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Therapeutic effect of pH-Responsive dexamethasone prodrug nanoparticles on acute lung injury

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ABSTRACT
Acute lung injury/inflammation (ALI) is usually caused by various injury factors inside and outside the lung, which can be transformed into acute respiratory distress syndrome (ARDS) in severe cases. Alveolar macrophages play a key role in the pathogenesis of ALI, which regulate inflammatory responses by secreting inflammatory mediators. Therefore, we prepared dexamethasone (DXM)/mannose co-modified branched polyethyleneimine (PEI) (DXM-PEI-mannose, DPM) prodrug nanoparticles, which could effectively target the mannose receptor (MR) on the surface of alveolar macrophages and be used for the treatment of ALI. The DXM-PEI (DP) prodrug was obtained by linking DXM with branched PEI through Schiff base reaction. Subsequently, the pH-responsive DPM prodrug was obtained by using mannose-targeted head modification. The DPM prodrug NPs with a particle size of 115 ± 1 nm, a polydispersity index (PDI) value of 0.054 ± 0.018, and a zeta potential of 31 ± 1 mV were obtained by cross-linking. The drug loading of DPM prodrug NPs measured by the acid hydrolysis method was 51.88%, which had good serum stability and biocompatibility. By comparing the stability and property release of prodrug NPs under different pH (7.4 and 5.0) conditions, it showed that DPM prodrug NPs had certain sensitivity to the micro-acid environment. To study the targeting of mouse mononuclear macrophages, mannose-modified prodrug NPs showed significant in vitro targeting. Moreover, prodrug NPs showed good anti-inflammatory activity in vitro, which was significantly different from free drugs. In vivo biodistribution experiments also showed that it had a long-term lung targeting effect. DPM prodrug NPs also had a good therapeutic effect on ALI. In conclusion, the mannose-modified DXM prodrug NPs delivery system could specifically target lung tissues and have a good therapeutic effect, which might be useful for the treatment of lung diseases.

1. Introduction
Acute lung injury/inflammation (ALI) and acute respiratory distress syndrome (ARDS) are usually caused by a series of pulmonary changes due to different internal and external lung injuries, leading to disruption of pulmonary endothelial cells and epithelial barriers, with high morbidity and mortality [1–3]. After infection or trauma, alveolar macrophages and alveolar epithelial cells are resulting in the secretion of a large number of inflammatory factors. Inflammatory factors secreted from the alveoli further aggravate pulmonary inflammation [4]. There are macrophages in 30–40% of pulmonary alveoli. Different from macrophages in other tissues, alveolar macrophages have sufficient locomotion capacity and can effectively clean particulate matter in the alveoli [5,6]. Alveolar macrophages play an important role in ALI, including initiating and maintaining an inflammatory response, as well as regulating lung injury to maintain pulmonary homeostasis [7]. Alveolar macrophages release cytokines (IL-1, IL-6, IL-8, IL-10, and TNF-α) that recruit and activate neutrophils in the lung [8], leading to further release of leukotriene, antioxidants, platelet-activating factor (PAF), and neutrophil elastase. Mannose receptor (MR, CD206) is highly expressed in macrophages during ALI [9] and contributes to the anti-inflammatory process. Meanwhile, MR also plays an important role in the pulmonary inflammatory cascade [10]. It has been pointed out that mannose itself can up-regulate the expression of MR on macrophage surface and protect against ALI at the same time [11–13].

Clinical treatment of ALI mainly includes mechanical ventilation,
inhale of vasodilators, and anti-inflammatory treatment of glucocorticoid (GC) et al. [14]. However, these treatments still have some drawbacks. First, they cannot be treated directly and effectively [15,16]. Second, multiple intervention treatments are easy to cause secondary injury to the lung [17]. GCs have serious side effects and cannot be specifically transmitted to inflammatory sites, limiting their application in anti-inflammatory therapy. Compared with high-dose GCs, a treatment with low-dose GCs can significantly reduce the release of pulmonary inflammatory mediators, while their toxic side effects (such as joint injury and cataracts) should not be ignored [18,19]. Studies have shown that dexamethasone (DXM) can stimulate the expression of MR in addition to anti-inflammatory therapy, and can up-regulate the activity of MR by increasing the expression of MR at the mRNA level, which can contribute to the efficient targeting of mannose prodrug nanoparticles (NPs) to alveolar macrophages [20,21].

Schiff base mainly refers to certain characteristics of imine and methylene amine groups (-RC= -N -) of a class of organic compounds [22,23]. These compounds and their metal complexes have antibacterial, bactericidal, antitumor, antiviral biological, and good catalytic activities. They have been widely used in medicine, catalysis, analytical detection, and functional materials [24–26]. Schiff base plays an important role in the application of medicine and drugs due to their biomedical activities, including anti-inflammatory [27], analgesic [28], anti-microbial [29], anti-convulsion [30], anti-tuberculosis [31], anti-cancer [32], anti-oxidation [33], and insecticidal properties [34]. In the present study, we selected mild reaction conditions to connect DXM with low-molecular-weight branched polyethyleneimine (PEI) by the Schiff base bond, and finally, a pH-responsive DP prodrug macromolecule was obtained.

Due to the high expression of MR in alveolar macrophages, Zhang et al. [35] have found that mannose-COOH modified polymers can actively target the MR on the cell surface, as well as shielding positive charges and improving the serum stability of the polymers. Therefore, we selected low-molecular-weight branched PEI (2 kDa) as hydrophilic carrier [36] to prepare the pH-responsive DXM-PEI-mannose (DPM) prodrug by Schiff base. The aggregation and retention of DPM prodrug NPs in the lung tissues of ALI were significantly enhanced by intravenous administration of MTC-mannose [37,38] and 0.16 g DP prodrug were accurately weighed and put into a round-bottom flask with stirring. Then, 4 mL DMSO was added, and the mixture was evenly stirred for 1 h.

2. Materials and methods

2.1. Materials

Branched PEI (MW, 2 kDa) was purchased from Sigma-Aldrich. DXM (AR, 98%), pyrene, bromobenzyl, and 2,2-bis (hydroxymethyl) propionic acid were obtained from Beijing Bailingwei Technology. Lipopolysaccharide (LPS) was supplied by Shanghai Solabel Reagent Company. TNF-α ELISA kit (JIM0635Ra) was provided by Jiyinmei, Wu Han, China. Absolute ethyl alcohol, glutaraldehyde, methanol, acetone, dimethyl sulfoxide (DMSO) were purchased from Chengdu Cologne Chemical. All reagents were of analytical or HPLC grade. Kunming mice were purchased from Chengdu Dashuo Experimental Company. All animal-related experiments were performed following the guidelines of the Animal Care and Use Committee of Sichuan University.

2.2. Cell culture

RAW264.7 cells were obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China) and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS, Bioseal) and 1% penicillin and streptomycin at 37 °C in a humidified atmosphere containing 5% CO₂.

2.3. Synthesis of DP and DPM prodrugs

Mannose-modified DXM prodrug macromolecules were prepared into prodrug NPs with lower toxicity and pH-response. First, 1.00 g PEI and 0.80 g DXM were evenly dissolved in 2 mL DMSO, and a proper amount of glacial acetic acid solution was added as a catalyst. The reaction lasted for 3.5 days at room temperature, then the excess impurities were removed by dialysis method, and the DP prodrug was obtained. The chemical structure of the DP prodrug was confirmed by FT-IR and ¹H NMR (400 MHz, Varian Inc., USA).

Briefly, 79.18 mg MTC-mannose [37,38] and 0.16 g DP prodrug were accurately weighed and put into a round-bottom flask with stirring. Then, 4 mL DMSO was added, and the mixture was evenly stirred for 1 h.

2.4. Preparation and characterisation of DPM

DPM prodrug NPs were prepared by glutaraldehyde cross-linking methods. At first, 5 mg DPM prodrug was accurately weighed and completely dissolved in 2 mL ultrapure water. Then, 7 mL absolute ethyl alcohol was slowly dripped to dehydrate. Subsequently, 75 μmol glutaraldehyde solution (30 μL) was added for cross-linking for 12 h. The organic solvent was removed by rotary evaporation, ultrapure water was added to the original volume to prepare 2.5 mg/mL DPM prodrug NPs solution. The particle size, polydispersity index (PDI), and zeta potential of DPM prodrug NPs were measured by dynamic light scattering (DLS) at 25 °C (Zetasizer Nano ZS90, Malvern Instruments, UK). Their morphology was observed by using transmission electron microscopy (TEM) after the samples were placed on a copper grid and negatively stained with uranyl acetate (1%, w/v).

2.5. Drug loading coefficient

The drug loading coefficient of DXM was determined by HPLC after hydrolyzing DPM prodrug NPs by the acid hydrolysis method. The pH of DPM prodrug NPs was adjusted to 1.0 with hydrochloric acid. Then the solution was hydrolyzed at 37 °C for 48 h at 100 rpm. The DXM loading coefficient was determined by HPLC under the conditions as follows: HPLC column (Dikma, C18 column), mobile phase acetonitrile: water = 60:40, flow rate 1.0 mL/min, column temperature 30 °C, and detection wavelength 240 nm.

2.6. pH responsiveness of NPs

The particle size and PDI of DPM prodrug NPs under different pH conditions were determined to illustrate the pH sensitivity of prodrug NPs. The DPM prodrug NPs solution was prepared under two different conditions, pH 7.4 and 5.0, respectively, and the two NPs solutions were incubated at 37 °C. By comparing the changes of particle size and PDI values, the stability of the NPs under different pH conditions was illustrated.

2.7. Serum stability

The increase of turbidity reflects whether there is an aggregate phenomenon of nanoparticles in FBS. The DPM prodrug NPs were mixed with 10% FBS, and the absorption value of the mixture within the range of 200–500 nm was scanned by ultraviolet spectrophotometer to identify its maximum value (λMax). The mixture was cultured at a constant temperature (37 °C, 100 rpm). Next, 0.1 mL sample was taken at 0.5, 1, 2, 4, and 7 h, and placed into 96-well plates. According to the results of λMax, the absorbance value of the mixture at λMax was determined by
using a microplate reader (THERMO FISHER 902-ULTS, USA).

2.8. Drug release profile in vitro

The release regularity of DPM prodrug NPs in vitro was investigated by the dialysis method. Briefly, 1 mL of the prepared prodrug NPs was accurately measured and placed into a 1 kDa dialysis bag containing 1 mL 2x PBS (pH 7.4 or 5.0). The dialysis bag was placed in 25 mL of release medium (PBS, pH 7.4 or 5.0), followed by slow release under horizontal vibration (100 rpm, 37 °C). Subsequently, 0.5 mL of release medium was absorbed at each preset time point (1, 2, 4, 7, 12, 24, 36, 48, 72, 96, 120, 144, 168, and 192 h), and then 0.2 mL of corresponding fresh medium was added. The HPLC method was used to determine the drug loading coefficient and calculate the cumulative release proportion of the drug.

2.9. Blood compatibility

The biocompatibility of DPM prodrug NPs was determined with 2% red blood cell (RBC) suspension, and whether NPs had significant hemolytic effects was observed. NPs were prepared at different concentrations (1–100 μg/mL). The mixture was centrifuged (5000 rpm/10 min, 4 °C) after 1 h of incubation with 2% RBC suspension. The supernatants were put into 96-well plates, and the ultraviolet absorbance of each well was measured at 540 nm. PBS and 1% Triton × 100 treated RBC were used as negative and positive controls, respectively. The formula of hemolysis was as follows:

\[
\text{Hemolysis(\%)} = \frac{A_{\text{sample}} - A_{\text{negative}}}{A_{\text{positive}} - A_{\text{negative}}} \times 100
\]

2.10. Cellular uptake

RAW264.7 cells were first passaged, seeded into 12-well plates at an initial density of 3 × 10⁴ cells/well, and cultured in a humid atmosphere containing 5% CO₂ for 24 h. The cells of the un-activated groups and activated groups (LPS stimulation for 24 h, 100 μg/mL) were treated with coumarin 6 solution, coumarin 6/DP prodrug NPs, and coumarin 6/DPM prodrug NPs at a final concentration of 5 μg/mL for 1 h. The cellular uptake was evaluated by flow cytometry and characterized by an inverted fluorescence microscope.

2.11. Cell cytotoxicity

The RAW264.7 cells were seeded into 96-well plates at a density of 5 × 10³ per well, and cultured overnight at 37 °C until the cells adhered to the wall. Sterile DP and DPM prodrug NPs at different concentrations (1–100 μg/mL) were added to each well, followed by co-incubation for 20 h. Subsequently, 20 μL MTT (5 mg/mL) was added to each well, followed by further incubation for 4 h. Then the medium was discarded, and DMSO was added to dissolve the formazan crystals. After horizontal vibration (100 rpm, 10 min), the optical density (OD) of each well was read using a microplate reader at a test wavelength of 570 nm.

2.12. Anti-inflammatory effect in vitro

The RAW264.7 cells were seeded into 48-well plates at a density of 5 × 10⁴ cells per well, and incubated in an incubator overnight (37 °C, 5% CO₂). RAW264.7 cells were then stimulated with 1 μg/mL LPS for 24 h, and then DPM prodrug NPs solution was added into each well at the final concentrations of 10 μg/mL, 5 μg/mL, and 1 μg/mL. DXM at a final concentration of 5 μg/mL was used as the positive control group. Cells without LPS challenge and cells stimulated with LPS were used as the control group and LPS group, respectively. The concentration of the inflammatory factor TNF-α in the cell supernatant was determined according to the instructions of the TNF-α ELISA kit.

2.13. ALI mouse model

The mouse model of ALI was established by intratracheal infusion of LPS solution. Kunming mice (male, 20–22 g) were anesthetized and fixed on a foam board, then their tongue were pulled out by tweezers. Subsequently, 10 μL/LPS (5 mg/mL) solution was quickly dripped into the throat wall of mouse’s throat, and the nasal cavity of mice was quickly covered to ensure that the LPS solution entered the lungs and was evenly distributed in the lungs. After 24 h, the ALI mice was successful modeled. The control group was given the same amount of sterile saline.

2.14. Biodistribution

In ALI model mice, free DiD, DiD/DP prodrug NPs and DiD/DPM prodrug NPs were injected into though the tail vein (injection amount: 3 μg DiD per mouse). The mice were sacrificed at 1, 2, 4, 12, and 24 h after the administration. The heart, liver, spleen, lung, and kidney were collected and fixed with 4% paraformaldehyde. The collected organs were fluorescently imaged by a living small animal imager (Hopkinton, MA, USA, Caliper), and untreated mouse tissues were collected as blank controls.

2.16. Streptococcus pneumoniae

Streptococcus pneumoniae were used to characterize the treatment effect of DPM prodrug NPs on ALI (2 mg/kg). The lung tissues of the control group, ALI group, and the group treated with DPM for 24 h were made into wax blocks. Hematoxylin and eosin (H&E) stained sections and TNF-α immunohistochemical sections were used to assess the lung physiology of mice.

2.17. Statistical analysis

All results were expressed as mean ± standard deviation (SD), and data were analyzed and graphed using Graphpad Prism 6.0 (GraphPad Software, USA). Differences between two groups were assessed for significance using Student’s t-test and comparisons among multiple groups were assessed for significance using one-way analysis of variance (ANOVA). Thresholds of statistical significance were defined as *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001, respectively.

3. Results and discussion

3.1. Synthesis and characterization of NPs

Fig. 1 shows the synthetic scheme of DP prodrug and DPM prodrug. The DXM and PEI reacted in DMSO (the reaction solvent) and glacial acetic acid (the catalyst) at room temperature, and finally, the DP prodrug was connected with Schiff base was obtained. Subsequently, MTG-mannose was modified on the functional group of DP prodrug by amide bond, and finally, the DPM was obtained.

Meanwhile, ¹H NMR and FT-IR were used to confirm the structure of the DXM related prodrug (Fig. 2). Fig. 2A showed ¹H NMR of DP: ¹H
NMR (400 MHz, DMSO) δ 7.29 (d, J = 10.1 Hz, 1H), 6.22 (dd, J = 10.1, 1.8 Hz, 1H), 6.01 (s, 1H), 5.30 (s, 1H), 4.96 (s, 1H), 4.49 (d, J = 19.2 Hz, 1H), 4.11 (dd, J = 27.1, 14.7 Hz, 3H), 3.61–3.05 (m, 9H), 2.94 (ddd, J = 11.0, 7.3, 4.0 Hz, 2H), 2.73–2.42 (m, 6H), 2.00–1.16 (m, 6H), 1.62 (d, J = 11.8 Hz, 1H), 1.57–1.21 (m, 6H), 1.16–0.94 (m, 2H), 0.99–0.67 (m, 7H). The multiple peaks at 2.00–2.73 in Fig. 2 A were the characteristic peaks of -CH$_2$ on the PEI, indicating that DXM and PEI macromolecules were successfully linked. In the same $^1$H NMR spectra, the characteristic absorption peak corresponding to DXM also appeared. For example, the characteristic absorption peak of -OH at 4.96 indicated that the DXM was successfully connected with PEI. Fig. 2C shows the FT-IR spectrum comparison chart of DP and PEI. The characteristic absorption zone (1600–1450 cm$^{-1}$) appeared due to the vibration of the benzene skeleton, which was caused by the function of the functional group on DXM. Moreover, 3440–3252 cm$^{-1}$ was decreased, indicating that -NH$_2$ which was originally highly expressed on PEI was decreased. Therefore, the stretching vibration peak intensity of -NH was significantly decreased. The strength of the characteristic absorption peak (1750–1700 cm$^{-1}$) was increased due to C=O stretching vibration, indicating that the MTC-mannose target was successfully connected to the DP prodrug. Besides, 1655–1590 cm$^{-1}$ was the bending vibration region of N-H, and the peak intensity of this region was decreased, indicating that -NH$_2$ on PEI was replaced by other functional groups. Moreover, 1000–1,300 cm$^{-1}$ was the stretching vibration of the -OH free hydroxyl group, forming a sharp absorption peak. The above-mentioned characteristics all indicated the successful preparation of DPM.

3.2. Preparation and characterisation

The DPM prodrug NPs solution was prepared by the glutaraldehyde cross-linking method, the DPM prodrug NPs (2.50 mg/mL) were
Fig. 2. $^1$H NMR and IR spectra of the macromolecular carrier fragments. (A) $^1$H NMR of DP prodrug. (B) $^1$H NMR of DP vs. DPM prodrug (top: DP; bottom: DPM). (C) PEI vs. DP infrared spectrum analysis (PEI: red curve; DP: purple curve). (D) DP vs. DPM infrared spectrum (DP: red curve; DPM: purple curve). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
obtained. The relative characterizations of the prepared DPM were measured by a DLS instrument as shown in Table 1 and Fig. 3A. Meanwhile, TEM was used to characterize the particle size and appearance of DPM NPs (Fig. 3B). The content of DXM attached to the branched PEI was determined by the acid hydrolysis method, and the drug loading (W/W) was calculated to be 51.88%, indicating that DXM was effectively linked with branched PEI.

The characterization of DPM.

Table 1

| Concentration (mg/mL) | Appearance | Size (nm) | PDI | Zeta potential (mV) | Drug loading (%) |
|-----------------------|------------|-----------|-----|---------------------|------------------|
| 2.50                  | Clear      | 115 ± 1   | 0.054 ± 0.018 | 31 ± 1             | 51.88            |

The Zₘₚₜ of produg NPs in PBS supplemented with 10% FBS was at 300 nm according to the UV scanning curve. As shown in Fig. 3C, the absorbance of produg NPs increased slightly in 7 h. The slight increase of absorbance indicated that the produg NPs could circulate stably in the blood to reach various tissues, and will not combine with various proteins or RBCs in the blood to make the drug ineffective. Fig. 3D showed that the hemolysis rate of produg NPs in 2% RBC solution. The hemolysis rate of all DP and DPM was less than 5% at concentrations ranging from 1 μg/mL to 1000 μg/mL, indicating that the produg NPs had good blood compatibility. Low-molecular-weight branched PEI did not cause identified damage to the cell membrane and hemolysis in a short time. The produg NPs solution prepared by us could be administered via the tail vein, and it had good biocompatibility, which would not affect the survival of mice.

To better understand the drug release characteristics of DPM, we selected PBS with different pH (pH 7.4 and 5.0) as the release solvent of the NPs solution to investigate the sensitivity of different produg NPs to the pH environment. Fig. 3E showed the experimental results, DPM could remain stable at pH 7.4 (37 °C), and there was no significant change in particle size and PDI values. When DPM was in a weak acid environment (pH 5.0), the particle size and PDI values of DPM stored at 37 °C would be suddenly increased after 4 days, indicating that DPM was sensitive to a weak acid environment. When the pH was changed to pH 5.0, most of the DPM NPs could still maintain their spherical morphology, but decompositions occurred in the internal part, leading to a hollow structure. At acidic environment, NPs size would increase rapidly with prolonged incubation time, which was in sharp contrast to the formulations kept in neutral medium (pH 7.4). These results suggested that NPs were sensitive to a pH value of 5.0. In vitro release under different pH conditions was determined by the dialysis method (Fig. 3F).

The cumulative release of DPM within 24 h at pH 7.4 was about 20%, while the cumulative release of DPM within 24 h at pH 5.0 was about 50%. The results showed that the DXM in the DPM produg NPs could be quickly released from the NPs in the weak acidic tissues, and the release of DXM in the NPs was affected by pH, again indicating that the DPM was a pH responsive produg macromolecule.

3.3. Cellular experiments

Next, we studied whether DPM produg NPs could specifically target mouse macrophages in vitro. To figure out whether produg NPs could target inflammatory pulmonary macrophages, the NPs were labeled with coumarin 6. The coumarin 6 fluorescent dye was selected as a marker, and the average fluorescence intensity of coumarin 6 was used to determine the drug uptake by the target cell. The content of DXM attached to the produg NPs was effectively linked with branched PEI.

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| 2.50                  | Clear      | 115 ± 1   | 0.054 ± 0.018 | 31 ± 1             | 51.88            |

In the research of IVIS, we used the near-infrared dye DiD as the fluorescent indicator. The results showed that other tissues, such as the liver and spleen, also exhibited different levels of fluorescence signals except for the lungs. It might be attributed to that MR on macrophages is still expressed in other tissues and organs, while its highest expression is in inflammatory tissues of the lung. However, the experimental results
still showed that the overall fluorescence intensity of the targeted prodrug NPs group was higher compared with the un-targeted prodrug NPs group and free DiD group. Meanwhile, we found that the fluorescence intensity tended to decrease at 24 h, which might be related to the quenching and degradation of fluorescent dyes. In summary, in the ALI mouse model, the targeted prodrug NPs group could effectively bind the MR on macrophages in the inflammatory lung tissues, thus targeting the inflammatory sites of the lung.

Fig. 3. Related characterization of DP and DPM prodrug NPs. (A) The size distribution diagram of DPM. (B) TEM of DPM prodrug NPs (scale bars: 100 nm) (C) Serum stability of DP and DPM within 7 h (n = 3, mean ± SD). (D) Erythrocyte hemolysis rates of DP and DPM in 2% RBC (n = 3, mean ± SD). (E) Under different pH conditions, the particle size of DPM was changed (n = 3, mean ± SD). F: Under different pH conditions, the in vitro release of DPM (n = 3, mean ± SD).

Fig. 4. DPM prodrug NPs targeting inflamed RAW264.7 in vitro. (A) Cytotoxicity of DP NPs and DPM NPs on the RAW264.7 cells after 24 h of incubation. (B) Quantitative analysis of RAW264.7 cells interacting with NPs using flow cytometry. (C) Uptake of un-activated RAW264.7 cells in different groups taken by an inverted fluorescence microscope (scale bars: 100 nm). (D) Uptake of activated RAW264.7 cells in different groups taken by an inverted fluorescence microscope (scale bars: 100 nm). (E) The anti-inflammatory study of DPM NPs in vitro. (n = 3, mean ± SD; P values: **P < 0.01, ***P < 0.001, ****P < 0.0001).
3.5. Improved ALI therapy

We evaluated the treatment effects of DPM NPs delivery system in ALI mice. Fig. 6A showed the results of H&E staining and TNF-α immunohistochemical sections of lung tissues. ALI induced by LPS could lead to the accumulation of inflammatory cells in the lungs, the increased permeability of alveolar capillary, diffuse pulmonary edema, pulmonary hemorrhage, and other symptoms [39]. H&E staining in the normal group showed that the alveoli were intact and uniform in size, and there was no obvious infiltration of inflammatory cells. The results of immunohistochemistry showed that there was no obvious secretion of the inflammatory factor TNF-α. The H&E staining results of the ALI group mainly showed thickening of the alveolar septum, collapsing of alveolar wall, infiltration of inflammatory cells in the lung parenchyma, as well as redness and swelling of the alveolar interstitium with red blood cells exudation. The immunohistochemical results of lung tissue showed obvious secretion of inflammatory factor TNF-α. Compared with the control group, the release of TNF-α was significantly increased. After DPM NPs were injected into the ALI mouse for 24 h, the alveolar septum became thinner, and the infiltration of inflammatory cells was significantly reduced. Moreover, the level of TNF-α in the DPM NPs treatment was significantly reduced. This finding suggested that DPM had an effective therapeutic effect on ALI. Fig. 6B illustrated a flow chart of the experimental design. LPS (5 mg/mL) was inhaled into the lung at 0 h, and the ALI model was successfully established after 24 h. Free DXM and DPM NPs were injected into ALI mice for 24 h. Refer to the official report of the American Thoracic Society on ALI assessment in animals [40]. We established evaluation criteria and evaluated the curative effect of each group. After LPS was dripped into the lung for 24 h, the wet/dry weight ratio (W/D) of lung tissues was increased, indicating the formation of pulmonary edema. Fig. 6C showed the calculated W/D results of lungs in each group. Compared with the control group, the W/D of the ALI group was significantly increased (###, p < 0.001). Compared with the ALI model group, the W/D of the free DXM group and DPM NPs treatment group was significantly decreased, indicating that DXM and DPM had a significant therapeutic effect on ALI. Meanwhile, the W/D ratio of the DPM NPs group was significantly lower than DXM group (**p < 0.01), and there was no significant difference between the DPM NPs group and the control group. It showed that the DPM NPs group had a better therapeutic effect on ALI than free DXM.

Fig. 5. DPM prodrug NPs selectively target the site of ALI. (A) Experimental protocol for evaluation of the targeting of NPs to inflammatory mouse lungs. (B) DiD in vivo imaging of the main organs in each group at different time points, from left to right: heart, liver, spleen, lung, kidney.

Fig. 6. (A) H&E-stained and TNF-α immunohistochemical sections of histopathological sections (Scale bar: 100 nm). (B) Experimental protocol for evaluating the therapeutic effect of free DXM, DP NPs, and DPM NPs. (C) Results of lung wet/dry weight ratio (W/D). (n = 3, mean ± SD; P values: ###p < 0.01 vs control, **p < 0.01 vs ALI).
4. Conclusions

In summary, we prepared a pH responsive DPM produg NPs delivery system. The mannose-modified DXM produg could effectively target to inflammatory tissues, and have good serum stability and biocompatibility. PEI is an effective cell permeabilizing agent, which can promote the entry of drugs into cells [41]. And PEI is mainly delivered to the lung tissue during tail vein injection therapy to improve the therapeutic effect of drugs [42]. The results of in vitro cellular uptake and ex vivo drug distribution tests showed that the DPM produg NPs could successfully target the lung tissue of ALL. Collectively, our current findings suggested that DPM was a potential drug delivery system for the treatment of ALL.

Author statement

Su Meiling: Methodology, Software, Formal analysis, Data Curation, Writing - Original Draft. Yang Bowen: Methodology, Software. Xi Mingrong: Supervision, Resources. Cheng Qiang: Resources, Formal analysis. Yin Zongning: Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors report no conflicts of interest in this work.

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