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Research paper

In vivo evaluation of the vaginal distribution and retention of a multi-particulate pellet formulation

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ABSTRACT

Non-disintegrating microcrystalline cellulose pellets (MCC) and disintegrating starch-based pellets were evaluated as new vaginal drug delivery forms and compared with a powder formulation. Pellets and powder were packed in a HPMC or hard gelatine capsule and vaginally administered to five series of five healthy volunteers. Distribution and retention of the multi-particulate formulation was monitored by colposcopy and swabbing. Capsule disintegration in the vagina was slow. MCC pellets clustered around the fornix 3 h after administration, and after 24 h only a few pellets were detected in the vaginal cavity. In contrast, starch-based pellets already started to disintegrate 6 h after administration, resulting in a complete coverage of the vaginal mucosa after 24 h in 8 out of 10 volunteers. The powder formulation had a better distribution after 6 h, although after 24 h almost no powder remained in the vagina. These results were confirmed by swabbing to determine the amount of riboflavin sodium phosphate (used as marker) distributed in the different vaginal regions.

1. Introduction

Vaginal drug delivery is a promising route for local and systemic drug delivery. By this delivery route the hepatic first-pass effect can be avoided and due to the presence of a dense network of blood vessels surrounding the vagina rapid absorption can be obtained [1,2]. Moreover, the vaginal mucosa has a high permeability for large molecular weight drugs, like peptides and proteins [3]. Despite the promising characteristics of the vagina for drug therapy, the development of a suitable vaginal delivery system remains an issue of concern. An optimal vaginal formulation should have (1) a long retention time to maximize drug release, (2) a proper spreading over the vaginal epithelium to obtain fast absorption or to maximize the effect in case of local treatment and (3) be easy to administer and not cause discomfort to improve patient compliance.

Different types of formulations are on the market each with their advantages and disadvantages [4,5]. The main group of commercially available vaginal delivery forms are semi-solid dosage forms like creams and gels, used to deliver hormones, antibiotics or fungicides. Gels and creams are easy to administer and have a fast drug release. However, these vaginal forms are messy to apply, can leak in the undergarments and give an uncomfortable feeling to the user [6–8]. Moreover, semi-solid formulations may not allow accurate drug dosing due to non-uniform distribution in and leakage from the vaginal cavity. Multiple administrations can be required as the retention time is limited [2]. Similar problems were reported for pessaries although they are easier to apply and provide accurate drug dosing. Large differences in retention of semi-solid formulations between individuals were reported, varying from 1% to 81% retained of the initial dose after 24 h [9]. Brown et al. [10] reported a loss varying from 9% to 97% 2 h post-dosing in post-menopausal women. Barnhart et al. [11] observed limited vaginal mucosal coverage 24 h after application of a microbicide C31G vaginal gel.

Vaginal tablets are easy to apply by the user, but vaginal disintegration of conventional tablets can be slow and due to gravity the tablets are rapidly cleared from the vagina. This can be reduced

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using bioadhesive vaginal tablets, but some studies reported the loss of bioadhesive tablets after vaginal application [12].

Vaginal rings are torus-shaped polymeric devices, most often silicone based, designed to release one or more drugs in a controlled fashion [1]. Compared to other vaginal delivery systems, they provide a more accurate and sustained drug dosing, but due to the specific production process not all drugs can be incorporated into vaginal rings.

In this study, pellets were evaluated as novel vaginal drug delivery system with the aim to achieve an acceptable retention time combined with a uniform spreading over the vaginal epithelium. Pellets are spherical particles with an average diameter of 300–1000 μm. It is anticipated that due to their small size pellets will evenly distribute in the vaginal cavity and will be less sensitive to gravity than tablets, resulting in a longer retention time. Pellets could be used as a vaginal drug delivery platform for conventional drugs (microbicides, fungicides, antimicrobial drugs) as well as for probiotic bacteria. The *in vivo* behaviour (vaginal distribution and retention) and patient acceptability (irritation, discomfort) of non-disintegrating microcrystalline cellulose pellets versus disintegrating starch-based pellets were evaluated following administration of these pellets packed in hydroxypropylmethylcellulose (HPMC) versus hard gelatine capsules. The vaginal distribution and retention of the pellets was also compared with a powder formulation.

2. Materials and methods

2.1. Materials

Microcrystalline cellulose pellets (MCC) (Cellets® 500 μm, Pharmatrans-Sanaq, Basel, Switzerland) were used as non-disintegrating pellets. Disintegrating starch-based pellets were prepared via extrusion/spheronisation using a high amylose, crystalline and resistant starch (Uni-Pure® EX starch, National Starch and Chemical Company, New Jersey, USA) as main excipient (concentration: 84.9%). Hydroxypropylmethylcellulose (HPMC) (Methocel® E15 LV EP Pharm, Colorcon, Dartford, UK) (4.9%) was used as a binder in the pellets and sorbitol (Sorbidex® P 16616, Cerestar, Vilvoorde, Belgium) (10.2%) was added to modify the consistency of the wet mass [13]. Demineralised water was used as granulation liquid. Riboflavin sodium phosphate (Certa, Braine-l'Alleud, Belgium) (concentration: 5%) was added as marker to visualise the spreading and to quantify the retention time of the formulation in the vaginal cavity.

2.2. Preparation of the pellets

Starch-based pellets were produced by extrusion/spheronisation according to Dukic et al. [13]. Dry mixing was performed in a Turbula® mixer (model T2A, W.A. Bachofen, Basel, Switzerland) for 15 min. The powder mixture was granulated with demineralised water for 10 min using a planetary mixer (Kenwood Chief, Hampshire, UK) with a K-shaped mixing arm. Water was added during the first 30 s of the wet massing phase. To ensure uniform water distribution, the material adhering to the mixing bowl was regularly removed. The wet mass was extruded at an extrusion speed of 50 rpm using a single screw extruder (Dome extruder lab model DG-L1, Fuji Paudal, Tokyo, Japan) equipped with a dome-shaped extrusion screen with 0.4 mm perforations. The extrudates were spheronised in a spheroniser having a friction plate with cross-hatched geometry (Caleva Model 15, Caleva, Sturminster Newton, Dorset, UK). Spheronisation time was 3 min and spheronisation speed 1000 rpm. The pellets were overnight dried in an oven (Memmert, Schwabach, Germany) at 40 °C. The size fraction of 315–800 μm was separated using a sieve shaker (Retsch, Haan, Germany). Pellets were packed into HPMC (size 00, Vcaps, Capsugel, Bornem, Belgium) or gelatine (size 00, Intercaps, Aca pharma, Waregem, Belgium) capsules. The number of pellets per capsule was about 7500.

In addition, HPMC or hard gelatine capsules (size 00) were also filled with a powder (about 450 mg), consisting of lactose (α-Pharma, Braine-l'Alleud, Belgium) and skim milk (Difco, Becton Dickinson, MA, USA) (ratio: 1/2, w/w).

2.3. In vitro disintegration of pellets and capsules

Simulated vaginal fluid [14] was used as test medium to evaluate the disintegration properties of the pellets using the watch glass method to simulate vaginal disintegration [15,16]: 1 g pellet was placed in the centre of a watch glass (diameter: 11 cm), which floated on a water bath at 37 °C. Simulated vaginal fluid of 4 ml (37 °C) was poured on the pellets and disintegration was evaluated by touching the pellets with dumb and index finger at regular time points. The disintegration time was defined as the time point at which the pellets consisted of a soft mass with no palpably firm, unmoistened core. Per test the pellets were only touched once to assess their disintegration. If the pellets still had a solid core upon touching, the test was repeated for a longer time using another set of pellets, to exclude interference of touching of the pellets on the disintegration time. The disintegration time of HPMC and hard gelatine capsules was assessed using the same procedure, the disintegration time was defined as the time point at which the pellets were released from the capsules.

2.4. In vivo experiments in healthy volunteers

Clinical trials were performed with healthy volunteers (five volunteers in each group) (Table 1). Volunteering women were screened to exclude gynaecological and systemic pathology, including cervico-vaginal infections or vaginal microflora alterations, according to a standardized protocol. Only nulliparous premenopausal healthy volunteers, aged between 18 and 50 year were included in the study. Study participants adhered to a strict protocol that involved an extensive list of behaviours they had to refrain from to avoid interference with the vaginal formulation, including abstinence from coitus for 48 h prior to and 24 h after administration of the product, no use of vaginal hygiene products (spray, foams, etc.) and no depilation of pubic hair. To avoid interference with menstrual or withdrawal bleeding, study participants adhered to a continuous oral contraceptive regimen during the

Table 1
Different study groups.

Group	Formulation	Capsule type	Colposcopy	RSP marker
1	Microcrystalline cellulose pellets	HPMC	3 h and 24 h	–
2	Starch-based pellets	HPMC	6 h and 24 h	+
3	Starch-based pellets	HPMC	24 h	+
4	Powder	HPMC	6 h and 24 h	+
5	Starch-based pellets	Gelatine	6 h and 24 h	+

study period. Two pellet and one powder formulations were administered: non-disintegrating MCC pellets, fast disintegrating starch pellets (containing 5% riboflavin sodium phosphate as marker) and a dried lactose/milk powder (containing 5% riboflavin sodium phosphate as marker). These products were administered using HPMC or hard gelatine capsules (Table 1).

All *in vivo* experiments were approved by the ethical committee of Ghent University Hospital.

Capsules were administered high in the vagina at the fornix posterior by a single investigator using a commercially available applicator (Infemin applicator, Pierre Fabre santé Benelux, Brussels, Belgium). All volunteers remained supine for 3 or 6 h after administration of the formulation and underwent colposcopy after 3, 6 or 24 h (Table 1). The *in vivo* behaviour of capsules and pellets (disintegration and spreading) was assessed via colposcopy using photographs ($n = 12$) to monitor the entire ectocervical and vaginal mucosa.

Using pellets or powder with riboflavin sodium phosphate (RSP) as marker, the vaginal discharge of pellets and powders was monitored over a period of 24 h using panty shields. After 6, 12 and 24 h the panty shields were replaced and the RSP amount on each panty shield was determined. To monitor the vaginal distribution of the formulation, the fornix, the mid portion of the vault mucosa and the introitus were swabbed during colposcopy and the fraction of RSP present at the different sites was assessed.

To determine the amount of riboflavin sodium phosphate, the panty shields or swabs were stirred for 24 h protected from light in 500 and 5 ml demineralised water, respectively. The RSP concentration in a filtered (Spartan 30/0.2 RC membrane filter, Whatman, Schleicher & Schuell, Dassel, Germany) sample was measured spectrophotometrically at 266 nm (UV-1650PC, Shimadzu, Antwerp, Belgium). The amount of RPS recovered was expressed as the percentage of the total administered RPS dose.

3. Results and discussion

Pellets, packed in HPMC or hard gelatine capsules, were evaluated as novel vaginal drug delivery system. The capsule disintegration time, spreading and retention of the different pellet formulations (non-disintegrating MCC pellets versus disintegrating starch-based pellets) and patient acceptability of this dosage form for vaginal application were assessed.

HPMC capsules filled with pellets (MCC or starch-based pellets) adhered well to the vaginal mucosa as no capsules were lost (Groups 1–3, $n = 15$). Although HPMC capsules disintegrated *in vitro* within 10 min, *in vivo* disintegration was much slower as even after 6 h three out of five capsules were still intact (Group 2). The lack of correlation between *in vitro* and *in vivo* results was probably due to the very low amount of vaginal fluid present. However, the capsule wall was weakened as even the slightest touch with the speculum during colposcopy opened the wetted capsules. After 24 h of administration all capsules had opened and no capsule remnants could be detected. In case of powder-filled HPMC capsules, all capsules (Group 4, $n = 5$) had disintegrated 6 h after administration.

Although *in vitro* disintegration was faster for hard gelatine capsules compared to HPMC capsules (3 versus 10 min), their *in vivo* behaviour was similar as two out of five were still intact 6 h after administration (Group 5). This slow capsule disintegration would limit the drug release rate, but this disadvantage could be eliminated if the pellets were administered in the vaginal cavity using an applicator with a different design that does not require that the pellets are packed in a capsule.

In those volunteers where the HPMC capsule (filled with non-disintegrating MCC pellets) (Group 1) had already disintegrated

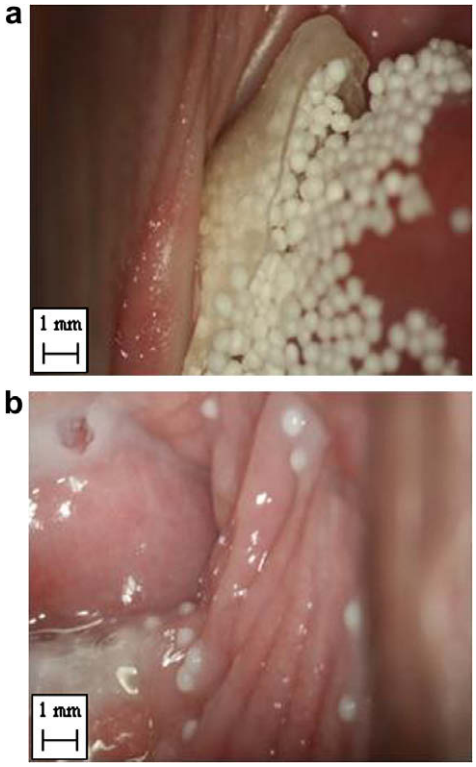

Fig. 1. Right side of fornix 3 h (a) and left side of the mid vagina 24 h (b) after administration of an HPMC capsule, filled with non-disintegrating MCC pellets.

3 h after administration, pellets clustered around the fornix and no spreading over the lateral walls to the introitus occurred (Fig. 1a). After 24 h a limited number of pellets (<50) were located

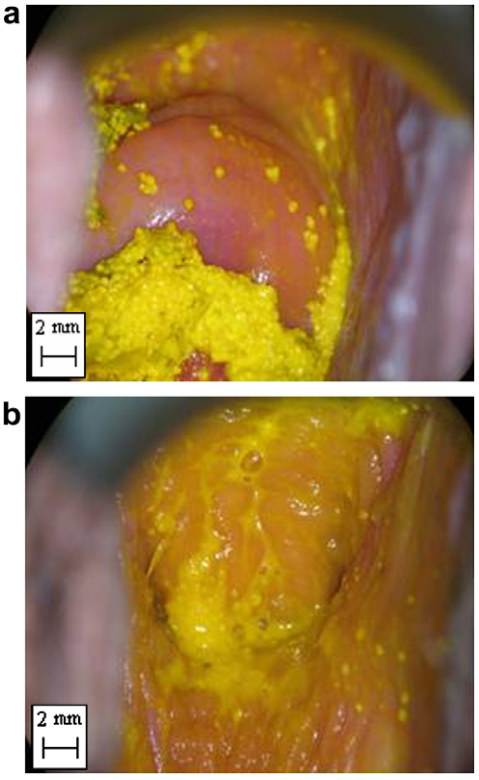

Fig. 2. The fornix anterior 6 h (a) and anterior side of the mid vagina 24 h (b) after administration of an HPMC capsule, filled with disintegrating starch-based pellets.

Table 2
% RSP of the administered dose detected by swabbing at the fornix, mid vagina and introitus 6 and 24 h after vaginal delivery of an HPMC capsule filled with starch-based pellets (Group 2) or powder (Group 4), with RSP as marker substance.

	6 h			Total	24 h			Total
	Fornix	Mid vagina	Introitus		Fornix	Mid vagina	Introitus	
Pellets								
Volunteer 1	0.14	0.01	0.00	0.15	0.70	0.25	0.04	0.99
Volunteer 2	0.00	0.00	0.00	0.00	0.49	0.10	0.10	0.69
Volunteer 3	0.60	0.00	0.00	0.60	0.15	0.02	0.00	0.17
Volunteer 4	0.00	0.00	0.00	0.00	0.14	0.01	0.00	0.15
Volunteer 5	0.48	0.02	0.00	0.50	0.34	0.13	0.02	0.49
Mean	0.24	0.01	0.00	0.25	0.37	0.10	0.03	0.50
Powder								
Volunteer 1	0.30	0.05	0.00	0.35	0.07	0.01	0.00	0.08
Volunteer 2	0.04	0.00	0.00	0.04	0.00	0.00	0.00	0.00
Volunteer 3	0.47	0.15	0.17	0.79	0.05	0.02	0.00	0.07
Volunteer 4	0.01	0.04	0.02	0.07	0.03	0.03	0.00	0.06
Volunteer 5	0.82	0.00	0.00	0.82	0.29	0.08	0.00	0.37
Mean	0.33	0.05	0.04	0.41	0.09	0.03	0.00	0.12

at the midportion of the vagina and around the introitus (Fig. 1b) as the volunteers reported that the majority of the pellets was discharged between 5 and 7 h after administration, mainly during toilet visit and showering. Chatterton et al. [9] also reported most loss of a vaginal cream formulation during urination. Hence, despite their small particle size and spherical shape, pellets did not spread evenly and vaginal retention was limited for MCC pellets.

Although starch-based pellets also clustered around the fornix 6 h after administration, the pellets already started to disintegrate and spreading of the pellets was observed (Fig. 2a) (Group 2). After 24 h for 8 out of 10 volunteers (Groups 2–3) complete coverage of the vaginal mucosa with disintegrated pellets was observed (Fig. 2b) as visualised via RSP as marker. Due to the disintegration of starch-based pellets this formulation probably spread more easily over the vaginal mucosa and was better retained, although high-amylose starch (the main ingredient of the starch-based pellets) did not have mucoadhesive properties. Vaginal swabbing showed that after 6 h, RSP was almost only detected around the fornix, but after 24 h RSP was recovered from all swabbed areas, although the highest concentration was still detected around the fornix (Table 2). In relation to the total amount of RSP administered, the amount recovered via swabbing was low, partly because a fraction of the RSP was already discharged from the vagina, but mainly because only a limited area of the vaginal mucosa was swabbed. In several volunteers, no RSP was detected on the swabs taken after 6 h as in these patients the capsules were not yet opened.

After 6 h the powder formulation was mainly spread around the fornix, but already some distribution to the middle anterior and posterior wall of the mid vagina was observed (Table 2) (Fig. 3a) (Group 4). No coverage of the side walls of the vagina midportion was seen. In one volunteer, the powder had formed a plug and no spreading occurred. Colposcopy after 24 h revealed only a small amount of powder ($n = 5$) at the posterior and anterior vaginal walls (Fig. 3b), indicating that the powder was faster cleared from the vagina in comparison to pellets. After dispersion of the powder it behaved as a liquid and was rapidly cleared from the vagina. In one volunteer, no powder was detected, whereas another volunteer had complete vaginal coverage after 24 h.

To evaluate the vaginal clearance of starch-based pellets compared to the powder formulation, pellets and powder discharged from the vagina were collected on panty shields. However, since most pellets and powder were lost during toilet visit and showering, these data did not provide meaningful information.

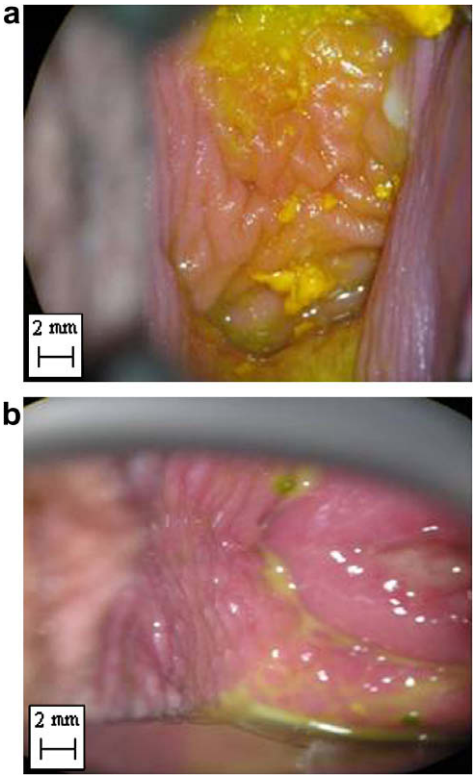

Fig. 3. The anterior side of the mid vagina 6 h (a) and right side of the mid vagina 24 h (b) after administration of an HPMC capsule, filled with powder.

During the clinical trials only minor side effects were reported by the volunteers ($n = 25$) (Groups 1–5): an irritated feeling by one woman and minor low belly pain shortly after administration of the capsule by three volunteers. As there was no control group and none of the side effects were severe, they could not be conclusively associated with product use.

4. Conclusion

Disintegrating starch-based pellets are a promising new vaginal drug delivery system, which resulted in complete coverage of the vaginal mucosa. A drawback of the pellet formulation was the slow disintegration time of the capsules, which could be eliminated

using a different applicator that does not require packing the pellets in capsules for vaginal delivery.

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