



Repairing Effect of New Dexamethasone Nanoparticles in the Treatment of Acute Lung Injury and Cluster Nursing

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ABSTRACT

The repairing effect of new dexamethasone nanoparticles in the treatment of acute lung injury was investigated in this study, as well as cluster nursing. In this study, new dexamethasone model drugs were prepared by the aqueous solvent diffusion method, such as anti-ICAM-I monoclonal antibody-modified anionic dexamethasone NLCs and anti-ICAM-I monoclonal antibody-modified cationic dexamethasone NLCs. Besides, the physical and chemical properties and repairing effects of cationic dexamethasone on acute lung injury were compared. A mouse model of acute lung injury was established, and the anti-inflammatory effect of dexamethasone was evaluated by intravenous injection of dexamethasone nanoparticles in the intervention group and normal healthy mice in the control group. A total of 100 patients with acute lung injury in First People's Hospital of Linping District were selected, of which 50 cases were given cluster nursing intervention and the other 50 cases were taken as the control group. A human vascular endothelial cell line was applied to establish the model cells, and a model of inflammatory endothelial cells in acute lung injury was constructed using lipopolysaccharide stimulation, to verify the cytotoxicity of dexamethasone NLCs. It was found that the anion particle size was 250.12 ± 20.15 nm, the cationic particle size was 245.7 ± 2.1 nm; their Zeta potentials were -31 ± 0.5 mV and 38 ± 0.6 mV in turn; their encapsulation rates were 91% and 83%, respectively; the drug loading was 3.7% and 3.4% in sequence; the release lowest rate was 60%. The 50% lethal dose of anionic cells was higher than 600 g/mL, while that of cationic cells was lower. The respiratory function of the cluster nursing intervention group was better markedly than that of the control group, and the lung infection rate was 2.5% in the intervention group and 15% in the control group. In conclusion, dexamethasone nanoparticles had good anti-inflammatory effects. Anionic ICAM NLCs were less toxic than cationic cells and could better bind to lung vascular endothelial cells, which might reduce adverse drug reactions. Therefore, the building of bundled nursing could effectively alleviate respiratory dysfunction and reduce the infection rate of patients.

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Introduction

Acute lung injury is acute/progressive respiratory failure caused by various internal and external lung factors other than cardiogenic factors, and its main feature is alveolar-capillary injury (1). Studies have shown that the key pathway of inflammation that leads to acute lung injury is the nuclear factor signaling pathway. In other words, when the nuclear factor is activated, it will cause the abnormal expression of certain regulatory cells and further promote various types of lung problems, such as inflammation, oxidative stress, and cell apoptosis, thus causing an inflammatory "cascade" reaction in the lungs; ultimately it will lead to acute lung injury in patients (2, 3). Another study has pointed out that the pathogenic factors of acute lung injury may be closely

related to infectious diseases, biochemical injuries, trauma or postoperative infection, sepsis, and shock. Once these conditions occur, they will lead to the damage of capillary epithelial cells of the alveoli and even the pulmonary barrier structure, resulting in the lung gas exchange not proceeding normally. In addition, the mortality rate of patients with acute lung injury is as high as 50% (4). Therefore, the direction of targeted drugs for acute lung injury was investigated in this study.

With the application of nanotechnology, nanosystems can coat drugs and substitute them into the human body for targeted treatment, thereby effectively improving the therapeutic effect of drugs on patients (5). Therefore, the nano drug-carrying controlled-release system composed of carrier

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materials and drugs is stable and convenient to use. Dexamethasone, a glucocorticoid drug, can bind to the glucocorticoid receptor in the cytoplasm and activate the tissue nuclear factor to achieve anti-inflammatory effects (6). In this study, the mice were applied to establish the acute lung injury models to explore the anti-inflammatory effect of dexamethasone on the occurrence of acute lung injury.

Materials and methods

Preparation of nanoparticles

Preparation of anionic dexamethasone nanoparticles

The medium-chain fatty acid glyceride (60 mg), monostearate (110 mg), polyethylene glycol monostearate (20 mg), dexamethasone (10 mg), and mono-amino-terminal polyethylene glycol monostearate (1 mg) were accurately weighed and added into 2 mL of ethanol, which was heated and dissolved in a water bath at 60°C. 0.1 mL of the dissolved organic phase was extracted by a syringe, which was mixed with an appropriate amount of deionized water at a speed of 5,000 r/min (7). After 5 minutes, it was taken out and cooled to 25°C. Then, 25 L of monoamino-terminal polyethylene glycol monostearate (NH₂-PEG2000-SA) (0.2 mg/mL) was added, and the mixture was cultured for 4 hours. Subsequently, 11 µg of anti-ICAM-1 monoclonal antibody (intercellular adhesion molecule-1) was added, and the culture was continued for 4 hours to finally obtain anionic ICAM / DEX / NLCs modified with anti-ICAM-1 monoclonal antibody.

Preparation of cationic dexamethasone nanoparticles

50 mg of medium-chain fatty acid glyceride, 90 mg of monostearate, 15 mg of polyethylene glycol monostearate vinegar, 5 mg of dexamethasone, 1 mg of monosamino-terminal polyethylene glycol monostearate, and 4 mg of carbide were weighed and added into 3 mL of ethyl fermentation for water bath treatment. 0.1 mL of the dissolved organic phase was extracted by a syringe, which was mixed with an appropriate amount of deionized water at a speed of 5,000 r/min. It was taken out after 5 minutes and cooled to 25°C. Then, 25 L of monoamino-terminal polyethylene glycol monostearate (NH₂-PEG2000-SA) (0.2 mg/mL) was added, and the mixture was cultured for 4 hours. Next, 11 g of anti-ICAM-1 monoclonal antibody was added and cultured for 4

hours to obtain cationic ICAM / DEX / ODA-NLCs modified by anti-ICAM-1 monoclonal antibody.

Establishment of the mouse model

51 male mice were provided by First People's Hospital of Linping District with a body weight of 120 ± 20 g; lipopolysaccharide (LPS) was produced by Jiangsu Simcere, China; Mouse interleukin (IL)-1β and tumor necrosis factor (TNF)-α enzyme-linked immunosorbent assay (ELISA) kit was produced by Nanjing Jiancheng Biological Reagents Co., Ltd (8). Besides, the mice were divided into the normal control group, the acute lung injury model group, and the dexamethasone intervention group. The anesthetized mice were placed in a supine position, the skin was cut and the subcutaneous tissue was separated bluntly to expose the trachea, and the tracheal tube was replaced with the venous cannula. In the acute lung injury model group, LPS solution was injected, and 0.5 mL of 0.9% sodium chloride solution was injected into the femoral vein 10 minutes later; LPS solution was instilled into the trachea of the dexamethasone intervention group, and dexamethasone nanoparticles (1 mg/kg) were orally administered 10 minutes later; the normal control group was given 0.9% sodium chloride solution, and 1 mL of 0.9% sodium chloride solution was injected 10 minutes later. Model evaluation criterion was that PaO₂ < 70 mmHg (1 mmHg = 0.133 kPa) and the ratio of wet/dry lung mass (W / D) was increased by more than 30% in the acute lung injury group; pathological observation showed that a large number of inflammatory cells and red blood cells were infiltrated in the lung interstitium, and the alveolar septum was significantly widened.

Establishment of the inflammatory endothelial cell model

ENhy926 cells were cultured in a 75cm² culture flask with high glucose Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum, which was put into a 37°C incubator (9). Besides, the culture medium was changed once every two days with phosphate buffer saline (PBS) to wash cell metabolic waste, and trypsin digestion was used for passage. 400 ng/mL LPS was adopted to stimulate EAhy926 cells for 24 hours to construct an in vitro inflammatory endothelial cell model.

Specimen collection and index detection and methods

(i) Determination of PaO₂: 1 mL of blood was drawn from the mouse through cardiac puncture and injected into a heparin anticoagulation tube, and PaO₂ was measured by a blood gas analyzer.

(ii) Pathological tissue staining: A 1 mm incision was made in the left atrial appendage of the mouse, and the right ventricle was perfused with 0.9% sodium chloride solution to clean the lung tissue. Then, the left lower lung tissue was placed in 4% paraformaldehyde, made into slices, and stained with hematoxylin and eosin.

(iii) Determination of W / D: The remaining left lung tissue was weighed wet and placed in a 60°C incubator. Afterward, it was weighed dry after 7 days (10).

(iv) Enzyme-linked immunosorbent assay method to determine the content of IL-1 β and TNF- α : The endotracheal intubation was inserted slowly into the right main bronchus, and the intubation was stopped when resistance appeared. 1 mL of 0.9% sodium chloride solution was absorbed with a syringe and the saline was slowly injected into the lung. When the right lung of the mouse gradually grew and became pale, the solution could be pumped back; the operation was repeated several times. Then, the pumped back solution was put into a 15 mL plastic centrifuge tube (placed in an ice bath), which was the first lavage, and then, the above operation was repeated 4 times. The whole irrigation process was about 20minutes. After 4 times of lavage, the samples were measured, centrifuged at 4°C at 1,500r/min for 10minutes, and the supernatant was stored in a refrigerator at -70°C for the determination of IL-1 β and TNF- α content in the alveolar lavage fluid.

Clinical data

A total of 100 patients who received laparotomy in the Department of General Surgery from June 2020 to May 2021 were selected as the research objects in this study. What's more, they were divided randomly into the control group and the intervention group according to the number table method, with 50 cases in each. The criteria for inclusion were defined to include patients who had a normal appearance after anesthesia awake; had normal body performance,

normal thinking performance, and no side effects. The criteria for exclusion were defined to include patients who were combined mental illness, were accompanied by respiratory disease, and suffered from motor dysfunction of the limbs. All patients and their family members gave informed consent to this study, and this experiment was reported to the hospital ethics committee for approval (11).

Nursing plan

(i) Routine nursing of the control group: Medical staff carried out ward environment management, provided health education and nutrition support for patients before surgery, and gave oral care, upper abdominal band, condition observation, and nursing after surgery, etc.

(ii) A clustered nursing plan established by the intervention group

For the training of medical staff, the mode of combining self-study and lectures was adopted to learn and master the relevant theories of clustered nursing and to standardize the nursing operations and other nursing behaviors of nursing staff. Furthermore, the above was assessed randomly by the head nurse. The standard process of cluster nursing intervention was formulated, as well as the quantitative evaluation project list. The front side of the quantitative evaluation project list was the project content, and the back side was the specific operation method of the project (12). The assistant head nurse was responsible for quality control, daily inspection, and feedback on the completion of the project, and continuous quality improvement.

Statistical methods

SPSS22.0 statistical software was used for statistical processing, and the measurement data were represented by ($\bar{x} \pm s$). One-way analysis of variance (ANOVA) was used for multiple group comparisons, and the Bonferroni-corrected t-test was used for pairwise comparisons between groups. In addition, $P < 0.05$ indicated that the difference was statistically substantial.

Results and discussion

Nanoparticles size and potential

Figure 1 below revealed that the particle size of ICAM / DEX / NLCs and ICAM / DEX / ODA-NLCs were 250.12 ± 20.15 nm and 245.7 ± 2.1 nm, respectively. Therefore, it showed that the modification of ODA with 3% mass ratio had no obvious influence on the particle size of nanoparticles.

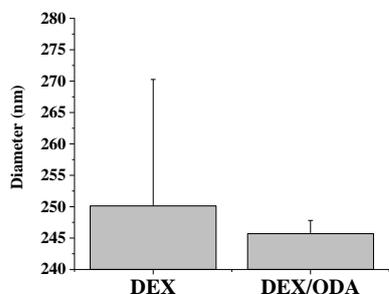


Figure 1. Particle size analysis.

As shown in Figure 2 below, the potential value was -31 ± 0.5 mV, and the Zeta potential value of ICAM / DEX / ODA-NLCs was 38 ± 0.6 mV, indicating the successful preparation of anionic and cationic nanoparticles.

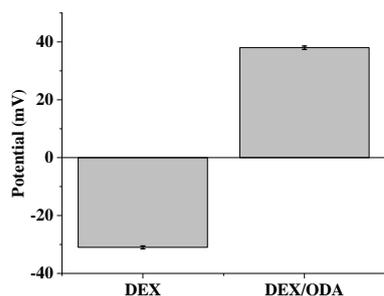


Figure 2. Potential analysis.

Figure 3 disclosed that the dexamethasone nanoparticle dispersion was diluted to 100 pg/mL with deionized water, and the sample was dropped on the carbon support membrane. After the sample was dried, it was negatively stained with 1% uranyl acetate solution for 1 minute, the excess dye solution was absorbed by filter paper, and the morphology of drug-loaded nanoparticles was observed through a JEM-1200EX transmission electron microscope. The results of electron microscopy meant that the particle size distribution of the prepared dexamethasone nanoparticles was relatively uniform and uniformly spherical.

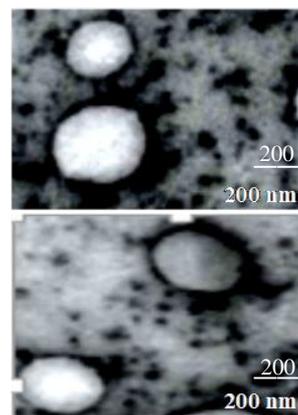


Figure 3. Microstructure. (Note: The image on the top: Cations; the image at the bottom: Anions.)

Determination of encapsulation rate and drug loading of nanoparticles

(1) The sample was placed in the ultrafiltration centrifuge tube and centrifuged at 10,000 r/min 3 times (15 minutes each time), in order to saturate the ultrafiltration centrifuge tube membrane. The drug peak area S in the ultrafiltrate after the fourth centrifuge was measured, and the drug concentration C was calculated according to the standard curve, and then the drug content in the ultrafiltrate was calculated. The encapsulation rates were 91% and 83%, and the drug loads were 3.7% and 3.4%, respectively.

(2) In vitro drug release characteristics of drug-loaded nanoparticles were investigated by using PBS (pH = 7.5) as the release medium. What's more, the drug was released in a time-dependent manner. Figure 4 showed that in vitro drug release lasted for 1 day, with the lowest cumulative release rate of 60%.

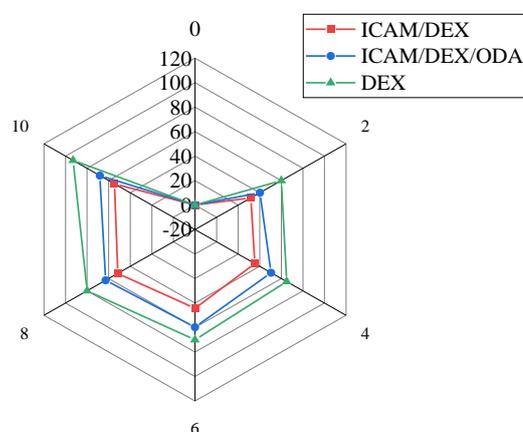


Figure 4. Drug release in vitro.

Evaluation of cytotoxicity

As shown in Figure 5 below, the cytostatic effect of blank nanoparticles on the inflammatory endothelial cell model was dose-dependent within a certain concentration range. As the concentration of blank nanoparticles increased, the inhibition rate of EAhy926 cells also rose. It was found that the median lethal dose of anion cells was higher than 600 $\mu\text{g/mL}$, but that of cations was lower than this value.

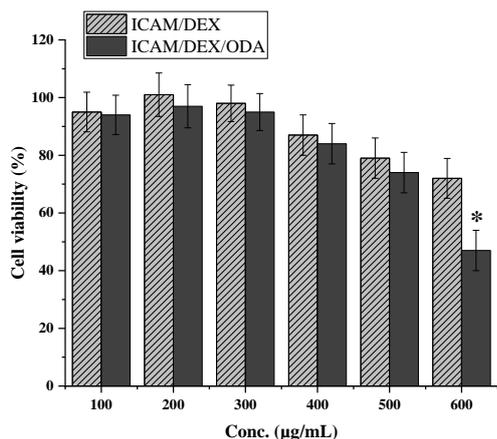


Figure 5. Comparison of cytotoxicity. (Note: * represents that the concentration of 600 $\mu\text{g/mL}$, the cell viability of ICAM/DEX/ODA is significantly different from that of ICAM/DEX ($P < 0.05$)).

The influence of dexamethasone nanoparticles on the dry-wet weight ratio of lung tissue in mice with acute lung injury

Figure 6 below indicated that the lung dry-to-wet weight ratio of mice from the model group elevated obviously after modeling in contrast to the ratio of the blank control group. Compared with the model group, the lung dry-to-wet weight ratio of the dexamethasone nanoparticle group was lower.

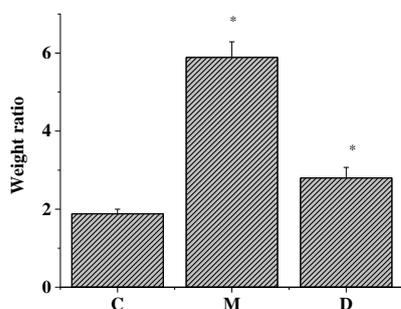


Figure 6. Comparison of the lung tissue dry and wet weight ratio. (Note: C: Blank group; M: Model group; D: Dexamethasone group; * indicated $P < 0.05$ compared with group C.)

The influence of dexamethasone nanoparticles on MDA in alveolar lavage fluid of mice with acute lung injury

Figure 7 showed that compared with the blank control group, the MDA content of mice from the model group increased after modeling. Compared with the model group, dexamethasone nanoparticles could sharply reduce the MDA content.

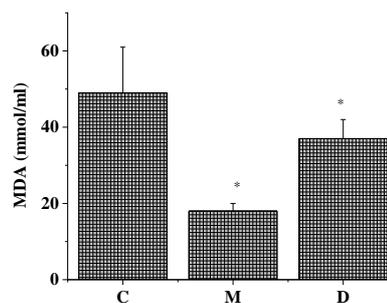


Figure 7. Comparison of MDA in alveolar lavage fluid. (Note: C: Blank group; M: Model group; D: Dexamethasone group; * indicated $P < 0.05$ compared with group C.)

The influence of dexamethasone nanoparticles on SOD in alveolar lavage fluid of mice with acute lung injury

As shown in Figure 8 below, the SOD activity of the model group mice was reduced after modeling compared with the blank control group. Compared with the model group, dexamethasone nanoparticles could significantly enhance the activity of SOD.

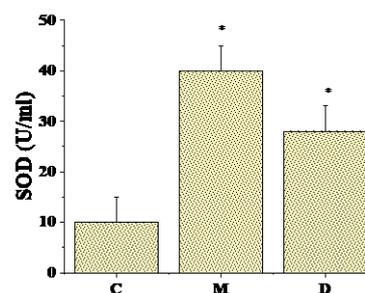


Figure 8. Comparison of SOD in alveolar lavage fluid. (Note: C: Blank group; M: Model group; D: Dexamethasone group; * indicated $P < 0.05$ compared with group C.)

The influence of dexamethasone nanoparticles on histopathology in mice with acute lung injury

Compared with the blank control group, the lung tissue of the model group showed obvious inflammatory cell infiltration after modeling (Figure

9). Compared with the model group, dexamethasone nanoparticles could steeply alleviate the inflammatory cell infiltration, showing a protective effect on mice with lung injury.

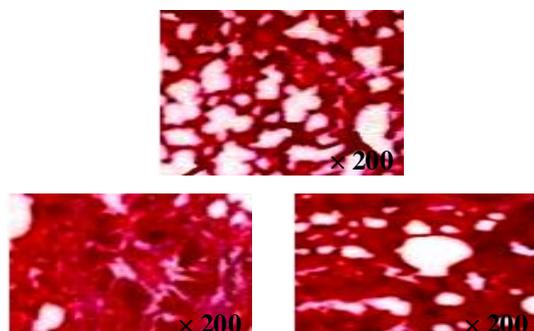


Figure 9. The influence of dexamethasone nanoparticles on tissue disease in mice with acute lung injury. (Note: The upper left was the blank group; the lower left was the model group; the lower right was the dexamethasone group.)

Comparison of respiratory function of patients from the two groups

The results in Figure 10 below showed that the degree of cough, sputum viscosity, and difficulty in expectorating from the clustered nursing intervention group was better substantially than those of the routine nursing group ($P < 0.05$), suggesting that clustered nursing intervention could effectively improve the respiratory function of patients.

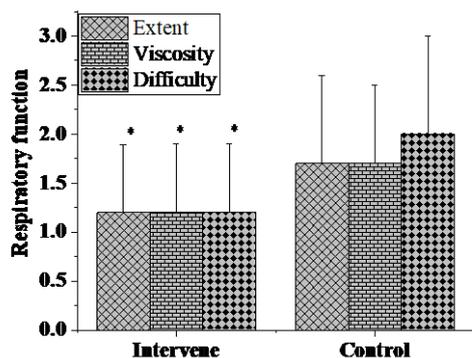


Figure 10. Comparison of respiratory function between the two groups of patients. (Note: * indicated $P < 0.05$ compared with the control group.)

Comparison of the infection rates of patients from the two groups

The results in Figure 11 below indicated that the incidence of postoperative lung infections in patients from the intervention group was lower than that of the control group ($P < 0.05$), while the patients'

satisfaction with nursing services was higher than that of the control group ($P < 0.05$).

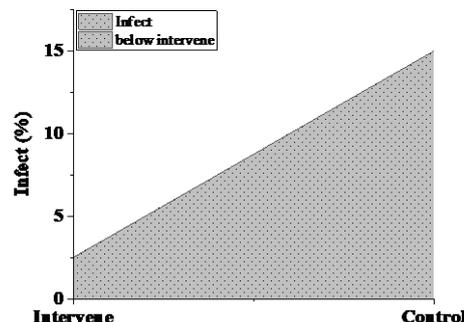


Figure 11. Comparison on the infection rates between the two groups of patients.

There is a multi-dispersion coefficient PI in the micro-evaluation of nano-targeted drugs, which represents the distribution of nanoparticle size. The smaller the PI value, the smaller the dispersion of nanoparticles is (13). Previous studies have shown that the value of PI is related to the stability and particle size distribution of nanoparticles (14). The PI value of dexamethasone nanoparticles prepared in this study was between 0.16 and 0.17, indicating that the particle size distribution of nanoparticles was relatively uniform. In addition, the stability of the nanoparticle system was characterized by Zeta potential. Generally speaking, the higher the Zeta potential, the stronger the repulsive force of the same charge on the particle surface, the less the particle agglomeration, and the better the dispersion stability of the system (15). In this study, the absolute value of the Zeta potential of the dexamethasone nanoparticles was between 29 mV-38 mV, which reflected the potential good dispersion stability of the system.

Nano-level drugs need the good safety required by the team drug delivery system. Besides, EAhy926 cells are immutable cell lines obtained by hybridization of human umbilical vein endothelial cells and human lung adenocarcinoma cell lines, which retain most of the characteristics of endothelial cells, so they can be used as a perfect object for cytotoxicity testing (16). The IC50 value of ICAM / ODA-NLCS is less than 600 g/mL, meaning that the toxicity of the prepared lipid nanocarrier to the inflammatory cell model is enhanced after replacing 3% MS with 3% ODA (17). The experimental results

have shown that blank nanoparticles without ODA modification have low cytotoxicity to activated EAhy926 and ICAM / DEX/ NLCs without ODA modification more satisfied the basic requirements of good cell safety required by the drug delivery system (18-21). Compared with free dexamethasone, the drug release rate of dexamethasone nanoparticles is slowed down significantly, which can be continuously released for more than one day, and the cumulative release rate has reached more than 60%. This is of great significance for reducing drug leakage in the systemic circulation of the drug administration system, reducing toxic and side effects, and improving the effective drug delivery in the inflammatory lung. The encapsulation efficiency of the dexamethasone nanoparticles prepared in this study was all above 90%, reflecting the good encapsulation ability of the nanoparticles to dexamethasone (22-24).

Based on the above, nursing for patients with cough and sputum discharge is the key to the prevention and treatment of postoperative pulmonary infection after acute lung injury. Postoperative pain and stress response caused by long-term inactivity of patients are important pathological manifestations of postoperative complications. Postoperative pain management is a critical guarantee for early activities and the core content of rapid rehabilitation surgery (25, 26). In this study, the synergistic effect of perioperative respiratory tract management and postoperative pain management intervention was adopted, which not only alleviated the pain of patients and reduced the stress response but also ensured the smooth progress of sputum nursing work and early activity intervention, thus effectively reducing the incidence of lung infection.

Conclusions

In this study, the reparative effect of the new dexamethasone nanoparticles and the advantages of cluster nursing were explored mainly through the acute lung injury mouse model. In the later research, the antiviral effect and pharmacological effect of the drugs should be further clarified through the health model, especially the regulation mechanism of immunity and the cluster-based nursing plan. It was found that the nanometer drug delivery system had a good sustained release and targeting properties. It could increase the efficacy of drugs and reduce the

adverse reactions of drugs and showed a unique resistance effect in animal models of acute lung injury, which brought a breakthrough for the clinical effective treatment of acute lung injury. However, the application of nanoscale drug delivery systems in acute lung injury is still in vitro and in animal experiments. It has not been used in clinical practice, and a large number of human trials and in-depth studies are needed to prove it. The toxicity and immunogenicity of nanocarriers, how to modify the surface of nanocarriers to avoid the clear encapsulation of the reticuloendothelial system and achieve better precise targeting, the stability of nanocarriers, and the size of drug loading need to be further studied and optimized. With the different research on the biological behavior of normal organs and diseased organs, a more intelligent and precise nanometer drug delivery system has been prepared, which can release characteristic drugs automatically and quantitatively and regularly, making “drug missile” become a reality.

Acknowledgments

Not applicable.

Interest conflict

The authors declare that they have no conflict of interest.

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