GALLIUM AND BONE PATHOLOGY

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ABSTRACT

Purpose: To fill this gap considering the incorporation of gallium into bone tissue, mechanisms of therapeutic activity of this element, as well as the formation, growth and solubility of hydroxyapatite in the presence of gallium salts.

Justification: In contrast to other calcium-saving drugs, salts of trace element gallium are effective in severe hypercalcemias. Gallium (most commonly in the form of its nitrate) enhances calcium and phosphorus content of the bone and has direct, non-cytotoxic effects on osteoclasts at markedly low doses. Although the details of gallium action on the bone are still uncertain, it is well established that the mechanism involves gallium insertion into the hydroxyapatite matrix protecting it from resorption and improving biomechanical properties of the skeletal system. The drug also acts on the cellular components of bone to reduce bone resorption by decreasing acid secretion by osteoclasts. Much has been published about the use of gallium in managing a series of clinical conditions in which this pathology is pronounced.

Conclusions: Due to its interesting and promising profile gallium merits further experimental and clinical evaluation as an antiresorptive agent in orthopaedics, traumatology and cancer-related conditions. Greater knowledge of the mechanisms involved may provide insights for therapeutic strategies aimed at diminishing hypercalcemia and bone loss. New gallium compounds are expected to be developed and tested clinically.

Keywords: *Gallium, Hypercalcemia, Hydroxyapatite, Bone resorption, Bone metabolism.*

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INTRODUCTION

It is estimated that more than 200,000 spine fusion procedures are performed each year in the United States. Posterolateral lumbar intertransverse process arthrodesis is the most common procedure performed, yet failure to achieve a solid bony union occurs in 10% to 40% of patients with only single-level fusions, and more frequently when multiple levels are attempted. This high rate of nonunion indicates that the physiological, biological and chemical events crucial to this process are not adequately understood. A nonunion frequently leads to unsatisfactory resolution of clinical symptoms and usually results in greater medical costs and morbidity, as well as the need for additional surgeries[1]. Unfortunately, the effect of metal ions on the mineralization process has not received considerable attention until recently, although interesting data on aluminum and gallium participation in bone metabolism had been published more than 15 years ago[2]. At present, the number of publications dedicated to the role of gallium in bone pathology is growing rapidly but no comprehensive reviews are available. The aim of the present work is to fill this gap considering the formation, growth and solubility of hydroxyapatite in the presence of gallium salts, the incorporation of gallium into bone tissue and mechanisms of therapeutic activity of this element.

Gallium properties

Gallium is a trace metallic element that is liquid near room temperature, expands on solidifying, and is found as a trace element in coal, bauxite, and other minerals. It is used in semiconductor technology and as a component of various low-melting alloys. It is mainly trivalent in its compounds; the ion Ga^{3+} is a hard acid, so it bonds strongly to strong Lewis bases, particularly to the hydroxyl OH^-. The cation $[Ga(H_2O)_6]^{3+}$ may act as proton donor giving $[Ga(H_2O)_5(OH)]^{2+}$, $[Ga(H_2O)_4(OH)]^{+}$, etc. As the pH gradually increases, this deprotonation of the mononuclear species leads to precipitation of hydrous oxide, and still further increase in pH leads to formation of a gallate $[Ga(OH)_4]^-$ ion, known as well as tetrahydroxygallate[3]. Thus there are two gallium containing ions suitable for metabolic pathways: $[Ga(H_2O)_5(OH)]^{2+}$ in a weak acid medium and $[Ga(OH)_4]^-$ in neutral to weakly alkaline solutions. The former may exist and probably get absorbed in stomach, while the latter is absorbed in duodenum owing to the bicarbonate present. Ionic radius of Ga(III) in tetrahedral coordination is 61 pm and 76 pm in octahedral coordination, so it is expected to be an analogue of Fe(III) (69.0 pm) and Al(III) (67.5 pm), in particular in combination with phosphorus. The affinity of gallium to this element is so high that gallium phosphates are among the most stable compounds[4].

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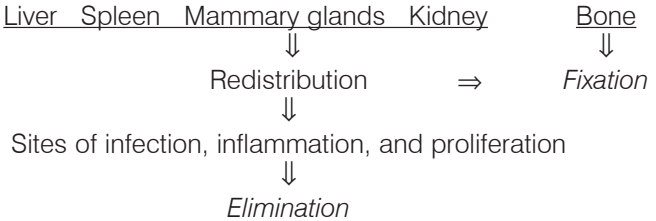
The main clinical interest derives from the observation that gallium metabolic properties are similar to those of iron. Together with comparable ionic radii, both elements show almost the same capability regarding protein and chelate binding. The most important iron transporters transferrin and lactoferrin do not distinguish gallium from iron, hence all gallium in blood is present in plasma in the form of complexes with these proteins.

Under normal circumstances, about one-third of transferrin iron-binding pockets are filled, and a large number of unoccupied sites are available for binding gallium; they amount to 2.7 mg/ml Ga^{3+}, which is a considerable value[5]. Lactoferrin shows higher affinity to gallium and may bind this element after removing from the transferrin complex. Its precursor, apolactoferrin (containing no metal atom), which possesses antibacterial activity, is concentrated in many epithelial secretions like milk, seminal fluid, tears and nasal secretions. Gallium is also observed to concentrate at sites of inflammation and infection, particularly in granulomatous neutrophils and polymorphonuclear leukocytes[6].

A broad body of literature now supports the concept that iron (gallium)-transferrin complex is internalized by receptor-meditated endocytosis. The plasma membrane oxidoreductase reduces transferrin bound iron from the Fe^{3+} state to Fe^{2+}, directly or indirectly facilitating the removal of iron from the protein. As gallium does not exist in the valence state 2^+, it cannot follow further iron pathways in the cell. The only conceivable suggestion is the formation of pentahydroxyoxygallium ion $[Ga(H_2O)_5(OH)]^{2+}$, which formally has the same charge 2^+ as bivalent iron and therefore would be able to imitate the manner of conducting of Fe^{2+} ions. The groups attached to maintain gallium octahedral coordination (H_2O, OH), in principle, in no way represent structural impediments to its reactive capacity. Even so, when cell redox phenomenon is a priority, e.g. in case of hemoglobin and cytochromes, gallium does not enter erythrocytes nor participates in the processes of oxygen transport [7].

Once inside the cell cytoplasm, gallium appears to be bound by ferritins, large proteins that show tissue specific variation depending on the combination of their subunits. Most of ferritin is concentrated in the Kupfer cells in the liver. Ferritin molecules aggregate over time to form clusters, which are engulfed by lysosomes and degraded. The end product of this process, hemosiderin, is an amorphous agglomerate of denatured protein and lipids interspersed with gallium hydroxide and its polymers. Little is known about how gallium is released from ferritins for use. In any case, metabolically inactive element stored in ferritins and hemosiderin is in equilibrium with exchangeable gallium circulating in plasma and bound to the low molecular weight carrier molecules.

Primary and secondary distribution of tissue gallium was traced using ^{67}Ga and ^{72}Ga as radioactive tags. The results are resumed in the following scheme:



Although these results are not entirely consistent, all gallium distribution studies find that the element concentrates in the same tissue site regardless of dose, with the relative proportion excreted in urine and retained by bone. Gallium accumulates avidly at sites of infection and inflammation, including those of granulomatous inflammation and synovitis associated with rheumatoid arthritis. It is found to concentrate in most tumors where large amounts of transferrin receptor are expressed[6].

Gallium in bone

Though bone is a known target for gallium[8,9] the kinetics, site and effects of gallium accumulation in this tissue are far from practical understanding. The usage of synchrotron X-ray microscopy allowed mapping the distribution of trace levels of gallium after short-term *in vivo* administration of gallium nitrate in rats. Trace (nanogram) amounts of gallium were found in the metabolically active regions, as well as the endosteal and periosteal surfaces of diaphyseal bone, regions where new bone formation and modeling are occurring. The lowest concentrations of gallium were observed in the relatively acellular, metabolically inactive mid-cortical regions. The amounts of gallium accumulated after *in vitro* exposure of bone explants was much greater than the levels achieved after *in vivo* administration. So it would appear that newly forming bone matrix has a large capacity to accumulate gallium[10]. After short-term (14 days) administration to rats, gallium nitrate produced measurable quantitative changes in bone mineral properties. Using atomic absorption technique, low levels of gallium were confirmed to preferentially accumulate in regions of active bone formation, 0.54 μg/mg bone in the metaphyses versus 0.21 μg/mg bone in the diaphyses, $P<0.001$. No detectable gallium was measured in the bones from control animals even when tenfold the amount of bone was tested as compared to the rats receiving the lowest dose of gallium nitrate[11]. Recent studies using X-ray fluorescence and proton-induced X-ray emission confirmed these observations showing that gallium is deposited both in organic and inorganic components of the cell. The accumulation of the element in the organic matrix indicates that the mechanism of gallium action is likely to be more complex than suggested by a simplistic idea of straightforward ion incorporation into crystalline lattice[12].

Gallium and hydroxyapatite

The most common misconception in relation to the bone is the idea that it is composed of stoichiometric hydroxyapatite (HA), while there is no clear evidence as to the true structure of calcium phosphate in living bone. It contains a large population of cells in close contact with the mineralized matrix, which surrounds them and there is a continuous exchange of substances throughout the tissue. Unlike a pearl, bone is a living structure, constantly changing, to be regarded in the same way as liver or brain[13].

Nevertheless, researchers still give preference to the apatite model because it seems to be the closest approximation to reality. Various metal ions that cause bone disease have been shown to interact with HA. As to the gallium usage, wide X-ray diffraction studies showed that a significant change of crystal

properties occurred in the bone from the metaphyseal regions of the treated rats. These changes, measured as a sharpening of the *c*-axis 002 reflection, indicated that an increase in crystal size and/or perfection along the larger axis of the newly formed HA crystallites had occurred in the treated bones. The fact that such changes appeared in the regions of active bone formation in which the highest levels of gallium were measured suggests that gallium may have a causative role in the transformations that were seen[11]. On the other hand, *in vitro* experiments showed that gallium delays HA formation and/or growth kinetics in a dose-related manner giving rise to the more perfect crystals. Also, in adsorption experiments, gallium reduced the dissolution kinetics of HA compared with Ga-free controls. The mechanism reported – the significant adsorption of gallium on forming and growing HA nuclei and on the surface of HA crystals – is believed to be responsible for the effects of the element on proliferation and solubility[14].

The relationship between gallium and phosphate phases was studied incorporating the element into rat bone-marrow stromal cell culture. The dominant phase of this culture constitutes the poorly crystalline HA. It was found that replacement of Ca^{2+} by Ga^{3+} yields a more distorted local environment in comparison with Sr^{2+} substitution for Ca^{2+}. The coordination of gallium atoms in the cell culture minerals was similar to that of Ga^{3+}, substituted for Ca^{2+}, in the Ga-doped synthetic brushite, $CaHPO_4$ $2H_2O$. Substitution for Ca^{2+} distorts locally the brushite-like configuration owing to the much smaller ionic radius of gallium. This replacement prevents the transformation of the initially deposited phase into HA and inhibiting the growth. Gallium supplementation at the later stages of mineralization does not influence the local environment of the deposited gallium and its ions seem to be loosely bound to the surface of the HA crystals[15].

In conclusion, gallium was shown to act as an inhibitor of bone mineralization in vitro. Two different mechanisms of its interaction with bone mineral could be distinguished: (1) substituting for Ca^{2+} and (2) rejection of Ga^{3+} by the apatite lattice with a possible adsorption on the surface of the HA crystals[14,15]. These data also were corroborated by the affect of gallium on seeded HA growth[16]. These *in vitro* results partly explain the *in vivo* action of gallium in treating hypercalcemia by decreasing bone apatite solubility.

Measurements of gallium in the density-separated fractions from treated metaphyseal bone confirmed that all particles, including newly formed, less dense particles and mature, more dense particles, were equally exposed to gallium as the gallium-to-calcium ratios were not different. The average specific density showed a slight shift to the right (to a higher density) in the curves, suggesting greater calcium content per packet of bone and indicating greater amounts of calcium being preserved or accumulated[17,18].

Gallium and bone diseases

Gallium appears to be a uniquely acting agent for treatment of bone resorptive conditions reducing accelerated bone loss in patients with cancer and metabolic bone disease. The concept of using certain medical therapies as adjuncts to traditional anticancer treatment in order to strengthen bone tissue against erosion from metastases has gained increased credence. Ideally, such therapy should not only minimize further bone loss but also restore bone that has been previously eroded, for instance in orthopedics. Other drugs, such as biphosphonates, fluoride and calcitonin, have previously been proposed for this use[19].

The clinical condition that is associated with the most rapid lost of calcium from bone tissue is cancer-related hypercalcemia. In this disorder, factors released from cancer cells cause a marked increase in osteoclastic bone resorption and calcium exits from bone so rapidly that it overwhelms renal excretory capacity. It was shown that low dose gallium nitrate could be successfully used for prevention of osteolysis in myeloma. Patients were treated with gallium nitrate for 6 months administrated in monthly cycles by daily subcutaneous injections for two weeks, supplemented by an intravenous infusion for 5 days every other month. Total-body calcium decreased in 4 of 7 patients during observation, but increased in 9 of 13 patients during gallium treatment. The group mean vertebral fracture index assessed by lateral spine x-rays decreased by 27% during observation compared with 2% during gallium nitrate treatment. It was concluded that adjuvant treatment with gallium nitrate attenuates the rate of bone loss in myeloma and may be useful for ameliorating the consequences of skeletal morbidity in patient with cancer-related osteolysis[20]. As to the mechanism involved, gallium nitrate seems to inhibit bone resorption by inhibiting parathyroid hormone-related protein[20].

One of the most successful applications of gallium therapy is the management of Paget's disease, which, at the same time may be considered as a condition representing numerous facets of osteometabolic disorder. It can be defined as an abnormal bone remodeling, e.g. a constant bone renewal or turnover without changes in the size and shape of bone. Disturbance of the bone remodeling processes changes the bone texture and gives rise to four phases of the disease observed radiologically: the osteolytic, mixed and osteoblastic stages and the inactive osteosclerotic phase characterized by normal or decreased bone activity. In Paget`s disease, disturbed modeling contributes to bone expansion that leads to spinal stenosis. The action of gallium nitrate seems to be long lasting and safe, although the response duration is variable, lasting from 6 to 42 weeks. Treatment significantly reduces serum levels of alkaline phosphatase and urinary excretion of hydroxyproline. A fall in hydroxyproline precedes alkaline phosphatase suppression, suggesting that suppression of osteoclastic bone resorption (reduced hydroxyproline) leads to bone formation by osteoblasts (decreased alkaline phoshatase). The effectiveness of treatment is proportional to the dose of the drug administrated. Response to IV treatment was more marked than to subcutaneous injection.

Bone destruction is the hallmark of multiple myeloma, with roughly 70% to 80% of patients manifesting bone involvement. In large series, about one to two thirds of patients with myeloma present with bone pain at the time of diagnosis. Plain skeletal radiographs are abnormal in approximately 80% of patients, with abnormalities including fractures, osteopenia, lytic lesions or a combination of these. Gallium plays a role in controlling

or reversing osteolysis produced by myeloma and reducing hypercalcemia. A pilot study randomized 14 myeloma patients to six months of subcutaneously administrated gallium nitrate versus observation and noted improvement in bone pain and stabilization of bone density in the treated group[20]. Important reservations also apply to interpretation of these results: although the study design used sophisticated technology and randomization, the sample size in this trial was small. The other difficulty is that the trial was not placebo-controlled, since it was considered doubtful whether 6 months of parenteral placebo injections could be justified[20]. Also, in a recent retrospective analysis, patients with advanced multiple myeloma treated with M-2 chemotherapy protocol plus gallium nitrate had markedly prolonged median survival compared with similar patients treated with M-2 chemotherapy alone.

After a series of ownership changes in the commercial rights and a period in which the drug has not been commercially available, gallium nitrate (Ganite, manufactured for Genta, Inc, Berkeley Heights, New Jersey) should be a valuable alternative treatment option for a variety of bone diseases. Because gallium nitrate also has non-overlapping toxicity profile with the newer agents, it evaluation in combination regimens with these agents merits consideration.

Due to the efforts from many laboratories and researchers evidence has accumulated that lends credit to the direct and relevant participation of gallium in bone metabolism. Although significant progress has been made, still more needs to be elucidated. Greater knowledge of the mechanisms involved may provide insights for therapeutic strategies aimed at diminishing bone loss in orthopaedics, traumatology and cancer-related conditions. New gallium compounds are also expected to be developed and tested clinically.

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