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Research Article

Paclitaxel-Loaded Mpeg-PLA-Lys(Fmoc) Nanomicelles: A Novel Formulation to Prevent Hypersensitivity Reactions

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Abstract

Commercial paclitaxel injections often induce severe Hypersensitivity Reactions (HSRs) in patients. Although lipid- and albumin-based paclitaxel formulations have been developed, they still require dexamethasone pretreatment and only partially mitigate HSRs. Here, we designed a paclitaxel-loaded nanomicellar drug delivery system based on mPEG-PLA modified with Fmoc-Lys(Boc)-OH. The introduction of Fmoc-Lys(Boc)-OH enhanced π-π conjugation and weak interactions (e.g., hydrogen bonding, van der Waals forces) between the micellar carrier and paclitaxel, improving blood stability and tissue permeability. Animal experiments confirmed that the paclitaxel-loaded micelles did not induce HSRs. Thus, this novel nanomicellar formulation can be administered intravenously without pretreatment.

Keywords: mPEG-PLA, Fmoc-Lys(Boc)-OH, Paclitaxel, Hypersensitivity, Nano-micelles

Introduction

Paclitaxel is a highly effective natural anticancer drug widely used in the treatment of breast, ovarian, and lung cancers. It exerts its therapeutic effects by binding to microtubules in cancer cells, inhibiting mitosis, and inducing apoptosis [1]. However, patients inevitably experience varying degrees of hypersensitivity reactions (HSRs) during paclitaxel administration, including dyspnea, vomiting, urticaria, hypotension, and erythema [2-4]. Current research proposes two mechanisms to explain paclitaxel-induced HSRs: (1) classical type I hypersensitivity mediated by immune system-stimulated IgE production, cytokine release reactions (CRR), or mixed responses [5]; and (2) direct binding of paclitaxel to mast cells and basophils, triggering the release of bioactive mediators such as histamine, leukotrienes, and kinins [6-7]. Severe allergic reactions, including bronchospastic dyspnea, urticaria, and hypotension, are associated with these mediators. The most common commercial paclitaxel formulation contains polyoxyethylated castor oil (CrEL) as an excipient, which may induce acute HSRs such as flushing, rash, hyperlipidemia, dyspnea, and hypotension [8-9]. The underlying mechanism involves interactions between anti-cholesterol antibodies and hydroxyl groups on CrEL, activating complement C3 and leading to mast cell degranulation and histamine release, significantly increasing patient discomfort and treatment risks [9]. Once an HSR occurs, re-exposure to the allergenic drug may trigger sudden release of inflammatory mediators from activated mast cells and basophils, resulting in life-threatening HSRs [10].

Dexamethasone, a glucocorticoid, is commonly used to mitigate paclitaxel-induced HSRs [11]. Clinically, dexamethasone is administered orally 12 and 6 hours before paclitaxel infusion or intravenously 30–60 minutes prior to reduce HSRs [12]. However, long-term dexamethasone use may lead to osteoporosis, gastrointestinal ulcers, and Cushing's syndrome. Studies indicate that current clinical doses of dexamethasone are excessive, and

increasing the dose does not enhance its anti-HSR efficacy [13-14]. Notably, pretreatment with dexamethasone, diphenhydramine, and cimetidine fails to completely eliminate paclitaxel-associated HSRs. Some patients still experience severe HSRs despite pretreatment [15-16], and may even develop allergies to the anti-allergic agents themselves [17]. Additionally, pretreatment imposes additional treatment burdens on patients.

Due to the adverse effects of paclitaxel injections, cumbersome pretreatment protocols are required before infusion. Consequently, developing paclitaxel alternatives with reduced HSR risks is critical. Paclitaxel liposomes and albumin-bound nanoparticles were designed to alleviate hematotoxicity and HSRs. However, their outcomes remain unsatisfactory: paclitaxel liposomes still require low-dose dexamethasone pretreatment, while albumin-bound formulations, though dexamethasone-free, suffer from poor blood stability, leading to premature drug release and subsequent HSRs. Thus, drug carriers that prevent direct contact between paclitaxel and blood components are necessary. The Fmoc-Lys(Boc)-OH, group is a lysine derivative containing both Fmoc (9-fluorenylmethoxycarbonyl) and Boc (tert-butoxycarbonyl) protective groups. The hydrophobicity and steric hindrance of the Fmoc group enhance micellar stability, while the acid-labile Boc group enables controlled drug release. Additionally, this molecule may act as a potential inhibitor of anti-apoptotic proteins, promoting cancer cell apoptosis [18]. The dual protective groups not only optimize micellar drug-loading capacity but also enable pH-responsive targeted drug release, offering greater flexibility in controlled-release mechanisms compared to single modifications (e.g., PEGylation or hydrophobic chain modifications). Fmoc-Lys(Boc)-OH exhibits excellent biocompatibility due to its amino acid backbone; its degradation products (lysine, carbon dioxide, etc.) are naturally biocompatible, minimizing immunogenicity risks, unlike some modifications (e.g., PLGA), which may induce inflammatory responses.

In this study, an mPEG-PLA-Lys(Fmoc) micellar carrier was designed for paclitaxel delivery. Compared to traditional mPEG-PLA, the incorporation of Fmoc-Lys(Boc)-OH strengthens π-π conjugation between paclitaxel and the micellar carrier. The Fmoc group exhibits strong π-π stacking with aromatic molecules, enabling more stable and efficient drug loading [19]. Furthermore, functional groups in paclitaxel (e.g., hydroxyl and carbonyl groups) interact with the micellar matrix via hydrogen bonding, which is further enhanced by the Fmoc-Lys(Boc)-OH group. These hydrogen bonds stabilize paclitaxel within the micelles, preventing premature release. Unlike other modifications (e.g., fatty acid chains or cholesterol), which rely solely on hydrophobic interactions, Fmoc-Lys(Boc)-OH forms stronger physical bonds with paclitaxel's benzene rings through combined π-π stacking and hydrophobic interactions, significantly improving drug encapsulation efficiency and loading capacity.

The paclitaxel-loaded micelles were prepared using the thin-film hydration method for animal HSR experiments. Vascular and muscular irritation tests in rabbits revealed no significant abnormalities at a dose of 12 mg/kg, with results comparable to the 0.9% sodium chloride control group. Additionally, no hemolysis or aggregation was observed in rabbit blood. These findings suggest that the micelles do not induce HSRs and can be administered intravenously without pretreatment. This novel paclitaxel nanomicellar formulation may provide a solution to HSRs caused by traditional paclitaxel formulations, thereby alleviating patient suffering during treatment.

Materials and Methods

Materials

Paclitaxel (PTX, purity >99.5%) was purchased from Jiangsu Hengrui Pharmaceuticals Co., Ltd. (China). Polyethylene glycol-polylactide (mPEG-PLA, Mn=2700, Mn(PEG): Mn(PLA)=3:1), pivaloyl chloride, and 4-pyrrolidinopyridine were obtained from J&K Technology Co., Ltd. (Beijing, China). Fmoc-Lys(Boc)-OH (purity >99.0%) was sourced from GL Biochemical Co., Ltd. (Shanghai, China). Triethylamine (TEA, purity >99.5%) was procured from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Tetrahydrofuran (THF, purity >99.0%) and ethanol (purity >99.5%) were supplied by Shanghai Titan Technology Co., Ltd. (Shanghai, China) and China National Medicines Chemical Reagent Co., Ltd. (Shanghai, China), respectively. Diethyl ether (purity >99.5%) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China).

Animals

New Zealand rabbits (conventional grade), weighing 2–3 kg (8 rabbits, half male and half female), were identified in accordance with the Standard Operating Procedure for Rabbit Identification (SOP-ZJGLP-DW05-12/3). The rabbits were supplied by the Zhejiang Experimental Animal Center.

Methods

Synthesis of mPEG-PLA-Lys(Fmoc)

In a flask, Fmoc-Lys(Boc)-OH (7.03 g, 15 mmol) and triethylamine (TEA, 2.08 mL, 15 mmol) were weighed and dissolved in 50 mL of tetrahydrofuran (THF), followed by cooling to -10°C. Pivaloyl chloride (1.83 mL, 15 mmol) was then added dropwise, and the mixture was magnetically stirred at 0°C for 2 h, then at room temperature for 1 h. After completion of the reaction, the mixture was filtered to remove insoluble residues. The filter cake was washed with a small amount of THF, and the filtrates were combined. The solvent was removed under reduced pressure using a rotary evaporator at 40°C, yielding a colorless viscous liquid, which was dissolved in 20 mL of dichloromethane.

Next, mPEG-PLA (12 g, Mn = 2700 g/mol), TEA (2.08 mL, 15 mmol), and 4-pyrrolidinopyridine (0.22 g) were weighed and added to a flask, followed by dissolution in 60 mL of dichloromethane. The colorless viscous liquid obtained from the previous step was added to the flask, and the mixture was magnetically stirred at 0°C for 1 h, then stirred at room temperature for 36 h. Subsequently, the solvent was removed under reduced pressure using a rotary evaporator at 30°C. After evaporation, 100 mL of ethanol was added to dissolve the residue, and the temperature was immediately raised to 45–

50°C. The solution was then transferred to an oil bath pre-cooled to -20°C and magnetically stirred. Crystallization occurred after approximately 10 minutes. The mixture was vacuum-filtered three times, with each residue washed using ethanol pre-cooled to -20°C. In the final step, the filtrate was washed with diethyl ether pre-cooled to -20°C. The product was vacuum-dried at room temperature for 48 h to yield mPEG-PLA-Lys(Fmoc) as a white solid.

The chemical reaction schemes for each synthesis step are illustrated in Figure 3-1. In the first step, the carboxyl group of Fmoc-Lys(Boc)-OH reacts with the acyl chloride group of pivaloyl chloride to form the Lys(Fmoc) intermediate, producing an anhydride structure. This step enhances the reactivity of Fmoc-Lys(Boc)-OH with other reagents. The second step involves the reaction of mPEG-PLA with the Lys(Fmoc) intermediate to generate mPEG-PLA-Lys(Fmoc). In this study, the mPEG-PLA used was a pre-synthesized product with a number-average molecular weight (Mn) of 2000 g/mol for mPEG and 700 g/mol for PLA. The high reactivity of the anhydride group and the catalytic role of 4-pyrrolidinopyridine (4-Py) ensured efficient reaction progression (Figure 1).

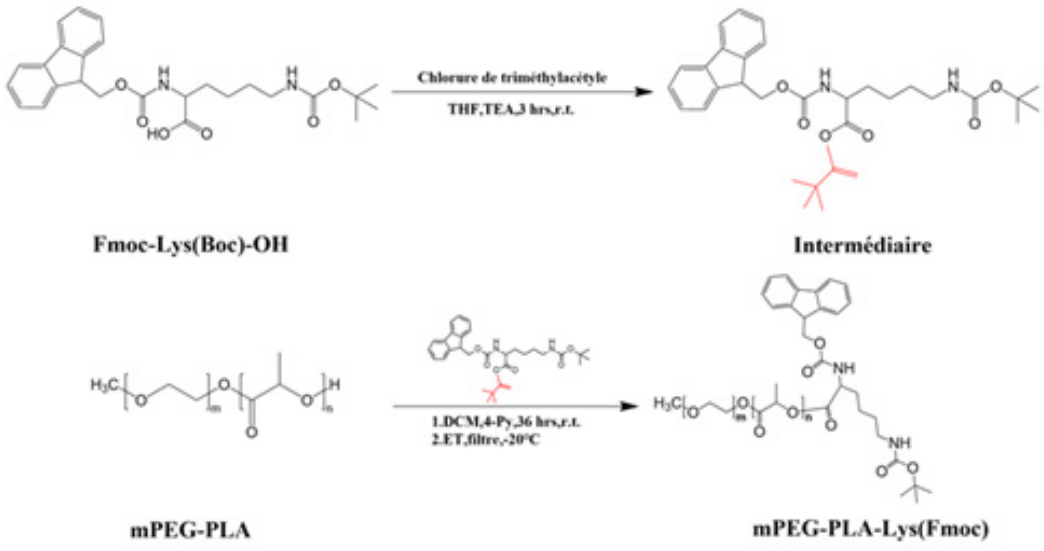

Figure 1: Preparation process of mPEG-PLA-Lys(Fmoc).

Micelle Preparation

Paclitaxel and mPEG-PLA-Lys(Fmoc) excipient at varying mass ratios were weighed and dissolved in a mixture of ethanol-water (1:3–1:5, v/v) under stirring [20]. For dialysis, the solution was transferred into dialysis bags (MWCO: 8000–10000 Da) and dialyzed in distilled water for 24-48 h with continuous stirring and water replacement. For the thin-film dispersion method, the drug and excipient were dissolved in a volatile solvent (e.g., chloroform), evaporated to form a thin film using a rotary evaporator, and then dispersed in distilled water via water bath sonication (200-400 W, 10-30 min) [21]. For the sonication method, the mixture was sonicated (20-40 kHz, 300–500 W) for 15-30 min under ice bath-controlled temperature (0–10°C). Dynamic Light Scattering (DLS) was used to measure particle size and zeta potential. Based on comprehensive evaluations of size, distribution, and zeta potential stability, the optimal formulation (particle size: 10–30 nm, zeta potential: |≥20 mV|, narrow size distribution) was selected for further studies [22].

Drug Loading and Encapsulation Efficiency:

a. **Ultrafiltration:** A suitable molecular weight cutoff (MWCO) membrane was used to separate micelles from free drug under specific pressure and temperature.

b. **Ultracentrifugation:** Separation was achieved by centrifugation at 10,000–50,000 rpm for 30–120 min.

Drug content was quantified via HPLC, and drug loading (DL) and encapsulation efficiency (EE) were calculated using standard formulas to evaluate micellar drug-carrying capacity.

Animal Studies for HSR Evaluation

HSR was evaluated through three assays: muscle irritation test, hemolysis test, and vascular irritation test.

i. Muscle Irritation Test

Eight healthy New Zealand rabbits (2–3 kg, half male and half female) with intact hindlimb muscles were randomly divided into two groups: the paclitaxel micelle formulation group and the 0.9% NaCl control group (4 rabbits per group). Prior to the test, hair over the quadriceps femoris muscles on both hindlimbs was shaved, and the skin was disinfected with 75% ethanol. A single dose of the test or control formulation was injected into each quadriceps muscle. Observations for erythema, edema, and systemic reactions (e.g., ruffled fur, lethargy, anorexia, or mobility impairment) were conducted at 24 and 48 hours post-injection. At 48 hours, rabbits were euthanized via air embolism through the ear vein. The quadriceps muscles were dissected for gross and histopathological

examination. If macroscopic irritation (e.g., inflammation, necrosis) was observed, 2 rabbits from each group were retained for histopathological re-evaluation at 14 days post-dose to assess reversibility. No recovery observation was performed if no irritation was evident.

ii. Vascular Irritation Test

Eight healthy New Zealand rabbits (intact ears, equal gender distribution) were divided into the paclitaxel micelle group and 0.9% NaCl control group (4 rabbits each). The left ear of each rabbit was disinfected with iodine and 75% ethanol. A single dose was slowly injected 1 cm from the tip of the marginal ear vein. Vascular and perivascular reactions (e.g., erythema, swelling) were monitored at 48–96 h post-injection. Using the Vascular Irritation Scoring Criteria (Gross Examination), reactions were graded. If irritation was observed, 2 rabbits per group underwent histopathological evaluation at 96 h. Three vascular segments (A, B, C) from the injected ear were fixed in 10% ASAF solution, processed for dehydration, embedding, sectioning, and staining, and scored via the Vascular Irritation Scoring Criteria (Histopathology). Remaining rabbits were observed for 14 days to assess reversibility. If no irritation was evident, all rabbits were euthanized at 96 h for histopathology.

Results and Discussion

Characterization of mPEG-PLA-Lys(Fmoc)

As shown in Figure 3-2, the proton nuclear magnetic resonance (^{1}H NMR) spectrum of mPEG-PLA-Lys(Fmoc) was analyzed. By identifying the characteristic peaks and correlating each peak with the structural formula, the successful synthesis of the compound mPEG-PLA-Lys(Fmoc) was confirmed (Figure 2).

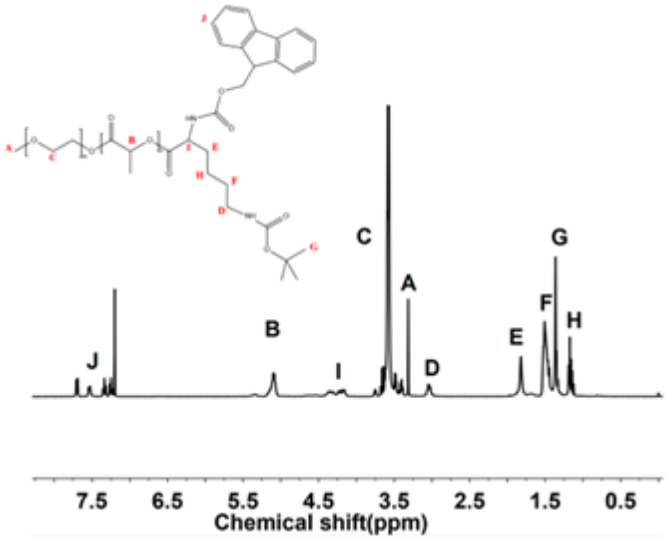

Figure 2: Proton nuclear magnetic resonance spectrum (^{1}H NMR) of mPEG-PLA-Lys(Fmoc).

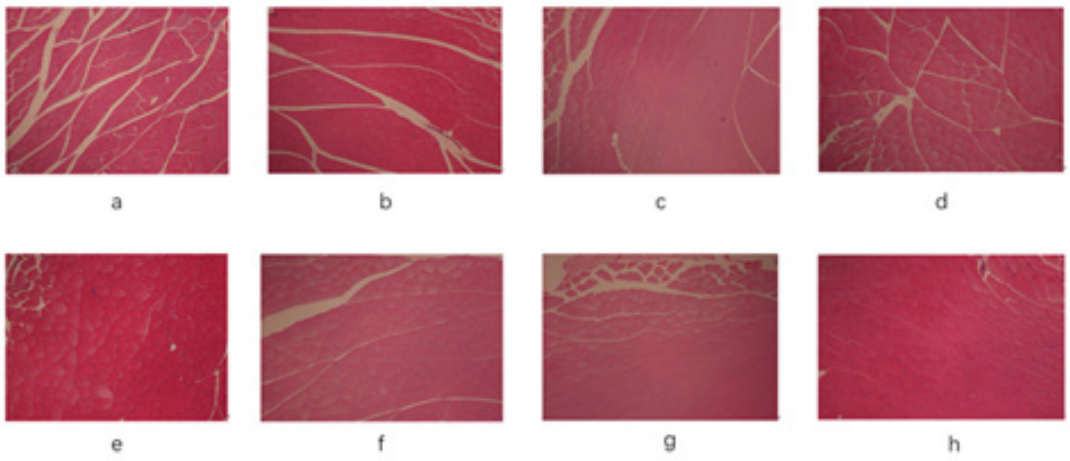

Figure 3: Histological images of brachial quadriceps muscle tissue in New Zealand rabbits:
(a-d) Tissue sections from rabbits injected with 0.9% NaCl saline solution (control group)
(e-h) Tissue sections from rabbits injected with paclitaxel-loaded micelles (treatment group

Analysis of Preparation Results

The particle size, zeta potential, drug loading, and encapsulation efficiency of micelles prepared by dialysis, thin-film dispersion, and sonication methods at varying excipient ratios are summarized in Table 1 (shown below). Key findings include:

1. Particle Size:

Dialysis-produced micelles exhibited broader size distributions across drug-to-excipient ratios.

Thin-film dispersion yielded more uniform particle sizes, while sonication showed similar trends but slightly larger sizes.

At a 1:20 drug-to-excipient ratio, all methods achieved ideal particle sizes, with thin-film dispersion demonstrating the narrowest distribution and likely superior stability.

2. Zeta Potential:

As the drug-to-excipient ratio decreased, the absolute zeta potential increased, enhancing stability.

Thin-film dispersion at a 1:20 ratio achieved the highest absolute zeta potential, indicating optimal stability.

3. Drug Loading and Encapsulation Efficiency:

Encapsulation efficiency increased while drug loading decreased with lower drug-to-excipient ratios.

Thin-film dispersion at a 1:20 ratio achieved the highest encapsulation efficiency with balanced drug loading.

After comprehensive analysis, the optimal formulation was determined as follows: mPEG polymerization degree (DP) = 45, PLA DP = 10, drug-to-excipient ratio = 1:20, and thin-film dispersion as the preferred preparation method (Table 1).

Table 1: Analysis of the results from the construction and optimization of self-assembled nanomicelles.

Preparation Methods	Drug/Excipient	Particle Size（nm）	Potential（mV）	Loading Capacity（%）	Encapsulation Efficiency（%）
Dialysis	1:05	40 ± 10	-15 ± 3	10	60
Dialysis	1:10	25 ± 8	-22 ± 4	8	75
Dialysis	1:20	18 ± 5	-30 ± 5	5	90
Film Dispersion	1:05	30 ± 6	-18 ± 4	12	65
Film Dispersion	1:10	20 ± 4	-25 ± 5	9	80
Film Dispersion	1:20	15 ± 3	-35 ± 6	6	95
Sonication Method	1:05	35 ± 8	-16 ± 3	11	62
Sonication Method	1:10	22 ± 5	-23 ± 4	8.5	78
Sonication Method	1:20	16 ± 4	-32 ± 5	5.5	92

Note*: Particle size and zeta potential were measured at 25°C using water as the dispersion medium.

Muscle Irritation Test Results

Histopathological examination at 48 h post-injection revealed microfocal lesions at the injection sites in the paclitaxel micelle group. These lesions were localized, with no significant muscle fiber degeneration, necrosis, interstitial inflammation, congestion, hemorrhage, or edema. Findings were comparable to the 0.9% NaCl control group (Figure 3). Thus, under the tested dose conditions, the paclitaxel micelles exhibited no significant muscle irritation.

Vascular Irritation Test

Following vascular irritation testing, rabbits were administered a single slow injection of paclitaxel micelles (12 mg/(kg·d), 10 mL/(kg·d)) or an equivalent volume of 0.9% NaCl into the marginal ear vein. At 96 h post-administration, gross examination revealed no abnormalities in rabbits 2, 3, 6, and 7 (paclitaxel micelle group) or rabbits 1, 4, 5, and 8 (0.9% NaCl control group). Histopathological analysis showed:

A. Rabbit 2 (micelle group): Normal structure of marginal ear vein segments A, B, and C, with intact surrounding connective tissue.

B. Rabbit 3 (micelle group): Mild dilation and congestion in segments B and C of the marginal ear vein; segment A appeared normal.

C. Rabbit 6 (micelle group): Mild dilation and congestion in segments A and C; segment B was normal.

D. Rabbit 7 (micelle group): Mild dilation and congestion in all segments (A, B, C).

Control group:

E. Rabbits 4 and 5: Mild dilation and congestion in all segments.

F. Rabbit 8: Normal segment A, mild dilation and congestion in B and C.

G. Rabbit 1: Normal vascular and perivascular structures in all segments.

No histological abnormalities, such as thrombosis, embolism, hemorrhage, edema, or necrosis, were observed in the injected ear veins or surrounding tissues of either group. These findings demonstrate that the paclitaxel micelles cause no significant vascular irritation at the tested dose, as shown in Figures 4 and 5).

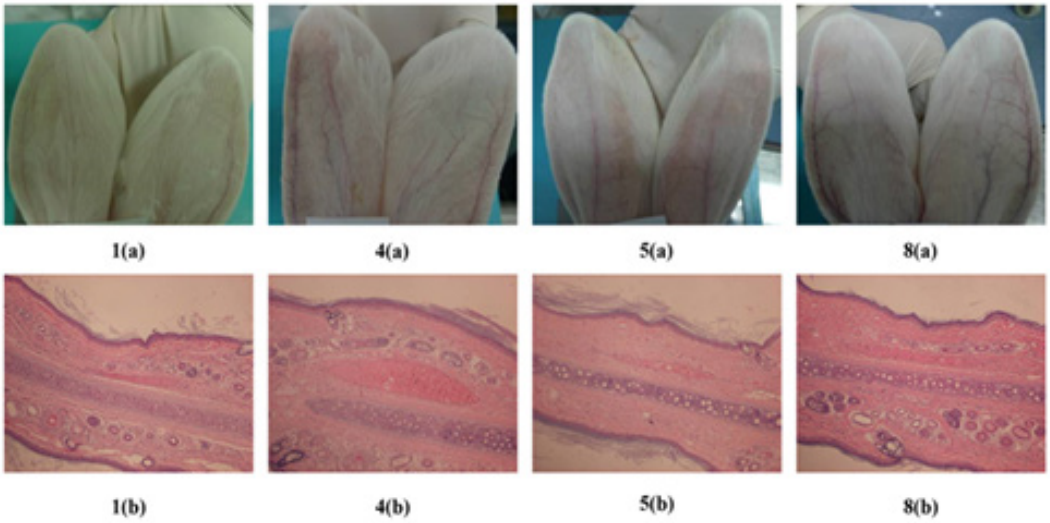

Figure 4: New Zealand rabbits injected with 0.9% NaCl solution (animals 1, 4, 5, 8):

(a) Macroscopic view of injection site (b) Histological section of auricular region

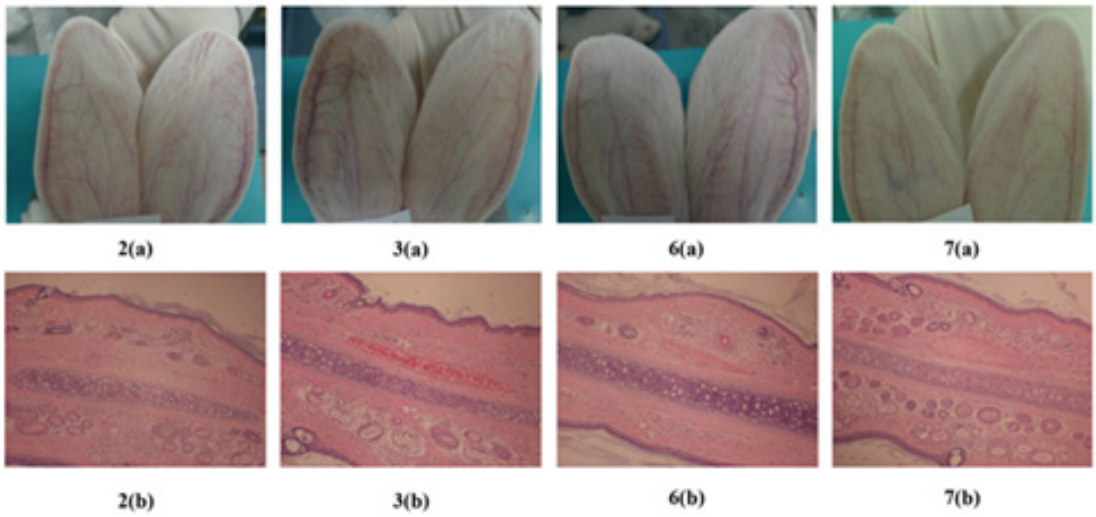

Figure 5: New Zealand rabbits administered paclitaxel nanomicelle formulation (animals 2, 3, 6, 7):

(a) Macroscopic examination of injection site

(b) Histopathological analysis of auricular region tissue

Conclusion

We designed and synthesized PEG-PLA-Lys (Fmoc) micelles for paclitaxel loading and precise in vivo delivery, and evaluated the in vitro and in vivo stability and hypersensitivity reactions (HSR) of the prepared paclitaxel micelle formulation. Compared to traditional paclitaxel injections, paclitaxel loaded in mPEG-PLA-Lys (Fmoc) micelles showed minimal HSR following intravenous administration. Thus, our micelle formulation offers a promising solution to address HSR associated with paclitaxel delivery, enhancing cancer therapy and reducing drug-related side effects. In the future, we will continue to explore potential micelle structures to further improve the safety of paclitaxel-based cancer treatment and investigate the feasibility of using micelles for delivering other traditional drugs.

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If any potential conflicts of interest arise in the future, I commit to disclosing them promptly to ensure transparency and maintain the integrity of this work.

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Conflict of Interest

None.

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