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A novel localized co-delivery system with lapatinib microparticles and paclitaxel nanoparticles in a peritumorally injectable in situ hydrogel

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ABSTRACT

The combination of high dose of oral lapatinib (LAPA), a HER2 tyrosine kinase inhibitor, with intravenous paclitaxel (PTX) exhibited a clinical survival advantage compared with PTX alone against HER2 positive breast cancer. However, localized delivery system with high regional drug level may greatly decrease the dose of drug, leading to higher safety and lower cost. In an attempt to imitate the fast and slow exposure of these two drugs in clinic use, we incorporated PTX nanoparticles and LAPA microparticles into a thermosensitive hydrogel (PL-gel) for peritumoral injection, using PTX-gel plus LAPA-oral (P-gel + L-oral) and so on as controls. To visually study in vitro or in vivo, PTX/DID and LAPA/DIR hybrid crystals were prepared. *In vitro* and *in vivo* studies demonstrated the fast and short-term release of PTX, as well as the slow and long-term release of LAPA from the PL-gel. The most synergistic effect was found between LAPA and PTX on the cell line overexpressing both HER2 and P-gp, and the mechanisms related to LAPA-induced inhibition on P-gp expression, more G2/M phase arrest of PTX and more uptake of PTX in tumor cells. With a dose of LAPA in PL-gel group only less than 5% of that in P-gel + L-oral group, PL-gel demonstrated significant tumor suppression similar to P-gel + L-oral group, and showed longer mice survival time. Besides, PL-gel achieved more steady LAPA accumulation in tumors and revealed significantly less toxicity compared with P-gel + L-oral group. To summarize, this localized co-delivery system with good synergistic effects between LAPA and PTX might offer a potential strategy for HER2 and P-gp positive breast cancer.

1. Introduction

About 15%–20% of breast cancers are detected with an overexpression of human epidermal growth factor receptor 2 (HER2), which predicts fast growth and recurrence of tumors [1]. Therefore, targeted therapy aiming at HER2 is identified as an important strategy for breast cancer treatment. At present, there are two main kinds of targeted therapeutic agents widely used in clinics including anti-HER2 monoclonal antibodies and tyrosine kinase inhibitors (TKIs) [2].

LAPA, an oral dual inhibitor to HER1 and HER2, is expected to have a more superior activity and less drug resistance compared with mono-targeted TKIs because of its improving targeted efficacy [3]. Besides, as a small molecular TKI, LAPA exhibits a potential advantage to antibodies, which bind to extracellular domain of HER2 and may be hampered if the receptors are truncated [4]. In recent years, combining LAPA with other conventional chemotherapies such as PTX has revealed a high anti-tumor efficacy [5]. In 2008 and 2013, two phase III studies were carried out to investigate the combination effect against HER2-positive metastatic breast cancer, and the combination group offered a significant survival advantage over single PTX administration group [6,7]. Another phase III study in 2014 showed that LAPA plus PTX also demonstrated better activity in HER2-positive advanced gastric cancer [8]. Since LAPA has a transient biological activity, it is orally administrated every day to maintain the therapeutic effects during the treatment [9,10].

Inspired by the clinical results of combination, several groups studied the novel delivery systems containing LAPA and PTX recently [11–13]. Y. Wei et al. synthesized a combined micelle system of LAPA and PTX, and showed an enhanced antineoplastic effect against SKBr-3 cells whose HER2 expressions were positive [11]. D. Vergara et al. prepared LAPA/PTX polyelectrolyte nanocapsules to overcome multidrug resistance in OVCAR-3 with the ability of LAPA to improve PTX cellular delivery [12]. Another micelle formulation, PEG-PCD, was synthesized by F. Li et al. to load efficiently both therapeutic agents, and the function of LAPA acted also as a P-glycoprotein (P-gp) inhibitor in the combination treatment of prostate cancer resistant cells [13].

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Since the clinical systemic use of PTX may produce severe side effects or only have limited drug distribution in tumor, localized delivery of PTX is thought to be a nice alternate [14,15]. In addition, Tykerb® (LAPA oral formulation) delivers a limited amount of drug to tumor site and is recommended to be administrated with a very high dosage of 1250 mg/d–1500 mg/d [16,17] which might mean high risks and high costs. Localized delivery of LAPA is expected to accumulate sufficient local concentration and obtain the anti-tumor effect with much less dose of LAPA. Hence, localized delivery of both drugs might offer us a new strategy to maintain or even improve combination therapeutic efficacy, reduce systemic toxicity and so on.

In this study, we adopt thermosensitive hydrogel of Pluronic F127 (F127) as the localized delivery system for its easy preparation, good temperature-sensitive characteristics, nice biocompatibility and high regional drug maintaining [18]. In order to imitate the clinical administration of injectable PTX and oral LAPA, short-term release of PTX and long-term release of LAPA were designed in thermosensitive hydrogel for the first time. We prepared PTX nanoparticles (PTX NPs) and LAPA microparticles (LAPA MPs), and then incorporated both of them into thermosensitive hydrogel (PL-gel). After characterization, this system and its related controls were evaluated in vitro and in vivo in different types of tumor cells with various expression levels of HER2 and P-gp, followed by some mechanism study. In the *in vivo* study, PL-gel was also compared with P-gel + L-oral.

2. Materials and methods

2.1. Materials

Pluronic F127 (F127, MW = 12,600, PEO_{99}-PPO_{67}-PEO_{99}) was purchased from Sigma-Aldrich Co. LLC. (St. Louis, USA). Paclitaxel (PTX) was obtained from Sciphar Natural Products Co. Ltd. (Shanxi, China) and lapatinib (LAPA) was from CHICO Pharmaceutical Co. Ltd. (Nanjing, China). Fluorescent probe 1,1′-Dioctadecyl-3,3,3′,3′-Tetramethylindotricarbocyanine Iodide (DIR) and 1,1′-Dioctadecyl-3,3,3′,3′-Tetramethylindotricarbocyanine Perchlorate (DID) were supplied from Biotium Inc. (Cambridge, USA). Antibodies of FITC Mouse Anti-Human P-glycoprotein were from BD Pharmingen (San Diego, USA). Anti-ErbB2 antibody (ab16901) and anti-CD31 antibody (ab7388) were purchased from Abcam (Cambridge, UK). All other chemicals and reagents were of analytical grade.

Human breast cancer cell lines MCF-7 and MCF-7/ADR were obtained from Institute of Basic Medical Science, Chinese Academy of Medical Science (Beijing, China), and BT474 cells were from China Center For Type Culture Collection (Wuhan, China). Cells were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C with 5% CO_2.

Nu/nu or BALB/c nude mice (female, 6–8 weeks) were from Vital River Laboratory Animal Center (Beijing, China) and kept under SPF

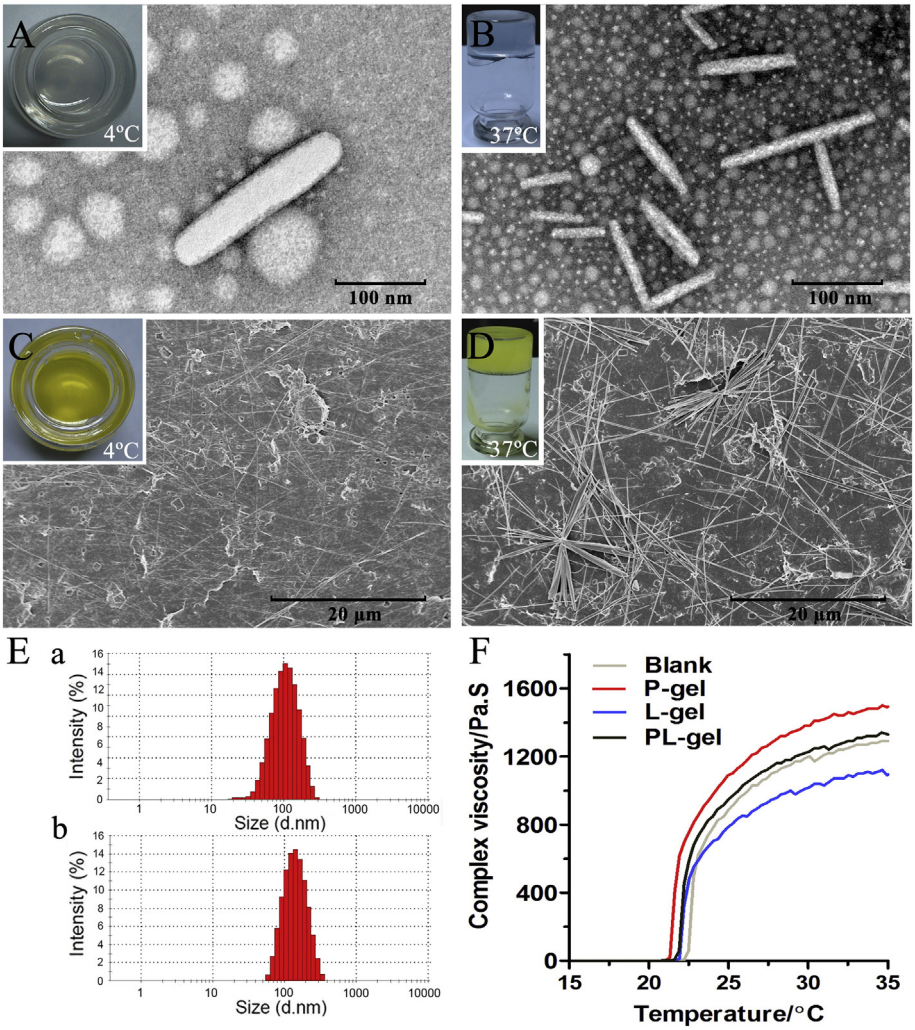

Fig. 1. Morphology, particle size and rheological study of PTX NPs, LAPA MPs, P-gel and L-gel. TEM images of PTX NPs before (A) and after (B) forming gels. Inserted pictures showed the appearance of PTX NPs suspension and P-gel. SEM images of LAPA MPs before (C) and after (D) forming gels. Inserted pictures showed the appearance of LAPA MPs suspension and L-gel. (E) Particle size of PTX NPs (a) and P-gel (b) analyzed by DLS. (F) Viscosity change of different gels from 4 °C to 40 °C.