In Situ Hydrogel for Intraperitoneal Delivery of Paclitaxel in Ovarian Cancer

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ABSTRACT SUMMARY
Injectable hydrogels for localized antitumor drug delivery were very attractive in recent years. They could provide high drug concentration within tumors and require no surgical procedures. In this study, we developed a novel paclitaxel loaded pH-responsive hydrogel and evaluated its feasibility for treatment of ovarian cancer.

INTRODUCTION
Ovarian cancer was the fifth most common cancer among women and most patients were diagnosed with advanced disease due to the lack of early-stage symptoms and reliable detection methods. Intraperitoneal chemotherapy could provide 20 to 1,000 folds higher peritoneal concentrations compared to plasma concentrations for several drugs, including cisplatin, doxorubicin, 5-fluorouracil, and paclitaxel¹. In the phase III trial by the Gynecological Oncology Group (GOG-172) demonstrated a significant survival advantage for intraperitoneal chemotherapy compared to intravenous chemotherapy in optimally debulked ovarian cancer patients. However, IP Taxol® was quickly cleared from the peritoneal cavity and it needed frequent dosing, which led to high risk of infection, pain, toxicity, or other catheter-related problems. Therefore, novel sustained-release formulations were developed for IP treatment of ovarian cancer².

In this study, a novel injectable paclitaxel loaded hydrogel (N-palmitoyl chitosan, NPCS) which showed a rapid structure transformation within a narrow pH range (pH 6.5-7.4) was developed for localized treatment of ovarian cancer.

EXPERIMENTAL METHODS
Synthesis of NPCS
Briefly, chitosan was dissolved in 1% acetic acid solution. The pH was adjusted to 6.0 by slow addition of 1 N NaOH. A solution of palmitic acid N-hydroxysuccinimide ester in anhydrous ethanol was added drop-wise to the chitosan solution at 98 ºC and reacted for 36 h. Subsequently, the prepared solution was cooled at room temperature, added acetone, and precipitated by adjusting its pH value to 9.0. The precipitate was then filtered, washed with an excess of acetone, and freeze-dried³.

Preparation of paclitaxel loaded NPCS hydrogel
Paclitaxel and TPGS were dissolved in t-butanol and the solvent was removed via freeze drying. Then the drugs were rehydrated and mixed with the NPCS hydrogel.

In-vitro release
Samples of 500 µL hydrogel were loaded into circular-shaped tube (diameter 8 mm) and then added with the 5mL release medium which comprised of 10 mM PBS (pH 7.4) containing 2.4% Tween 80 and 4% Cremophor EL. The bottles were placed in a shaking incubator at 37 ºC and 25 rpm. At different time periods, 5mL release medium was analyzed for drug conc. via HPLC. The release medium was replaced with fresh media after each removal.

In vivo pharmacokinetic studies
Paclitaxel loaded NPCS hydrogels were injected into peritoneal cavity of BALB/c mice. At day 1, 7, 14, 21 and 28 after IP injection, mice were sacrificed and the peritoneal muscle and intestine tissues were collected. The tissues homogenates were extracted and paclitaxel concentrations were analyzed by LC-MS/MS.

In vivo efficacy assessment
NOD-SCID mice were IP injected with 2 × 10⁶ SKOV-3-Luc and the treatment was initiated 7 days post SKOV-3-Luc inoculation. Taxol® (10mg/kg) and paclitaxel hydrogels(10mg/kg and 50mg/kg) were injected into peritoneal cavity and NPCS hydrogel was injected for control groups. Kaplan–Meier survival curves were constructed.
RESULTS AND DISCUSSION

The Paclitaxel loaded NPCS can be rapidly transformed into hydrogel which triggered by its environmental pH through a balance between charge repulsion and hydrophobic interaction. The formulations with different grafting ratio of palmitic acid on NPCS revealed similar release behaviors. Paclitaxel hydrogels continuously released 60% of initial loaded drugs over a period of 30 days by diffusion.

![Figure 1. In vitro release of paclitaxel loaded NPCS hydrogel.](image1)

After intraperitoneal administration of paclitaxel hydrogel, paclitaxel conc. in peritoneal cavity and intestine were lasting for 28 days at least. The plasma concentrations of paclitaxel were between 5 and 23.6 ng/mL after administration (data not shown). The localized IP delivery approach leads to high local drug concentrations and much lower systemic exposure which should theoretically result in reduced toxicity to healthy tissues such as the bone marrow.

![Figure 2. Paclitaxel level in peritoneal layer and intestine.](image2)

The median survival for the control groups and Taxol® was 69 days and it indicated that Taxol® do not have survival advantage. On the other hand, median survival was extended to 82 days and 93 days in paclitaxel hydrogel group at dose of 10 and 50 mg/kg, respectively. The results showed that continuous therapy is more efficacious in ovarian cancer.

![Figure 3. Efficacy of locally delivered paclitaxel in a murine model of ovarian cancer.](image3)

CONCLUSION

Paclitaxel loaded NPCS hydrogels were developed for treatment of ovarian cancer. This novel sustained-release paclitaxel formulation could maintain drug levels in peritoneal cavity over 28 days and demonstrate survival advantages in a murine model of ovarian cancer.

REFERENCES

3. Chiu YL, Chen SC, Su CJ, Hsiao CH, Chen YM, Chen HL, Sung HW. *Biomaterials* 2009,30, 4877-4888

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