Research Article

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Treatment activity of the injectable hydrogels loaded with dexamethasone In(III) complex on glioma by inhibiting the VEGF signaling pathway

Abstract: In this work, a novel In(III) coordination polymer, i.e., \([\text{In}(\text{L})(\text{im})(\text{H}_2\text{O})](\text{H}_2\text{O}))_n\) (1) was produced from the synthetic reactions in water and dimethylformamide mixed solvents and using methyl-3-hydroxy-5-carboxy-2-thiophenecarboxylic acid (H2L) and imidazole (Him) as the co-ligands, and then successfully loaded with dexamethasone. The injectable hydrogels were prepared from natural polysaccharide hyaluronic acid with good biocompatibility and successfully loaded with In(III) complex. The suppressive inhibitory effect of hydrogels toward U251 human glioma cell line viability was assessed using the Cell Counting Kit-8 assay kit, and the relatively expressed levels of the vascular endothelial growth factor signaling pathway were determined by real-time RT-PCR.

Keywords: hydrogels, dexamethasone, glioma, complex

1 Introduction

As economic and social development and the standard of living of people increase, the prevalence of glioma is on a decreasing trend in most countries all around the world [1,2]. However, due to the large population in our country and the continuous increase in the proportion of aging population, the burden of glioma in our country is still heavy [3]. In 2014, there were about 410,000 new cases of glioma in our country, accounting for about 40% of all new cases of glioma in the world. At the same time, there are large regional differences in morbidity and mortality [4–6].

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The incidence and mortality of glioma in rural areas are 1.3 and 1.4 times compared with those in urban areas.

The construction and design of supramolecular architectures based on crystallographic engineering with metal participation is currently receiving much attention in the field of coordination and supramolecular chemistry. The growing interest in the above areas is attributed to their commendable unitary structures, as well as their promising potential in the fields of biochemistry, catalysis, and luminescence, mostly in modern medicinal chemistry [7–10]. Within the range of compounds that have been produced, functional complexes have drawn great interest, thanks to the underlying medicinal value applications [11–14]. Therefore, the selection of bio-compatible, effective, and secure ligands has become a key component of architectural engineering, pharmacotherapy, and clinical applications [15–19]. Multi-dentate ligands like heterocyclic ligands with nitrogen or polycarboxylic acids are broadly employed in the rationally designed and controlled generation of these versatile complexes [20–22]. Recently, coordination polymers containing both carboxylic acid and N-heterocyclic ligands have aroused significant interest among the biologist and chemist due to its rich functional properties and coordination modes, in addition to their role as H bond acceptors and donors under solution conditions [23,24]. Indium is of great interest in the synthesis of metal–organic ligands complexes because of its good plasticity, chemical stability, low toxicity, and non-irritation to skin [25,26]. Indium ion has been selected as an important component in the construction of In-MOFs because of its high stability and tunability in In-MOF’s [27,28]. The synthesized complexes have some applications in the medical field, such as indium complexes can be used as components of anti-tumor drugs with certain anti-tumor activity [29]. In this work, a novel In(III) CP has been successfully generated from the synthetic reactions in a water and dimethylformamide (DMF) mixed solvents with the Him and H2L as the co-ligands.

In recent years, with the development of biomedical science and materials science, tissue engineering has been gradually applied to the treatment of glioma [30–32].
Biological scaffolds are combined with bioactive drug factors and delivered to the lesion site of glioma to inhibit the activity of cancer cells through the slow release of drugs, so as to achieve functional protection and even repair of damaged organs [33,34]. Hydrogels have become the preferred material for tissue engineering scaffolds due to their good biocompatibility and structural diversity [35,36]. Among them, injectable hydrogels are implanted in the body as a minimally invasive way, causing little trauma to tissues and relatively simple operation, which has clinical application prospects and advantages [37–39].

Hydrogels for injection can be classified into natural and synthetic polymeric materials depending on their sources [40]. Natural polymer materials, such as hyaluronic acid, gelatin, chitosan, and sodium alginate, have been extensively studied. These materials have good biocompatibility, and their internal porous structure can carry therapeutic drugs [41]. After implantation, with the degradation of hydrogels, local long-lasting controlled drug release can be achieved, greatly improving the therapeutic effect of glioma [42,43].

In this study, the injectable hyaluronic acid hydrogels were prepared by Schiff base reaction and successfully loaded with In(III) complex. The microstructure of hydrogels was studied and its application value in glioma treatment was explored. Cell Counting Kit-8 indicated that hydrogel can dramatically decrease the survival rate of U251 human glioma cells, and the expression of vascular endothelial growth factor (VEGF) modeling in cancer cells was markedly inhibited.

2 Experimental details

2.1 Materials and instrumentation

All chemicals are available from commercial suppliers and were not further refined. Fourier transform infrared spectra were documented with KBr particles (5 mg sample in 500 mg KBr) in a region from 400 to 4,000 cm\(^{-1}\). Elemental analysis of N, H, and C was performed with a Perkin Elmer 240C analyzer. The current work applied ethylene glycol (AR, Sinopharm Chemical Reagent Co., Ltd), sodium periodate (AR, Sinopharm Chemical Reagent Co., Ltd), hyaluronic acid (AR, Sinopharm Chemical Reagent Co., Ltd), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), (AR, Shanghai Aladdin Biochemical Technology Co., Ltd), carbonyl dihydrazin (CDH) (AR, Sinopharm Chemical Reagent Co., Ltd), and 1-hydroxybenzotriazole (HOBt) (AR, Shanghai Aladdin Biochemical Technology Co., Ltd).

2.2 Preparation and characterization for \{[\text{In}(L)(\text{im})(\text{H}_2\text{O})](\text{H}_2\text{O})_n\} (1, \text{C}_{10}\text{H}_{10}\text{InN}_2\text{O}_{6.5}\text{S})

A mixture generated from 20.2 mg and 0.1 mmol H\(_2\)L, 0.1 mmol and 30 mg In(NO\(_3\))\(_3\)-6H\(_2\)O, 13.62 mg and 0.2 mmol imidazole, 0.2 mL and 2 M HNO\(_3\), and H\(_2\)O/DMF (4 mL, 1:3) was enclosed in a Pyrex glass tube (20 mL) and later warmed at a temperature of 80°C for 3 days, subsequently cooling to RT at a 5°C h\(^{-1}\) rate. Massive light yellow crystals were gathered (with a yield of 52% according to In(m)). Elemental analysis calcd. (%) for C\(_{10}\)H\(_{11}\)InN\(_2\)O\(_7\)S: N, 6.70; H, 2.65; C, 28.73. Found (%): N, 6.69; H, 2.89; C, 28.81. Selected IR peaks (KBr, cm\(^{-1}\)): 686 (m), 715 (m), 734 (m), 755 (m), 836 (m), 920 (m), 1,166 (m), 1,342 (s), 1,375 (s), 1,433 (s), 1,464 (s), 1,536 (s), 1,593 (s), 2,639 (m), 3,296 (m), 3,357 (m), 3,517 (s).

X-ray result was taken from the Xcalibur E diffractometer at Oxford University. CrysAlisPro was chosen to investigate the intensity data, followed by conversion to the HKL files. The SHELXS procedure based on straight mean together with a least squares-based SHELXL-2014 was adopted for the creation and revision of primary structure patterns, separately [44]. In the end, the whole H-atoms can be anchored to C-atoms to which they are joined by AFIX commands [45]. Details in optimization of the CP as well as its crystallographic parameters are given in Table 1.

2.3 Preparation of oxidized hyaluronic acid (OHA)

First, prepare 100 mL hyaluronic acid solution with concentration of 1 wt% and add 10 mL (0.5 mol/L) sodium periodate solution away from light for reaction for 3 h. Immediately, glycol (1.5 mL) was applied to stop the reactions. The solution was dialyzed utilizing deionized water for 72 h and later lyophilized. The sample was labeled as OHA.

2.4 Preparation of CDH-HA

First, 100 mL of hyaluronic acid solution at a concentration of 1 wt% was prepared and stirred for 3 h after the addition of 0.5 g CDH. Then, the solution pH was tuned to 7.0, followed by 0.2 g HOBt and 0.3 g EDC, then the pH was adjusted to 4.8, and the mixture was allowed to stir overnight at room temperature. The mixture was freeze-dried...
Table 1: Details in optimization of the CP 1 and its crystallographic parameters

<table>
<thead>
<tr>
<th>Empirical formula</th>
<th>C₁₀H₁₀InN₂O₆.₅S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula weight</td>
<td>409.08</td>
</tr>
<tr>
<td>Temperature (K)</td>
<td>293.0</td>
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<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
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<tr>
<td>Space group</td>
<td>C2/c</td>
</tr>
<tr>
<td>a (Å)</td>
<td>18.2369(12)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>18.2547(8)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>8.3369(4)</td>
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<tr>
<td>α (°)</td>
<td>90</td>
</tr>
<tr>
<td>β (°)</td>
<td>101.623(2)</td>
</tr>
<tr>
<td>γ (°)</td>
<td>90</td>
</tr>
<tr>
<td>Volume (Å³)</td>
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</tr>
<tr>
<td>Z</td>
<td>8</td>
</tr>
<tr>
<td>ρ calc (g/cm³)</td>
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</tr>
<tr>
<td>μ (mm⁻¹)</td>
<td>1.923</td>
</tr>
<tr>
<td>Reflections collected</td>
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</tr>
<tr>
<td>Independent reflections</td>
<td>2,527 [R_{int} = 0.0423, R_{sigma} = 0.0344]</td>
</tr>
<tr>
<td>Data/restraints/parameters</td>
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</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.064</td>
</tr>
<tr>
<td>Final R indexes [I ≥ 2σ (I)]</td>
<td>R₁ = 0.0340, wR₂ = 0.0761</td>
</tr>
<tr>
<td>Final R indexes (all data)</td>
<td>R₁ = 0.0430, wR₂ = 0.0796</td>
</tr>
<tr>
<td>Largest diff. peak/hole (e Å⁻³)</td>
<td>0.72/-0.47</td>
</tr>
</tbody>
</table>

after 7 days of dialysis in deionized water, and the sample was labeled CDH-HA.

2.5 Preparation of the injectable HA hydrogels

OHA sample of 200 mg, 400 mg CDH-HA, and In(m) complex were dissolved in 10 mL PBS, respectively. When the solution was fully dissolved to colorless and transparent, it was transferred to a double-cylinder syringe and quickly extruded into a mold to form hydrogels.

2.6 Morphology observation

The micromorphology of hyaluronic acid hydrogels were viewed by employing a SEM (FEI Inspect F35). The samples were sprayed with gold before testing.

2.7 Cell Counting Kit-8

Following treatment of U251 human glioma cells with the new compound, their survival was quantified using the Cell Counting Kit-8 assay kit. The preformation was carried out following the instructions with minor modifications. Briefly, U251 human glioma cells in logistic growing phages were collected and then seeded in 96-well plates at 10⁵ cells/well. Following 12 h of cultivation in an oven at 37°C under 5% CO₂, the compounds were added sequentially at different concentrations (1, 2, 4, 8, 10, 20, 40, and 80 μM). Then the medium was removed and the incubation was continued by adding new media with CCK-8 reagent. Lastly, the absorbance of individual wells was determined using a microplate reader.

2.8 Real time RT-PCR

The relative expression of VEGF in U251 human glioma cells after compound treatment was detected by real time RT-PCR. This protocol was performed exactly according to the manufacturer’s directions, with minor modifications. Briefly, U251 human glioma cells in logic growing phages were gathered and seeded in cell culture plate at a concentration of 10⁵ cells/well. The cells were cultured at 37°C and 5% CO₂ for 12 h and then treated with various levels of compounds. Subsequently, U251 human glioma cells were gathered and total RNA was collected using TRIZOL reagent, and the RNA levels were quantified and transcribed into cDNA. Finally, the total VEGF levels in the U251 human glioma cells were measured by real-time RT-PCR, and GAPDH was determined as the internal standard reference gene.

3 Results and discussion

3.1 Structural characterization of complex 1

Complex 1 was obtained as block light yellow crystalline materials by the reaction of H₂L and In(NO₃)₃·6H₂O with imidazole in the mixed solution of water and DMF medium. Notably, the addition of HNO₃ in the starting reaction solutions is essential to successfully prepare the compound. Attempts to synthesize 1 without HNO₃ failed, which only gave unknown deposition. The reason may be the easy hydrolysis of In(NO₃)₃·6H₂O. Complex 1 is crystallized in monoclinic C2/c space group; in the unsymmetrical unit, there is a imidazole ligand, a L²⁻ anion, and a In(m) ion coordinated together with a lattice water molecule (Figure 1a). In(m) ion exhibits a twisted octahedral geometry, which is coordinated
with an imidazole N atom from a coordinated H2O molecule together with a terminal imidazole ligand and four O atoms derived from 3 $L^2$ anions. In 1, each $L^2$ anion in a tridentate $\mu^3-(\eta 2)-(\eta 1)-(\eta 1)$ bridging mode links adjacent In(m) ions, hence yielding a two-dimensional layer structure running parallel to the bc plane, which is further decorated by the terminal imidazole ligand. The coordination mode is similar to the previously reported indium complexes [46–50]. In 1, a pair of In(m) ions is bridged by a pair of hydroxyl oxygen atoms to give a dinuclear In$_2$O$_2$ cluster with a shorter In–In separation of 3.5500 Å (Figure 1b). In topology, such a In$_2$O$_2$ cluster can be regarded as a 4-linked node to bridge four other same clusters via four $L^2$ anions, thereby generating a 4-linked $\text{sq}_4$-type net with topology of $4^4\text{sq}_2$ (Figure 1c). Besides, such two-dimensional layers are extended into a whole 3D net through intermolecular O–H–O interactions between the lattice water (O(7)) and oxygen atoms of $L^2$/or coordinated water molecules O(7)–H(4 W)–O(5), O(7)–H(3 W)–O(6), and O(7)–H(3 W)–O(5). In addition, this structure also contains intramolecular hydrogen-bonding involving carbonyl and terminal imidazole moieties (N(2)–H (2)–O(4) and O(6)–H(2 W)–O(5), which further facilitate the integrity and stability of the three-dimensional supramolecular net. The above data can also be further verified by IR characterization, and the typical characteristic peaks at 1,464, 3,296 and 3,357 cm$^{-1}$ can be attributed to imidazole ligands. The typical characteristic peak at 1,375 cm$^{-1}$ can be attributed to NO$_3^-$.

Based on the above characterization we can tell that the obtained product is the target product.

### 3.2 Synthesis and micromorphology of injectable hydrogels

Hyaluronic acid has good biocompatibility and is the main component of extracellular matrix, which has obvious advantages as injectable hydrogels. The principle of injectable hydrogels is shown in Figure 2a. First, hyaluronic acid was prepared by the oxidation of hyaluronic acid with sodium periodate. Second, amidinated hyaluronic acid was prepared by substituting hyaluronic acid with diacyl carbonate well. Finally, injectable hydrogels were formed in situ by Schiff base reaction of aldehyde group and amino group in hyaluronic acid.

### 3.3 Morphology of the injectable hydrogels

An injectable gel system was constructed by mixing OHA and CDH-HA with natural polysaccharide hyaluronic acid. The efficient Schiff base reaction between aldehyde and amino groups in hyaluronic acid gives hydrogels the ability...
of rapid in situ gelling. The injection diagram of hydrogels is shown in Figure 2b. To further test the stabilization of the obtained complexes and hydrogels, we first conducted relevant tests on the stability of the obtained complexes. Specifically, as follows: first, considering the practical application scenario of human body fluid circulation, the stability of the complexes was verified by dispersing them in simulated body fluids at pH 4–8 and temperature 0–40°C for 24 h. There was no obvious variation in crystal structure of the treated complexes, indicating that the complexes have good stability of drug loading in body fluids. On this basis, to further examine the stability of the formed hydrogels, sodium hydroxide or hydrochloric acid at a concentration of 5 mmol/L was added to the prepared hydrogels, respectively, and after 24 h, no significant effects of both reagents were observed on the formed hydrogels, indicating that the gels have good stability. On this basis, the stability of the gels was further examined by adding an equimolar amount of urea, a hydrogen bond-breaking factor, to the gel preparation, and after 24 h, it was observed that it had no effect on the formation of the gels. The results indicated that the formed hydrogels had good stability. Afterward, they were tested by injection. The solution of OHA and CDH-HA can be easily extruded from the double-barrel syringe, mixed evenly with the needle and quickly formed into glue in the mold. There is no fracture during extrusion, indicating that the gel has good stability. Figure 2c shows a hydrogel with a strongly porous shape, with excellent inter-pore commutability, which is critical for drug loading.

Figure 2: (a) Preparation of OHA and CDH-HA, (b) molding of injectable hydrogels, and (c) morphology of injectable hydrogels.

Injectable hydrogels, glioma

Figure 3: Viability of U251 human glioma cells was dramatically decreased by hydrogel processing. U251 human glioma cells were collected and then hydrogel processed using 5-Fu as a positive control. The viability of U251 human glioma cells was determined using the Cell Counting Kit-8.
3.4 Hydrogel greatly reduces the U251 human glioma cell viability

Following the synthesis of a novel hydrogel with a new construction, the value of its application to gliomas was determined. Therefore, the Cell Counting Kit-8 assay kit was used in this research to evaluate the survival of U251 human glioma cells under the treatment of the novel hydrogel. As demonstrated in Figure 3, the hydrogel could considerably decrease the survival rate of U251 human glioma cells, even better than that of the positive control drug 5-Fu.

3.5 Hydrogel significantly suppressed the VEGF signaling channel in U251 human glioma cells

The aforementioned experiments demonstrated that hydrogel dramatically reduced the survival rate of U251 human glioma cells. Moreover, the suppressive activities of hydrogels on the level of VEGF signaling channel activation in U251 human glioma cells remain to be explored. The findings of Figure 4 show that the level of VEGF signaling channel was higher in U251 human glioma cells as compared to HEB derived from regular glial cells. The activated levels of vascular endothelial growth factor signaling channel were obviously reduced under the processing of the novel hydrogel.

4 Conclusion

Taken together, we have produced a novel In(III) CP with the synthetic reactions in a water and DMF mixed solvents utilizing the Him and H2L as the co-ligands, and subsequently loaded with dexamethasone. Structure analyses exhibit that the CP 1 is established with dinuclear In(III) clusters that were linked with two hydroxyl oxygens of L2− anions, which is the characteristics of a two-dimensional net with 4-bridged sq1 topology. Aldehyde and amidation of hyaluronic acid were prepared by chemical modification, and injectable hydrogels were rapidly prepared in situ using Schiff base reaction. SEM showed that the injectable hydrogels presented a continuous porous structure, which could be further loaded with In(III) complex for the treatment of glioma. The findings of Cell Counting Kit-8 showed that hydrogel dramatically reduced the viability of U251 human glioma cells. In addition, the expression level of VEGF signaling channel in cancer cells was significantly inhibited. Finally, we conclude that hydrogel can treat glioma by inhibiting the activation of VEGF signaling channel in carcinoma cells.

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Conflict of interest: All authors declare that there do not exist conflict of interest in the publication of this study.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References


