Preparation and in vitro/in vivo evaluation of gestodene (GEST) intravaginal ring

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Abstract: Preparation and in vitro/in vivo evaluation of gestodene (GEST) intravaginal ring (IVR) formulations which can release a constant dose of GEST during 3 weeks were investigated. In present study a reservoir gestodene intravaginal ring, including a gestodene silicone elastomer core and the non-active silicone layer, was reported, which was manufactured by reaction injection moulding at 80°C for 20 min. The raw materials compatibility experiments showed that the silicone elastomer core carrier wouldn’t interact with drugs. In vitro release samples were determined by HPLC and the experiment was performed under sink conditions. The equation of cumulative release verse time was \( Y=64.76x+5.44 \) \( (r=0.9998) \), performing zero-order release at about the target dose of 60 µg/day over 21 days. Drug release increased with temperature elevating from 45 to 55°C, which could be attributed to optimizing the prescription. In addition, the pharmacokinetic and safety studies of gestodene intravaginal ring were evaluated in female New Zealand White rabbits. The GEST in plasma was analyzed by LC-MS/MS and the results proved that the correlation between in vitro and in vivo was relatively well.

Keywords: Intravaginal ring; silicone elastomer; gestodene; pharmacokinetics.

INTRODUCTION

In this paper, gestodene (GEST) is selected as the object of the study. GEST, which is one of the third generation of progestogens, has the ever strongest progestational activity of all the steroidal hormone. At the same time, GEST does not have any estrogenic activity, and shows a strong resistance to estrogen as well as extremely weak androgenic effects (Kaplan, 1994, Jaithitivit et al., 2012, Wilde et al., 1995). Currently, the commercially pharmaceutical preparation of GEST (Minulet®) is oral tablet together with ethinylestradiol (EE), which is produced by the Wyeth. Minulet® has the characteristics of safety, effectiveness and good tolerance, meanwhile its side effects are extremely low (de Andrade, 1989, Brill et al., 1991, Düsterberg et al., 1990), and Minulet® has obvious clinical advantages compared with other commonly used short-acting oral contraceptives (Lihu et al., 2008, Aguiar et al., 1994, Zichella et al., 1994, Endrikat et al., 1997).

However, the bioavailability of GEST is comparatively low due to the hepatic first pass effect (Kuhl, 1987). In addition, the inconvenience from daily intake of Minulet® results in the low compliance and the awkward in case of a forgotten pill as well as all of oral contraceptives (OCs). The fluctuation of plasma level would lead to some side-effects including stroke, migraine, breast cancer and so on (Kerns et al., 2011, Lidegaard et al., 2012). As a result, non-oral intake way of steroids has been developed, offering a long-period effect with good compliance (Kirkman et al., 1999, Gaspard et al., 2000), which involves the intrauterine contraceptive devices (Haihao, et al., 2010, Thonneau, et al., 2008, Backman et al., 2004), patches (Wan et al., 2007, Lipp, 1998), implants (Dieben et al., 2002, van den Heuvel et al., 2005) and injections (Yi et al., 2008, Lipp, 1998). However, all these methods demand the assistance of the professional staff restricting the place and the time of usage. IVR has satisfactory contraception effect with lower dose of steroids by avoiding the hepatic first pass effect, and allows a more stable plasma level to minimize side effects (Kerns et al., 2011, Sitruk-Ware et al., 2013). Moreover, it also realizes long-period effect and good compliance by reducing the need for frequent dosing (Kerns et al., 2011, Sitruk-Ware et al., 2013).

We have developed the IVRs, which can deliver GEST at controlled rates of 60µg/day for at least 21 days. To our knowledge, this is the first intravaginal ring that can achieve controlled release of gestodene at the target dose. We also tried to combine ethinyl estradiol into gestodene vaginal ring, which showed that ethinylestradiol could achieve the pseudo-zero-order release and the average daily release was 15µg (unpublished). Next, our research group will continue to improve related research of GEST/EE compound intravaginal ring.

In present study, the compatibility of GEST and MED-6382 silicone elastomer, which was used as the
intravaginal ring carrier material of the drug core was examined. In vitro study, we investigated the drug release of the IVRs with different drug loadings. Meanwhile, in order to perform quick prescription screening, the drug release was accelerated with the elevating temperatures, according to the Arrhenius law. The in vitro/in vivo correlation was investigated with female New Zealand White rabbits, and the tolerability of GEST IVR in tissue level was also performed to inspect if this drug delivery system was safe or not.

MATERIALS AND METHODS

Materials
Gestodene (99.25%) was supplied by Beijing Zizhu Tiangong Science and Technology Company. Norgestrel (99.26%) as internal standard (IS) was also obtained from Beijing Zizhu Tiangong Science and Technology co., LTD. Petroleum ether (analytical-reagent grade) was purchased from Beijing Chemical Works. Acetonitrile (LC grade) was purchased from Fisher Scientific. Heparin sodium salt (BR) was purchased from Chinese Chemical Reagent co., LTD. New Zealand White rabbits [general grade, kg (2.5±0.5), female, Beijing Keyu animal breeding center. license: SCXK (Beijing), 2012-0004]. Silicone elastomer base (MED-6382) and tetrapropoxysilane (n-proethyl poylorthosilicate) were purchase from Nusil Technology (Carpinteria, USA).

Methods
Compatibility study of gestodene and silicone elastomer
Gestodene and silicone elastomer mixed with 1:20 and 1:50 (w/w) and then the mixtures were performed the compatibility test under the condition of 4500 Lx, 60°C and relative humidity of 92.5% for 5 d and 10 d respectively, at which time weight changes were recorded as well as drug extraction and analysis were performed.

The mixtures of silicone elastomer and gestodene were cut into 0.5 mm, extracted with magnetic stirring for 36 h in 50 ml of methanol, the efficiency of which was about 93%. Changes of impurities from extracting solution, diluted for 1000 times to be about 300 ng/mL, were detected by HPLC.

Preparation of the reservoir-type IVR containing GEST
Manufacture of silicone intravaginal devices
Silicone elastomer base (a condensation-cure RTV2-type silicone) and the crosslinking agent tetrapropoxysilane (ratio 100:0.5) of MED-6382 were blended to be the homogeneous mix. IVRs loaded with appropriate dosage of GEST were prepared on the basis of the following steps (Woolfson et al., 2011, Malcolm et al., 2010, Moss et al., 2012). The needed amount of GEST was added in 10.0 g of the silicone elastomer mix, and stirred until blended. Stannous octoate (0.5% w/w) was then dispersed and mixed for 30 s, after which the mixture was injected into the stainless steel moulds of a laboratory-scale reaction injection mould specifically designed for IVR manufacture (fig. 1). The injection mix was cured at 80°C for 20 min producing GEST-loaded silicone elastomeric matrix rings with the following dimensions: 4.0 mm cross-sectional diameter, 42.0 mm internal diameter, 50.0 mm external diameter.

Fig. 1: Injection moulds for manufacture of intravaginal ring

Fig. 2: Technological processes of reservoir-type IVR

Fig. 3: Structure diagram of reservoir-type IVR with block structure, 1/4 containing GEST, 3/4 was blank

In the outside of the matrix IVR, the non-active blank sheath with the thickness of 1.0 mm was wrapped, and then heated for 10 min at 130°C on a vulcanizing machine to be cured. The flow chart was provided (in fig. 2).
**Preparation of block structure reservoir-type GEST intravaginal ring**

As we used the block structure to achieve controlled release of the drug, the 1/4 GEST matrix IVR and 3/4 blank matrix IVR were cut to make up a complete intravaginal ring, outside of which was wrapped with 0.8, 0.9 or 1.0 mm inactive blank sheath. And the two blocks became an integrity as the sheath was cured at 130°C on a vulcanizing machine for 10 min (fig. 3) (ChunXiao et al., 2014).

**In vitro drug release studies and high performance liquid chromatography**

Each reservoir-type intravaginal ring was fixed on a paddle of dissolution tester with a fine nylon. The dissolution cup containing 200 ml water was kept at 37°C. The paddles rotating at a constant rate of 50 rpm, could effectively minimize the thickness of the hydrostatic layer around the ring. The dissolution medium was replaced every 24 ± 0.5h over the 21-day period. About 1.0 ml of the used dissolution medium was removed for being analyzed by HPLC (Waters, USA), which consisted of a 2695 auto injector and a 2487 detector. An injection volume of 100 µl was isolated by a C18 column (250*4.6 mm, 5 µm, Diamonsil®, DIKMA) with a mobile phase of acetonitrile/water (50:50) at a flow rate of 1.0 ml·min⁻¹ and detected at the wavelength of 239 nm. Based on these conditions, quantitation of GEST release was calculated by peak area at the retention time of 10.71 min. A linear relationship between peak area (A) and concentration (C) was A=315.63C-168.32, r=0.9998, during 75–750 ng·mL⁻¹.

**Accelerated drug release testing for GEST intravaginal rings (Maeda et al., 2004, Externbrink et al., 2013)**

Releasing experiments were performed with a whole ring in 200 mL of water at 37°C and the rate of the shaker was 160 rpm. Elevated temperature experiments were performed in water at 45 and 55°C (±0.2°C). Sampling was performed in adjusted intervals, as well as the media was renewed to maintain sink conditions. All experiments were performed in triplicate and mean values with standard deviations were both reported in the release profiles. The concentration of GEST in the release medium was determined by HPLC.

**In vivo study in rabbits (Moss et al., 2012)**

Six female New Zealand White rabbits were housed in the laboratory animal center of National Research Institute...
for Family Planning (IACUC Issue No. NRIFP14031601) according with recommendations in the Guide for the Care and Use of Laboratory Animals of the NIH. At the onset of experiment, the rabbits were approximately 5 to 7 months old with the mean body weight of 2.50±0.10kg. About 1/2 segment (7~10cm) of whole IVR comprising a smooth drug-containing end, lubricated with normal saline, was inserted through the exterior vaginal opening. Insertion was performed as a clean procedure by trained technical staff. Once the drug-containing end of the segment reached the cervix, then the other end of the implant without drug was anchored with silk braided suture to the vagina wall around the vaginal opening. The surgery didn’t require laparotomy. The IVR segments were inserted into the vaginal vaults on day 0 and retained for a period of 21-day. Blood samples (about 1.5 ml each) were gathered from marginal ear vein predose on days 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 17, 19 and 21. Blood samples were treated with heparin sodium salt before plasma was separated by centrifugation (3,500 rpm, 10 min) at 4°C and stored (-80°C) prior to analysis.

**Fig. 6**: Cumulative release of GEST from IVR at 45°C(a) and 55(b), -○-37; -ϒ-45 or 55; -∆-, mimetic cumulative release of GEST based on the Arrhenius equation.

The blank group was performed with blank IVR segments without any drug and sham-operated rabbits with normal diet were the control group.

**In vivo drug release analysis and LC-MS/MS (YanKun Wang et al., 2014)**

The internal standard norgestrel was blended with 0.5 ml plasma sample and then 50µL acetonitrile was added. After the plasma samples were mixed for 1 min, 5 ml petroleum benzine was added to extract the drug. And then the plasma samples were centrifuged at 3,500 rpm for 10 min. The upper solution was transferred into the inner glass sample tube following 10 µL injected into the LC/MS-MS.

**Fig. 7**: Mean (±SD) in vitro daily release-time profile and in vivo plasma concentration-time profile of gestodene from intravaginal rings (n=6), -○-, in vitro; -□-, in vivo.

**Fig. 8**: Morphology of vaginal tissues of the rabbits after application of gestodene IVR. A sham-operated rabbits with normal diet (control group); B blank IVR administrated group (blank group); C drug-loaded IVR administrated group (experimental group).

The LC system was coupled with a triple quadrupole mass spectrometer, which was equipped with electrospray ionization (ESI) source made by Agilent (California, USA) for detection. The mass spectrometer was operated under the multiple reaction monitoring (MRM) mode while mass transition ion-pair was followed as m/z from 313.10 to 309.10 for norgestrel and 311.10 to 309.10 for gestodene. Agilent Mass Hunter Workstation Software was used for data acquisition and processing. A degassed mobile phase comprising acetonitrile (A) and 0.1% formic acid in water (B) was delivered at the flow rate of 0.3
mL/min with a time and solvent composition gradient. The total run time was 5.0 min for each sample analysis. Peak area ratios of GEST/IS were calculated and standard curve was constructed as following, \( R=0.147C+0.119 \), \( r=0.9995 \). The equation was used to calculate the GEST concentrations in the samples with their peak area ratios.

The rabbit plasma samples were processed and analyzed using validated methods established by our laboratory (YanKun Wang et al., 2014).

**Table 1**: The results of API excipients compatibility testing under strong light exposure (4500 LX)

<table>
<thead>
<tr>
<th>Light exposure</th>
<th>Gestodene</th>
<th>Gestodene+ silicone elastomer (1:20)</th>
<th>Gestodene+ silicone elastomer (1:50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 d</td>
<td>0.25</td>
<td>0.37</td>
<td>0.26</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.28</td>
<td>0.45</td>
<td>0.26</td>
</tr>
<tr>
<td>T (%)</td>
<td>0.25</td>
<td>0.21</td>
<td>0.43</td>
</tr>
<tr>
<td>( \Delta M ) (%)</td>
<td>-0.45</td>
<td>-0.53</td>
<td>-0.54</td>
</tr>
<tr>
<td>10 d</td>
<td>0.22</td>
<td>0.2</td>
<td>0.33</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.31</td>
<td>0.57</td>
<td>0.80</td>
</tr>
<tr>
<td>T (%)</td>
<td>-0.2</td>
<td>-0.01</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**Table 2**: The results of API excipients compatibility testing under high temperature (60°C)

<table>
<thead>
<tr>
<th>High temperature</th>
<th>Gestodene</th>
<th>Gestodene+ silicone elastomer (1:20)</th>
<th>Gestodene+ silicone elastomer (1:50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 d</td>
<td>0.25</td>
<td>0.37</td>
<td>0.26</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.28</td>
<td>0.45</td>
<td>0.26</td>
</tr>
<tr>
<td>T (%)</td>
<td>0.42</td>
<td>0.17</td>
<td>0.45</td>
</tr>
<tr>
<td>( \Delta M ) (%)</td>
<td>-1.78</td>
<td>-0.74</td>
<td>-0.75</td>
</tr>
<tr>
<td>10 d</td>
<td>0.21</td>
<td>0.36</td>
<td>0.62</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.34</td>
<td>0.59</td>
<td>0.82</td>
</tr>
<tr>
<td>T (%)</td>
<td>-0.14</td>
<td>0.04</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Table 3**: The results of API excipients compatibility testing under high humidity.

<table>
<thead>
<tr>
<th>High humidity</th>
<th>Gestodene</th>
<th>Gestodene+ silicone elastomer (1:20)</th>
<th>Gestodene+ silicone elastomer (1:50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 d</td>
<td>0.25</td>
<td>0.37</td>
<td>0.26</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.28</td>
<td>0.45</td>
<td>0.26</td>
</tr>
<tr>
<td>T (%)</td>
<td>0.43</td>
<td>0.28</td>
<td>0.48</td>
</tr>
<tr>
<td>( \Delta M ) (%)</td>
<td>1.31</td>
<td>-0.13</td>
<td>-0.09</td>
</tr>
<tr>
<td>10 d</td>
<td>0.28</td>
<td>0.145</td>
<td>0.36</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.38</td>
<td>0.26</td>
<td>0.46</td>
</tr>
<tr>
<td>T (%)</td>
<td>0.34</td>
<td>-0.01</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

**Morphology study of vaginal tissues after application of GEST IVR**

In order to observe and study the biological compatibility of IVR device, morphology study of vaginal tissues after insertion of GEST IVR was conducted. After the 21 days’ operation, the vaginal tissues of the sham-operated rabbits with normal diet (A control group), the blank IVR received group (B blank group), and the drug-loaded IVR received group (C experiment group) were handled with...
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10% neutral carbonated-buffered formaldehyde, embedded in paraffin, and cut into slices. The slices, after hematoxylin-eosin staining, were observed under a light microscope (×50, ×400).

RESULTS

Compatibility Study of API and Excipients
The single and total impurities changes from the mixtures of silicone elastomer and gestodene were calculated as area percent (%) of total peak area after extracted by solvent and detected by HPLC. Meanwhile the changes in weight were also recorded (tables 1, 2 and 3).

Drug degradation were studied under high illumination, temperature and humidity conditions as this would indicate long-term drug degradation around ambient conditions and might imply degradation mechanisms if possible. The results indicated no significant degradation of gestodene in polymer on 5 and 10 day compared to day 0. Besides, there were no new peaks appearing in the sample chromatograms, which could indicate possible degradation. And the weight changes were also acceptable. Totally, these results indicated that gestodene was stable in silicone elastomer and might be formulated into silicone elastomer intravaginal ring for 21-day period sustained and controlled release.

In vitro release for GEST from reservoir-type IVR
The mean daily release profiles of GEST from reservoir-type IVR with the controlling sheath thickness of 0.8, 0.9 and 1.0 mm, each core loaded with 3.5% (w/w) GEST, were shown in (fig. 4a). Release profiles describing the daily release of gestodene from reservoir IVR with different drug loadings from 3.5% to 4.5% (w/w), each IVR wrapped with 1.0 mm controlling sheath, were presented in (fig. 5b). All of the release profiles were not common reservoir systems with the ‘burst effect’ followed by a reduced steady curve. Instead, the drug release of first three days was gradually increased to the average level.

Reservoir devices with a constant activity source (i.e., excess solid permeate within the reservoir), presented linear profiles of cumulative release (CR) versus time (t). Fig. 4a and 5b demonstrated these linear relationships for GEST, indicating that zero order release kinetics were being obeyed as well as constant activity sources were being kept during 21 days. The cumulative release equations couple with respective r coefficients, were displayed in (table 4).

Elevated temperature release studies
All the elevated temperature experiments were performed in water while the cumulative release profiles at 37°C and elevated temperatures were shown in (fig. 6). During elevated temperature release experiments, samples took with shorter intervals, and the experiments were ended when the cumulative release equaled to that at 37°C after 21 days. Fig. 6 demonstrated that drug release increased with elevating temperature. And the zero-order release constants were calculated for each temperature in (table 5) according to the cumulative release versus time graph.

In vivo studies in rabbit
The above mentioned LC-MS/MS method was applied in the plasma sample analysis for in vivo study of GEST IVR. The LC-MS/MS method was selective, accurate and sensitive, which had been validated in our previous study (YanKun Wang et al., 2014) and it also displayed excellent linearity within the concentration range of 0.5–6.0 ng·mL⁻¹. Fig. 7 showed the mean (±SD) plasma levels of GEST tested after the vaginal insertion of GEST IVR in 6 rabbits. Results showed that after being implanted the GEST IVR made in house, the rabbits’ plasma concentrations of GEST were kept constant in observing day, and the releasing-concentration fitting results showed that the correlation between in vivo and in vitro analysis was perfect.

From fig. 7 we could see mean concentrations were below 2 ng·mL⁻¹, but maintained constant for 21 days, indicating sustained and controlled release from the IVR devices. Rabbit GEST plasma levels were within the scope of testing: mean ± standard deviation (SD), 1.36±0.16 ng·mL⁻¹; peak value, 1.50 ng·mL⁻¹.

Tolerability of GEST IVR in Tissue Level
Fig. 8 showed the pathological changes of the vaginal mucosa after vaginal administration of IVR. When compared to the control group with no treatment, the blank group and the IVR-treated group presented no visible indication of inflammation or necrosis. IVR didn’t change the morphology of vaginal mucosas, which indicated that IVR device had good biocompatibility and such drug delivery systems might be safe for vaginal delivery.

DISCUSSION
In reservoir IVR, the solid drugs disperse within the silicone elastomer core and wapped by a drug-free silicone elastomer sheath. As a result, gestodene molecules within the core must first dissolve into the surrounding silicone elastomer, then diffuse through the silicone core and sheath and finally distribute into the dissolution medium around the device. To the block structure reservoir-type intravaginal ring, drug will also diffuses to the blank carrier on both sides to form a temporary reservoir. The reservoir IVR can perform zero-order drug release with rational design, and most important is that their release behaviours can be readily modified as needed (Woolfson et al., 2003).
Based on Eq. (1) (Malcolm et al., 2003), presents the cumulative release per unit area, CR presents verified from the release profiles in (fig. 4a). Then the release rate will be enhanced. It had also been shorter diffusional access for the drug is provided, and release rate. When decreasing the sheath thickness (h), a can easily find that changing the thickness of the silicone elastomer, we presents the diffusivity of the drug in the elastomer, we couldn’t be mixed homogeneously in the silicon elastomer conclusion was obtained. This might be because the drug 3.5% or 4.0%. We repeated the experiment, and the same corroborating results in Dapivirine polyurethane vaginal rings release conformed to zero order equation. However in present study, the release rate of the intravaginal ring studies, that drug loading and release rate were proportional to the vaginal ring, in the case of intravaginal rings release conformed to zero order equation. However in present study, the release rate of the intravaginal ring comprising 4.5% drug was obviously less than either 3.5% or 4.0%. We repeated the experiment, and the same conclusion was obtained. This might be because the drug couldn’t be mixed homogeneously in the silicon elastomer as the relatively small drug loading.

Extrembrink et al. (2013) demonstrated that the rate constants of drug release from intravaginal rings increased with temperature could be appropriated for the Arrhenius equation (Eq. (2)). The required sampling interval (t) at any temperature could be calculated according to the ratio of the real-time zero-order release constant and the elevated temperature zero-order release constant (Eq. (3)).

\[ Q = \frac{D_{SIL} C_{SIL}}{h_{2 sheath}}, t \]  
\[ k = A \cdot e^{\frac{-E_A}{RT}} \]  
\[ t(h)_{TF} = \frac{k_{37^\circ C}}{k_{TF}} \cdot 24 \text{ h} \]

With Eq. (3), sampling intervals of 11.71 and 5.91 h were calculated for experiments performed at 45 and 55°C which were used to represent 24h at 37°C. As the slope of the release equation k can generally reflect the average rate of drug release, therefore, the accelerated drug release tests can not only judge whether the release of the drug conforms to zero order equation or not by the correlation coefficient, but also can estimate the rate of drug release. The application of accelerated drug release tests will greatly enhance the rate of prescription screening. As shown in (table 5), the slopes of the mimetic zero-order release equation k of 45°C and 55°C, 62.83 and 62.67, respectively, almost equaled to the slope of the zero-order release profile of 37°C, which was 62.63.

From the above in vivo data, we achieved the sustained-release of GEST in rabbits over the process of the study and this finding also standed for the first report of a GEST IVR releasing drugs in vivo as a controlled release model.

The rabbit model was used in this study, as it was easy to keep and control. The use of rabbit vaginal pharmacokinetic model in preclinical testing of intravaginal rings containing microbicide was supported (Clark et al., 2011). The rabbit model had also been used to test the IVIVC and plasma levels of ethynodiol diacetate from silicone devices (Chien et al., 2013) and was employed to evaluate the capability of a novel, dual-protection, pod IVR platform for the delivering of ACV and TFV (Moss et al., 2012). However, the rabbit vagina

### Table 4: Release data for GEST IVR reservoir devices with different thickness of sheath and drug loadings (n=3)

<table>
<thead>
<tr>
<th>GEST intravaginal rings</th>
<th>Zero-order release equation /µg</th>
<th>r</th>
<th>Mean daily release (MDR)/µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8 mm sheath</td>
<td>CR=62.63r+19.13</td>
<td>0.999</td>
<td>61.17</td>
</tr>
<tr>
<td>0.9 mm sheath</td>
<td>CR=58.79r+4.24</td>
<td>0.996</td>
<td>57.72</td>
</tr>
<tr>
<td>1.0 mm sheath</td>
<td>CR=55.17r+20.72</td>
<td>0.999</td>
<td>53.85</td>
</tr>
<tr>
<td>3.5% drug loading</td>
<td>CR=58.16r+10.40</td>
<td>0.999</td>
<td>57.22</td>
</tr>
<tr>
<td>4.0% drug loading</td>
<td>CR=64.76r+5.44</td>
<td>0.998</td>
<td>64.42</td>
</tr>
<tr>
<td>4.5% drug loading</td>
<td>CR=54.78r+0.94</td>
<td>0.999</td>
<td>54.31</td>
</tr>
</tbody>
</table>

CR: cumulative release.

### Table 5: Release data for GEST IVR reservoir devices at different accelerated temperature (n=3)

<table>
<thead>
<tr>
<th>GEST intravaginal rings</th>
<th>Zero-order release equation /µg</th>
<th>r</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>CR=62.63r+19.13</td>
<td>0.999</td>
<td>62.63</td>
</tr>
<tr>
<td>45°C</td>
<td>CR=120.95r+14.99</td>
<td>0.998</td>
<td>62.83</td>
</tr>
<tr>
<td>Mimetic (45°C)</td>
<td>CR=62.83r+14.99</td>
<td>0.998</td>
<td>62.83</td>
</tr>
<tr>
<td>55°C</td>
<td>CR=238.76r+19.31</td>
<td>0.997</td>
<td>62.67</td>
</tr>
<tr>
<td>Mimetic (55°C)</td>
<td>CR=62.67r+19.31</td>
<td>0.997</td>
<td>62.67</td>
</tr>
</tbody>
</table>

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is longer than the human vagina, with an urovaginal sphincter separating the lower urovagina and upper two thirds of the vagina, the cervicovagina, which is stratified squamous, similar to the human endocervix, and a columnar cell monolayer respectively. The drug-loaded IVR segment was placed in the rabbit cervicovagina, at which drug had to be absorbed through the single layer of columnar cells, since it would be exposed to urine, washing away the drug and influencing pharmacokinetics studies when placed in the urovagina (Moss et al., 2012). Based on above reasons, we would also consider the use of sheep as a model in our following pharmacokinetic trial, because it absorbed drug releasing from the IVR through stratified squamous epithelium just like human (Moss et al., 2012).

The IVIVC is firstly used to control quality by pharmaceutical industry, while most IVIVCs explore to mimic the dissolution of oral medicines in the gastrointestinal tract. However in present study, IVIVC was attempted to guide in vitro experiments during the development of sustained and controlled release formulations.

CONCLUSION

In this paper, intravaginal ring was designed to achieve control release of GEST in vitro for the first time, with the release rate of 60 µg/d for 21 days. Meanwhile it also achieved stable drug absorption in rabbits. The compatibility test results of raw materials and biological tissues showed that silicone elastomer was an ideal carrier material. We also confirmed the accelerating effect of temperature on drug release, this findings could be used in formulation screening. Our laboratory is currently working on a combination of gestodene and ethinyl estradiol intravaginal ring, which will serve as the basis for the development of the compound gestodene intravaginal ring.

REFERENCES


