International Journal of Nanomedicine

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ORIGINAL RESEARCH

In situ Preparation of a Phospholipid Gel Co-Loaded with Methotrexate and Dexamethasone for Synergistic Rheumatoid Arthritis Treatment

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by arthrocele, cartilage damage and disability. Although several anti-RA drugs have been developed for long-term treatment, they require frequent local injection and lead to multiple adverse effects such as osteoporosis and myelosuppression.

Purpose: Reducing the amount and frequency of anti-RA drugs methotrexate (MTX) and dexamethasone sodium phosphate (DSP) by local injection of phospholipid-based phase separation gel (PPSG) coloaded the two drugs, which presented PPSG-(+).

Methods: First, We characterized PPSG-(+). And we used UV spectrophotometry and high performance liquid chromatography (HPLC) to detect drug concentration, which can clarify the drug release in vitro and in vivo, respectively. We also injected PPSG-(+) into the joint cavity of healthy rabbits to prove the safety of PPSG-(+). Then, we injected PPSG-(+) into the joint cavity of RA modeled rabbits to demonstrate the effect in anti-RA of PPSG-(+) including the thickness of joints, tumor necrosis factor (TNF)-α and interleukin (IL)-1β detection, hematoxylin–eosin (H&E) staining and computed tomography (CT) of joints.

Results: Suspended particles show a tight and uniform arrangement in PPSG-(+). The gel underwent a phase transition at 20 min in vitro and 8 h in vivo, and vesicular structures reflecting its degradation and phase transition were observed in vivo. PPSG-(+) released both drugs in a sustained and fixed ratio for more than 14 days, while it proved to be safe for intra-articular injection and did not induce inflammation in a rabbit. Eventually, PPSG-(+) showed a good anti-RA effect and its potency can be maintained for 3 weeks.

Conclusion: PPSG-(+) is a drug delivery system offering good biocompatibility and sustained release of MTX and DSP, leading to long-lasting anti-RA effect.

Keywords: drug delivery system, in situ gel, sustained release, anti rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that occurs mainly in women and can lead to severe deformation and loss of function in the joints, significantly affecting daily life.[1] The clinical treatment of RA is based mainly on symptomatic therapy such as pain relief, anti-inflammatory treatment and slowing of disease progression.[1–3] Dexamethasone sodium phosphate (DSP) is widely used in the acute phase of RA, and it shows good efficacy against inflammation and swelling. However, its long-term administration can lead to many serious adverse reactions such as myelosuppression, osteoporosis and aggravation of RA symptoms.[2] Methotrexate (MTX), a dihydrofolate reductase inhibitor, has also been widely used as a first-line drug to delay RA progression.[4] However, its application is significantly

Received: 2 August 2022
Accepted: 25 October 2022
Published: 2 November 2022

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limited due to its high toxicity, slow onset of action and narrow therapeutic window.[5,6] MTX and DSP can be given as combination therapy to patients with RA, but this requires separate, multiple dosing via oral and injectable routes.[2–4,7]

Sustained drug release systems have been developed for MTX or DSP.[8–13] For example, a previously reported formulation released MTX in a sustained manner for more than 40 days.[8] However, 50% of the drug was released in the first 12 h in vitro. Although this initially high concentration may relieve symptoms during the acute phase of RA, it may lead to toxicity during the non-acute phase.[14] Similarly, higher doses of glucocorticoids can be used to slow the rapid progression of symptoms in acute RA, but they may lead to severe adverse effects after the acute phase.[2,3] This systemic toxicity can be avoided by locally administering glucocorticoids.[15,16] Therefore, we hypothesized that administering a sustained-release formulation co-loaded with MTX and DSP locally to the joint cavity might improve therapeutic efficacy and reduce the required dose of both drugs while limiting health care costs and improving patient compliance.[15,17]

Phospholipid-based phase separation gels (PPSGs) can release drugs slowly over a few weeks without obvious burst release. PPSGs are prepared from phospholipids, anhydrous ethanol and medium-chain triglycerides or soybean oil.[18–21] Phospholipids are amphoteric surfactants, they can increase the solubility of some hydrophobic drugs, and they are the main component of the cell membrane and can be easily absorbed and degraded with good biocompatibility, while medium-chain triglycerides or soybean oil are used to minimize the irritation caused by ethanol.[22] When PPSGs enter the body, ethanol is exchanged with water, leading to gel formation in situ, which acts as a reservoir for drug delivery.[20] Nowadays, PPSG has been used as a sustained drug delivery system for chemical drugs, proteins and peptides. It can also be applied as the reservoir of antigens with adjuvant.[18–21]

In this study, we used PPSG to construct a sustained drug release system co-loaded with a hydrophobic MTX and a hydrophilic DSP for the treatment of RA (Figure 1). The co-loaded formulation, termed PPSG-(+), was prepared by dispersing MTX and DSP into PPSG. Next, we examined its rheological, degradation, and drug release properties as well as the phase transition mechanism in vivo. PPSG-(+) was injected into the joints of healthy rabbits to assess its ability to induce irritation and rejection, while its anti-inflammatory activity was evaluated in a rabbit model of RA. The results indicate that PPSG-(+) is a promising drug delivery system offering sustained release of the drugs in a fixed ratio during three weeks, both in vitro and in vivo. The developed formulation also shows a low risk of inflammation and toxicity after intra-articular injection in vivo. The gel system appears to provide better therapeutic efficacy of anti-RA than the free drugs at a lower dosing frequency.

Materials and Methods

Materials

Egg yolk lecithin (E80, phospholipid content >80%) and medium-chain triglyceride (MCT) were donated by the West China School of Pharmacy, Sichuan University (Chengdu, China). Chicken serum albumin, DSP and Karl-Fischer reagent were purchased from Macklin (Shanghai, China). MTX was purchased from Shyuanye (Shanghai, China) and complete Freund's adjuvant (10 mg/mL) from Chondrex (Woodinville, WA, USA). Enzyme-linked immunosorbent assay (ELISA) kits for tumor necrosis factor (TNF)-α and interleukin (IL)-1β were obtained from FineTest (Wuhan, China).

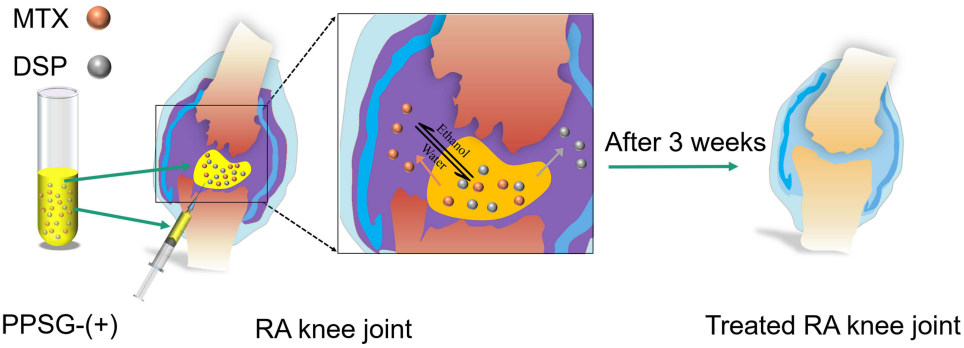

Figure 1 Schematic illustration of PPSG-(+) administration into an inflamed joint.
Abbreviations: DSP, dexamethasone sodium phosphate; MTX, methotrexate; PPSG-(+), phospholipid-based phase separation gel co-loaded with MTX and DSP; RA, rheumatoid arthritis.

Injectable methotrexate (1000 mg/10 mL; Pfizer, New South Wales, Australia) served as a control for assessing the effects of PPSG. All other reagents were of analytical grade or higher.

Animals

Healthy female New Zealand rabbits (2.5 ± 0.2 kg) were purchased from Chongqing Tengxin Biotechnology (Chongqing, China), were free access to water and food, and housed in a temperature-controlled environment in the Laboratory Animal Center of North Sichuan Medical College (Nanchong, China). All animal procedures followed the Guidelines for Care and Use of Laboratory Animals of North Sichuan Medical College, and experiments were approved by the Animal Care and Ethics Committee of North Sichuan Medical College (NSMC-AEC 2021[87]).

Preparation of PPSG-(+)

E80 (2.1 g) and MCT (0.45 g) were dissolved in 0.57 mL ethanol and stirred at room temperature for 2 h.[18] MTX and DSP were then dispersed by ultrasonication in an ice bath for 560 s at 400 W (JY 92-II, Scientz, Ningbo, China), generating PPSG-(+). The concentrations of MTX and DSP in PPSG-(+) were 25 mg/mL.

Characterization of PPSG-(+)

The scanning electron microscope (SEM) (GeminiSEM 300, ZEISS, Germany) was used to observe the micro-structure of PPSG-(+) at 3.00 kV accelerating voltage. MTX, DSP, PPSG, gelled PPSG, PPSG-(+) and gelled PPSG-(+) were analyzed by Fourier transform infrared reflection (FTIR, IS50, Thermofisher Nicolet, USA).

PPSG-(+) (4 mL) was added to a dialysis bag (molecular weight cut-off, 8–14 kDa; Solarbio, Beijing, China) and soaked in phosphate-buffered saline (PBS, pH 7.4) at 37°C.[18] At 0, 4, 8, 12 and 20 min, PPSG-(+) was placed on a plate (20 mm) of an AR 2000ex Rheometer (TA Instruments, New Castle, DE, USA), and the initial viscosity (η), storage/elasticity modulus (G') and loss/viscosity modulus (G") were measured at 25°C using a time scan pattern, frequency of 1 Hz, and constant strain of 1%.[18,23]

Phase Transition of PPSG-(+) in vivo

Rabbits were subcutaneously injected with PPSG-(+) and sacrificed at 0.5, 2, 4, 8 and 48 h post-injection. The formed gels were carefully collected, frozen, and sectioned to a thickness of 10 μm. The phase transition and degradation of PPSG-(+) were observed at different time points using an optical microscope (BX43, Olympus, Tokyo, Japan). The collected gels were also dissolved in 20 mL of absolute methanol, centrifuged and filtered. The ethanol content was determined by high-performance gas chromatography as described on an Agilent 7890A instrument (Santa Clara, CA, USA) equipped with a CP-Wax 57 CB column and a flame ionization detector. The water content was determined using a Karl-Fischer titrator (V20, Mettler Toledo, Zurich, Switzerland) following the manufacturer's instructions.[24]

Drug Release from PPSG-(+)

Drug release in vitro. PPSG-(+) (0.3 mL, 25 mg/mL) was added to a dialysis bag and dialyzed against 10 mL of PBS (pH 7.4) at 37°C with stirring at 40 rpm. At 5 min, 30 min, 1 h, 4 h and 8 h during the first day and once daily until day 14, the entire amount of PBS was collected and added to 10 mL of methanol, followed by centrifugation at 9168 g for 5 min and filtered. The concentration of MTX was measured with a UV/Vis spectrophotometer (SP-756, Spectrum Shanghai, Shanghai, China) at 306 nm. The concentration of DSP was determined by HPLC (Agilent 1260, C18 column, 4.6 × 250 mm) connected to a UV/Vis detector (G 4228 C, Agilent). The mobile phase was a 31:69 mixture of acetonitrile and a 0.5% aqueous solution of phosphoric acid, and DSP was detected at 245 nm.[25] Column temperature was kept at 25°C, the injection volume was 20 μL and the flow rate was 1.0 mL/min.

Drug retained in vivo. PPSG-(+) (0.3 mL, 25 mg/mL) was injected into the knee articular cavities of healthy rabbits. On days 1, 3, 5, 7 and 14, the rabbits were sacrificed and PPSG-(+) from the knee articular cavities was collected and extracted with 100 mL of methanol. The resulting mixtures were centrifuged, and the concentrations of MTX and DSP in the filtered supernatants were determined by HPLC using 0.5% aqueous phosphoric acid and acetonitrile as the mobile phase (gradient elution: 0–8 min, 15% acetonitrile, with detection at 306 nm; then 8–8.5 min, 31% acetonitrile, with

detection at 245 nm; 17.5–18 min, same conditions as at 0–8 min, until to 25 min before the next injection). The column temperature was kept at 25°C, the flow rate was 1.0 mL/min and the injection volume was 20 μL.

Irritation Potential of PPSG-(+) in vivo

PPSG-(+) (0.4 mL) was injected into the articular knee cavities of healthy rabbits. On days 1, 7, 14 and 21 after injection, all animals were sacrificed, and articular cavity samples were collected and stained with H&E. The irritation caused by PPSG-(+) in joint tissues was observed using an optical microscope.

Establishment of a Rabbit Model of RA

The rabbit model of RA was established based on a reported protocol.[26] First, 10 mg of albumin and 0.5 mL of complete Freund's adjuvant were injected subcutaneously into the back of rabbits once a week for two weeks to induce an immune response. Then, 5 mg of albumin in saline was injected into the knee joint cavities once a week for four weeks.

Anti-RA Efficacy of PPSG-(+) in the Rabbit Model

Rabbits with RA were randomly divided into five groups (five animals per group), which were intra-articularly injected with saline, free MTX, free DSP, a mixture of free MTX and DSP, or PPSG-(+). RA was not induced in a sixth group of five animals, which served as healthy controls. The total dosage of each drug was 10 mg. The free drug solutions were administered once a week for three weeks, while PPSG-(+) was injected only once. The size of knee joints was measured once a week with a vernier caliper. At 3 weeks after PPSG-(+) injection, all rabbits were sacrificed, and their knee joints and plasma were collected. Tissue and bone lesions in the knee joints were assessed using 16-slice computed tomography (CT; Somatom Emotion, Siemens, Munich, Germany). Histology slices were stained with H&E and analyzed under an optical microscope.

Plasma samples after 3 weeks of PPSG-(+) injection were stored at −80°C and analyzed using ELISA kits. Briefly, washed plate 2 times before adding standard sample (diluted 1/2 with sample dilution buffer) and control wells were designed as negative controls. Added 100 μL standard or sample to each well and incubate for 90 minutes at 37°C. Aspirated and washed plates 2 times. Added 100 μL biotin-labeled antibody working solution to each well and incubated for 60 minutes at 37°C. Aspirated and washed plates 3 times. Added 100 μL horse radish peroxidase–streptavidin conjugate working solution into each well and incubated for 30 minutes at 37°C. Aspirated and washed plates 5 times. Added 90 μL tetramethylbenzidine substrate solution. Incubated 15 minutes at 37°C. Added 50 μL stop solution. Recorded the optical density (O.D.) absorbance at 450 nm in microplate reader (iMark, BIO-RAD, USA) immediately after adding the stop solution.

Statistical Analysis

All experiments were conducted at least three times, and all results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using the two-tailed Student's *t*-test. The intergroup differences associated with $P < 0.05$ were considered statistically significant.

Results

Preparation and Characterization of PPSG-(+)

For the preparation of PPSG-(+), MTX and DSP were dispersed in PPSG to form a heterogeneous, golden-yellow suspension (Figure 2A), which became darker after gelation (Figure 2B). Solid particles in PPSG-(+) showed a tight and uniform closely (Figure 2C). The η, G' and G" values fell substantially during the first 4 min (Figure 2D) due to the high osmotic pressure, which accelerated ethanol/water exchange. Between 4 and 12 min, G" increased from 136.82 ± 14.59 Pa to 465.19 ± 82.46 Pa, while the G' value was 2.7 times higher at 12 min than at 4 min, suggesting greater diffusion of ethanol and onset of gelation. Moreover, the difference between G' and G", an index of elasticity, gradually increased from 4 to 20 min, consistent with gelation.[27] This result was similar to reported work, which indicated that suspension

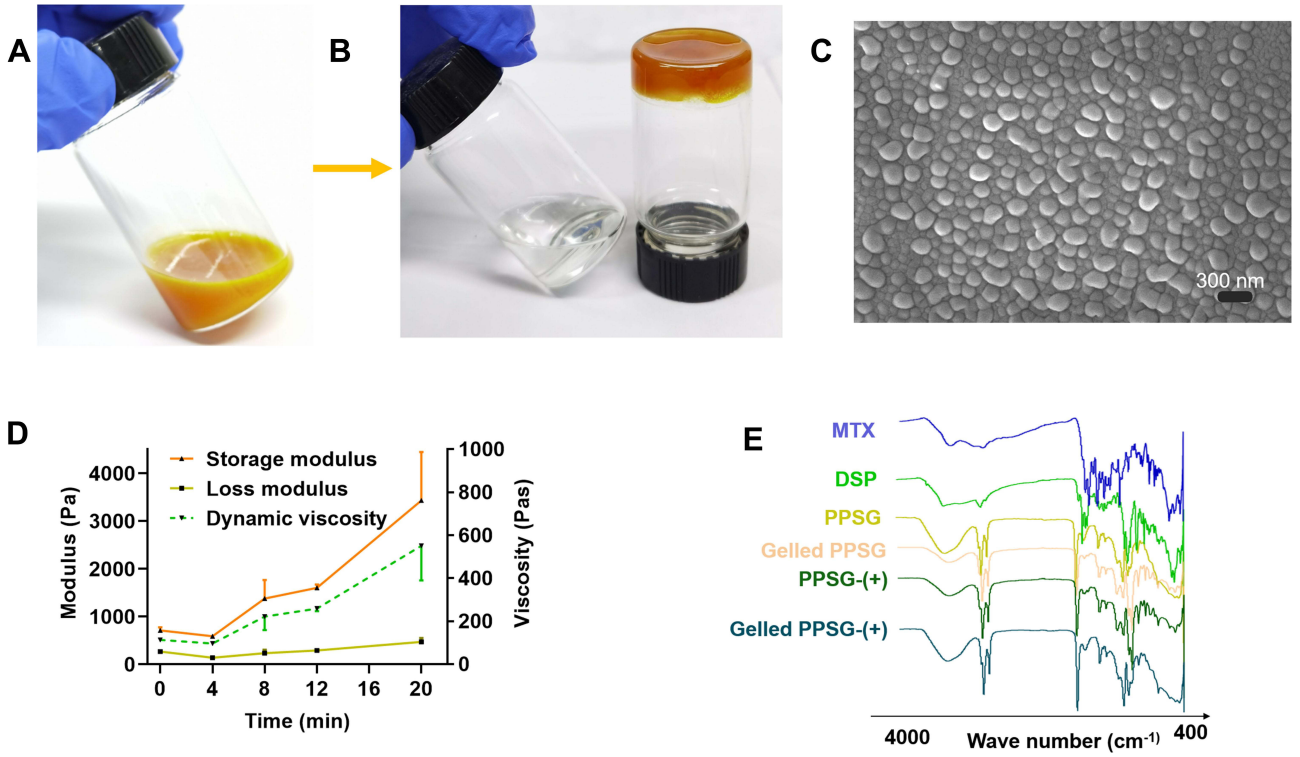

Figure 2 (**A** and **B**) Photographs of (**A**) suspended and (**B**) gelled PPSG-(+). (**C**) SEM of PPSG-(+). (**D**) Rheological characterization of PPSG-(+) in phosphate-buffered saline (pH 7.4). (**E**) FTIR of MTX, DSP, PPSG, PPSG-(+), gelled PPSG and PPSG-(+). Data are shown as mean ± SD (n = 3).

state did not affect PPSG's rheological properties.[22] FTIR showed that the particles in PPSG-(+) were loaded with PPSG in physical action (Figure 2E).

Phase Transition of PPSG-(+) in vivo

In order to elucidate the phase transition mechanism of PPSG-(+) in vivo, we observed the changes in the appearance and structure of the gel over time after subcutaneous injection into rabbits. The color of the residual gel did not change within 48 h (Figure 3A), probably because of the strong yellow color of MTX. However, the number of vesicle-like structures increased from 4 to 48 h after injection, which may reflect phase transition and gel degradation.

To further explore the phase transition tendency of PPSG in vivo, we measured the content of ethanol and water in the gel after subcutaneous injection of healthy rabbits. In the first 4 h, the amount of ethanol in the injected gel decreased sharply and that of water increased, reaching 0.206 ± 0.01 mg and 29.6 ± 3.49%, respectively, at 8 h post-injection (Figure 3C). These amounts did not change significantly thereafter, indicating that the phase transition of PPSG-(+) was completed by 8 h after subcutaneous injection. Interestingly, gelling of PPSG-(+) took significantly longer in vivo than in vitro, probably due to the higher water content in vitro. These suggested that PPSG in suspension undergoes phase transition and degradation similar to a homogeneous PPSG solution.[18]

Irritation Potential of PPSG-(+) in vivo

No obvious inflammatory lesions were observed in the joints of the injected rabbits (Figure 3B), suggesting that PPSG-(+) was safe for intra-articular injection in vivo.

Drug Release from PPSG-(+) in vitro and in vivo

Drug release from PPSG-(+) was studied in vitro using the dialysis method, and in vivo by measuring the residual amount of MTX and DSP after intra-articular injection in healthy rabbits. In vitro, the cumulative release of both drugs was about 13% at 8 h and was barely 20% over 24 h, which indicated that there was no obvious burst release of PPSG-(+). The

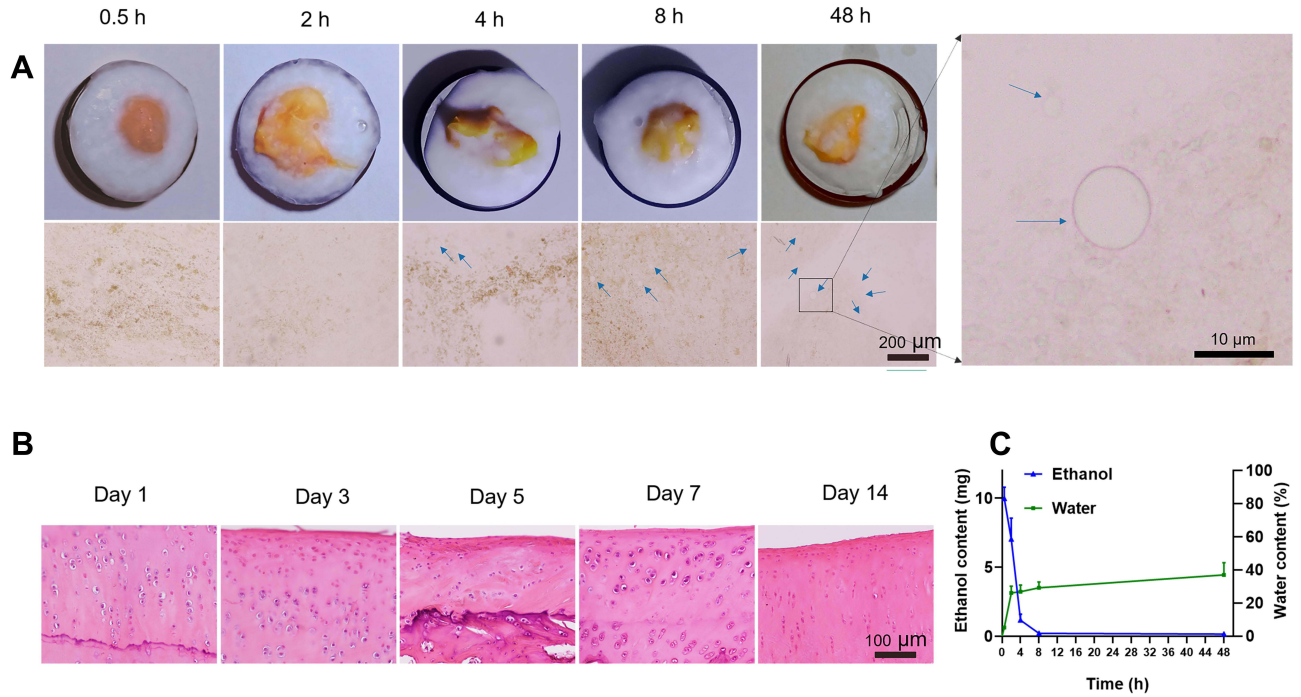

Figure 3 (**A**) Changes in the appearance and structure of PPSG-(+) over time in vivo, arrows point at vesicular structures associated with phase transition and degradation. (**B**) Histopathological changes in the knee joints of healthy rabbits after intra-articular injection of PPSG-(+). (**C**) Changes in ethanol and water content during phase transition in vivo. Data are shown as mean ± SD (n = 3).

cumulative release of MTX and DSP within 14 days reached 49.32 ± 6.20% and 34.73 ± 2.76%, respectively (Figure 4A). Similar release was observed in vivo for both drugs (Figure 4B): the amounts of residual drug in the joint cavity were 12.94 ± 1.82% for MTX and 38.9 ± 3.57% for DSP. These results indicate that PPSG-(+) can sustainably release both drugs in a fixed proportion without obvious initial burst release. MTX was released faster than DSP, probably because of the latter's higher water solubility, which favours retention in the formed gel.

Anti-RA Effects of PPSG-(+) in Arthritic Rabbits

To examine the anti-RA effect of PPSG-(+) in vivo, RA-induced rabbits were injected intra-articularly with the dual drug-loaded formulation (Figure 5A). After the first week of RA induction, redness and swelling were observed in joints, indicating the onset of the disease. PPSG-(+) reduced the joint thickness to 21.5 ± 1.12 mm after 3 weeks of treatment, similar to the thickness in the healthy group (Figure 5B).

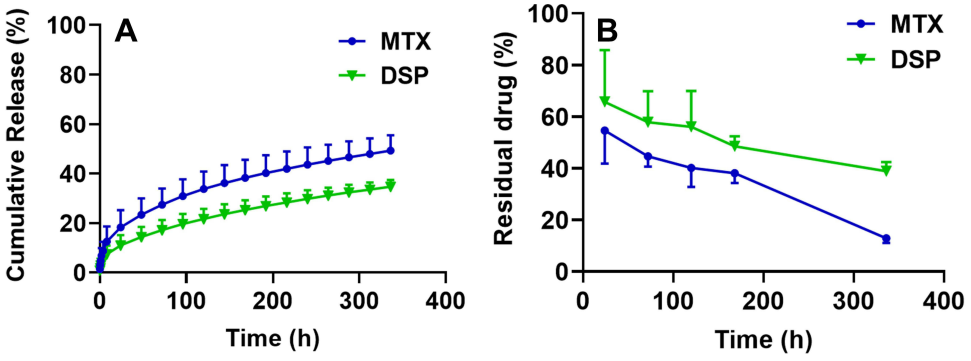

Figure 4 (**A**) Cumulative release in vitro and (**B**) residual amount in vivo of methotrexate (MTX) and dexamethasone sodium phosphate (DSP) during 14 days.

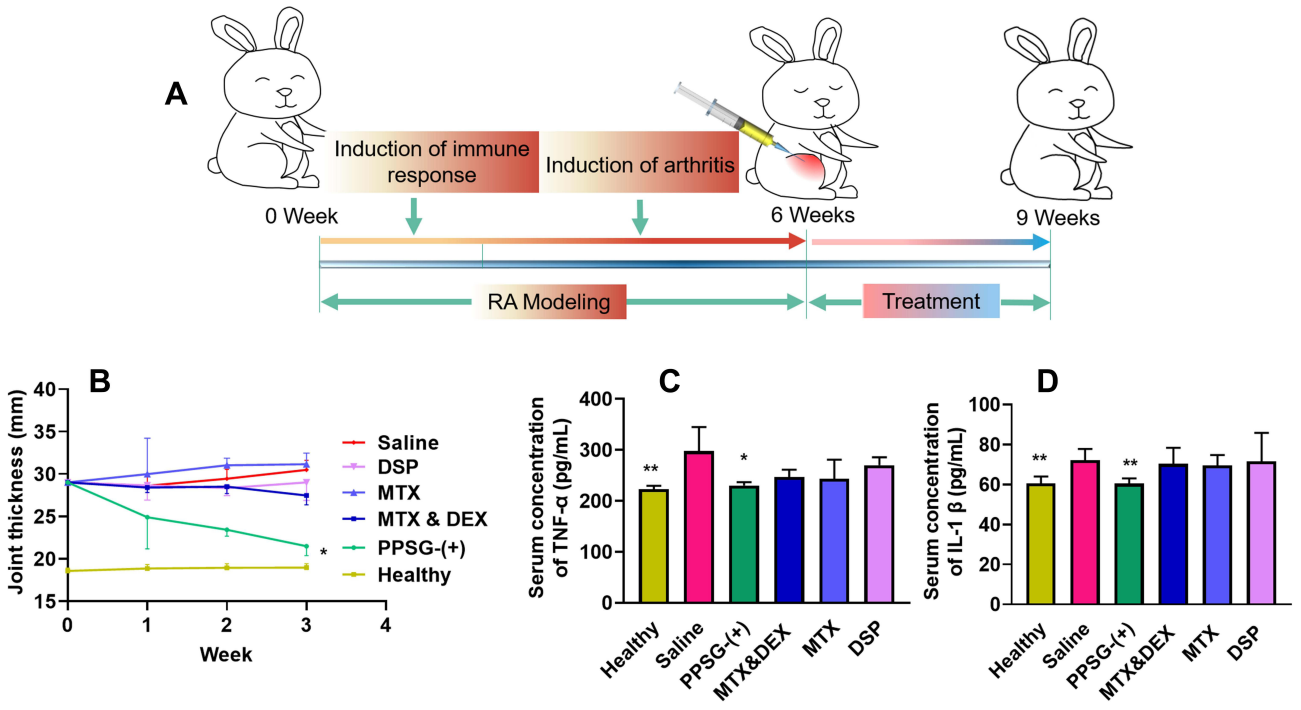

Figure 5 (**A**) Schematic illustration of rheumatoid arthritis (RA) development and treatment in vivo. (**B**) Thickness of rabbit knee joints after treatment with different formulations. (**C** and **D**) Levels of (**C**) tumor necrosis factor-α (TNF-α) and (**D**) interleukin (IL)-1β after three weeks of treatment with various formulations. Data are shown as mean ± SD (n = 5). *P < 0.05, **P < 0.01 vs saline group.
Abbreviations: DSP, dexamethasone sodium phosphate; MTX, methotrexate.

MTX can block the proliferation of T lymphocytes, playing an immunosuppressive role.[28] Similarly, glucocorticoids can strongly inhibit inflammatory responses in RA.[2] Therefore, we evaluated the anti-RA effect of PPSG-(+) by measuring the serum levels of TNF-α and IL-1β, two indices of inflammation in RA.[29–32] PPSG-(+) significantly reduced the concentrations of both TNF-α and IL-1β compared to the saline group, such that the levels were similar to those in healthy controls (Figure 5C and D). These results suggest good anti-RA-effects by PPSG-(+) delivered once every three weeks.

The development of bone and tissue lesions was evaluated by H&E staining and CT (Figure 6A and B). H&E staining revealed severe joint tissue injury and a completely destroyed articular cartilage surface in the saline group, along with extensive inflammatory cell infiltration in the soft tissues around the joint capsule, synovial hyperplasia, consolidation of the joint capsule cavity, severe bone tissue destruction and necrotic bones. Animals treated with free MTX, free DSP or their mixture also showed moderate or severe soft tissue injury and bone lesions. In contrast, the articular surface in the PPSG-(+) group was smooth, with 12 to 15 neatly arranged layers of chondrocytes with clearly visible nuclei, and the tissue showed only slight damage. Soft tissues around the joint capsule showed limited infiltration by inflammatory cells, and the synovium was mildly hyperplastic, while the bone tissue was normal (Figure 6A). These results suggest that PPSG-(+) can inhibit bone erosion in an RA model.

CT showed smooth articular surfaces and bone margins in healthy rabbits, with uniform density and no soft tissue swelling. In contrast, RA rabbits treated only with saline showed severe bone tissue damage and soft tissue swelling. These signs were somewhat milder in rabbits treated with free drugs or their mixture, and significantly milder in animals treated with PPSG-(+). Nevertheless, animals treated with the dual-loaded gel still showed some tissue damage, indicating the potential for improving the gel.

Our results in a rabbit model suggest that even a single administration of PPSG-(+) can significantly slow RA progression during three weeks, leading to considerably stronger therapeutic effects than free MTX and DSP.

Discussion

One limitation of delivering drugs in injectable PPSG is that they must first be dissolved in phospholipids, ethanol and MCT. We are aware of only a handful of drugs that have been loaded into phospholipid gels: doxorubicin, octreotide

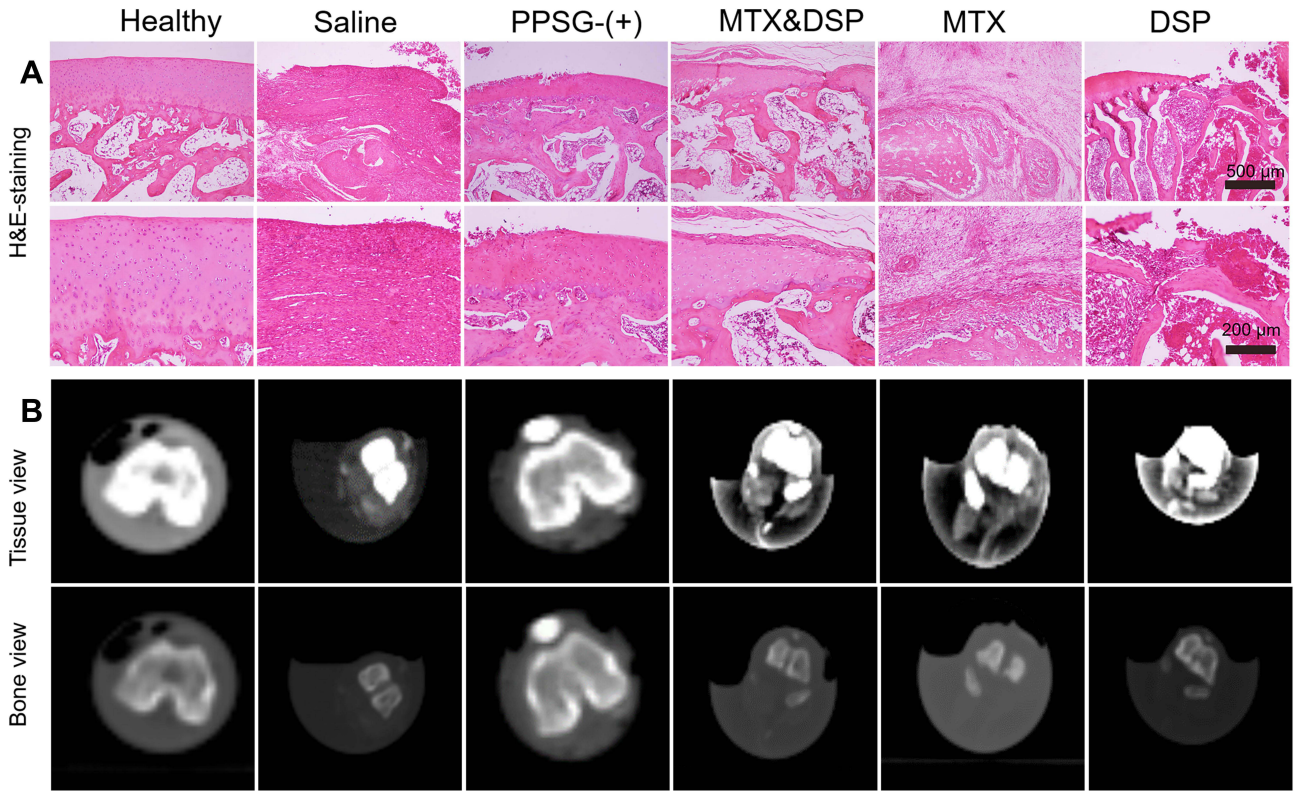

Figure 6 (**A**) Histopathological analysis and (**B**) computed tomography of knee joints from rabbits with rheumatoid arthritis after various treatments.
Abbreviations: DSP, dexamethasone sodium phosphate; H&E, hematoxylin-eosin; MTX, methotrexate; PPSG-(+), phospholipid-based phase separation gel co-loaded with MTX and DSP.

acetate, insulin, paclitaxel, and 5-fluorouracil with magnesium oxide.[18–22] In this study, two common anti-RA drugs, MTX and DSP, were loaded for the first time into a suspended phospholipid-based gel to improve their anti-RA efficacy and thereby permit lower doses and less frequent injection.

The initial viscosity and elasticity of PPSG-(+) were higher than those reported in previous studies,[18–20] probably because MTX and DSP were suspended and not dissolved in PPSG. In contrast, the phase transition mechanism, degradation and drug release from the gel were similar to those reported for insulin-loaded PPSG, suggesting that these characteristics do not depend on whether the drugs are suspended or dissolved.[18] Moreover, we found that the phase transition of PPSG-(+) was completed by 8 h after subcutaneous injection. Since water content was higher in the joint cavity of RA animals than in subcutaneous tissue, we hypothesize that phase transition occurs faster in the joint cavity.

The developed formulation released the two drugs in a sustained manner both in vitro and in vivo. Both MTX and DSP showed a release behavior near zero-order release, and it can be caused by the suspended state of drugs. And the zero-order release of PPSG can favour the sustained-drug release, maintain a duration drugs' concentration over the therapeutic window for an extended dosing cycle, and achieve a better anti-RA effective with lower side effect.[33] Usually, hydrophobic drugs release slower than hydrophilic. However, in PPSG, the carrier materials, phospholipids and MCT are hydrophobic. MTX can diffuse from the inside to the surface and then release into body fluids, while DSP is a hydrophilic drug that is difficult to diffuse in hydrophobic materials and can be released mainly by dissolution of the carrier, so MTX release is faster than DSP.

The recommended clinical dosage of MTX is 25 mg per week for RA treatment, which corresponds to 1.59 times the weekly dosage of DSP (15.75 mg).[2,34] Here, we found that the release rate of MTX in vitro was 1.42 times that of DSP, which is quite close to the desired ratio in the clinic. This suggests the potential of PPSG to achieve differential dosing regimes in combination therapy.

The concentrations of MTX and DSP were measured in the blood of rabbits based on a previously reported method.[35] Interestingly, the amounts of both drugs were below the detection limit at all time points after injecting PPSG-(+) into the knee joint cavities, which suggests that the drugs were not released or thimbleful into the blood or other organs. This supports the safety of PPSG-(+) in vivo.

MTX, a classic anti-RA drug, has a slow onset of action, but may play a crucial role in the treatment of RA.[1–4] Adenosine signaling is popular among explanations. MTX can increase adenosine levels. Then, bags of adenosine bind to the extracellular adenosine receptors and inhibit nearly all inflammatory cells.[36] Also, the binding on monocytes can suppress its synthesis and release of TNF and IL factors.[37] TNF-α plays a very vital role in regulating immune responses.[36] IL-1β is closely associated with acute and chronic inflammatory reactions.[38] Dexamethasone, as a long-acting glucocorticoid, has the efficacy of rapid relieving RA symptoms in the acute phase of RA.[1,2] Generally, DSP can play a vital role in reducing IL-1β, and the combination of MTX and DSP can be a better anti-RA efficacy.

PPSG-(+) showed good anti-inflammatory and osteoprotective effects in our animal model of RA. As we expected, all the above anti-RA effect tests, including ELISA tests, H&E staining and CTs, sustain that free MTX or DSP and the mixture of MTX and DSP were ineffective against RA, and PPSG shows much better anti-inflammatory and osteoprotective effects. This may be caused by co-delivery of the two drugs in PPSG, which keep a sustained-release action and obtain a potentiate combination therapeutic effect.

Conclusion

In conclusion, dispersion of solid drug in PPSG did not much affect its rheological characters, phase transition, degradation and drug release. Thus, the dispersion of drugs in phospholipid gel platforms is a promising strategy for the construction of sustained drug release systems with synergistic anti-RA effects, which may reduce drug-related toxicity and the need for frequent dosing. This can, in turn, improve patient compliance. PPSG also showed high safety in joint cavity injection. The sustained and fixed ratio release properties of PPSG-(+) may help personalize therapy based on disease severity, as well as avoid the drawbacks of separately administering each drug in combination therapy.

Abbreviations

RA, rheumatoid arthritis; PPSG, phospholipid-based phase separation gel; MTX, methotrexate; DSP, dexamethasone sodium phosphate; PPSG-(+), MTX and DSP coloaded in PPSG; E80, egg yolk lecithin; MCT, medium-chain triglyceride; ELISA, enzyme-linked immunosorbent assay; TNF, tumor necrosis factor; IL, interleukin; HPLC, high-performance liquid chromatograph; H&E, hematoxylin-eosin; CT, computed tomography.

Acknowledgments

This work was financially supported by the North Sichuan Medical College Doctoral Research Project (CBY19-QD06) and Research Project of Sichuan Traditional Chinese Medicine Administration (2021MS196).

Disclosure

The authors report no conflicts of interest in this work.

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