Ain Shams University
Faculty of Science
Biochemistry Department

Biochemical Study on the Effect of a Novel Gallium Complex and Its Derivatives on Metallo-Collagenase Activity *In Vitro*

A Thesis submitted by

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For the requirement of the Ph D Degree in

Biochemistry

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This Thesis has not been submitted to this or any other University.

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It was shown in the 1980s that gallium (III) ions may have anti-tumor activity in animals. Unfortunately, clinical trials with simple gallium salts failed because of the low bioavailability of oral gallium salts, renal toxicity and the rapid onset of gallium resistance. Furthermore, gallium salts hydrolyse rapidly to insoluble gallium hydroxide. A solution to these problems consists in the complexation of the metal by a ligand. A number of effective ligands have been proposed, demonstrated promising results in preclinical studies and have entered clinical trials but with limited water solubility. In this context there is a need to identify a gallium ligand able to preserve the gallium from the hydrolysis while maintaining water solubility.

To address this issue, the present study was designed to prepare, synthesize and characterize the chemical structure of water soluble bioavailable gallium complex(es) by using different chemical analyses methods. This work was also pre-designed to evaluate the anti-invasion capabilities of the novel synthesized gallium complex(es) through inspecting their inhibitory effect over some collagenase enzymes such as (MMP-2 and MMP-9 and MM-14) as a possible promising mechanism to control the invasion of some metastatic tumors where, studies that touched that goal were scarce. Moreover, an intensive biological study was performed to evaluate the antitumor activity, possible mode of action including reactive oxygen species activity, early and late stage apoptotic, Phosphoinositide-3 kinase/Protein kinase B pathways and collagenases inhibitory action of the tested gallium complex(es).

Abstract

Abstract

It was shown in the 1980s that gallium salts may have anti-tumor activity in animals. Unfortunately, clinical trials with either nitrate or gallium chloride have failed for a variety of reasons including low bioavailability, renal toxicity, and rapid onset of gallium resistance. So, the main task of our work was to prepare and characterize the chemical structure of some new water-soluble bioavailable gallium complexes derived from aspartic acid and to study their possible biochemical effects on the activity of metalloproteinases (collagenases) as a possible promising mechanism to control the invasion of some metastatic tumors.

In the present study, two water soluble gallium complexes with formula [Ga(III)LCl], where L stands for the deprotonated form of N-2-hydroxybenzyl aspartic acid derivatives that were synthesized and characterized by 1H NMR, 13C NMR, FT-IR, mass spectrometry and elemental analysis. The analytical data are consistent with a mononuclear structure in which the gallium (III) cation is liganded by one of the two carboxylic acid groups, the phenol oxygen and the nitrogen atom of the 2-hydroxybenzylamino group. In such a structure, the tridendate ligand secures the binding of the metal ion whereas the carboxylic appendage provides the water solubility. The cytotoxicity of the gallium complex of (R)-2-(5-chloro-2-hydroxybenzylamino) succinic acid (GS2) was evaluated against human breast carcinoma MDA-MB231 and fibrosarcoma HT-1080 cell lines. The 5-chloro derivative GS2 was found to be more cytotoxic than the unsubstituted derivative and $GaCl_3$. GS2 induces apoptosis through down-regulation of AKT phosphorylation, G2M arrest in cell cycle *via* activation of the caspase3/7 pathway. Although, many molecular and cell effects of Ga

have been described, including proteasome inhibition and osteoclastic activities, GS2 appears as the first gallium compound able to decrease AKT phosphorylation in cancer cells. The activity of GS2 on cell invasion and on the expression and activity of Matrix Metalloproteinases (MMPs) was investigated using modified Boyden chamber coated with type I collagen. The activity of MMPs was analyzed by zymography and enzymatic assay using high affinity fluorogenic substrates. A selective inhibition of MMP-14 has been reported to block tumor cell migration and invasion. The expression of MMPs mRNA was analyzed by qRT-PCR. GS2 induces a decrease in cell invasion. A dose dependent inhibition effect was observed on MMP-2, MMP-9 and MMP-14 activities. A decrease in MMP-14 mRNA expression was observed in both cell lines, whereas MMP-2 and MMP-9 mRNA expression was decreased only in MDA-MB231 cells. Thus, the present study proposes that GS2 compound may be a potential candidate to decrease the MMP-14 activity in cancer metastatic diseases presenting high level of MMP-14 expression and activity. Taken together, these data show that the possible future combination of GS2 with cytotoxic chemotherapy may be considered one of the promising treatment modes for anti-invasive and anticancer therapy.

Keywords: gallium, coordination complex, anticancer agent, metastasis, matrix metalloproteinase

List of Abbreviations

Abz	2-Aminobenzoic acid
Ala	Alanine
AKT	Protein kinase B
ANOVA	Analysis of variance
APL	Acute promyelocytic leukemia
Arg	Arginine
ASTM	American standard for testing materials
ATP	Adenosine triphosphate
ATPas	Adenosine triphosphatase enzyme
BCA	Bicinchoninic acid
bp	Base pair
BSA	Bovin Serum Albumin
CCDP	*cis*-Diamminedichloridoplatinum(II)
Cdc2, 25c	Cell Division Cycle phosphatase 2 or 25c
cDNA	Cellular deoxyribonucleic acid
CML	Human chronic myelogenous leukemia
Cp	Cyclopentadienyl
CSC	Cancer stem cells
C_t	Cycle threshold
CTLA-4	Cytotoxic T-lymphocyte-associated protein-4
CTR-1, 2	Copper transporter receptor
Cys(Me)	S-Methyl cystine
Dap	2,3-Diaminopropionic acid
DNA	Deoxyribonucleic acid
DNP	3,4-Dinitropheny
DFO	Desferrioxamine
Dpa	3’,3’-Diphenylalanine
ECL	Estimated chemiluminescent
ECM	Extracellular matrix
EDTA	Ethylenediamine tetraacetic acid
EEF1a1	Eukaryotic translation elongation factor 1 alpha 1
EGFR	Epidermal growth factor receptor
EMT	Epithelial mesenchymal transition
ErbB-2	Receptor tyrosine-protein kinase-2
ERK	Extracellular signal-regulated kinase