Effect of component of organogel on E2 release
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ABSTRACT SUMMARY
An optimizing of estradiol (E2) loaded L-amino acid derivatives organogel formulations was developed aimed at of resulting in improved the high initial release problems and sustained release of E2. The change of the gelator structure and concentration could affect significantly the stiffness of the implants and the release behavior.

INTRODUCTION
The design of synthetic oil-based gels systems with organogelators which can form network structures in hydrophobic solvents has received considerable research interest (1, 2). The system is injectable fluid that can be introduced into the body, which lessens the risk of implant migration and minimizes surgical defect. These kinds of organogels present a very interesting advantage as drug delivery formulations for their ease of preparation and administration. Another advantage of amino acid derivate organogels is that their metabolites are harmless completely. However, the oil miscible organic solvent (e.g. N-Methyl-2-pyrrolidinone [NMP]) was added into the organogel systems in order to disrupt interactions between organogelator molecules at room temperature. Thus, after subcutaneous administration, the drug diffused freely with such a solvent to the surrounding tissues which produced the initial burst.

Our major scientific purposes in this study was investigated the effect of composition of formulation on the initial burst of drugs and the sustained release behavior of the organogel systems.

EXPERIMENTAL METHODS
Amino acid derivate organogelators (N-Lauroyl L-lysine methyl ester (LLM) and N-Lauroyl L-alanine methyl ester (LAM)) were synthesized according to react acyl chloride with esterified L-amino acid and resulting in the formation of an amide bond. The estradiol orgaogels were prepared in three steps. First, NMP was mixed with pharmaceutical soybean oil containing E2. Second, an organogel was prepared by dissolving gelators in the oil. Finally, this mixture was mixed using a standard vortex at maximum speed for 3 min. A control formulation consisting of drug oil solution without the organogelator was also prepared.

In vitro release studies were performed in 10 mM ionic strength phosphate-buffered saline aqueous solution of pH 7.4 containing 0.1% (w/v) sodium dodecylsulfate (SDS). 1 ml of each formulation was loaded into dialysis bags (sigma) with a molecular mass cut-off of 12,000 Da. The bag was then suspended in a vial with 50 ml of the release medium. The vial was placed in shaker water bath at 37 °C with 100 rpm. At various time points, the releasing medium was emptied from the vials and replaced with 50 ml of fresh releasing medium. The releasing solution samples were filtered with 0.45 μm syringe tip filter and the concentration of E2 in different formulations were measured by reverse - phase - HPLC. Release experiments were performed in triplicate.

In vivo studies were carried out in female Sprague Dawley rats (220 - 250 g, n = 4 per group). The rats were operated of removing the ovaries and housed for 1 week under controlled conditions (12 hours light/dark schedule, 25 °C) prior to the experiment. The rats were given a single s.c. injection of one of the three gel formulations (0.4 ml, 1.2 mg/kg E2) in the dorsal area. As the control formulations, E2 was dissolved in oil solution to obtain the same dosage as the organogels. After administration, blood samples were collected (200 – 300 μ l) from the retro-orbital plexus at 0.5, 1, 2, 6, 12, 24, 48, and 72 hours. After Day 3, blood samples were taken at interval of two days. In order to obtain the serum, the blood samples were placed at the obstacle light condition (25 °C) for 2 hours, centrifuged at 3000g for 15 min. Then, the serum was determined using ELISA kit.

RESULTS AND DISCUSSION
Fig.1 showed the percentage of E2 released over time for different LLM Formulations. The initial fast release phase lasted about two days for the organogels. Increasing the proportion of LLM from 5% to 10% in the formulation decreased the release rate greatly. LLM organogels (5% and 10%) released 95.1% and 72% of total drug in 25 days at a nearly constant rate, respectively. Furthermore, there was a decrease in the initial fast release with the increase of the concentration of LLM. At the
first two days, the accumulated E2 release was 20.2% and 12.8% for 5% and 10% LLM formations.

Figure 1. In vitro release profiles of E2 from different LLM organogel formulations. Mean ± SD (n = 3).

The nature of the organogelator was also one of the key factors affecting the drug release. Fig.2 showed the percentage of E2 released from different L-amino acid derivatives. LPM formulation displayed the faster release rate and much higher initial burst than that of the others. Owning more hydrogen bond donors in molecular structure of gelator, as a consequence of forming multiple hydrogen bond interactions, can form the lower porosity and the greater stiffness gels at the same concentration.

Figure 2. In vitro release profiles of E2 from oil solution, organogels with 10% LPM, 10%LAM or 10% LLM (g/g). Mean ± SD (n = 3).

Fig.3 showed comparative in vivo profiles of different organogel formulations. The system containing higher concentration of gelator showed the longer release behavior and also improved the initial burst problems. The nature of the organogelator was also found to strongly influence the performance in vivo. Implants prepared with LAM released E2 more rapidly than those obtained with LLM in vivo (15 days vs. 30 days). The results were consistent with in vitro release of E2.

Figure 3. Comparative in vivo profiles of different organogel formulations after s.c. administration of E2. “Formulation A”: the formulation of 9%LLM (circles); “Formulation B”: the formulation of 5%LLM (triangles); “Formulation C”: the formulation of 10%LAM (squares) (n = 4).

CONCLUSION
The developed optimal gel formulation with gelator of 10% LLM displayed the lower initial drug release, showed a much lower blood drug level since the second day and maintained a steady state for nearly one month. All of these displayed the concentration and molecular structure of gelator can heavily influence the initial release of the organogel systems.

REFERENCES

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