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In vivo evaluation of an *in-situ* hydrogel system for vaginal administration

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The vaginal retention and local irritation of a carrageenan, poloxamer 407 and carbopol-based thermosensitive hydrogel system for vaginal drug delivery was assessed. Results showed that the residence of hydrogel in the mouse vagina following intravaginal administration was prolonged by carrageenan and further prolonged by the addition of a mucoadhesive component (carbopol). The optimal hydrogel formulation was proven to be safe for vaginal use in rabbits.

1. Introduction

Nowadays, *in-situ* thermosensitive hydrogels have attracted increasing interest in vaginal drug delivery due to their extraordinary properties such as easy administration and slow clearance from the vagina. For example, a poloxamer 407-based thermosensitive hydrogel has been widely used for vaginal drug delivery (Bouchemal et al. 2013; Fu et al. 2002; Zhou et al. 2013). Previously we reported a new thermosensitive hydrogel system based on the combination of poloxamer 407 and κ-carrageenan. This *in-situ* hydrogel system showed excellent sustained drug release properties *in vitro* as well as prolonged vaginal residence (Liu et al. 2009). A Chinese patent for this invention (CN 200810200716.0) has been granted. In the current study, a mucoadhesive polymer-carbopol was added into the poloxamer 407 and κ-carrageenan hydrogel system to further prolong its vaginal retention time. The *in vivo* vaginal retention and local irritation of this hydrogel system were reported.

2. Investigations, results and discussion

A change with the time of near infrared (NIR) fluorescence signal can be clearly observed using the *in vivo* NIR imaging technique after intravaginal administration of fluorescent-labeled formulations (Fig.). Mice were selected as experimental animals due to: *1)* the requirement of the commercially available whole-mouse imaging system and *2)* the possibility to compare with our previous data (Liu et al. 2009). Semi-quantitative data of the fluorescence intensity showed that the order of vaginal retention capability was: 0.2% κ-carrageenan-20% poloxamer 407–0.2% carbopol hydrogel (CPCbH) > 0.2% κ-carrageenan-20% poloxamer 407 (CPH) > 20% poloxamer 407–0.2% carbopol hydrogel (PCbH) > 20% poloxamer 407 hydrogel (PH) > normal saline solution.

κ-Carrageenan was known to be able to confer the poloxamer 407 hydrogel considerable resistance to its erosion in an aqueous environment (Liu et al. 2009), thus resulting in prolonged vaginal retention of the CPH. Thy is may be explained by the interaction between κ-carrageenan and cationic ions in the physiological environment (Pekcan and Tari 2008), for which we have also found some evidence (unpublished). The addition of mucoadhesive polymer-carbopol (De Araujo Pereira et al. 2013) into the CPH further increased its vaginal retention. However, the addition of carbopol into the PH only showed limited prolonging effect, probably due to the fast hydrogel erosion caused by carbopol previously observed by us (Liu et al. 2009).

The good accordance of the intravaginal hydrogel retention data from NIR fluorescent imaging with our previous intravaginal drug residence data supports the feasibility of the NIR fluorescent whole-mouse imaging technique for preliminary *in v*ivo assessment of vaginal formulation. This technique is charming for its convenience (no need for time-consuming quantitative analysis) and non-invasiveness (continuous observation of one animal).

Rabbits were used in evaluating the local safety of the CPCbH (the formulation with the best vaginal retention result) since rabbits are the unique animal model approved by FDA for vaginal safety evaluation and the RVI assay was the most widely accepted indicator reflecting vaginal irritancy and inflammation potential (Li et al. 2012; Yang et al. 2012). In the RVI assay, visual observations and the subjective parameters of erythema, edema, and discharge are used to evaluate injury and inflammation, and histological cross-sections from exposed tissues are scored for epithelial exfoliation, leukocyte infiltration, thickening of the lamina propria (edema) and vascular congestion.

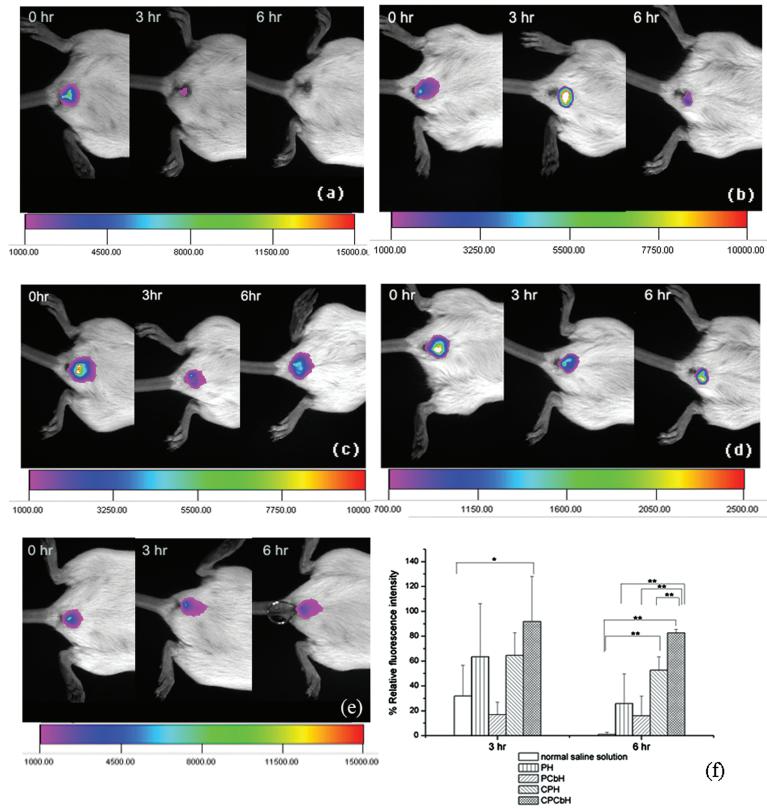

Fig.: Typical *in vivo* NIR fluorescence images of mices intravaginally administered with IR820-labeled control solution (a), PH (b), PCbH (C), CPH (d) and CPCbH (e). (f) Semi-quantitative analysis of relative fluorescence intensity around the administration site after 3 hours and 6 hours (intensity at time 0 as 100%). n = 3, mean ± SD).

After seven consecutive day's treatment, the body weight data for all three CPCH doses showed no obvious change, which was similar to that of the normal saline control group. It was noted that RVI scores for CPCbH (the medium dose) and normal saline (control) were similar, indicating its good safety profile for vaginal use. Although the CPCbH had a higher RVI score at an extreme high dose, irritation was significantly reduced after another 7-day recovery period (Table).

In summary, the prolonged vaginal retention and the acceptable vaginal irritation data demonstrated that the thermosensitive hydrogel system based on the combination of poloxamer 407, κ-carrageenan and carbopol is a promising vaginal drug delivery vehicle.

3. Experimental

3.1. Materials and animals

Poloxamer 407 was from BASF (Ludwigshaften, Germanry). κ-Carrageenan was from FTA Co. (Shanghai, China). Carbopol® 934P-NF (carbopol) was from Noveon, Inc. (Cleveland, OH, USA). Fluorescence dye IR820 and 6-aminocaproic acid were purchased from Sigma (St. Louis, MO, USA). All chemicals were analytical grade and used as received.

Female ICR mice (21∼25 g) were provided by the Department of Laboratory Animal Science, Fudan University, Shanghai, China. Female New Zealand rabbits (2.2∼2.35 kg) were provided by Chedun Experimental Animal Seed Farm, Shanghai, China.

3.2. Methods

Hydrogels, 0.2% κ-carrageenan-20% poloxamer 407–0.2% carbopol hydrogel (CPCbH), 0.2% κ-carrageenan-20% poloxamer 407 (CPH), 20% poloxamer 407–0.2% carbopol hydrogel (PCbH) and 20% poloxamer 407 hydrogel (PH) were prepared using a so-called cold method (Liu et al. 2009). Concentration of poloxamer 407, carbopol and κ-carrageenan in the in-site hydrogel was always expressed as the weight percentage (wt. %).

IR 820 fluorescent dye was modified with 6-aminocaproic acid to provide ideal fluorescence signals as previously reported (Meng et al. 2010). Mice had free access to standard food and tap water and acclimated for at least one week before use. Mice (n = 15) were randomly divided into five groups (3 mice per group). Each group was intravaginally treated with 20 μL of IR 820 fluorescent dye-containing CPCbH, CPH, PCbH, PH and normal saline (control) (10 μg/g), separately. NIR fluorescent images were obtained immediately (time 0), 3 h and 6 h after administration with a whole-mouse imaging system (Imaging Station IS2000MM, Kodak) at Ex: 690 nm/Em: 730 nm. Images were captured by the CCD camera embedded in the imaging system and analyzed using Kodak ID3.6.3 imaging software. For each mouse, residual signal intensity after 3 and 6 hours was expressed as the percentage of correspondingly initial signal intensity (time 0).

Table: Body weights and average RVI (rabbit vaginal irritation) scores for CPCbH and normal saline (control group) in rabbits after consecutive 7-day administration (once per day) (n = 6, ± SD)

	Administration dose (mL/kg)	Body weight (kg)		Irritation scores
		Before treatment	After treatment	After treatment
Normal saline	1.0	2.90 ± 0.26	2.80 ± 0.28	5.5 ± 1.64
CPCbH	0.5	2.93 ± 0.31	2.86 ± 0.29	3.67 ± 0.52*
CPCbH	1.0	2.87 ± 0.25	2.80 ± 0.12	5.00 ± 1.10
CPCbH	1.5	2.80 ± 0.22	2.78 ± 0.11	7.83 ± 1.47*

* Statistically different from the normal saline group (t-test, $p < 0.05$)

Local irritation of CPCbH was tested in healthy female rabbits which had been acclimated for 10 days before use. Rabbits were randomly divided into four groups: the control group (normal saline, $n = 6$) and three treatment groups with different doses (*i.e.* 0.5, 1.0, 1.5 mL/kg body weight, $n = 6$ per group). All the tested formulations were sterilized at 121 °C for 20 min before use and were injected into vagina once per day for seven consecutive days using a sterilized polyethylene tube attached to a syringe. The weights of each rabbits were recorded to monitor their general health condition. By the end of the 7-day treatment period, 4 out of 6 rabbits of each group were sacrificed and the vagina wall was harvested for irritation study (*e.g.* gross observation of vagina appearance and rabbit vaginal irritation (RVI) score assessment). The remaining two rabbits of each group were allowed to recover for another seven days and then sacrificed for further examination. All animal experiments were carried out in accordance with guidelines evaluated and approved by the Ethics Committee of Fudan University. Differences of the mean values were evaluated by the Student's unpaired t-test. A $p < 0.05$ was considered significant.

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References

Bouchemal K, Frelichowska J, Martin L, Lievin-Le Moal V, Le Grand R, Dereuddre-Bosquet N, Djabourov M, Aka-Any-Grah A, Koffi A, Ponchel G (2013) Note on the formulation of thermosensitive and mucoadhesive vaginal hydrogels containing the miniCD4 M48U1 as anti-HIV-1 microbicide. Int J Pharm 454: 649–652.

De Araujo Pereira RR, Ribeiro Godoy JS, Stivalet Svidzinski TI, Bruschi ML (2013) Preparation and characterization of mucoadhesive thermoresponsive systems containing propolis for the treatment of vulvovaginal candidiasis. J Pharm Sci 102: 1222–1234.

Fu YM, Du LN, Wang Q, Liao WX, Jin YG, Dong AJ, Chen C, Li ZL (2002) *In vitro* sustained release of recombinant human bone morphogenetic protein-2 microspheres embedded in thermosensitive hydrogels. Pharmazie 67: 299–303.

Li N, Yu M, Deng L, Yang J, Dong A (2012) Thermosensitive hydrogel of hydrophobically-modified methylcellulose for intravaginal drug delivery. J Mater Sci - Mater Med 23: 1913–1919.

Liu Y, Zhu YY, Wei G, Lu WY (2009) Effect of carrageenan on poloxamer-based in situ gel for vaginal use: Improved *in vitro* and *in vivo* sustained-release properties. Eur J Pharm Sci 37: 306–312.

Meng Q, Yu M, Gu B, Li J, Liu Y, Zhan C, Xie C, Zhou J, Lu W (2010) Myristic acid-conjugated polyethylenimine for brain-targeting delivery: *in vivo* and ex *vivo* imaging evaluation. J Drug Target 18: 438–446.

Pekcan O, Tari O (2008) Cation effect on gel-sol transition of kappa carrageenan. Polymer Bull 60: 569–579.

Yang J, Li L, Jin H, Tan S, Qiu J, Yang L, Ding Y, Jiang Z-H, Jiang S, Liu S (2012) Vaginal Gel formulation based on theaflavin derivatives as a microbicide to prevent HIV sexual transmission. Aids Res Human Retrovir 28: 1498–1508.

Zhou QN, Zhong L, Wei XH, Dou W, Chou GX, Wang ZT (2013). Baicalein and hydroxypropyl-gamma-cyclodextrin complex in poloxamer thermal sensitive hydrogel for vaginal administration. Int J Pharm 454: 125–134.