Heterometallic coordination polymers: Treatment activity on diabetic foot by reducing the excess inflammatory response in the plantar tissue

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Abstract
Two new heterometallic coordination polymers formulated as [(LaCo(2.5-pydc)2(H2O)4]Cl·4H2O]n (1, 2.5-H2pydc = pyridine-2,5-dicarboxylic acid) and [(LaCo2Cl3(4,4′-bpdc)2(H2O)3]·H2O]n (2, 4,4′-H2bpdc = 2,2′-bipyridyl-4,4′-dicarboxylic acid) have been solvothermally synthesized using the coordination between rare earth/transition metal ions and pyridinedicarboxylic acids. To develop new candidates for enhancing the therapeutic effect of improved negative pressure suction for diabetic foot, the activities of compounds 1 and 2 were studied. First, the ELISA (enzyme linked immunosorbent assay) detection kit was used and the inflammatory cytokines in the plantar tissue were measured. Besides, the TLR-4-NF-κB signaling pathway was measured with real-time reverse transcription-polymerase chain reaction.

Keywords
coordination polymer, diabetic foot, heterometallic

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Introduction
Diabetes (DM) has become a common clinical disease with many complications, and the diabetic foot (DF) is one of the most serious complications.1 DF lesions include diabetic neuropathy, peripheral vascular disease, neuroarticular disease, and ulcer formation. The literature reports show that in recent years, in developed countries, DF has a very high morbidity and mortality, and the incidence of DF in DM patients is as high as 25%.2,3 It is estimated that every year, there are more than 1 million DM patients worldwide who receive surgery because of DF, which has a significant impact on individual, social, and economic development.4 Therefore, studying the pathogenesis of DF and improving the treatment level of DF has become a topic of common concern worldwide.

The structure and design of metallic coordination polymers (CPs) based on crystal engineering are currently of interest in the field of supramolecular chemistry and coordination chemistry.5–8 The increasing interest in this field is justified not only by their valuable structures, but also their potential applications in luminescence, catalysis, and biochemistry, and particularly in modern medicinal chemistry.9–15 Among the series of compounds fabricated, functional complexes attract great attention due to their potentially valuable drug applications. Metal complexes, particularly those containing sp2 hybrid nitrogens as part of the aromatic system of the ligands, find significant applications as drugs.16 Besides, CPs can interact with DNA through noncovalent interactions, including intercalation and groove binding for large molecules and serve as external electrostatic binding sites for cations.17 On the other hand, cobalt is an essential human element at the active site of vitamin B12, which indirectly regulates the synthesis of DNA, and has attracted many biological and organometallic chemists who have investigated cobalt complexes with the aim of
developing medical applications due to their significant bioactivity. According to the literature, cobalt complexes have been shown to possess antibacterial, antifungal, antiviral, antiparasitic and antioxidant activity, and antitumor and antiproliferative activity. To study further the potential drug value of the Co(II)-based CPs, in this study, two new heterometallic coordination polymers (CPs) formulated as \([\text{LaCo(2.5-pydc)\text{H}_2\text{O})_4\text{Cl\cdot4H}_2\text{O}}\)\(_n\) (1, 2,5-pydc = pyridine-2,5-dicarboxylic acid) and \([\text{LaCoCl}(4,4'\text{-bpdc}\text{H}_2\text{O})_3\text{H}_2\text{O}}\)\(_n\) (2, 4,4'-bpdc = 2,2'-bipyridyl-4,4'-dicarboxylic acid) have been solvothermally synthesized on the basis of the coordination between rare earth/transition metal ions and pyridinedicarboxylic acids. In biological research, the enhancement of compounds 1 and 2 on the therapeutic effect of improved negative pressure suction for DF was assessed. The results of the ELISA (enzyme linked immunosorbert assay) detection kit suggested that compound 1 showed stronger inhibitory activity on the inflammatory cytokines releasing the plantar tissue than compound 2. The data from the reverse transcription-polymerase chain reaction (RT-PCR) revealed that compared with compound 2, compound 1 has a better effect on TLR-4-NF-κB signaling pathway activation.

**Results and discussion**

**Molecular structure**

The single crystal X-ray diffraction study shows that complex 1 with the chemical formula of \([\text{LaCo(2.5-pydc)\text{H}_2\text{O})_4\text{Cl\cdot4H}_2\text{O}}\)\(_n\) belongs to the monoclinic space group C2/c and features a three-dimensional cationic framework \([\text{LaCo(2.5-pydc)\text{H}_2\text{O})_4\text{Cl\cdot4H}_2\text{O}}\)\(_n^+\) whose positive charges are balanced by Cl\(^{-}\) anions. The asymmetric unit of 1 consists of 1/2 La\(^{3+}\) ion, 1/2 Co\(^{2+}\) ion, one 2,5-pydc ligand, 1/2 CT\(^{-}\) ion, two coordinated water molecules, and two lattice water molecules. The La\(^{3+}\) ion of 1 coordinates with six different carboxyl groups from six 2,5-pydc ligands and two coordinated water molecules. The Co\(^{2+}\) ion adopts an octahedral geometry by bonding to two O atoms and two N atoms from two 2,5-pydc ligands, and two coordinated water molecules. The Co-O bond lengths range from 2.0602(18) to 2.088(2) Å, which are comparable with those observed in other La(III)-based coordination polymers based on the carboxylate-pyridine ligands. As shown in Figure 1(b), the Co\(^{2+}\) ion adopts an octahedral geometry by bonding to two O atoms and two N atoms from two 2,5-pydc ligands, and two coordinated water molecules. The Co-O bond lengths range from 2.0602(18) to 2.088(2) Å. The Co-N bond length is 2.017(2) Å. One carboxyl group of 2,5-pydc ligand coordinates with one La\(^{3+}\) ion and one Co\(^{2+}\) ion, while the other links two La\(^{3+}\) ions. One Co\(^{2+}\) ion connects two 2,5-pydc ligands and two water molecules to form a \([\text{Co(H}_2\text{O)}_2(2,5\text{-pydc})_2]_n\) subunit, while La\(^{3+}\) ions link these \([\text{Co(H}_2\text{O)}_2(2,5\text{-pydc})_2]_n\) subunits to set up a three-dimensional cationic framework \([\text{LaCo(2.5-pydc)\text{H}_2\text{O})_4\text{Cl\cdot4H}_2\text{O}}\)\(_n^+\) with \((4\text{.13})^2(4\text{.6})^8\) topology (Figure 1(c)). A type of channel with a size of 11.0 × 11.7 Å is formed in this cationic framework along the c-axis, where Cl\(^{-}\) anions are located in the channels (Figure 1(d)). Since lattice water molecules in the channels of 1 were highly disordered and could not be fully located from the difference-Fourier map, a SQUEEZE procedure was carried out in the structure refinement of 1 to remove the scattering contributions from the lattice water molecules.
The structural solution and refinement results based on the single crystal data collected around room temperature show that complex 2 with the chemical formula of \{[LaCo_2Cl_3(4,4′-bpdc)_2(H_2O)_3]·H_2O\}_n features a two-dimensional neutral layer and crystallizes in the monoclinic space group P21/n. The asymmetric unit of 2 contains one La^{3+} ion, two Co^{2+} ions, two 4,4′-bpdc ligands, three Cl− ions, three coordinated water molecules and one lattice water molecule. Each La^{3+} ion adopts a distorted decahedral geometry by bonding to seven O atoms from four different 4,4′-bpdc ligands and two water molecules (Figure 2(a)). The La-O bond distances are in the range of 2.425(2)–2.670(2) Å. The Co^{2+} ions in the structure of 2 exhibit two different coordination modes: the Co(1) atom is octahedrally coordinated with one Cl atom, two N atoms, and three O atoms from three 4,4′-bpdc ligands and a water molecule (Figure 2(b)), while the Co(2) atom displays a five coordination mode by bonding to two Cl atom, two N atoms and one O atom from the two 4,4′-bpdc ligands. The Co(1)-O and Co(1)-N bond lengths are in the ranges of 1.954(2)–2.365(3) Å and 2.004(3)–2.007(3) Å, respectively. The Co(1)-Cl bond length is 2.2791(9) Å. The Co(2)-O bond length is 1.998(2) Å and Cu(2)-N bond lengths range from 1.996(3) to 2.013(3) Å. The Co(2)-Cl bond lengths range from 2.2778(9) to 2.5922(10) Å. In the structure of 2, there are two types of 4,4′-bpdc ligands, namely L(1) and L(2), which possess different coordination modes with La and Co atoms. L(1) connects one Co(2) and two La atoms through three oxygen atoms from the two carboxylate groups, while the two nitrogen atoms from the pyridine rings chelate one Co(1) atom. L(2) connects two Co(1) and two La atoms through four oxygen atoms from the two carboxylate groups, while two nitrogen atoms from the pyridine rings chelate one Co(2) atom. Complex 2 consists of three distinct types of building units: [LaO_5(H_2O)_2], [CoO_2(H_2O)(4,4′-bpdc)Cl] and [CoO(4,4′-bpdc)Cl_2]. The LaO_5(H_2O)_2 are connected by the building units of CoO_2(H_2O)(4,4′-bpdc)Cl and CoO(4,4′-bpdc)Cl_2 to form a 2D layer along the c-axis (Figure 2(c)). The 2D layers are further extended in to 3D supramolecular network via the H-bond interactions as shown in the Figure 2(d).

To check the phase purity of the products, powder X-ray diffraction (PXRD) experiments have been carried out for these complexes (Figure 3). The peak positions of the experimental and simulated PXRD patterns are in good agreement with each other, indicating that the crystal structures are truly representative of the bulk crystal products. The differences in intensity may be owing to the preferred orientation of the crystal samples. Considering the following bioactivity tests, it is important to study the framework integrality of both complexes in their stock solutions in DMSO that were used in their in vivo injection. A small amount (nearly 80–100 mg) of the synthesized complexes 1 and 2 was taken separately in a mortar. It was then ground manually for 30 min using a pestle, which was followed by ultrasonic treatment in the DMSO solution for 1 hour at 70 W to make a stock solution with good dispersity. The fine
powders could be recollected by centrifugal treatment and then their PXRD patterns were collected, which reflected a similar PXRD patterns with those samples prepared as described above, indicating that the framework integrity was retained in the stock solutions.

Reduction of the release of inflammatory cytokines in the plantar tissue

After the synthesis of compounds 1 and 2, their enhancement activity on the therapeutic effect of improved negative pressure suction for DF was assessed. The DF is usually combined with an increased level of inflammatory response in the plantar tissue. Thus, the ELISA was conducted to evaluate the level of the inflammatory cytokines. As the results in Figure 4 show, after the addition of compound 1, the level of inflammatory cytokines released in the plantar tissue was significantly reduced and the activity of the modified negative pressure suction for DF treatment was improved. However, compound 2 had a much weaker effect on the inflammatory cytokine level in the plantar tissue.

Inhibition of the activation of the TLR-4-NF-κB signaling pathway in the plantar tissue

In the above research, the inhibitory activity of the compound on inflammatory cytokines in the plantar tissue was confirmed, while the detailed mechanism still needed to be explored. So, the real-time RT-PCR was conducted and the activation level of the TLR-4-NF-κB signaling pathway in the plantar tissue was measured. From the results in Figure 5, compound 1 showed excellent suppression of the TLR-4-NF-κB signaling pathway activation. Consistent with the previous experiment, compound 2 showed almost no effect on the inflammation level.
Conclusion

In summary, we have successfully prepared two new heterometallic coordination polymers on the basis of the coordination between rare earth/transition metal ions and pyridinedicarboxylic acids. These two complexes were characterized by single crystal X-ray diffraction and elemental analysis, which showed that complex 1 shows a 3D channel-type framework with the point symbol of (413.62)(48.66.8), and complex 2 demonstrates a two-dimensional layered structure based on three distinct types of building units: [LaO5(H2O)2] and [CoO2(H2O)(4,4′-bpdc)], [CoO(4,4′-bpdc)Cl] and [CoO(4,4′-bpdc)Cl2]. Furthermore, the enhancement activity of the compounds on the therapeutic effect of improved negative pressure suction for DF was assessed. The results of the ELISA detection kit suggested that compound 1 showed stronger inhibitory activity on the inflammatory cytokines releasing the plantar tissue than compound 2. The data from the RT-PCR revealed that compared with compound 2, compound 1 has a significantly improved effect on the TLR-4-NF-κB signaling pathway activation. In conclusion, compound 1 has much stronger inhibitory effect on the inflammatory response in the plantar tissue than compound 2, indicating that compound 1 could enhance the therapeutic effect of improved negative pressure suction for DF.

Experimental

Chemicals and measurements

All of the chemicals were commercially available and were used without further purification. Elemental analyses (C, H, & N) were performed using a PerkinElmer 240C analyzer. Infrared spectra were recorded in the range 4000–400 cm\(^{-1}\) on a Bruker ALPHA spectrometer using the pure solid samples.

Preparation and characterization for \[\text{[LaCo(2.5-pydc)_2(H_2O)_4]Cl}_24H_2O \text{ (1)}\] and \[\text{[LaCo}_2Cl_3(4,4′-bpdc)_2(H_2O)_3]_nH_2O \text{ (2)}\]

For complex 1, a mixture of Co(NO\(_3\))\(_2\)·6H\(_2\)O (0.497 g, 1.71 mmol), LaCl\(_3·6\)H\(_2\)O (0.363 g, 1.03 mmol), pyridine-2,5-dicarboxylic acid (0.200 g, 1.20 mmol), [HMIm]Cl (1.191 g, 5.88 mmol), H\(_2\)O (1 mL) and acetonitrile (1 mL) was mixed and sealed in an autoclave equipped with a Teflon liner (25 mL). Then the autoclave was heated at 120 °C for 5 days. The products were filtered and washed several times with ethanol and water, and pink block-like crystals of 1 were obtained with a yield of 36% based on the pyridine-2,5-dicarboxylic acid ligand. Elemental analysis calcd (%) for 1 (C\(_{14}H_{14}ClCoLaN\(_2\)O\(_{12}\)): C, 26.46; H, 2.22; N, 4.41; found (%): C 26.62, H 2.11, N 4.17. FTIR (4000–400 cm\(^{-1}\)): 3415 (brs), 3082 (w), 2928 (w), 2116 (s), 1634 (s), 1573 (s), 1446 (s), 1352 (s), 1091 (w), 1022 (w), 932 (w), 887 (w), 774 (s), 692 (w), 414 (s).

For complex 2, a mixture of Co(NO\(_3\))\(_2\)·6H\(_2\)O (0.29 g, 1.01 mmol), LaCl\(_3·6\)H\(_2\)O (0.710 g, 2.01 mmol), 2,2′-bipyridyl-4,4′-dicarboxylic acid (0.245 g, 1.00 mmol), [DMIm]Cl (2,2′-bipyridyl-4,4′-dicarboxylic acid ligand. Elemental analysis calcd (%) for 2: C, 31.35; H, 2.19; N, 6.09; found (%): C, 31.28; H, 2.15; N, 6.24. IR (4000–400 cm\(^{-1}\)): 3424 (brs), 3078 (vw), 2928 (w), 1632 (s), 1547 (s), 1346 (s), 1098 (w), 932 (w), 887 (w), 774 (s), 692 (w), 414 (s).
The X-ray data were obtained by utilizing the Oxford Xcalibur E diffractometer. The intensity data was analyzed by utilizing the CrysAlisPro software and converted to the HKL files. The SHELXS program on the basis of direct approach was utilized to create the initial structural models, and the SHELXL-2014 program on the basis of the least-squares approach was modified. The whole non-H atoms were mixed with anisotropic parameters. Then we utilized the AFIX commands to fix the whole H atoms geometrically on the C atoms that they attached. Table 1 details refinement details as well as crystallographic parameters of the two complexes.

### Inflammatory cytokine determination

The DF mice model was constructed for this experiment according to the established protocols with some modification. In brief, the mice used in this experiment were purchased from Model Animal Research Center of Nanjing University (Nanjing, China), and kept at the standard environment of 45% humidity and 20–25 °C temperature. All the animal experiments in this research were approved by the Ethics Committee of the Affiliated Hospital of Nanjing University (Nanjing, China). The mice were divided into five different groups: the control group, the model group, the improved negative pressure suction group, compound 1 + improved negative pressure suction group, and the compound 2 + improved negative pressure suction group. After treatment the plantar tissue in the mice was collected and the release of IL-1β and TNF-α was detected with ELISA detection kit.

### TLR-4-NF-κB signaling pathway activation

The real time RT-PCR was performed to detect the activation level of the TLR-4-NF-κB signaling pathway in the plantar tissue. This experiment was finished under the guidance of the instructions with a little modification. In short, the DF mice model was constructed and compound 1 or 2 was injected into mice for the treatment. After that, the plantar tissue was collected and the total RNA was extracted with TRIZol Reagent (Sigma, St. Louis, MO, USA). The quality and quantity of the RNA was measured with the OD ratio, and then reversely transcripted into cDNA with RNA reverse transcription kit. Finally, the RT-PCR was performed to detect the relative expression level of the tlr-4 and nf-κb in the plantar tissue. The results were calculated using 2−ΔΔCt method from triplicate preformation.

### Declaration of conflicting interests

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### Supplemental material

Supplemental material for this article is available online.

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### Table 1. Refinement details and crystallographic parameters for complexes 1 and 2.

| Identification code | 1                                                                 | 2                                                                 |
|---------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|
| Empirical formula   | C$_{14}$H$_{14}$ClCoLaN$_2$O$_{12}$                           | C$_{24}$H$_{20}$Cl$_3$Co$_2$LaN$_4$O$_{12}$                     |
| Formula weight      | 635.56                                                         | 919.56                                                         |
| Temperature/K       | 150.0                                                          | 150.0                                                          |
| Crystal system      | monoclinic                                                     | monoclinic                                                     |
| Space group         | C2/c                                                           | P2$_1$/n                                                        |
| a/Å                 | 12.2854(2)                                                     | 11.5529(6)                                                     |
| b/Å                 | 17.9811(3)                                                     | 13.3267(2)                                                     |
| c/Å                 | 10.67300(10)                                                   | 17.6658(4)                                                     |
| α/°                 | 90                                                             | 90                                                             |
| β/°                 | 102.626(2)                                                     | 94.2450(10)                                                    |
| γ/°                 | 90                                                             | 90                                                             |
| Volume/Å$^3$        | 2300.70(6)                                                     | 2712.40(16)                                                    |
| Z                   | 4                                                              | 4                                                              |
| ρ$_{calc}$/g/cm$^3$  | 1.835                                                          | 2.252                                                          |
| μ/μm$^{-1}$         | 2.727                                                          | 3.128                                                          |
| Data/restraints/parameters | 2730/6/156               | 6452/12/439                                                     |
| Goodness-of-fit on F$^2$ | 1.133                | 1.103                                                          |
| Final R indexes [I$>$2σ (0)] | R$_1$ = 0.0240, ωR$_2$ = 0.0572     | R$_1$ = 0.0365, ωR$_2$ = 0.0913                                 |
| Final R indexes [all data] | R$_1$ = 0.0246, ωR$_2$ = 0.0575     | R$_1$ = 0.0409, ωR$_2$ = 0.0947                                 |
| Largest diff. peak/hole / e Å$^{-3}$ | 0.74/-0.99 | 1.33/-1.45                                                     |
| CCDC                | 1987285                                                         | 1987286                                                         |
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