

Gallium maltolate has *in vitro* antiviral activity against SARS-CoV-2 and is a potential treatment for COVID-19

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Abstract

Background: Gallium has demonstrated strong anti-inflammatory activity in numerous animal studies, and has also demonstrated direct antiviral activity against the influenza A H1N1 virus and the human immunodeficiency virus (HIV). Gallium maltolate (GaM), a small metal-organic coordination complex, has been tested in several Phase I clinical trials, in which no dose-limiting or other serious toxicity was reported, even at high daily oral doses for several months at a time. For these reasons, GaM may be considered a potential candidate to treat coronavirus disease 2019 (COVID-19), which is caused by the SARS-CoV-2 virus and can result in severe, sometimes lethal, inflammatory reactions. In this study, we assessed the ability of GaM to inhibit the replication of SARS-CoV-2 in a culture of Vero E6 cells.

Methods: The efficacy of GaM in inhibiting the replication of SARS-CoV-2 was determined in a screening assay using cultured Vero E6 cells. The cytotoxicity of GaM in uninfected cells was determined using the Cell Counting Kit-8 (CCK-8) colorimetric assay.

Results: The results showed that GaM inhibits viral replication in a dose-dependent manner, with the concentration that inhibits replication by 50% (EC₅₀) being about 14 μM. No cytotoxicity was observed at concentrations up to at least 200 μM.

Conclusion: The *in vitro* activity of GaM against SARS-CoV-2, together with GaM's known anti-inflammatory activity, provide justification for testing GaM in COVID-19 patients.

Keywords

SARS, replication, Coronaviridae, compounds, virus

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Introduction

Coronavirus disease 2019 (COVID-19) can produce severe, life-threatening symptoms due to hyperinflammatory reactions.¹ Treatments that suppress such excessive inflammatory responses, and that also act against the causative virus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2), would be particularly useful.

Gallium, a metallic element with chemical similarities to ferric iron (Fe³⁺) and to zinc, is known to have both anti-inflammatory and antiviral activity. Its anti-inflammatory activity has been recently reviewed.² Most relevant to COVID-19, gallium was found effective in a murine model of septic shock, in which it attenuated lipopolysaccharide-induced hepatitis and suppressed the production of nitric oxide, but had no

effect on TNF- α production.³ The hyperinflammatory reactions observed in severe cases of COVID-19 have many characteristics in common with septic shock.⁴

Limited data have demonstrated antiviral activity of gallium against influenza A virus⁵ and human immunodeficiency virus (HIV).⁶ Further antiviral activity of gallium can be hypothesized due to gallium's chemical similarity to zinc. Zinc has efficacy against some RNA

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viruses, including coronaviruses and hepatitis E virus, possibly in part by acting against the RNA-dependent RNA polymerase.^{7,8} SARS-CoV-2 is dependent on the host enzyme furin,⁹ which can be inhibited by Zn.¹⁰ On the other hand, Zn is essential for entry into cells by RNA viruses, including coronaviruses^{11,12} and it is probable that Ga would interfere with Zn-dependent cell entry.

Furthermore, iron deprivation has been proposed as an effective means to inhibit the replication of SARS-CoV-2.¹³ Because Ga is a potent competitor of ferric iron,^{14,15} this is another mechanism by which Ga may act against the virus. It is noted that Ga does not enter heme,¹⁴ accounting in part for the low observed clinical toxicity of GaM.

Gallium is therefore hypothesized to be an effective treatment for COVID-19, because it could reduce inflammation, act directly against the causative virus, and deprive the virus of iron by competing against Fe³⁺ in the host.

In this study, a screening assay was used to determine whether gallium maltolate (GaM) is effective at inhibiting SARS-CoV-2 replication in cell culture. GaM was selected as the gallium compound because it has already completed several Phase 1 clinical trials, in a total of 94 subjects, in which it demonstrated a high level of safety and tolerability, even when administered at high daily doses for many weeks at a time.^{16,17} No dose-limiting or other serious adverse effects were reported at doses of up to 3500 mg/day for repeated 28-day cycles.¹⁷ At the highest doses (>2000 mg/day) there was an increase in diarrhea, likely due to osmotic effects in the gut, and transient, mild iron-deficiency anemia, due to the competition of Ga³⁺ with Fe³⁺. GaM can be administered orally once per day, is readily synthesized in large quantities at low cost, and is stable over a wide temperature range, making it suitable for widespread distribution and use.

Materials and methods

Test compound

Gallium maltolate, manufactured under current FDA Good Manufacturing Practice (cGMP) protocols, was obtained from Gallixa LLC (Menlo Park, California, USA). The purity was >99%, based on HPLC, UV spectroscopy, FTIR, ICP-MS, and powder x-ray diffraction results.

SARS-CoV-2 replication assay in vero E6 cells

A culture of African green monkey kidney Vero E6 cells was obtained from the American Type Culture Collection (ATCC, CRL-1586), with authentication

performed by a microscopic morphology examination and growth curve analysis. The cells, which tested mycoplasma-negative, were maintained in minimum Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere containing 5% CO₂.

A clinical isolate of SARS-CoV-2 (hCoV-19/Wuhan/WIV04/2019; GISAID EPI_ISL_402124) was propagated in the Vero E6 cells, and viral titer was determined by 50% tissue culture infective dose (TCID₅₀) using immunofluorescence assay. All the infection experiments were performed in a biosafety level-3 (BSL-3) laboratory.

Pre-seeded Vero E6 cells (5 X 10⁴ cells per well) were pre-treated with different concentrations of GaM for 1 h, and the virus was then added at a multiplicity of infection (MOI) of 0.01, with 1 h allowed for infection. Next, the supernatant virus-drug mixture was removed and the cells were further cultured with fresh GaM-containing medium. At 24 h post infection, a volume of 80 µL cell supernatant was collected from each well for viral RNA measurement by quantitative real-time reverse-transcriptase polymerase chain reaction analysis (qRT-PCR). All experiments were performed in triplicate.

Statistical analysis was performed using Prism software. The EC₅₀ value was calculated using an inhibitor vs. normalized response, variable slope equation:

$$Y = 100 / (1 + 10 \exp((\text{LogEC}_{50} - X) \times \text{HillSlope})).$$

Cytotoxicity

The cytotoxicity of GaM to uninfected Vero E6 cells was determined in triplicate with the Cell Counting Kit-8 (CCK-8) colorimetric assay (Beyotime, China), using WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt). The Vero E6 cells were suspended in growth medium in 96-well plates, and on the next day, different concentrations of GaM were added to the medium. After 24 h, the relative numbers of surviving cells were measured with the CCK8 assay in accordance with the manufacturer's instructions.

Results

GaM produced dose-dependent inhibition of viral replication, with an EC₅₀ (concentration producing 50% inhibition of viral replication) of about 14 µM (CI 95% 8.9–22.8 µM), Hill Slope of 0.6 (CI 95% 0.42–0.77), and r² = 0.88 for Goodness of Fit (Figure 1). The gallium maltolate was not toxic to the cells at concentrations up to at least 200 µM (cell viability at 200 µM was 109(±3)% relative to untreated cells) (Figure 2).

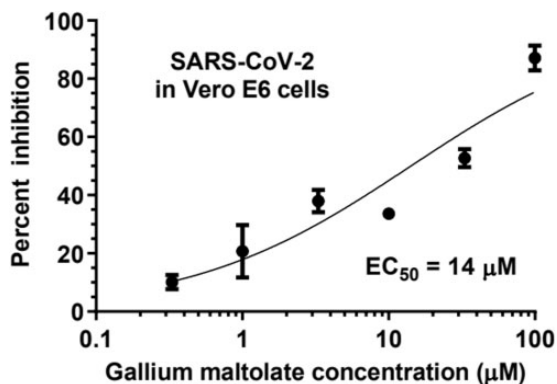


Figure 1. Inhibition of SARS-CoV-2 replication in Vero E6 cells by gallium maltolate at several concentrations.

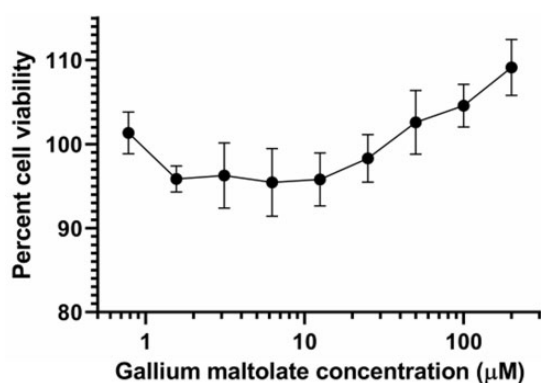


Figure 2. Viability of Vero E6 cells at several concentrations of gallium maltolate.

Discussion

Replication of SARS-CoV-2 in Vero E6 cells was strongly inhibited by GaM. Clinical studies have shown that the *in vitro* gallium concentrations required to inhibit SARS-CoV-2 replication are attainable with oral dosing¹⁸ (Titan Pharmaceuticals, unpublished clinical trial data, 2005). We therefore suggest that orally administered GaM enter randomized, placebo-controlled, double-blinded clinical trials in COVID-19 patients, due to its activity against SARS-CoV-2, its anti-inflammatory activity, its Fe³⁺-competitive activity, and its reported clinical safety and tolerability.

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Declaration of conflicting interests

The author(s) declared following potential conflicts of interest with respect to the research, authorship, and/or

publication of this article: LRB has applied for a patent that involves the use of gallium to treat coronavirus disease.

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