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Study on the effect of polyethylene glycol emodin in the treatment of severe acute pancreatitis in rats

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ABSTRACT

Severe acute pancreatitis (SAP) is a common gastrointestinal disorder requiring hospitalization. Pancreatic edema, hemorrhage, and pancreatic necrosis are potentially fatal complications. Therefore, the development of new drugs for the treatment of SAP is essential. We developed a nanocomposite drug (polyethylene glycol [PEG]-loaded emodin [EMD] liposomes [PEG-EMD Lip]) for the treatment of SAP that was constructed based on the excellent biocompatibility, non-toxicity, moisture retention, and favorable dispersive properties of PEG. *In vitro* experiments revealed that the effect of PEG-EMD Lip (15 μg/mL) modified by PEG on the activity of mononuclear macrophages (RAW264.7) remained above 93.3%, which was 1.12 times higher than that of EMD Lip alone. This suggests that PEG-modified EMD can effectively reduce the side effects observed with the parental drug. In addition, PEG–EMD Lip significantly decreased the levels of serum inflammatory factors, oxidative stress induced by SAP, and the degree of damage to the pancreas and lung organized system.

Keywords: Severe Acute Pancreatitis, Emodin, Polyethylene Glycol, Nanodrug, Associated Lung Lesions.

1. INTRODUCTION

Severe acute pancreatitis (SAP) is an aggressive pancreatic inflammatory disease, characterized by edema and necrosis of pancreatic tissues. It is associated with a dangerous rapid onset and a high mortality rate [1–3]. SAP is accompanied by severe pancreatic injury, gastrointestinal bleeding, septicemia, lung injury, and multiple organ failure [4–6]. The mortality rate from SAP has reached approximately 15%–20% over the past decade and remains a serious threat to human health [7, 8]. Therefore, improving the effectiveness and prognosis of SAP treatment is an important step to prolong the survival of patients.

Until recently, the treatment of SAP has focused primarily on the maintenance of organ function and the use of drugs for adjuvant therapy [9, 10]. Enzyme inhibitors (e.g., Gabexate [11], ulinastatin [12, 13]) are used widely to treat SAP, and their effects are significant. However, the intravenous injection of gabexate axetil may cause local venous inflammation, allergy, chest tightness and dyspnea, as well as other symptoms. Ulinastatin is often accompanied by nausea, vomiting, diarrhea, and other adverse reactions, and affects the normal function of the vascular system. Therefore, it is essential to design and develop specific drugs to safely and effectively slow the progression of pancreatitis.

Emodin (EMD), a natural onion derivative, is the active ingredient of several medicinal plants, including palm, polygonum multiflorum, and cassia seeds [14–17]. EMD exhibits diverse pharmacological properties including anti-inflammation, bacteriostasis, antioxidant, and nerve repair activities [18–21]. However, the side effects of EMD including hepatotoxicity are particularly significant at high doses and frequent administration. Polyethylene glycol (PEG) is a high-molecular-weight polymer with good water solubility, biocompatibility, moisture retention, and

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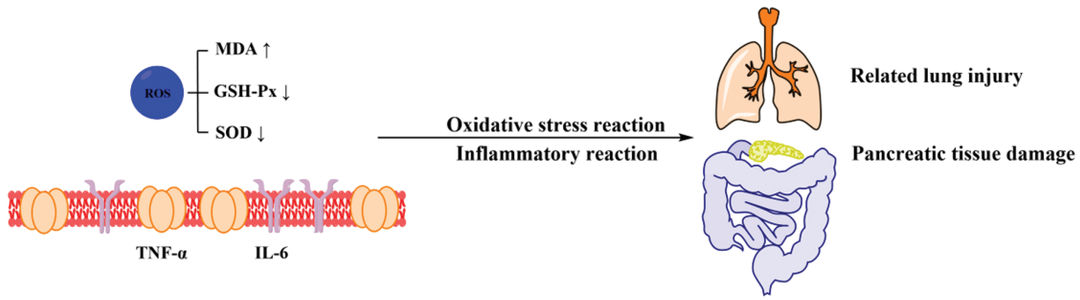

Fig. 1. Inflammatory reaction and associated lung injury in severe acute pancreatitis.

dispersion [22–24]. These excellent physical and chemical properties have clinical benefits in the field of nanopharmaceutical development.

Based on its favorable hydrophilicity properties, PEG was grafted with EMD to form a PEG–EMD liposome composite nano-drug to reduce the toxicity observed with unmodified EMD. We evaluated PEG–EMD Lip in a rat model of SAP with respect to its anti-inflammatory and protective effects on pancreatic and lung tissue (Fig. 1).

2. MATERIALS AND METHODS

2.1. Reagents and Instruments

EMD, anhydrous ethanol, normal saline, glucose, sodium chloride injection, and tetrazolium (MTT) were obtained from the Shanghai McLean Biochemical Technology Co., Ltd. (Shanghai, China). PEG was obtained from the Jinan Century Tongda Chemical Co., Ltd. (Shandong, China). RAW264.7 mononuclear macrophages were obtained from the Hunan Fenghui Biotechnology Co., Ltd. (Hunan, China). Phosphate-buffered saline (PBS) solution and dimethyl sulfoxide (DMSO) solution were obtained from the Shandong Johnson Chemical Co., Ltd. (Shandong, China). Wistar rats were obtained from the Kaixue Biotechnology Co., Ltd. (Shanghai, China). Caerulein (Cn) was obtained from the Beijing Solebo Technology Co., Ltd. (Beijing, China). Lipopolysaccharide (LPS) was obtained from the Shanghai Jizhi Biochemical Technology Co., Ltd. (Shanghai, China). The ELISA kit was obtained from the Quanzhou Ruixin Biotechnology Co., Ltd. (Fujian, China). The Multiskan Sky full-wavelength enzyme-labeling instrument was obtained from Semel Fisher Technologies (Massachusetts, USA). The oven was obtained from the Shanghai Jinwen Instrument and Equipment Co., Ltd. (Shanghai, China).

2.2. Preparation of PEG-EMD Lip

PEG–EMD Lip was prepared by a thin-film dispersion ultrasonic method. Briefly, PEG and EMD were dissolved in 10 mL anhydrous ethanol at a mass ratio of 5:2, respectively. The uniform thin film was obtained by the decompression and rotary evaporation of the solvent at 45 °C, and then hydration at normal pressure for 16 min with normal saline. PEG-EMD Lip was obtained by ultrasonic filtration for 5 min (power: 300 W).

2.3. *In Vitro* Studies

2.3.1. Cell Culture

Frozen RAW264.7 mononuclear macrophages were cultured in Dulbecco's modified Eagle's medium at 37 °C and 5% CO_2. After continuous culture for 36 h, the medium was changed every other day and cell growth was monitored each day. When the cells reached 80% confluence, the cells were subcultured and used for experiments.

2.3.2. Cell Viability Tests

RAW264.7 cells in the logarithmic growth phase were seeded in 96-well plates at a density of 1.0×10^5 cells/well. After 20 h, the culture medium was removed and a PEG–EMD Lip solution was added at a range of concentrations (5.0, 10, 15, 20, 25 μg/mL). The EMD Lip and PEG–EMD Lip groups were established and cultured for 1 day. After removing the supernatant, 200 μL of 0.50% MTT solution was added to the wells. After incubation for 3 h, DMSO solution was added at a concentration of 150 μL/well. The plates were shaken evenly, the OD values at 490 nm were measured with an enzyme-labeling instrument, and the cell viability was calculated.

2.4. *In Vivo* Studies

2.4.1. Construction of SAP Model Rats

Forty-eight wistar rats aged 5–6 weeks were selected for the study. After the rats were adaptively fed for 1 week, the SAP model was established. Twelve rats were assigned randomly to a control group. The remaining rats were injected intraperitoneally with Cn at a concentration of 40 μg/kg once every 2 h for a total of six times. After the sixth injection, LPS was injected intraperitoneally at a concentration of 8 mg/kg. The handling and disposal of the rats were consistent with international ethical and moral standards.

2.4.2. Treatment of Rats

The SAP model rats were distributed randomly into three groups based on their body weight: a model group, an

EMD Lip group, and a PEG-EMD Lip group, with 12 rats in each group. The rats were treated immediately. Rats in the control and model groups received an intraperitoneal injection of glucose and sodium chloride (2.0 mg/kg). The rats in the EMD Lip group were given equivalent doses of EMD Lip. The rats in the PEG–EMD Lip group were administered equivalent doses of PEG-EMD Lip intraperitoneally.

2.4.3. Detection of Inflammatory Cytokines

Fresh blood (0.1 mL) was collected from the retroorbital venous plexus before and after treatment. The blood was centrifuged at high speed and the supernatant was extracted. The assay was performed by following the ELISA kit instructions for the inflammatory factor content, which included tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6).

2.4.4. Detection of Oxidative Stress Index in Pancreatic Tissue

After anesthesia, the pancreatic tissue of the treated rats was removed surgically. After rinsing with PBS solution, a tissue homogenate was prepared. The supernatant was obtained by centrifugation and the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and the content of malondialdehyde (MDA) were measured by chemical colorimetry.

2.4.5. Determination of Wet-Dry Weight Ratios of Pancreas and Lung Tissues

After 6 days of treatment, the rats were decapitated. The pancreas and lung tissues were dissected and collected into a dish containing normal saline. After absorbing water from the surface of the tissue, the pancreas and lung tissues were weighed to obtain the wet weights. The tissue was placed in a 60 °C oven for 2 days and the mass was reweighed to obtain their dry weight. The wet–dry weight (W/D) ratio of the pancreas and lung tissue was calculated.

2.5. Statistical Methods

SPSS 26.0 statistical software (IBM SPSS Inc., Chicago, IL, U.S.A.) was used for data processing and analysis. A t-test was used to compare the data (mean $\pm$ SD). $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Cell Activity

The effects of PEG-EMD Lip on the activity of cultured RAW264.7 cells were determined by the MTT assay, and the results are shown in Figure 2. The results indicated that a single EMD Lip dose exhibited greater cytotoxicity. The activity of RAW 264.7 was associated with the concentration of EMD Lip. However, EMD Lip modified by PEG exhibited a decreased effect on the activity of RAW264.7 cells ($P < 0.05$), which suggested that the PEG–EMD Lip treatment of SAP is associated with reduced toxicity and side effects.

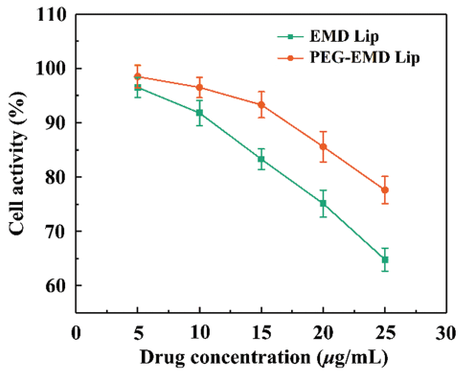

Fig. 2. Effects of various concentrations of pegylated emodin liposomes (PEG-EMD Lip) on the activity of RAW264.7 cells.

3.2. Measurement of Inflammatory Markers

Inflammatory cytokines (TNF-α, IL-6) are important chemical factors involved in vascular and leukocyte reactions. They mediate the inflammatory response by damaging organs and affecting their normal physiological function. The TNF-α and IL-6 levels in the serum of rats were measured by ELISA, and the results are presented in Figure 3. Compared with the model group, the level of TNF-α in the blood decreased in the EMD Lip and PEG–EMD Lip groups ($P < 0.05$), particularly in the PEG–EMD Lip group (Figs. 3(A and B)). Compared with the model group, the expression level of IL-6 also showed a downward trend in the EMD Lip and PEG-EMD Lip groups, and PEG-EMD Lip was the most effective at controlling the expression of inflammatory factors ($P < 0.05$).

3.3. Detection of Oxidative Stress in Pancreatic Tissue

Pancreatic injury affects the antioxidant function of tissues. The oxidative stress indices of pancreatic tissue (MDA, GSH-Px, and SOD) were measured to evaluate the effect of PEG-EMD Lip on SAP rat pancreatic tissue (Fig. 4). Compared with the control group, the activities of SOD and GSH-Px in the pancreatic tissue of the model group were decreased with a high MDA status ($P < 0.05$). In SAP rats treated with EMD Lip and PEGEMD Lip, the activities of SOD and GSH-Px in the pancreatic tissue were increased significantly and the secretion of MDA was controlled (Figs. 4(A–C)). However, the variation of each index was larger in the EMD Lip and PEG-EMD Lip groups ($P < 0.05$), whereas PEG-EMD Lip was more effective in relieving oxidative stress in rat pancreatic tissue.

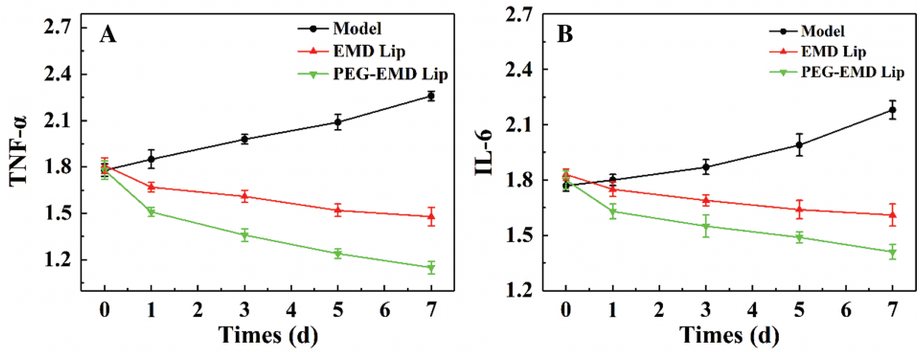

Fig. 3. Detection of factors involved in inflammation: (A) tumor necrosis factor-α (TNF-α) and (B) interleukin-6 (IL-6).

3.4. Determination of W/D in Rat Pancreas and Lung Tissues

The W/D values of the pancreas and lung tissues were determined to evaluate the degree of damage (Fig. 5). Compared with the control group, the wet weight of the pancreas and lung tissues in the model group increased significantly and resulted in a significant increase in the W/D ratio ($P < 0.05$). The analysis demonstrated that the water content in the pancreas and lung tissues was increased, whereas the pancreas and lung tissues were damaged significantly (Figs. 5(A and B)). After the SAP rats were treated with EMD Lip and PEG-EMD Lip, the W/D ratio decreased significantly ($P < 0.05$), and the drug had a significant effect on protecting the pancreas and lung tissues. PEG–EMD Lip exhibited significantly reduced damage in the SAP rats' pancreas and lung tissue.

3.5. Discussion

The complexity of SAP often results in unpredictable damage in patients. In recent years, the treatment of SAP has evolved toward a multidisciplinary, minimally invasive, rapid, and timely approach. Yet the fatality ratio of SAP remains high. Therefore, we prepared PEG-loaded EMD Lip to explore their potential application in the treatment of SAP.

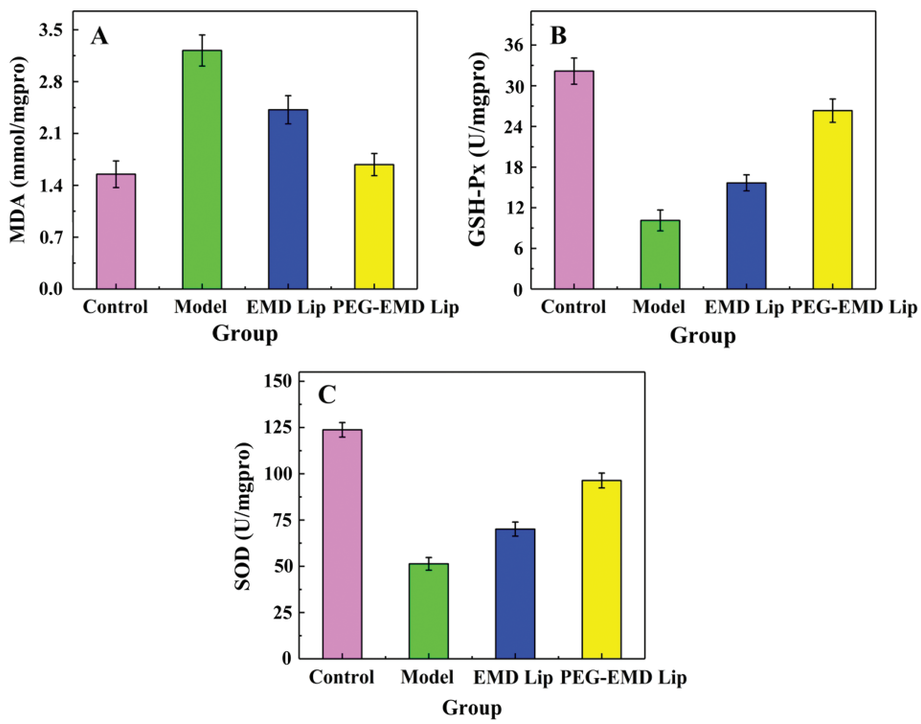

Fig. 4. Detection of oxidative stress indices of pancreatic tissue: (A) malondialdehyde (MDA); (B) glutathione peroxidase (GSH-Px); and (C) superoxide dismutase (SOD).

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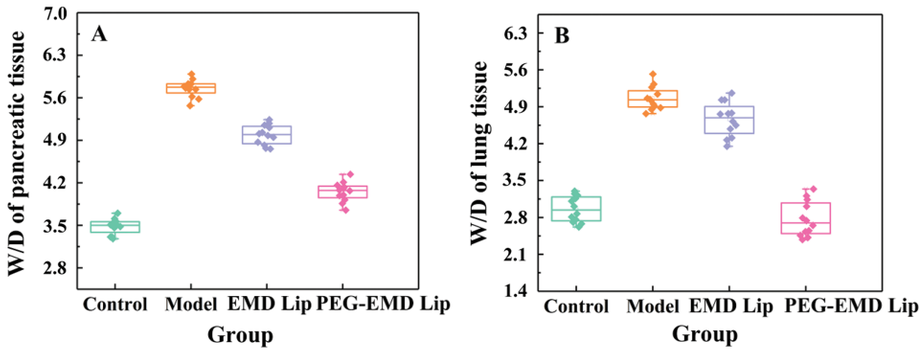

Fig. 5. Determination of the wet-dry (W/D) weight ratio of pancreas and lung tissue in rats: (A) W/D of pancreatic tissue and (B) W/D of lung tissue.

Previous studies have shown that an imbalance in immune regulation is a key factor in the death of patients with SAP. TNF-α, which is produced mainly by activated macrophages and lymphocytes, is a common inflammatory mediator that regulates the inflammatory response. IL-6, a cytokine with a wide range of functions, is a key player in regulating the immune imbalance. In the present study, high levels of TNF-α and IL-6 in the serum of SAP rats predicted the severity of the inflammatory response. However, TNF-α and IL-6 levels decreased in rats treated with PEG–EMD Lip, indicating that PEG–EMD Lip inhibits inflammation effectively.

The pancreas is a vital organ in the body [25–28]. Many components, such as islets, pancreatic cells, and pancreatic ducts, are scattered on the surface of the tissue and renders it extremely sensitive to oxidative stress [29, 30]. We found that the activities of SOD and GSH-Px in the SAP rats were very low and the MDA level was markedly elevated, which indicated that oxidative stress was a significant problem in the rats. PEG–EMD Lip treatment significantly increased the activities of SOD and GSH-Px and reduced MDA levels, which resulted in an enhanced antioxidant capacity of pancreatic tissue and reduced the damage of SAP to vital organs.

SAP not only causes damage to the pancreatic tissue but is also associated with lung injury and other complications, which if serious enough, will lead to organ failure. Treatment with PEG–EMD Lip can alleviate the inflammatory and oxidative stress reactions, which ameliorate damage to the local pancreatic tissue and distant organs, while reducing SAP-induced mortality.

In summary, PEG–EMD Lip has significant activity in the treatment of SAP. It affects immune regulation, alleviates inflammatory and oxidative stress, and provides a strategy for physicians to treat SAP. Through additional studies, we hope to provide an effective treatment for clinical use.

4. CONCLUSION

We prepared polyethylene glycol–emodin liposomes (PEG–EMD Lip). These composite nano-drugs exhibited reduced cytotoxicity and ameliorated the serious side effects of the standard EMD drug. PEG–EMD Lip was evaluated in SAP model rats with respect to the production of inflammatory factors that regulate the immune system. The composite nano-drug exhibited a significant effect in alleviating oxidative stress in pancreatic tissue and protected the pancreas and lung tissue from injury.

Ethical Compliance

Research experiments conducted in this article with humans were approved by the Medical Ethics Committee of the Hwa Mei Hospital, University of Chinese Academy of Sciences following all guidelines, regulations, legal, and ethical standards as required for animals.

Conflicts of Interest

There are no conflicts to declare including any competing financial interest.

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