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

Chunxiao Li[1,2], Meiying Ning*[1,2], Xiaodong Yao[1,2], Yankun Wang[3] and Ying Liu[1]

[1]Research Center of Biomaterial and Novel Drug Delivery Systems, National Research Institute for Family Planning, Da Hui Si Haidian District, Beijing, PR China

[2]Graduate School of Peking Union Medical College; No. 9, Dongdan No. 3 Avenue, Beijing, PR China

[3]Shenyang Pharmaceutical University, Shenyang, China

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 progestogen, 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 (Lihua *et al.,* 2008, Aguiar *et al.,* 1994, Zichella *et al.,* 1994, Endrikat *et al.,* 1997).

However, the bioavailability of GEST is comparitively 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

*Corresponding author: e-mail: mayning999@126.com

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 MEATHODS

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-proepithelial pylorthosilicate) 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 stired 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

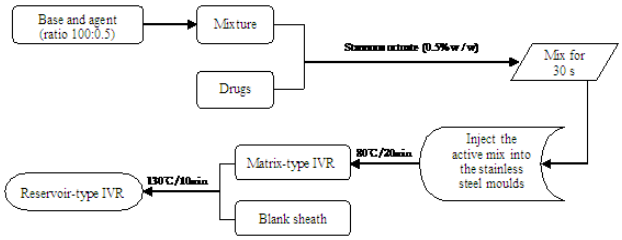

Fig. 2: Technological processes of reservoir-type IVR

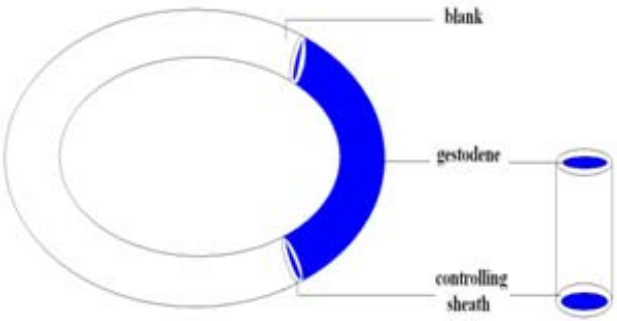

Fig. 3: Structure diagram of reservoir-type IVR with block structure, 1/4 containing GEST, 3/4 was blank

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).

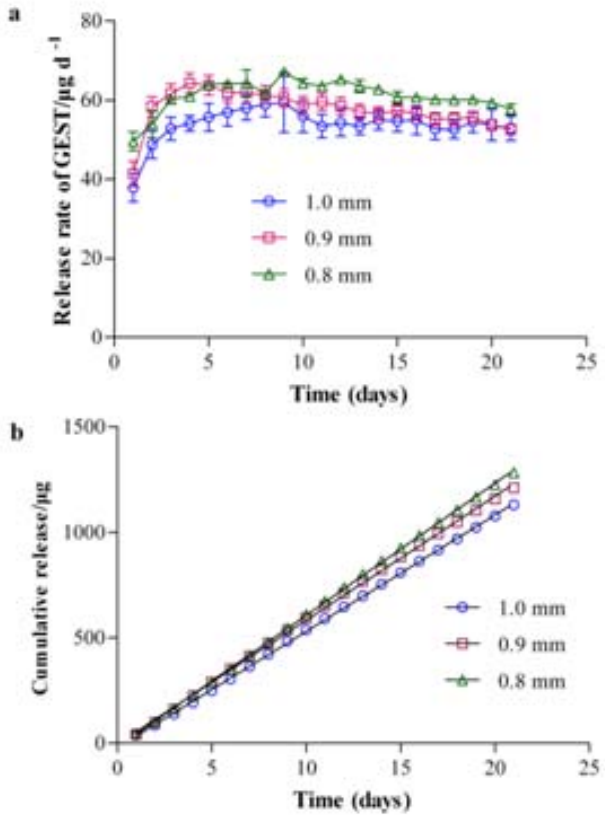

Fig. 4: Release profile (a) and zero order release profile (b) of GEST reservoir-type IVR with different thickness of sheath for 21 d (*n*=3). -◁-, 1.0 mm; -ϒ-, 0.9 mm; -Δ-, 0.8 mm

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}.

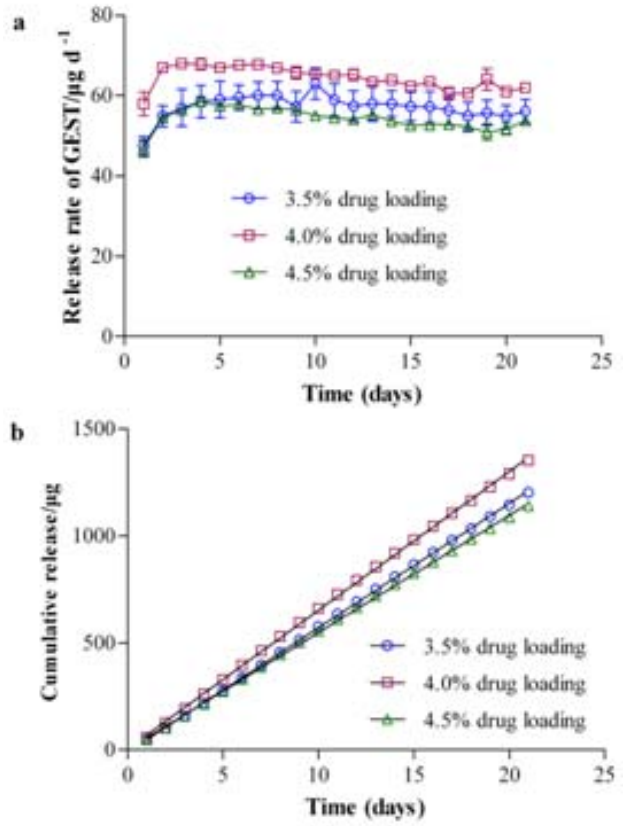

Fig. 5: Release profile (a) and zero order release profile (b) of GEST reservoir-type IVR with different drug loadings for 21 d (*n*=3). -◁-, 3.5%; -ϒ-, 4.0%; -Δ-, 4.5%

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.

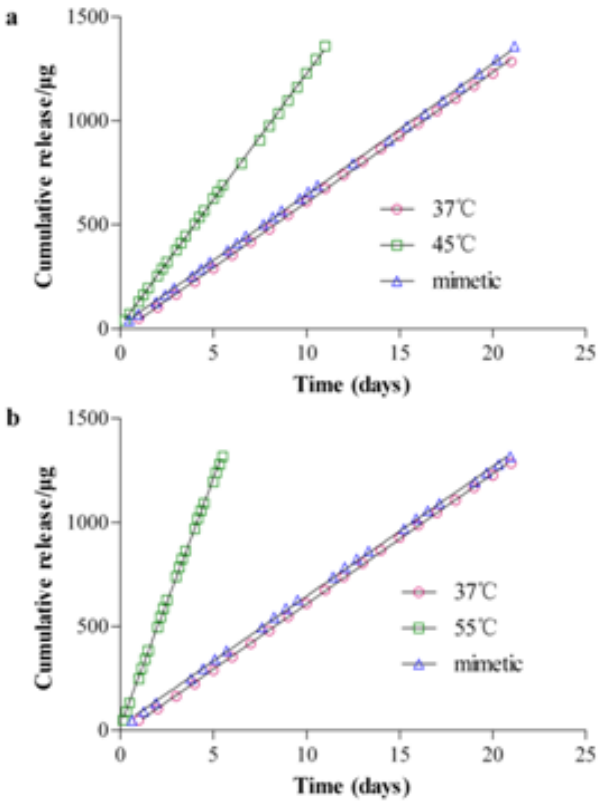

Fig. 6: Cumulative release of GEST from IVR at 45□(a) and 55(b), -○-, 37; -ϒ-, 45 or 55; -Δ-, mimetic cumulative releae of GEST based on the Arrhenius equation

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.

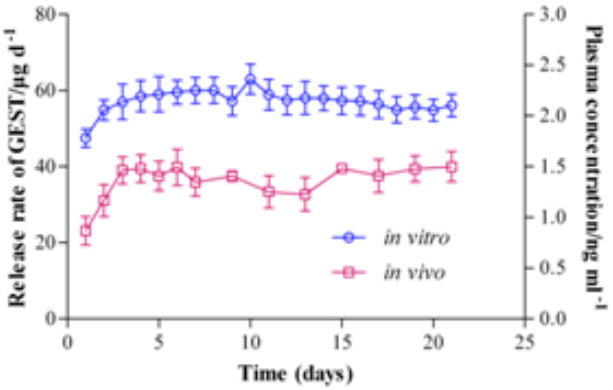

Fig. 7: Mean (±SD) *in vitro* daily release-time profile and *in vivo* plasma concentration-time profile of gestodene from intravaginal rings (n=6), -○- , *in vitro*; -□-, *in vivo*

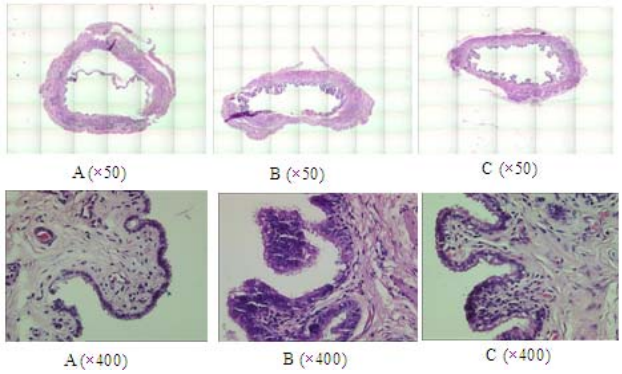

Fig. 8: Morphology of vaginal tissues of the rabbits after application of gestodene IVR. A sham-operated rabbits with normal diet (control group); B blank IVR administrated group (blank group); C drug-loaded IVR administrated group (experimental group)

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

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

Light exposure	Gestodene	Gestodene+ silicone elastomer (1:20)	Gestodene+ silicone elastomer (1:50)
0 d			
S (%)	0.25	0.37	0.26
T (%)	0.28	0.45	0.26
5 d			
S (%)	0.2	0.10	0.22
T (%)	0.25	0.21	0.43
△m (%)	-0.45	-0.53	-0.54
10 d			
S (%)	0.22	0.2	0.33
T (%)	0.31	0.57	0.80
△m (%)	-0.2	-0.01	0.08

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

High temperature	Gestodene	Gestodene+ silicone elastomer (1:20)	Gestodene+ silicone elastomer (1:50)
0 d			
S (%)	0.25	0.37	0.26
T (%)	0.28	0.45	0.26
5 d			
S (%)	0.42	0.04	0.14
T (%)	0.42	0.17	0.45
△m (%)	-1.78	-0.74	-0.75
10 d			
S (%)	0.21	0.36	0.62
T (%)	0.34	0.59	0.82
△m (%)	-0.14	0.04	0.01

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

High humidity	Gestodene	Gestodene+ silicone elastomer (1:20)	Gestodene+ silicone elastomer (1:50)
0 d			
S (%)	0.25	0.37	0.26
T (%)	0.28	0.45	0.26
5 d			
S (%)	0.43	0.28	0.48
T (%)	0.65	0.49	0.60
□m (%)	1.31	-0.13	-0.09
10 d			
S (%)	0.28	0.145	036
T (%)	0.38	0.26	0.46
□m (%)	0.34	-0.01	-0.05

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).

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

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^{o}C and elevated temperatures were shown in (fig. 6). During elevated temperature release experiments, samples were took with shorter intervals, and the experiments were ended when the cumulative release equaled to that at 37^{o}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).

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

GEST intravaginal rings	Zero-oder release equation /μg	*r*	Mean daily release (MDR)/μg
0.8 mm sheath	*CR*=62.63*t*-19.13	0.9999	61.17
0.9 mm sheath	*CR*=58.79*t*-4.24	0.9996	57.72
1.0 mm sheath	*CR*=55.17*t*-20.72	0.9999	53.85
3.5% drug loading	*CR*=58.16*t*-10.40	0.9999	57.22
4.0% drug loading	*CR*=64.76*t*+5.44	0.9998	64.42
4.5% drug loading	*CR*=54.78*t*+0.94	0.9998	54.31

CR: cumulative release.

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

GEST intravaginal rings	Zero-oder release equation /μg	*r*	*k*
37°C	*CR*=62.63*t*-19.13	0.9999	62.63
45°C	*CR*=120.95*t*+14.99	0.9998	
Mimetic (45°C)	*CR*=62.83*t*+14.99	0.9998	62.83
55°C	*CR*=238.76*t*+19.31	0.9997	
Mimetic (55°C)	*CR*=62.67*t*+19.31	0.9997	62.67

$$Q - \frac{D_{SIL} C_{SIL}}{h_{sheath}} \cdot t \quad (1)$$

Based on Eq. (1) (Malcolm *et al.,* 2003), where Q presents the cumulative release per unit area, C_{SIL} presents the solubility of the drug in silicone elastomer, D_{SIL} presents the diffusivity of the drug in the elastomer, we can easily find that changing the thickness of the silicone sheath (h) would be an effective way to regulate drug release rate. When decreasing the sheath thickness (h), a shorter diffusional access for the drug is provided, and then the release rate will be enhanced. It had also been verified from the release profiles in (fig. 4a).

Early studies of our laboratory showed that the drug loading in the range of 0.48~13.5%, had a positive correlation with the average release rate of the drug, Q/t = 1.292 8A + 8.598 1 (r=0.992 2) (Chun Xiao *Li et al.,* 2014). Gupta *et al.* (Gupta *et al.,* 2008) had come to the corresponding results in Dapivirine polyurethane vaginal 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 could't be mixed homogeneously in the silicon elastomer as the relatively small drug loading.

Externbrink *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)).

$$k = A \cdot e^{\frac{-E_A}{RT}} \quad (2)$$

$$t(h)_{T\uparrow} = \frac{k_{37°C}}{k_{T\uparrow}} \cdot 24\ h \quad (3)$$

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

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

Aguiar L, Aldrighi J, Deandrade R, Barbosa I, Barreto C, Bossemeter R and Bosque V (1994). Clinical comparison of monophasic oral contraceptive preparations of gestodene/ethinyl estradiol and desogestrel/ethinyl estradiol. *Contraception,* **50**(3): 201-214.

Backman T, Rauramo I, Huhtala S and Koskenvuo M (2004). Pregnancy during the use of levonorgestrel intrauterine system. *Am. J. Obstet. Gynecol.*, **190**(1): 50-54.

Brill K, Norpoth T, Schnitker J and Albring M (1991). Clinical experience with a modern low-dose oral contraceptive in almost 100,000 users. *Contraception,* **43**(2): 101-110.

Chien YW, Mares SE, Berg J, Huber S, Lambert HJ and King KF (1975). Controlled drug release from polymeric delivery devices III: *In vitro-in vivo* correlation for intravaginal release of ethynodiol diacetate from silicone devices in rabbits. *J. Pharm. Sci*., **64**(11): 1776-1781.

ChunXiao Li, YanKun Wang and Mei Ying Ning (2014). Preparation and release mechanism of gestodene reservoir-type intravaginal rings. *Yao. Xue. Xue. Ba.,* **49**(3): 399-405.

Clark MR, Kiser PF, Loxley A, McConville C and Malcolm RK and Friend DR (2011). Pharmacokinetics of UC781-loaded intravaginal ring segments in rabbits: a comparison of polymer matrices. *Drug Deliv. Trans. Res.,* **1**(3): 238-246.

de Andrade RP (1989). A multicenter clinical evaluation of a new monophasic combination: Minulet (gestodene and ethinyl estradiol). *Int. J. Fertil. Steril.,* **34**: 22-30.

Dieben TO, Roumen FJ and Apter D (2002). Efficacy, cycle control, and user acceptability of a novel combined contraceptive vaginal ring. *Obstet. Gynecol.*, **100**(3): 585-593.

Düsterberg B and Brill K (1990). Clinical experience with a low-dose oral contraceptive containing gestodene. *Adv. Contracept.,* **6**: 37-49.

Endrikat J, Müller U and Düster berg B (1997). A twelve-month comparative clinical investigation of two low-dose oral contraceptives containing 20μg ethinylestradiol /75μg gestodene and 30μg ethinylestradiol /75μg gestodene, with respect to efficacy, cycle control and tolerance. *Contraception,* **55**(3): 131-137.

Externbrink A, Clark MR, Friend DR and Klein S (2013). Investigating the feasibility of temperature-controlled accelerated drug release testing for an intravaginal ring. *Eur. J. Pharm. Biopharm.,* **85**(3): 966-973.

Gaspard U, Buicu C and van den Brûle F (2000). New forms of hormonal contraception. *J. Gynecol. Obst. Bio. R.,* **29**(3): 288-291.

Gupta KM, Pearce SM, Poursaid AE, Aliyar HA, Tresco PA, Mitchnik MA and Kiser PF (2008). Polyurethane intravaginal ring for controlled delivery of dapivirine, a non nucleoside reverse transcriptase inhibitor of HIV-1. *J. Pharm. Sci.,* **97**(10): 4228-4239.

Haihao Shao, Lining Yu, Mengning Lan, Fang Li and Jianxing Chen (2010). Development of Gestodene-releasing intrauterine device. *Reproduction & Contraception,* **30**(7): 469-472.

Jaithitivit L, Jaisamrarn U and Taneepanichskul S (2012). Cycle control, safety and acceptability of a new oral contraceptive containing ethinylestradiol 15 micrograms and gestodene 60 micrograms. *J. Med. Assoc. Thai.,* **95**(5): 630-635.

Kaplan B (1994). Desogestrel, norgestimate, and gestodene: The newer progestins. *Ann. Pharmacother*., **29**(7-8): 736-742.

Kerns J and Darney P (2011). Vaginal ring

contraception. *Contraception,* **83**(2): 107-115.

Kirkman RJ, Bromham DR, O'Connor TP and Sahota JE (1999). Prospective multicentre study comparing levonorgestrel implants with a combined contraceptive pill: final results. *Br. J. Fam. Plann.,* **25**(2): 36-40.

Kuhl H (1987). Progestagens in contraception. *Wiener medizinische Wochenschrift,* **137**(18-19): 433-440.

Lidegaard Ø, Løkkegaard E, Jensen A, Skovlund CW and Keiding N (2012). Thrombotic stroke and myocardial infarction with hormonal contraception. *New Engl. J. Med.,* **366**(24): 2257-2266.

Lihua Peng, Li Wang, Linrui Cai, Yue Meng and Youping Li (2008). Contraceptive effect and safety of ethinyl estradiol versus desogestrel: A systematic review. *Chin. J. Evid-based. Med.,* **8**(4): 252-260.

Lipp R (1998). Pharmaceutics: Selection and use of crystallization inhibitors for matrix-type transdermal drug-delivery systems containing sex steroids. *J. Pharm. Pharmacol.,* **50**(12): 1343-1349.

Maeda H, Sugie T, Sano A, Kawasaki H and Kurosaki Y (2004). Study on accelerated evaluation system for release profiles of covered-rod type silicone formulation using indomethacin as a model drug. *J. Control Release,* **94**(2): 337-349.

Malcolm K, Woolfson D, Russell J, Tallon P, McAuley L and Craig D (2003). Influence of silicone elastomer solubility and diffusivity on the *in vitro* release of drugs from intravaginal rings. *J. control release,* **90**: 217-225.

Malcolm RK, Edwards KL, Kiser P, Romano J and Smith TJ (2010). Advances in microbicide vaginal rings. *Antivir. Res.,* **88**: S30-S39.

Moss JA, Malone AM, Smith TJ, Kennedy S, Kopin E, Nguyen C and Baum MM (2012). Simultaneous delivery of tenofovir and acyclovir via an intravaginal ring. *Antimicrob. Agents Ch.,* **56**: 875-882.

Sitruk-Ware R, Nath A and Mishell Jr DR (2013). Contraception technology: Past, present and future. *Contraception*, **87**(3): 319-330.

Thonneau PF and Almont TE (2008). Contraceptive efficacy of intrauterine devices. *Am. J. Obstet. Gynecol.,* **198**(3): 248-253.

van den Heuvel MW, van Bragt AJM, Alnabawy AKM and Kaptein MCJ (2005). Comparison of ethinylestradiol pharmacokinetics in three hormonal contraceptive formulations: The vaginal ring, the transdermal patch and an oral contraceptive. *Contraception*, **72**(3): 168-174.

Wan GJ, Barnowski CE, Ambegaonkar BM, Bolge SC and McDonnell DD (2007). Treatment satisfaction with a transdermal contraceptive patch or oral contraceptives. *Contraception,* **75**(4): 281-284.

Wilde MI, Balfour JA (1995). Gestodene. *Drugs,* **50**: 364-395.

Woolfson AD, Malcolm RK and Gallagher RJ (2003). Design of a silicone reservoir intravaginal ring for the delivery of oxybutynin. *J. Control Release,* **91**: 465-476.

YanKun Wang, Ying Liu, Mei Ying Ning and Chun Xiao Li (2014). Determination of plasma concentration of gestodene from sustained releasing intravaginal ring in rabbits by LC-MS/MS. *Lat. Am. J. Pharm.,* **33**(5): 753-758.

Yi Sun, Jian Cheng Wang, Xuan Zhang, ZhiJun Zhang, Yan Zheng, Da Wei Chen and Qiang Zhang (2008). Synchronic release of two hormonal contraceptives for about one month from the PLGA micro spheres: *in vitro* and *in vivo* studies. *J. Control Release,* **129**(3): 192-199.

Zichella L, Sbrignadello C, Tomassini A, Di Lieto A, Montoneri C, Zarbo G and Perrone G (1999). Comparative study on the acceptability of two modern monophasic oral contraceptive preparations: 30µg ethinyl estradiol combined with 150µg desogestrel or 75µg gestodene. *Adv. Contracept.,* **15**(3): 191-200.