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Analysis of the effect of a new type of nano-drug carrier preparation on the pathological changes in lung and inducible nitric-oxide synthase expression in severe sepsis

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ABSTRACT

To explore the effect of a new type of nano-drug carrier preparation on the pathological changes in severe sepsis lung and inducible nitric oxide synthase (iNOS) expression, a new type of nano-drug carrier preparation was prepared based on RNA interference technology in this experiment. The new type of nano-drug carrier preparation was applied to the control group consisting of 120 rats and the experimental group consisting of 90 rats. The nano-drug carrier preparation group was given a drug injection, and the other group was injected with 0.9% sodium chloride injection. The data of mean arterial pressure, lactic acid, nitric oxide (NO) concentration, and iNOS expression level were recorded during the experiment. The results showed that the survival time of rats in each group was less than 36 hours before 24 hours, the mean arterial pressure of severe sepsis rats continued to decrease, while the mean arterial pressure and survival rate of rats given nano-drug carrier preparation increased significantly in the later stage of the experiment. The concentration of NO and lactic acid in severe sepsis rats increased significantly within 36 hours, while the concentration of NO and lactic acid in rats of the nano group decreased in the later stage of the experiment. The expression level of iNOS mRNA in lung tissue of rats in the severe sepsis group increased significantly during 6-24 hours and began to decrease after 36 hours. The expression level of iNOS mRNA in rats injected with nano-drug carrier preparation decreased significantly. In summary, the new type of nano-drug carrier preparation can improve the survival rate and mean arterial pressure of severe sepsis rats, decrease the concentration of NO and lactic acid and the expression level of iNOS, selectively silence the inflammatory factors in lung cells, reduce the inflammatory reaction, inhibit the synthesis of NO, and correct the oxygenation reaction in the body, which was of great significance for the clinical treatment of severe sepsis lung pathological changes.

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Introduction

Sepsis, which is dangerous and has a high mortality rate, is a systemic inflammatory response syndrome caused by infection. It has become the main cause of death in an intensive care unit (ICU) except for heart disease (1). Severe sepsis is usually accompanied by malperfusion of surrounding tissues, decreased blood pressure, and organ dysfunction. The most common lung pathological changes are acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) (2), whose main causes are the damage to alveolar epithelial cells and capillary endothelial cells. The damage can cause diffuse pulmonary interstitial and alveolar edema, resulting in progressive hypoxemia and respiratory distress (3).

The scientific research conclusion showed that nitric oxide (NO) and nitric oxide synthase (NOS) can not only control mitochondrial respiratory capability but also lead to lung decompensated respiratory failure, thus they are important organ toxic substances (4). When the toxin and the proinflammatory cells gather in large quantities in the body, the inducible nitric oxide synthase (iNOS), one of the subtypes induced by NOS after injury, will cause hypotension in the case of severe sepsis (5). The results are the blood circulation between the pulmonary artery and venous capillary and multiple organ dysfunction syndromes (6). iNOS is an important reason for the production of NO. To restore the hemodynamic impairment in severe sepsis, it is necessary to reduce the production of NO and avoid pulmonary pathological changes such as pulmonary edema and respiratory distress (7).

In recent years, the research of RNA interference technology has made a breakthrough, and the nano-drug carrier preparation developed with interference molecules is also widely used in the field of medical treatment (8). It is a phenomenon inhibiting the expression of specific genes in normal organisms. When the double-strand RNA homologous with the coding region of endogenous mRNA is introduced into cells, the mRNA will be degraded, resulting in gene expression silencing (9). It is a specific, efficient, and economical method of inhibiting gene expression, which can specifically knock down or silence inflammatory factors in alveolar cells and reduce the inflammatory reaction, so as to treat acute lung injury and acute respiratory distress syndrome. However, the present nano preparations can't be decomposed in the body, so there is biological toxicity (10). Moreover, its interface charge is quite high, and it is often expelled from the body before the nano preparation reacts. Therefore, a new type of nano-drug carrier preparation was designed in this experiment, which can reduce the biological toxicity to the minimum. The surface of the nano-drug carrier preparation is covered with Mannatide, which can reduce the interface charge. Moreover, Mannatide can quickly process and interpret the mannose receptor around the tissue cells and improve the absorption of tissue cells to nano-drug carrier preparation. It is beneficial to the treat-

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ment of lung pathological changes in severe sepsis (11).

The purpose of this study was to investigate the clinical application value of this new nano-drug carrier preparation in the lung pathological changes of severe sepsis and the expression of iNOS.

Materials and Methods

Experimental animals

Adult male Wistar rats, weighing 200-250g, aged 8-11 weeks, were provided by Jingjiang People's Hospital Experimental Animal Center, which did not carry the main zoonotic pathogens and fulminating infectious disease, and did not carry the pathogens that were harmful to animals and interfered with the research. This experiment had been approved by the Ethics Committee of Jingjiang People's Hospital, which was in line with the standard of experimental animal feeding management and use.

Main experimental reagents and instruments

The multi-parameter monitor was manufactured by Ruibo Technology Co., Ltd., China. The blood gas analyzer was manufactured by Easy Diagnosis Biomedicine Co., Ltd., China. Ultracentrifuge was manufactured by Beckman Coulter, Inc., USA. Constant- temperature water tank was manufactured by HerryTech Co., Ltd., China. Biomicroscopy was manufactured by Dianying Optical Instrument Co., Ltd., China. 5℃ Low-temperature freezer was manufactured by Fuyi Electric Appliance Co., Ltd., China. - 70℃ ultra-low temperature freezer was manufactured by Lneya constant temperature refrigeration technology Co., Ltd., China. 4% Polyformaldehyde was manufactured by Linyi Shengyang Huagong Co., Ltd., China. The nitric oxide test box was manufactured by Haling Shengwu Co., Ltd., China. Hematoxylin and Eosin staining kit was manufactured by Gefan Biotechnology Co., Ltd., China.

Experimental methods

Preparation of nano-agents

Firstly, N, N-dimethylaniline was synthesized, and then new decomposable poly β-amino ester, low-sensitivity base-pair polymer, and linear polymer were synthesized by Michael polyaddition reaction, respectively. The short interfering RNA was mixed with these substances to form three kinds of nanocomposites, which were fully mixed with Mannatide to obtain a new type of nano-drug carrier preparation. N, N-dimethylaniline and poly β-amino ester were characterized by the method of subtracted infrared spectrum peak separation, and each polymer and the final synthesized nano-drug carrier preparation were characterized by thermal fractionation-differential scanning calorimetry and gel permeation chromatography. The results confirmed that N, N-dimethylaniline was successfully synthesized, and there was no significantly different among the three polymers. The characterization of the interface charge of the nano-drug-loaded preparation showed that the tissue cells under this structure can well absorb the nano-drug, and also had excellent blood stability.

Experimental grouping

120 Wistar male rats were randomly divided into 5 groups according to administration time at 0, 6, 12, 24, and 36 hours after cecal ligation puncture (CLP). Each rat was subcutaneously injected with 1mL100g 0.9% sodium chloride injection and intravenously injected with 2mg/100g of the new type of nano-drug carrier preparation at different times. The survival rate of each group was observed in 100 hours after the operation.

Ninety male Wistar rats were randomly divided into 5 groups according to the administration time at 0, 6, 12, 24, and 36 hours after CLP. Each group was further divided into sham operation group (A), severe sepsis group (B), and nano-drug carrier preparation group (C), with 6 rats in each group. Rats in group C were intravenously injected with 2mg/100g nano-drug carrier preparation by tail at 0, 6, 12, 24, and 36 hours after the operation, and rats in groups A and B were given the same dose of 0.9% sodium chloride injection.

Establishment of sepsis model

CLP method was used to construct a severe sepsis rat model. The rats were fasted 12 hours before the operation but drank water freely. The establishment of the sepsis model was as follows: 2% tribromoethanol 4mL/100g was injected intraperitoneally under general anesthesia. After the drug took effect, the rats were fixed on the operating table in the supine position, and the skin was prepared and disinfected routinely. The incision was about 2cm along the medioventral line. The subcutaneous tissues such as skin and muscle were cut layer by layer, and the cecum was found and removed out of the abdominal cavity. The mesenteric vessels were carefully stripped and 1/2 of the end of the cecum was ligated with line 4. The cecum was punctured once with a sterile needle 18, and a drop of feces was squeezed out at the two holes, then the cecum was returned and the abdominal cavity was sutured layer by layer. In the sham operation group, only the abdominal cavity was opened. After the operation, each rat was subcutaneously injected with 5mL/100g sodium chloride injection and put back into the cage without the restriction of activity and diet. The nano-drug carrier preparation groups were intravenously injected with 2mg/100g nano preparation by tail according to the administration time at 0, 6, 12, 24, and 36 hours after the operation and the severe sepsis group was given the same dose of sodium chloride injection.

Measurement of mean arterial pressure

After 6 hours of administration, the rat was anesthetized by intraperitoneal injection of 2% tribromoethanol 4mL/100g. After the drug took effect, the neck hair was removed and the rat was fixed on the operating table in the supine position, and the skin was prepared and disinfected routinely. The longitudinal incision was 2cm long along the middle line of the neck. The subcutaneous tissues were carefully separated to expose the right common carotid artery. The peripheral tissues and vagus nerve were carefully stripped, avoiding the common carotid artery. Line 4 was selected to ligate a breakpoint away from the heart, and the catheter was placed near the heart of the common carotid artery. The blood vessels were gently lifted by the left hand and the No. 24 yellow cannula needle was used by the right hand through the common carotid artery. After the needle remained in the blood vessels, the needle core was slid out and the needle was fixed with a wire. Then, the electrocardiogram monitor was connected, the relevant values were recorded, and the cannula needle was removed after the situation was stable.

Collection of specimens

A 2mL syringe was selected to extract 1-1.5mL arterial blood from each rat for blood gas analysis and the values were recorded. 0.2mg/100g of phenylephrine was injected into the left jugular vein, and the peak means arterial pressure was recorded. 5mL blood was extracted from the right common carotid artery by vacuum blood collection tube. The blood was slowly shaken

and placed in the refrigerator at 5°C for 1 hour. The blood was centrifuged at 2,900r/min for 10 minutes, and the plasma was sucked and placed in a sterile centrifuge tube, which was kept in the refrigerator at -15°C. The rats were killed and their chests were cut. The left lower lobe of the lung was divided into small pieces, and each piece was about 100mg. The pieces were put into the strain preservation tube, and stored at -70°C, then fixed with 4% polyformaldehyde, and stained with hematoxylin and eosin.

Detection of specimens

1-1.5mL of right carotid artery blood was extracted. A blood gas analyzer was used to detect the concentration of lactic acid in rat artery blood, and relevant values were recorded. Then, three test tubes were taken, 50μL standard solution was injected into tube 1, 50μL sample was injected into tube 2, and 50μL distilled water was injected into tube 3. 100μL of reagent A and 100μL of reagent B was injected into each tube. After completely mixed, they were placed in the constant temperature tank for 1 hour. Afterward, 100μL of reagent C and 100μL of reagent D were injected into each tube. After the solution was mixed completely and stood, 3000r/ min centrifugation was performed for 15 minutes. 300μL of upper layer plasma was sucked and 500μL of substrate solution was added, mixed completely and stood. After the transmittance was adjusted to zero with distilled water, the transmittance of each tube was detected under T550 light, and the serum NO concentration was calculated. After 6 hours of administration, the lung tissue of each rat was collected. Liquid nitrogen was added, the lung tissue was ground into powder, and then 1mL phenol extraction reagent was added. The lung tissue was completely shaken up and pyrolyzed at a low temperature for 10 minutes. After that, 0.1mL trichloromethane was added to make the lung tissue stand at a low temperature, which was centrifuged at 10,000r/min for 10 minutes. The upper layer of plasma was sucked and then transferred to the EP tube. After precipitation, it was centrifuged at 10,000r/min for 10 minutes. The upper plasma was removed, and the RNA precipitate appeared.

Statistical analysis

SPSS 24.0 was used for statistical analysis, and the data were expressed as mean ± standard deviation ($\bar{x}\pm s$). The Cox regression method was used for survival analysis, and the χ^2 test was used for comparison between groups. The test level of $\alpha = 0.05$ or $P < 0.05$ was statistically significant.

Results

General situation

After the establishment of the model, the rats were in poor spirits, curled up, and the amount of diet and activity decreased. Six hours later, a dark red tissue block appeared in the mouth and nose of some rats. After 12-24 hours, the area of tissue block increased, the color deepened, and edema appeared on the head and face of rats. In the process of dissection, the abdominal erosion, a large amount of bleeding, caecum black, and adhesive with peripheral tissue were observed.

Effect of the new type of nano-drug carrier preparation on the survival rate of rats with severe sepsis at different administration times

There were no significant differences in the average survival time and survival rate among groups during 0-12 hours, the average survival time and survival rate of different groups during 24-36 hours were significantly improved, and the survival rate among the groups was statistically significant ($P< 0.01$), as shown in Figure 1.

The effect of the new type of nano-drug carrier preparation on MAP of rats with severe sepsis at different administration time

At 0hour after the operation, the MAP in group B was slightly lower than that in group A, but there was no significant difference ($P> 0.05$). As time went by, the MAP of group B decreased slowly during 6-12hours after the operation, and increased gradually during 24-36 hours, but was still lower than group A. The difference between the groups was statistically significant ($P< 0.01$). The MAP of rats in group C during 12-36hours was higher than that in group B, and the difference between the groups was statistically significant ($P< 0.01$), as shown in Figure 2.

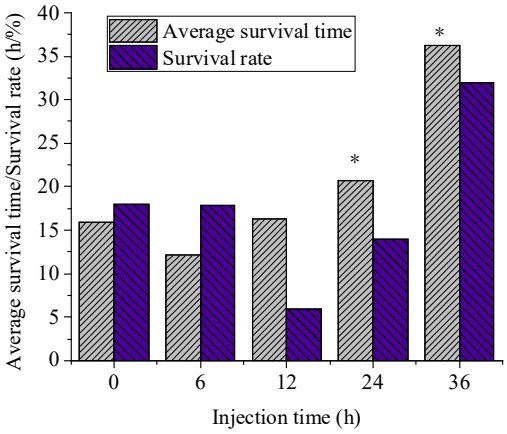

Figure 1. Effect of the new type of nano-drug carrier preparation on the survival rate of rats with severe sepsis at different administration times. Note: * indicated that there were differences in the average survival time and survival rate among groups during 24-36hours ($P< 0.01$).

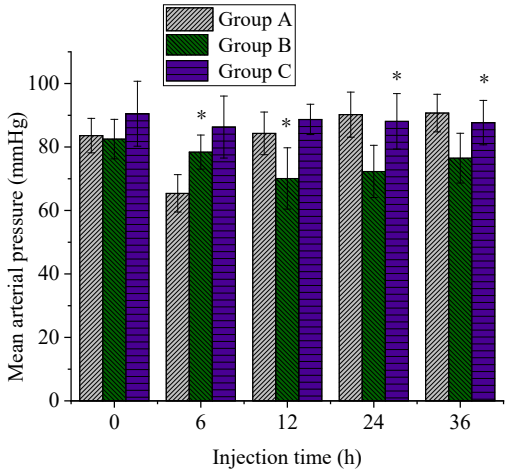

Figure 2. Effect of the new type of nano-drug carrier preparation on MAP of rats with severe sepsis at different administration times. Note: group A represented the sham operation group, group B represented the severe sepsis group, and group C represented the nano-drug carrier preparation group, the same as below. * indicated that there were differences in MAP among groups during 6-36 hours ($P< 0.01$).

The effect of the new type of nano-drug carrier preparation on MAP change of rats with severe sepsis at different administration time

There were no significant differences in MAP change among groups A, B and C during 0-6 hours after the operation ($P > 0.05$). The MAP change of rats in group B was significantly decreased after 12-24 hours, with statistical significance ($P < 0.05$). The MAP change in group B was slightly lower than that in group A at 36 hours, but there was no statistical significance ($P > 0.05$). The MAP change in group C during 12-24hours was higher than that in group B, with statistical significance ($P < 0.01$). MAP change of rats in group C was slightly higher than that in group B at 36 hours, but the difference between the groups was not statistically significant ($P > 0.05$), as shown in Figure 3.

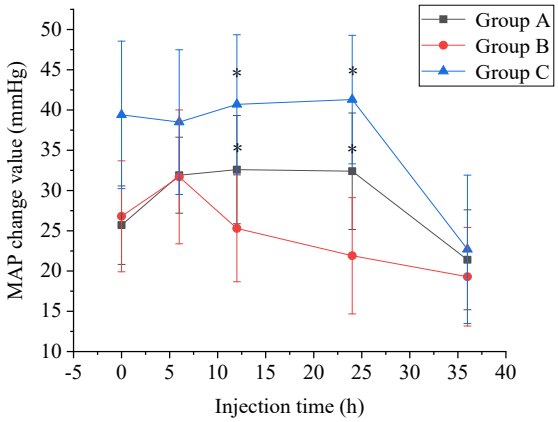

Figure 3. Effect of the new type of nano-drug carrier preparation on MAP change of rats with severe sepsis at different administration times. Note: * indicated that the MAP change of groups B and group C were different from that of group A during 12-24 hours ($P < 0.05$).

The effect of the new type of nano-drug carrier preparation on the concentration of lactic acid of rats with severe sepsis at different administration time

At 0 hours after the operation, there were no significant differences in the concentration of lactic acid among group A, group B, and group C ($P > 0.05$). From 6-36 hours, the concentration of lactic acid in group B gradually increased, which was significantly higher than that of group A, with statistical significance ($P < 0.05$). The concentration of lactic acid in group C was significantly lower than that in group B from 6-36 hours after the operation, with statistical significance ($P < 0.05$). The concentrations of lactic acid at 24 hours and 36 hours in group C were higher than that in group A, and the difference was statistically significant ($P < 0.01$), as shown in Figure 4.

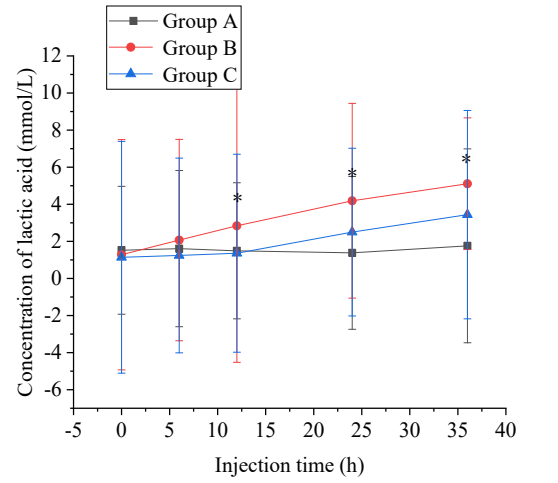

Figure 4. Effect of the new type of nano-drug carrier preparation on the concentration of lactic acid of rats with severe sepsis at different administration times. Note: * indicated that the concentration of lactic acid of group B was different from that of the other groups during 6-26 hours ($P < 0.01$).

The effect of the new type of nano-drug carrier preparation on NO concentration of rats with severe sepsis at different administration time

After the operation, the NO concentration of rats in group B was higher than that in group A, and the difference was statistically significant ($P < 0.01$). The NO concentration in group C was lower than that in group B from 6-36 hours but was significantly higher than that in group A, and the differences were statistically significant ($P < 0.05$), as shown in Figure 5.

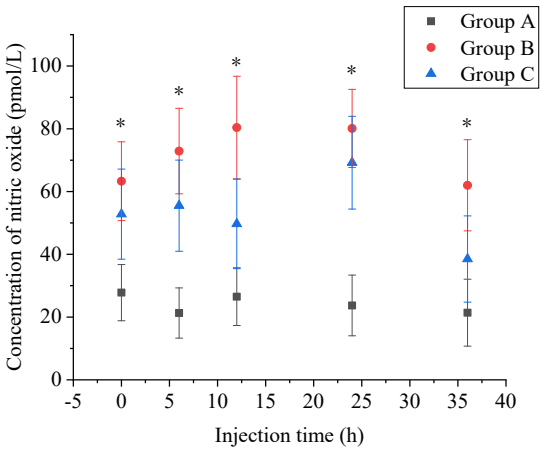

Figure 5. Effect of the new type of nano-drug carrier preparation on NO concentration of rats with severe sepsis at different administration times. Note: * indicated that the NO concentration of group B was different from that of the other groups during 6-36 hours ($P < 0.05$).

The effect of the new type of nano-drug carrier preparation on the expression of iNOS mRNA in lung tissue of rats with severe sepsis at different administration time

At 0 hours after the operation, there were no significant differences in the expression level of iNOS mRNA among the lung tissues of rats of group A, group B, and group C ($P > 0.05$). The expression level of group B was significantly higher than that of group A during 6-24 hours, with statistical significance ($P < 0.01$). The expression level of group C from 6-24 hours was significantly lower than that of group B, with statistical significance ($P < 0.05$). The expression level of group C was lower than that of group B from 12-24 hours but was still significantly higher than that of group A, and the differences were statistically significant ($P < 0.01$), as shown in Figure 6.

The effect of the new type of nano-drug carrier preparation on the expression of iNOS mRNA and protein in lung tissue of rats with severe sepsis at different administration time

Within 6 hours after the operation, the expression of iNOS mRNA and protein in lung tissue of rats in group B and group C were not significantly increased, and the difference was not statistically significant ($P > 0.05$). The expression level of group B gradually increased from 12-24 hours. The expression level of group C was lower than that of group B, but was always higher than that of group A during the same period, with statistical significance ($P < 0.01$). At 36 hours, there was no significant

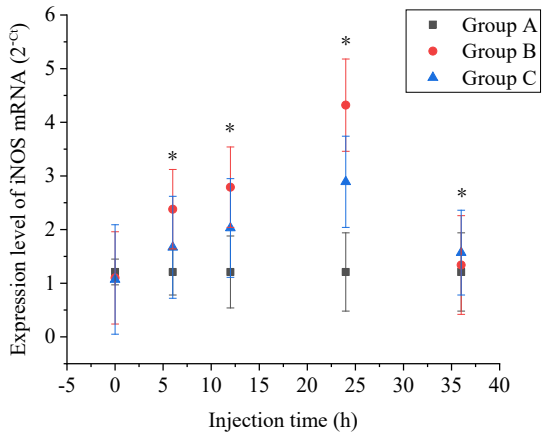

Figure 6. Effect of the new type of nano-drug carrier preparation on the expression level of iNOS mRNA of rats with severe sepsis at different administration times. Note: * indicated that the expression level of iNOS mRNA of group B was different from that of the other groups during 6-24 hours ($P < 0.01$).

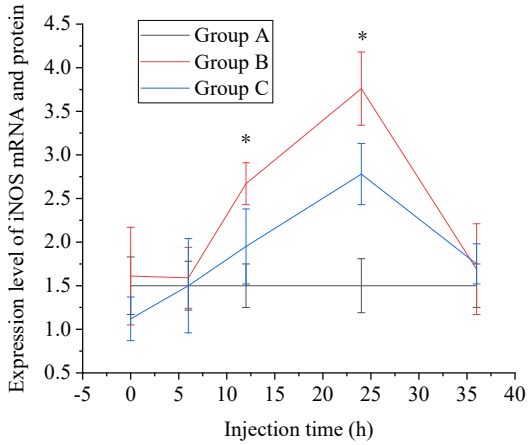

Figure 7. Effect of the new type of nano-drug carrier preparation on the expression of iNOS mRNA and protein in lung tissue of rats with severe sepsis at different administration times. Note: * indicated that the expression level of iNOS mRNA and protein of group B was different from that of the other groups during 12-24 hours ($P < 0.01$).

increase in the expression level of all groups, and the differences were not statistically significant ($P > 0.05$), as shown in Figure 7.

The detection results of blood routine and blood biochemical of rats in the control group and nano-drug carrier preparation group

The results showed that there was no significant difference in indicators of blood routine and blood biochemical between the rats injected with the new type of nano-drug carrier preparation and the normal control group. It was proved that the nano-drug carrier preparation had high drug safety and no obvious biological toxicity to the body, as shown in Figure 8.

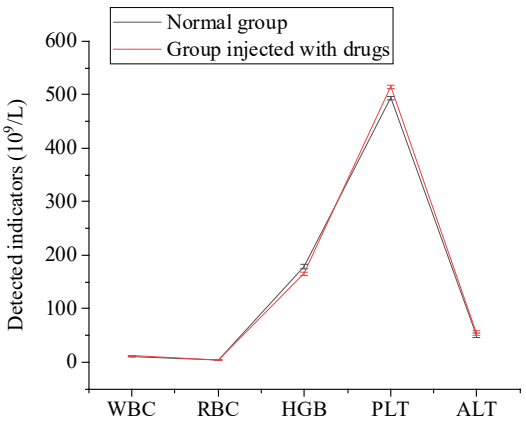

Figure 8. The detection results of blood routine and blood biochemical of rats in the control group and nano-drug carrier preparation group.

The effect of the new type of nano-drug carrier preparation on the pathological changes in lungs of rats with severe sepsis at different administration time

The light microscope of Figure 9 showed that there were a small number of red blood cells in the lung vesicles of rats in group A at 0, 6, 12, 24, and 36 hours. The structure of the vesicles was normal. At 0 hours, there were some red blood cells, and the structure of the vesicles was roughly normal. During 6-24 hours, a large number of red blood cells were exuded from the lung vesicles of rats in group B, and the lung parenchyma was seriously swollen. Inflammatory cells were gathered in the inflammatory foci, with vesicles collapsing at the same time. At 36 hours, red blood cells appeared in the vesicles of group B, and swelling and inflammatory cell aggregation still existed. During 0-6 hours, micro red blood cells appeared in the pulmonary vesicles of rats in group C, and the alveolar structure was roughly normal. During 12-24 hours, there was a large number of red blood cells exudation, with swelling of lung parenchyma. Inflammatory cells gathered in inflammatory foci and vesicles collapsed, and still existed at 36 hours. The lung pathological damage was not significantly improved compared with that in group B during the same period.

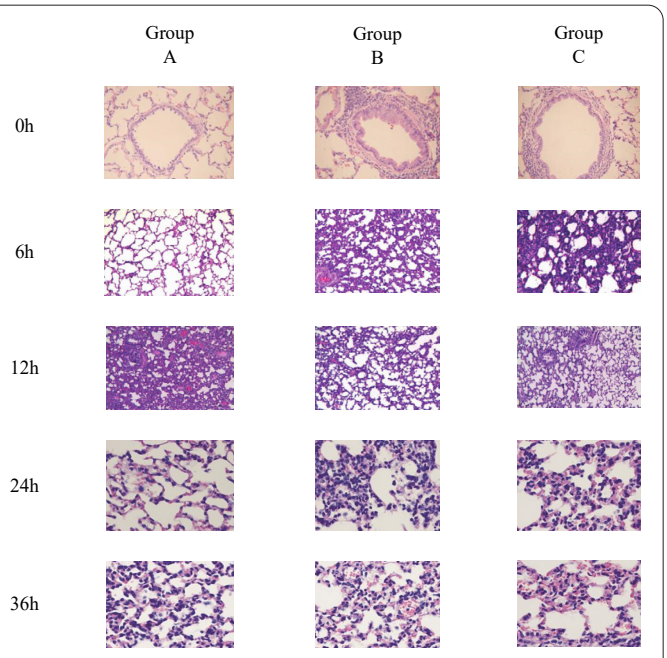

Figure 9. Pathological changes in lungs of rats in three groups under a light microscope (100x magnification).

Discussion

Sepsis is a systemic inflammatory response syndrome caused by infection. The clinical mortality rate is high. Its pathogenesis involves many factors such as cell endotoxin, inflammatory mediators, and immune dysfunction, which is closely related to the pathophysiological changes of many systems and organs in the human body (12). Severe sepsis patients often have multiple organ dysfunction. The first organ damaged is the lung, which

mainly shows acute lung injury and acute respiratory distress syndrome. Studies showed that severe sepsis is mainly caused by cell death in lung tissue, which is due to the increase of energy between white blood cells and the expression level of inducible nitric oxide synthase. That affects the blood circulation between arterioles and venules in the body, causing lung injury and respiratory distress (13).

In this experiment, a new type of nano-drug carrier preparation was prepared based on RNA interference technology. Ninety rats were randomly divided into five groups according to different postoperative administration times, and each group was further divided into three subgroups. One group was injected with 2 mg/100 g nano-drug carrier preparation by tail vein, and the other two groups were given 0.9% sodium chloride injection. The experimental result showed that the survival time of rats in the pre-24 hours groups was less than 36 hours, indicating that the new type of nano-drug carrier preparation could improve the survival rate of rats with severe sepsis. During the experiment, the MAP of rats with severe sepsis continued to decrease, and the MAP of the rats given the new type of nano-drug carrier preparation significantly increased in the later stage of the experiment. The concentration of nitric oxide and lactic acid in rats with severe sepsis significantly increased within 36 hours, and the concentration of nitric oxide and lactic acid in rats treated with the nano-drug carrier preparation decreased at the later stage of the experiment. The expression level of iNOS mRNA in lung tissue of rats with severe sepsis group significantly increased during 6-24 hours, which began to decrease after 36 hours. The expression level of iNOS mRNA in rats injected with the new nano-drug carrier preparation significantly decreased. Simeone et al. (2018) (14) pointed out that some calmodulin-dependent on enzymes, such as myosin light chain kinase, can catalyze the phosphorylation of myosin to activate ATP enzyme, causing smooth muscle contraction and aggravating pulmonary edema, leading to acute respiratory failure. Some micro RNAs in RNA interference technology can inhibit the mRNA expression of these enzymes, and then restore the blood barrier between venules and arterioles caused by respiratory failure (16). This experiment also confirmed the reliability of this statement, which using nanoparticles can be considered for this purpose (17-18). The new type of nano-drug carrier preparation can selectively silence inflammatory factors in lung cells, reduce the inflammatory reaction, control the amount of nitric oxide production, make gas conversion unobstructed, and stabilize the average pulse pressure (19). It can also increase the absorption of drugs for lung cells, which was of great significance for the clinical treatment of lung pathological changes with severe sepsis.

Depending on the gene silence of RNA interference technology, a new type of nano-drug carrier preparation was designed and applied in the study of lung pathological changes and iNOS expression in severe sepsis. A total of 120 rats were selected for CLP and were grouped according to different administration times. The rats were observed for 100 hours after the injection of nano-drug carrier preparation. Then, 90 rats were randomly grouped according to a different administration. Each group was further divided into three subgroups. One group was injected with nano-drug carrier preparation for the experimental study. The result showed that the new type of nano-drug carrier preparation can improve the survival rate and MAP, reduce the concentration of nitric oxide and lactic acid, and reduce the expression level of iNOS in rats with severe sepsis. However, due to the complicated production process and a small amount of preparation, it can't be used in animal experiments with large samples. Moreover, the drug characteristics and biotoxicity of the preparation were still unclear, and further clinical research was needed. It was believed that this new type of nano-drug carrier preparation would play an important role in the treatment of severe sepsis with the exploration of scientific research and experiments.

References

1. Usman OA et al. (2018). Comparison of SIRS, qSOFA, and NEWS for the early identification of sepsis in the Emergency Department. Am J Emerg Med. 2019 Aug;37(8):1490-1497. doi: 10.1016/j.ajem.2018.10.058. Epub 2018 Nov 7. PMID: 30470600.
2. Fowler AA 3rd et al. (2019). Effect of Vitamin C Infusion on Organ Failure and Biomarkers of Inflammation and Vascular Injury in Patients With Sepsis and Severe Acute Respiratory Failure: The CITRIS-ALI Randomized Clinical Trial. JAMA. 2019 Oct 1;322(13):1261-1270. doi: 10.1001/jama.2019.11825. Erratum in: JAMA. 2020 Jan 28;323(4):379. PMID: 31573637; PMCID: PMC6777268.
3. Komiya K et al. (2017). A systematic review of diagnostic methods to differentiate acute lung injury/acute respiratory distress syndrome from cardiogenic pulmonary edema. Crit Care. 2017 Aug 25;21(1):228. doi: 10.1186/s13054-017-1809-8. PMID: 28841896; PMCID: PMC6389074.
4. Barrington KJ et al. (2017). Nitric oxide for respiratory failure in infants born at or near term. Cochrane Database Syst Rev. 2017 Jan 5;1(1):CD000399. doi: 10.1002/14651858.CD000399.pub3. PMID: 28056166; PMCID: PMC6464941.
5. Tunctan B et al. (2018). Bexarotene, a Selective RXRα Agonist, Reverses Hypotension Associated with Inflammation and Tissue Injury in a Rat Model of Septic Shock. Inflammation. 2018 Feb;41(1):337-355. doi: 10.1007/s10753-017-0691-5. PMID: 29188497.
6. Guo Yet al. (2019). Inducible nitric oxide synthase contributes to insulin resistance and cardiac dysfunction after burn injury in mice. Life Sci. 2019 Dec 15;239:116912. doi: 10.1016/j.lfs.2019.116912. Epub 2019 Oct 18. PMID: 31634465.
7. Wang TTet al. (2019). Overexpression of inducible nitric oxide synthase in the diabetic heart compromises ischemic postconditioning. J Mol Cell Cardiol. 2019 Apr;129:144-153. doi: 10.1016/j.yjmcc.2019.02.011. Epub 2019 Feb 21. PMID: 30797815.
8. Li Wet al. (2016). Overcoming ABC transporter-mediated multidrug resistance: Molecular mechanisms and novel therapeutic drug strategies. Drug Resist Updat. 2016 Jul;27:14-29. doi: 10.1016/j.drup.2016.05.001. Epub 2016 May 13. PMID: 27449595.
9. Han H (2018). RNA Interference to Knock Down Gene Expression. Methods Mol Biol. 2018;1706:293-302. doi: 10.1007/978-1-4939-7471-9_16. PMID: 29423805; PMCID: PMC6743327.
10. Bencsik Aet al. (2017). Nano- and neurotoxicology: An emerging discipline. Prog Neurobiol. 2018 Jan;160:45-63. doi: 10.1016/j.pneurobio.2017.10.003. Epub 2017 Nov 3. PMID: 29108800.
11. Dwyer Bet al. (2020). Expression, purification, and characterization of human mannose-6-phosphate receptor - Extra cellular domain from a stable cell line utilizing a small molecule biomimetic of the mannose-6-phosphate moiety. Protein Expr Purif. 2020 Jun;170:105589. doi: 10.1016/j.pep.2020.105589. Epub 2020 Feb 3. PMID: 32027983.
12. Li Zet al. (2019). Methane alleviates sepsis-induced injury by inhibiting pyroptosis and apoptosis: in vivo and in vitro experiments. Aging (Albany NY). 2019 Feb 18;11(4):1226-1239. doi: 10.18632/aging.101831. PMID: 30779706; PMCID: PMC6402521.
13. Hess CNet al. (2019). Acute Limb Ischemia in Peripheral Artery Disease. Circulation. 2019 Aug 13;140(7):556-565. doi: 10.1161/

CIRCULATIONAHA.119.039773. Epub 2019 Jun 26. PMID: 31238713.

14. Simeone KAet al. (2018). Respiratory dysfunction progresses with age in Kcna1-null mice, a model of sudden unexpected death in epilepsy. Epilepsia. 2018 Feb;59(2):345-357. doi: 10.1111/epi.13971. Epub 2018 Jan 12. PMID: 29327348; PMCID: PMC7641387.
15. Colburn TDet al. (2016). Effect of sodium nitrite on local control of contracting skeletal muscle microvascular oxygen pressure in healthy rats. J Appl Physiol (1985). 2017 Jan 1;122(1):153-160. doi: 10.1152/japplphysiol.00367.2016. Epub 2016 Oct 27. PMID: 27789769; PMCID: PMC5283855.
16. Alavi M, Rai M. Antisense RNA, the modified CRISPR-Cas9, and metal/metal oxide nanoparticles to inactivate pathogenic bacteria. Cellular, Molecular and Biomedical Reports. 2021;1(2):52-9.
17. Iqbal MJ, Ali S, Rashid U, Kamran M, Malik MF, Sughra K, Zeeshan N, Afroz A, Saleem J, Saghir M. Biosynthesis of silver nanoparticles from leaf extract of Litchi chinensis and its dynamic biological impact on microbial cells and human cancer cell lines. Cellular and Molecular Biology. 2018 Oct 1;64(13):42-7.
18. Ibrahem KH, Ali FA, Sorchee SM. Biosynthesis and characterization with antimicrobial activity of TiO2 nanoparticles using probiotic Bifidobacterium bifidum. Cellular and Molecular Biology. 2020 Oct 31;66(7):111-7. https://doi.org/10.14715/cmb/2020.66.7.17.
19. Butt ASet al. (2018). A review: Therapeutics potentials of phytochemical drugs and their loading in pH specific degradable Nano-drug carrier targeting colorectal cancer. J Pak Med Assoc. 2018 Apr;68(4):607-614. PMID: 29808052.