemia cell lines)	0.85–2.5 (96 h)	[64]
Ga chloride ($GaCl_3$)	L-1210 leukaemic cells	174 (48 h) 35 (72 h) 16 (96 h)	[65]
$[Ga(L^X)_2]ClO_4$ (X= nitro, chloro, bromo, iodo)	BE(2)-C neuroblastoma cells	13.3–23.8 (24 h)	[48]
$[Ga(L^X)_2]ClO_4$ (X= methoxy)	BE(2)-C neuroblastoma cells	245.4 (24 h)	[48]
Ga(III)-*N*-phtaloyl glycine ($Me_2Ga(\mu\text{-}O_2\text{-}CCH_2N(CO)_2C_6H_4)]_2$)	8505C anaplastic thyroid cancer, A253 head and neck tumour, A549 lung carcinoma, A2780 ovarian cancer, DLD-1 colon carcinoma	5.72–26.31 (96 h)	[36]
Ga(III)-*N*-phtaloyl DL-alanine (*RS*-$[Me_2Ga(\mu\text{-}O_2CCHMeN(CO)_2C_6H_4)]_2$)	8505C anaplastic thyroid cancer, A253 head and neck tumour, A549 lung carcinoma, A2780 ovarian cancer, DLD-1	6.59–25.58 (96 h)	[36]
Kenpaullone (9-bromo-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5*H*)-one)	Human tumour cell lines (leukaemia, non-small cell lung, colon, melanoma, ovarian, breast)	1.5-18 (48 h)	[51]
Me_2-Ga(S-imi)]$_2$	8505C anaplastic thyroid cancer, A253 head and neck tumour, A549 lung carcinoma, A2780 ovarian cancer, DLD-1	5.15–13.79 (96 h)	[55]
Me_2-Ga(S-oxa)]$_4$	8505C anaplastic thyroid cancer, A253 head and neck tumour, A549 lung carcinoma, A2780 ovarian cancer, DLD-1	4.73–10.75 (96 h)	[55]
Organogallium(III) dinuclear complex (noted 1 in ref. 56)	Squamous cell carcinoma: HN –soft palate, Cal27, Cal33 – tongue, FaDu – hypopharynx and A253 – submandibular duct	7.38–18.52 (96 h)	[56]
Organogallium(III) tetranuclear complex (noted 10 in ref 56)	Squamous cell carcinoma: HN – soft palate, Cal27, Cal33 – tongue, FaDu – hypopharynx and A253 – submandibular duct	10.75–19.37 (96 h)	[56]

3.2. *In vivo studies*

In experimental studies on animal models various inorganic compounds of gallium were used. General data regarding such experiments are presented herein.

Gallium trinitrate. Studies were performed on rats following daily injection of 0.9, 1.8 and 3.6 mg / kg body weight (b.w.) for a duration of 21 days or 3.5 mg / kg b.w. for 33 days – the concentration was calculated for elemental gallium [66]. Changes were observed only at the dose of 3.5 mg / kg b.w., consisting of a decrease of serum calcium and decreased vitamin D in blood. High doses of Ga nitrate in rats revealed pathobiochemical and pathophysiological effects [67]. Nevertheless, the low efficiency of intestinal absorption and renal toxicity place some limits on the applicability of this simple form of gallium [68].

Gallium chloride. It has been administered as a treatment for some pulmonary cancers. The effects of oral administration as compared with i.v. administration were studied. Oral administration was more efficient, especially in cases of metastasis [69, 70].

Gallium sulphate. Only the acute toxicity of Ga sulphate has been studied in rats and mice. According to the findings of Domingo et al., 1987, also cited by Collery et al. (2002) [65], the median dose lethal in tested rats (LD_{50}) was 2 g/kg, higher than that for Ga nitrate.

Gallium arsenide. Investigating the antineoplastic activity of Ga, the effect of intratracheal administration of gallium arsenate in rats was also studied. The doses used experimentally in rats, according to a review by Chitambar (2010) [71], varied widely, from 0.6 mg/kg b.w. administered twice per week and lasting 4 weeks, up to 100 mg/kg b.w. in a single administration with investigation at 2 weeks. The effects on lungs, kidneys, testicles and haeme biosynthesis as well as immunological effects were evaluated in this context. Many authors performed studies by orally administering doses from 10 to 1000 mg/ gallium arsenide [71].

Gallium maltolate, chemical name tris(3-hydroxy-2-methyl-4*H*-pyran-4-onato) gallium. Administration of Ga maltolate (injected i.v.) Ga^{3+} revealed that it intervenes in the hydro-electrolytic metabolism of bones, decreasing calcemia in cases of parathyroidian carcinomas [72]. The mechanism involed inhibits the activity of osteoclasts, and a decrease in hydroxyapatite by the absorption of Ga at the surface of its crystalline structure.

Gallium quinolinolate (abbreviated KP46), chemical name is tris(8-quinolinolato)Ga(III). Studies regarding its effects on rats with carcinosarcoma revealed a decrease in hypercalcemia [73]. Other experiments in healthy mice showed good tolerance at lower doses while at higher doses it caused leucopoenia and even death.

Gallium protoporphyrin IX (abbreviated GaPPIX). Its effects were studied with focus on on Gram-positive and Gram-negative bacteria and also in mice by i.p. administration [74].

A series of thiosemicarbazone derivatives of gallium, including 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine®), were also examined and shown to have cytotoxic activities, with gallium more active than iron in this respect and with an involvement in oxidative stress [75,76].

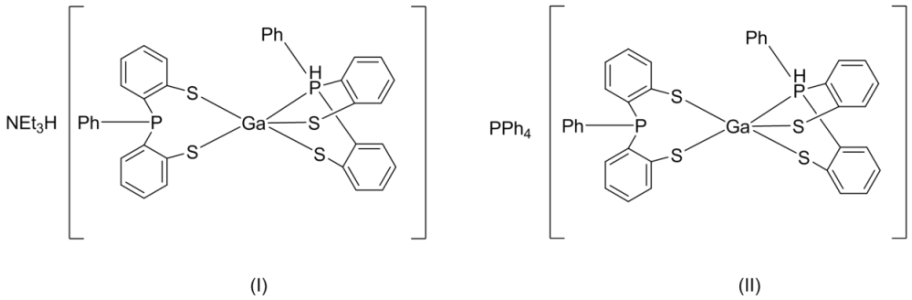
Our own efforts are concentrated on anionic gallium complexes [77, 78] of the following types:

– ammonium gallium complex of phosphinobisthiolato *P,S,S* pincer ligand, noted hereafter as C(24), with the general formula:

$$[NEt_3H][Ga\{PPh(2\text{-}SC_6H_4)_2\text{-}\kappa^3S,S',P\}\text{–}\{PPh(2\text{-}SC_6H_4)_2\text{-}\kappa^2S,S'\}]$$

–phosphonium gallium complex of phosphinobisthiolato *P,S,S* pincer ligand, noted hereafter as C(85), with the general formula:

$$[PPh_4][Ga\{PPh(2\text{-}SC_6H_4)_2\text{-}\kappa^3S,S',P\}\text{–}\{PPh(2\text{-}SC_6H_4)_2\text{-}\kappa^2S,S'\}]$$



(I) (II)

Fig. 3. Molecular structure of gallium complexes C(24), noted as I and C(85), noted as II.

A chronobiological study regarding the actions of C(24) and C(85) Ga complexes on some haematological and biochemical parameters was performed, analysing the morning and evening serum concentratio of nonprotein nitrogenous compounds, haematological parameters and the kidney tissue concentrations of Fe, Cu and Zn. The results revealed an increase in non-protein nitrogenous compounds (uric acid, creatinine, etc) analysed in the morning and evening (except the concentration of uric acid in the latter case.) and an increase in haematological parameters under the influence of C(85) in both the morning and evening. An interesting behaviour was observed in the case of C(24),

where some haematological parameters either increased (red blood cells, haemoglobin and white blood cells) or decreased (platelets) in the morning, white blood cells being the only parameter which increased in the evening, the other values being seen to decrease. Also, monitoring the kidney tissue concentrations of Fe, Zn and Cu, decreases were observed in all experiments, with iron the most affected [78].

Literature data are presented in Table 2 regarding the major experiments performed on laboratory animals, with details on the inorganic compounds administered, doses, route of administration, and duration of experiments.

T a b l e 2

Gallium compounds administered to laboratory animals

Compound	Species	Used dose	Period of admin.	Route of admin.	References
Gallium nitrate	rats	1.75 g/kg b.w. (i.e. 0.48 g/kg b.w. Ga)	not specified	per os	[79]
		67.5 mg/kg/24 h	days 1–10	i.p. daily	[80]
		67.5 mg/kg	single dose	i.p.	[6]
	mice	2.15 g /kg (i.e. 0.59 g/kg b.w. Ga)	not specified	per os	[79]
		80.0 mg/kg/24 h	days 1–10	i.p.	[80]
		80.0 mg/kg	single dose	i.p.	[6]
	dog (8–11 kg)	10 mg/ml (i.e. 1.5 mg/kg b.w. Ga)	single dose	gavage	[81]
	dog (8–11 kg)	25 mg/ml Ga NO_3, în sodium citrate solution (i.e. 1.5 mg/kg b.w. Ga)	single dose	i.v.	[81]
Gallium sulphate	rats	2.0 g/kg b.w. (i.e. 0.33 g/kg b.w. Ga)	not specified	per os	[79]
	mice	2.33 g /kg (i.e. 0.38 g/kg b.w. Ga)	not specified		
	rats	0.74 and 1.48 mg Ga/kg b.w.	not specified	not specified	[10]
	common carp (12 weeks)	4; 8; 12; 16; 20; 24; 28 mg/l	96 hours	administered in aquarium water	[82]
	guinea pigs	2.5 mg/kg-day	5 weeks	not specified	[10]
Gallium chloride	mice	200 and 400 mg/kg/24h	30 days	per os	[83]
	rats	200 mg/kg/24h	20 days	per os	[84]
Gallium arsenide	rats F344/N	1; 10; 37; 75; 150 mg/m^2	6 hrs/day, 5 days/week,14 weeks	inhalation	[85]
	rats Ficher-344	10; 100; 1000 mg/kg/b.w.	single dose	per os	[86]
	rats Wistar strain	10; 200; 500 mg/kg/b.w.	single dose	per os	[87]
	mice B_6C_3F1	1; 10; 37; 75; 150 mg/m^2	6 hrs/day, 5 days/week,14 weeks	inhalation	[85]
Gallium maltolate	dog (8–11 kg)	10 mg/ml (i.e. 1.5 mg/kg b.w. Ga)	single dose	gavage	[81]
Gallium 8-quinoli-nolate	rats	24 mg/kg/day	3 and 9 days	gavage	[73]
	mice	62; 5; 125 mg/kg/day	14 days	gavage	[88]
Gallium pro-toporphyrin IX	mice	25-30 mg/kg/ 1st day; 10-12 mg/kg/2–5 days	5 days	i.p.	[74]

4. DATA CONCERNING BIOMEDICAL EFFECTS

Nowadays research on the use of organometallic compounds in oncology are focused, in addition to Pt(II) and Pt(IV) compounds, on other metal compounds like Ru(III), Ga(III), Sn(IV), Au(I), Fe(II), and Co(II) a.o. [89-92]. Issues referring to the interactions of various chemical compounds with biological systems are of interest for xenobiochemistry and pharmacology [93, 94].

Early studies in the domain of biology and medicine regarding Ga compounds pursued the effects of this metal after oral administration of Ga

salts or the injection of Ga citrate. Following such administration, Ga accumulated in bones, liver and kidneys [95].

The antineoplastic activity of Ga was described for the first time by Hart and Adamson (1971). Regarding inorganic Ga compounds, it was observed that only the Ga^{3+} cation has toxic activity. Thus, comparing the action of sulphates and chlorides, similar cytotoxic effects were evidenced [96].

It was shown that all salts of Group (III)a metals (aluminium, gallium, indium and thallium) exhibited antitumor activity, and the toxicity increased (LD_{50} decreases) as one moved down the periodic table ($TlCl_3$ > $In(NO_3)_3$ > $Ga(NO_3)_3$ > $Al(NO_3)_3$) [97].

The mechanism of action of Ga(III) presents peculiarities depending on the ionic status. Thus, it is known that Ga^{3+} interacts competitively with Fe^{2+} towards transferrin, which leads to an intracellular increase in Ga. Also, in metabolic processes Ga^{3+} has antagonistic effects with various other divalent metal ions, e.g. Mg^{2+}, Ca^{2+} and Zn^{2+}.

For such experiments, in vitro and in vivo, bioavailability must also be taken into account. Pre-clinical studies on the action of gallium compounds in cancer therapy evidenced a synergy with hydroxyurea, gemcitabine, vinorelbine, interferon etc. [2]. In clinics their cytostatic effects were investigated on humans [6, 98]. In this context the first confirmations of the anti-cancer activity of Ga compounds were demonstrated by studying the effects of Ga nitrate, and Ga chloride a.o.

The i.v administered gallium nitrate intervenes in bone metabolism, attested to by the depression in hypercalcemia associated with neoplastic processes [99]. Gallium inhibits the activity of osteoclasts and reduces hydroxyapatite crystal formation [72]. This effect was explained by the adsorption of gallium on the surface of hydroxyapatite crystals [100]. Under such conditions, an increase in collagen concentration occurs as a consequence of increased Ga in bones [2].

In the case of a combined treatment with vinblastine, ifosfamide and gallium nitrate, haematologic toxicity and a positive effect on advanced urothelial carcinoma were observed [101].

The biochemical and pharmacological effects of Ga chloride were studied after oral administration compared with i.v. injection in patients with lung tumours. It was determined that, when associated with radiotherapy and chemotherapy, oral administration is preferable [69].

At present inorganic compounds of Ga(III) like gallium nitrate are used in oncology [2, 72, 102]. This Ga compound was administered in non-Hodgkin's lymphoma by i.v. infusion [102]. Another inorganic gallium compound used in oncotherapy was Ga chloride administered orally in lung cancer [69]. Gallium chloride has also been administered in polychemotherapy alongside cisplatin and etoposide [103].

Also studied were other inorganic compounds of Ga in the form of complexes, e.g. Ga maltolate and Ga quinolinolate a.o. [104]. The bioavailability of Ga maltolate was studied in healthy subjects receiving doses of 100, 200, 300 and 500 mg/day as a single oral administration. The obtained results showed a 25–57% bioavailability, higher than in the case of Ga chloride [6]. The effects of Ga maltolate were also studied in the chemotherapy of hepatocellular carcinoma [105].

As to Ga quinolinolate (also known as KP46 or FTC11), it was administered to patients with phase I solid tumours and showed a good bioavailability and activity in renal cell carcinoma [98]. Research with gallium protoporphyrin IX (GaPPIX) was performed initially on bacteria, in mice and since 2004 it has also been used in humans [6].

A particular aspect of competitive effects between Ga^{3+} and Mg^{2+} related to their binding to DNA was noted in studies by Manfait and Collery (1984) [106], i.e., a 100 fold higher affinity for Ga^{3+} as compared to Mg^{2+}.

Antineoplastic activity was noted in the case of certain inorganic Ga compounds but, in some cases, proliferative effects were also seen, depending on the dose administered [2].

5. BIOMEDICAL USE OF GALLIUM ISOTOPES

Regarding Ga isotopes, the biological and biomedical effects of the isotopes ^{67}Ga and ^{71}Ga were studied.

In medical practice radioisotope ^{72}Ga has been used in the treatment of primary bone cancers as well as metastatic ones [107]. ^{72}Ga was concentrated mainly in tumours, which may then be destroyed by the local irradiation it produces.

The radioisotope ^{67}Ga has also been studied experimentally. It was administered as citrate (doses less than 10^{-4} mg/kg body weight) in rats and after 5 days it was found to be retained predominantly in bones and soft tissues [107]. Another study on ^{67}Ga revealed its accumulation at the level of inflammatory lesions [108].

In vitro studies also revealed that ^{67}Ga may be transferred from transferrin to the iron-storage protein ferritin [109]

A comparative study regarding transferrin complexes with ^{59}Fe, ^{111}In and ^{69}Ga was performed on Wistar rats. The aim was to find a good macromolecular tracer for the evaluation of vascular permeability in tumours and other body tissues. The authors concluded that the transferrin-indium complex could be a good tracer to estimate vascular permeability [110].

^{67}Ga has also been used as a tumour scanning agent. Imaging studies made by Edwards and Hayes (1969) [111] revealed that upon injecting gallium citrate (as ^{67}Ga), the radioactive substance was localised not only in known but also in unknown tumour cells. Hayes et al. carried on studies in 1981 on the effects of ^{67}Ga in normal and tumour tissues [112]. Experiments on mice with ^{67}Ga citrate evidenced the localization of Ga in bones (femoral bone), bone marrow, spleen, liver and muscles [113].

A study carried out in patients with cancer and treated with ^{67}Ga citrate came to the conclusion that Ga was retained in higher quantities in neoplasmic bone tissue [114].

Detailed aspects regarding the usage of Ga radioisotopes were carried on by Bernstein [10]. They pointed out that the radioisotope ^{67}Ga (with a half life of 14.1 hrs) was obtained by proton irradiation of ^{68}Zn, while ^{72}Ga (with a half life of 78 hrs) was obtained by neutron irradiation of the stable isotope ^{71}Ga.

Administration of the above-mentioned substances as citrates was possible in experimental biological and pharmacological studies because citrate ions could cross over more easily (as compared to complexes) in the context of tricarboxylic acid (TCA) cycle metabolism.

Some studies tried to evaluate the contribution of gallium-67 scintigraphy to detect new or additional infectious sites (particularly bone, joint and soft tissues) in patients from intensive care units; significant differences were not observed between patients with negative and positive findings on Ga-67-scintigrapy in their characteristics and other outcomes [115].

Recently, the introduction of Ga-68-DOTA instead of In-111 for positron emission photography (PET) imaging has brought a significant improvement in the quality of imaging neuroendocrine tumours (NET), somatostatin analogues being the preferred labelled peptides in this case [116]. Also, two new ^{68}Ga-labeled prostate specific membrane antigen (PSMA) inhibitors are considered to have significant commercial potential, serving as an alternative to positron emission photography (PET), such as ^{18}F or ^{124}I [117].

6. CONCLUSION

Studies on metal and metalloid compounds in biological systems are major issues in metallomics, not only with regard to their biochemical aspects (i.e. the interaction of biometals and biometalloids with metabolites resulting from the breakdown of nutrients) but also xenobiochemical aspects (i.e. the interaction of metals and/or metalloids with potentially toxic compounds which enter into the organism by food, air, or water intake or as chemotherapeutic agents).

In this context, research on gallium initiated since the middle of the 20th century may be argued to play a very important issue in metallomics. Gallium compounds may be found as inorganic salts or obtained by synthesis as organo-metallic complexes. Both in vitro and in vivo studies attest that various Ga complexes may interact with metalloproteins (e.g. metalloenzymes, nucleoproteins, and chromoproteins a.o.) and interfere competitively with Mg^{2+}, Fe^{2+}/Fe^{3+}, Zn^{2+}, Ca^{2+} etc.

The most studied inorganic Ga compounds have been nitrate, chloride, sulphate and arsenide. Their effects have been examined on laboratory animals, especially on rats, mice, hamsters and dogs a.o.

Among organo-metallic compounds of Ga, complexes with maltolate, quinolinolate and protoporphyrin IX a.o. have been studied. Literature data revealed that some gallium compounds have better bioavailability than other metal complexes.

Preclinical studies confirmed a synergistic action between some gallium complexes used in cancer therapy and other cytostatic drugs such as hydroxyurea, interferon, gemcitabine, etc.

Experimental and therapeutic findings lead us to the conclusion that gallium is the second metal ion, after platinum, with positive results in cancer therapy. In this context studies on humans were initiated – some of which were discussed in this review – evidencing their cytostatic activity against renal tumours, bone neoplasms etc.

On the whole, one can say that metals may induce toxic effects too. This statement refers not only to biometals (when they are in excess) but also to metals with toxic potential. The toxic actions of metals may be evaluated by the appearance of changes in biochemical homeostasis (dyshomeostasis) expressed initially by pathobiochemical changes (discreet at the beginning and with progressive evolution) and thereafter by pathophysiological (perceivable) changes.

The existing pharmacological data referencing gallium compounds in chemotherapy encourage the synthesis of new organo-metallic gallium complexes and their in-depth investigation as a very important problem in metallomics.

In recent years, modern medical techniques have been developed using Ga isotopes for the detection of various tumour or occult sepsis, all of which has brought significant improvement in nuclear imaging practices. Important progress has also been made in proteomics and through techniques such as CP-MS, CE-MS, ESI-MS and other.

Acknowledgements. The present work was elaborated with the financial support of the Romanian Ministry for Education, Research, Youth and Sports – National Authority for Scientific Research, PN – II - ID – PCCE – 2008 – 1 (Project Nr.2 / 2010, code CNCSIS ID–PCCE 140), entitled "Biomedical application of metal compounds – Metallomics"). Prof. L. Silaghi-Dumitrescu is thanked for helpful discussions.

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