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Intravaginal ring delivery of the reverse transcriptase inhibitor TMC 120 as an HIV microbicide

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Abstract

TMC 120 (Dapivirine) is a potent non-nucleoside reverse transcriptase inhibitor that is presently being developed as a vaginal HIV microbicide. To date, most vaginal microbicides under clinical investigation have been formulated as single-dose semi-solid gels, designed for application to the vagina before each act of intercourse. However, a clear rationale exists for providing long-term, controlled release of vaginal microbicides in order to afford continuous protection against heterosexually transmitted HIV infection and to improve user compliance. In this study we report on the incorporation of various pharmaceutical excipients into TMC 120 silicone, reservoir-type intravaginal rings (IVRs) in order to modify the controlled release characteristics of the microbicide. The results demonstrate that TMC 120 is released in zero-order fashion from the rings over a 28-day period and that release parameters could be modified by the inclusion of release-modifying excipients in the IVR. The hydrophobic liquid excipient isopropyl myristate had little effect on steady-state daily release rates, but did increase the magnitude and duration of burst release in proportion to excipient loading in the IVR. By comparison, the hydrophobic liquid poly(dimethylsiloxane) had little effect on TMC 120 release parameters. A hydrophilic excipient, lactose, had the surprising effect of decreasing TMC 120 burst release while increasing the apparent steady-state daily release in a concentration-dependent manner. Based on previous cell culture data and vaginal physiology, TMC120 is released from the various ring formulations in amounts potentially capable of maintaining a protective vaginal concentration. It is further predicted that the observed release rates may be maintained for at least a period of 1 year from a single ring device. TMC 120 release profiles and the mechanical properties of rings could be modified by the physicochemical nature of hydrophobic and hydrophilic excipients incorporated into the IVRs.

Keywords: TMC 120; Microbicide; Reverse transcriptase; Intravaginal ring; HIV/AIDS

1. Introduction

In the continued absence of an effective HIV vaccine, there is much interest in the development of vaginal microbicides as a strategy to reduce the heterosexual transmission of the human immunodeficiency virus (HIV) (Stone, 2002; Malcolm et al., 2004; Shattock and Moore, 2003; Shattock and Solomon, 2004). Microbicides are chemical substances that, when formulated and delivered appropriately to the vagina prior to intercourse, have the potential to prevent or decrease the likelihood of viral transmission and subsequent infection. Although a vaginal microbicide may potentially act at any stage of the HIV infectivity cycle, it is preferable to disrupt the cycle as early as possible so as to minimise the risk of systemic infection. Various mechanistic strategies are being pursued, including development of compounds that: (i) inhibit binding/attachment/entry of HIV to host $CD4^+$ cells, (ii) inhibit the reverse transcription step in the viral replication cycle and (iii) maintain or restore the naturally protective vaginal environment (Malcolm et al., 2004).

TMC 120 (Dapivirine, Fig. 1), is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with high potency against wild-type HIV-1 (EC_{50} = 0.3 ng/ml, EC_{90} = 0.9 ng/ml, CC_{50} (MT4 cells) = 0.7 mg/ml, selectivity index = 2400) (Herrewege et al., 2004). The antiretroviral activity of TMC 120 when administered orally as monotherapy to HIV-positive patients has already been established (Gruzdev et al., 2001). With respect to its use as a vaginal microbicide, experiments in rabbits

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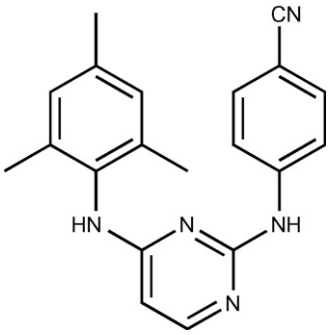

Fig. 1. Chemical structure of TMC 120 (MW = 329.41).

showed no vaginal irritation, and systemic absorption was only detected with very high (up to 1 M) concentrations (Tibotec, 2004). The potential effectiveness of TMC 120 as a vaginal microbicide has recently been demonstrated in the humanised severe combined immunodeficient (hu-SCID) mouse model, where intravaginal application of a TMC 120 gel formulation administered 20 min prior to non-invasive vaginal challenge with cell-associated HIV prevented systemic infection (Di Fabio et al., 2003). Importantly, the work provides the first *in vivo* evidence that a vaginally administered non-nucleoside reverse transcriptase inhibitor is capable of acting as an HIV microbicide.

Most vaginal microbicides under development are formulated as conventional, single-dose vaginal gels/creams. However, there is a strong clinical rationale for pursuing prolonged, controlled release formulations. Conventional semi-solid formulations generally exhibit poor vaginal retention, thus requiring administration immediately prior to each act of intercourse in order to afford optimal protection against HIV infection. This has particular implications for commercial sex workers who have multiple partners daily. The time-dependent variability in vaginal concentrations associated with the typical 'peak-and-trough' profile of multiple applications of a single-dose delivery system, coupled with issues relating to patient compliance and user-acceptability, are likely to compromise the effectiveness of semi-solid HIV microbicide formulations.

A number of reservoir (core)-type (Malcolm, 2003) intravaginal ring (IVR) drug delivery systems have already been commercialised for the long-term, controlled release of steroids for contraception and hormone replacement therapy (Woolfson et al., 2000; Ballagh, 2001; Johansson and Sitruk-Ware, 2004). The potential for exploiting vaginal ring technology for the prolonged delivery of HIV microbicides is now being considered (Malcolm and Woolfson, 2001; Malcolm et al., 2003, 2005; Moore and Shattock, 2003). Of critical importance is the ability to modify daily release of the vaginal microbicide to ensure that sufficient drug is present to block vaginal transmission of the virus, and to moderate, if necessary, any abnormal initial burst release that may lead to undesirable systemic absorption. Thus, in this study, a range of release-modifying excipients is studied in TMC 120 IVR systems, in order to provide flexibility in dosing regimens to be employed in subsequent clinical trial evaluations.

2. Materials and methods

2.1. Materials

TMC 120 (99.1%, HPLC) was kindly provided by Tibotec Pharmaceuticals (Mechelen, Belgium). Silicone elastomer base (MED-6382) was supplied by Warner Chilcott (UK) Ltd., Larne, UK. Tetrapropyl orthosilicate (95%), tin(II) 2-ethylhexanoate (stannous octoate, 95%), isopropyl myristate (IPM, 98%), poly(dimethylsiloxane) fluid (PDMS, 20 cSt), isopropanol (99.8%) and HPLC-grade acetonitrile were purchased from Sigma–Aldrich (Gillingham, UK). α-Lactose monohydrate was purchased from BDH (Poole, UK). Ultra-pure water was obtained using an Elga Purelab Maxima system. Analytical-grade phosphoric acid was purchased from Davidson and Hardy (Belfast, UK).

2.2. Preparation of TMC 120-loaded silicone core-type vaginal rings

Silicone elastomer base MED-6382 and the crosslinking agent tetrapropyl orthosilicate were blended in the ratio 40:1. The required weight of TMC 120 was added to this blend, followed by stannous octoate catalyst (0.5%, w/w), to produce an active mix. IVR devices of reservoir design were manufactured using a laboratory-scale, electrically heated reaction injection moulding machine (Warner Chilcott (UK) Ltd., Larne, UK) specifically designed for this purpose. Briefly, an active mix was cured at 80 °C for 2 min, producing TMC 120-loaded silicone elastomeric cores having the following dimensions: 2.0 or 4.5 mm cross-sectional diameter, 48.0 and 45.0 mm internal diameter, respectively, 52.0 and 54.0 mm external diameter, respectively. The TMC 120-loaded silicone cores were then encapsulated with a non-medicated silicone elastomer sheath. All of the TMC 120-loaded silicone IVR formulations had the same external dimensions (7.6 mm cross-sectional diameter, 43.0 mm internal diameter, 58.0 mm external diameter), irrespective of the sheath and internal core dimensions. The mean ring weight was 7.6 ± 0.1 g. A detailed description of the various IVR formulations is provided in Table 1.

2.3. In vitro TMC 120 release from reservoir vaginal rings

TMC 120-loaded IVRs were individually placed into stoppered conical flasks containing 100 ml of a 1:1 mixture of isopropanol/water in an orbital shaking incubator (60 rpm, 32 mm orbital diameter, Sanyo Gallenkamp IOX400.XX2.C) at 37 °C. The mixed solvent release medium, in which the solubility of TMC 120 was determined to be 1535 μg/ml, was selected to provide sink conditions for TMC 120 release. Daily sampling with complete replacement of the release medium was performed for 14 days, followed by twice-weekly sampling/replacement for a further 2 weeks. After the 4-week release period, the vaginal rings were removed from the release medium, blotted to dryness and subsequently weighed to quantify water uptake and/or release of substances.

Table 1
Description of TMC 120 intravaginal ring formulations

No.	TMC 120-loaded core				Non-medicated sheath		
	Drug Loading (%, w/w, mg)	Dimensions length/CSD (mm)[a]	Filler loading (%, w/w)	Excipient[b] (name, %, w/w)	Sheath thickness (mm)	Filler loading (%, w/w)	Excipient[b] (name, %, w/w)
1	7.5, 200	Full, 4.5	22.0	–	1.55	22.0	–
2	7.5, 200	Full, 4.5	10.0	–	1.55	10.0	–
3	37.0, 200	Full, 2.0	22.0	–	2.8	22.0	–
4	7.5, 200	Full, 4.5	22.0	IPM, 1.0	1.55	22.0	IPM, 1.0
5	7.5, 200	Full, 4.5	22.0	IPM, 5.0	1.55	22.0	IPM, 5.0
6	7.5, 200	Full, 4.5	22.0	IPM, 10.0	1.55	22.0	IPM, 10.0
7	7.5, 200	Full, 4.5	22.0	PDMS, 1.0	1.55	22.0	PDMS, 1.0
8	7.5, 200	Full, 4.5	22.0	PDMS, 5.0	1.55	22.0	PDMS, 5.0
9	7.5, 200	Full, 4.5	22.0	PDMS, 10.0	1.55	22.0	PDMS, 10.0
10	7.5, 200	Full, 4.5	22.0	Lactose, 1.0	1.55	22.0	Lactose, 1.0
11	7.5, 200	Full, 4.5	22.0	Lactose, 5.0	1.55	22.0	Lactose, 5.0
12	7.5, 200	Full, 4.5	22.0	Lactose, 10.0	1.55	22.0	Lactose, 10.0
13	7.5, 200	Full, 4.5	22.0	Lactose, 20.0	1.55	22.0	Lactose, 20.0

Mean weight of 2.0 mm full-length cores = 0.54 g.
[a] Mean weight of 4.5 mm full-length cores = 2.67 g.
[b] IPM = isopropylmyristate; PDMS = polydimethylsiloxane oil.

For all *in vitro* drug release data, the coefficient of variation was ≤2.23% for mean release rates of replicates of the same design and formulation ($n = 4$). This high degree of reproducibility in *in vitro* studies is typical for IVRs produced in our laboratory, which are manufactured by reaction injection moulding using precision-engineered stainless steel moulds identical to those used in the commercial manufacturing process. As a result, error bars have been omitted from the release plots (Figs. 2–5) as, in most cases, they are of a similar size to the plot symbols.

2.4. Quantification of release of TMC 120 by high performance liquid chromatography

TMC 120 released from IVR formulations was quantified using reverse-phase high performance liquid chromatography with ultraviolet detection (Waters Breeze HPLC system; Phenomenex Synergi 4 μ Fusion-RP80 column 4.6 mm i.d. × 150 mm; run temperature 25 °C; isocratic mode; mobile phase 1:1 acetonitrile/0.01 M pH 2.7 phosphate buffer; flow rate 1.0 ml/min; detection wavelength 290 nm; injection volume 10 μl; TMC 120 retention time 2.9 min). A linear calibration plot for TMC 120 was obtained over the range 0.1–100 μg/ml ($R^2 = 0.999$).

2.5. Compression testing of vaginal rings

The compressional force (Newtons) required to depress the vaginal rings through a specified distance was measured on a modified TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, UK). The ring was placed vertically in a holder and subjected to a compression cycle (distance 5.0 mm, rate of compression 2 mm/s, 5 cycle counts) using an aluminium probe. The rings were tested before and after the 4-week dissolution period. Error bars for replicate determinations ($n = 4$) were between ±0.01 N, values that are too small for graphical representation.

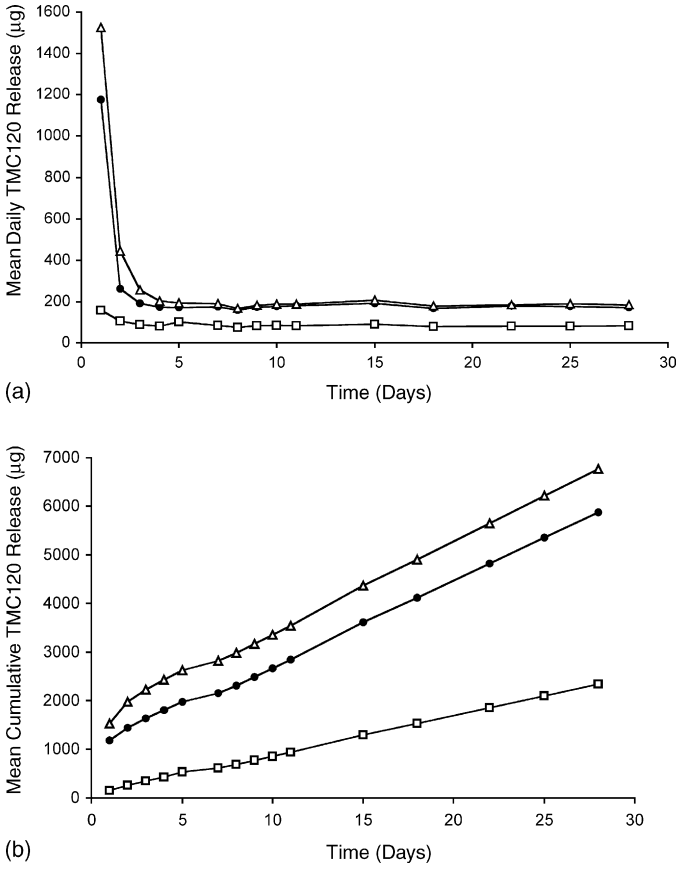

Fig. 2. Mean *in vitro* daily (a) and cumulative (b) release of TMC 120 from reservoir-type silicone vaginal rings: (●) 22% filler and 4.5 mm full core, (△) 10% filler and 4.5 mm full core and (□) 22% filler and 2 mm full core (Formulations 1, 2 and 3, respectively).

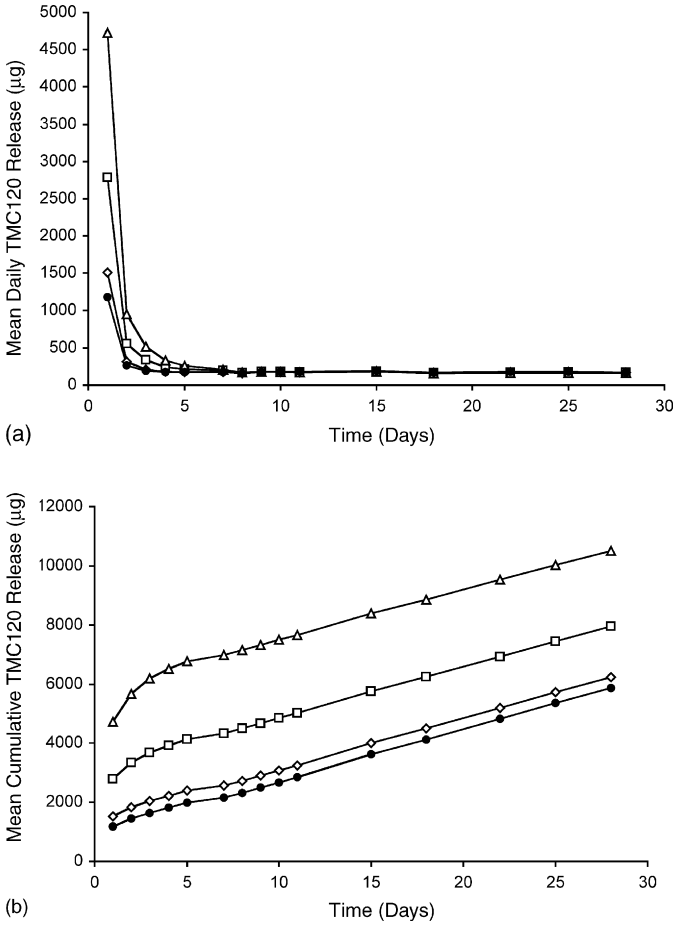

Fig. 3. Mean *in vitro* daily (a) and cumulative (b) release of TMC 120 from reservoir-type silicone IVRs containing various IPM loadings: (●) 0% (w/w) (◊) 1.0% (w/w), (□) 5.0% (w/w), and (△) 10.0% (w/w) (Formulations 1, 4, 5 and 6, respectively).

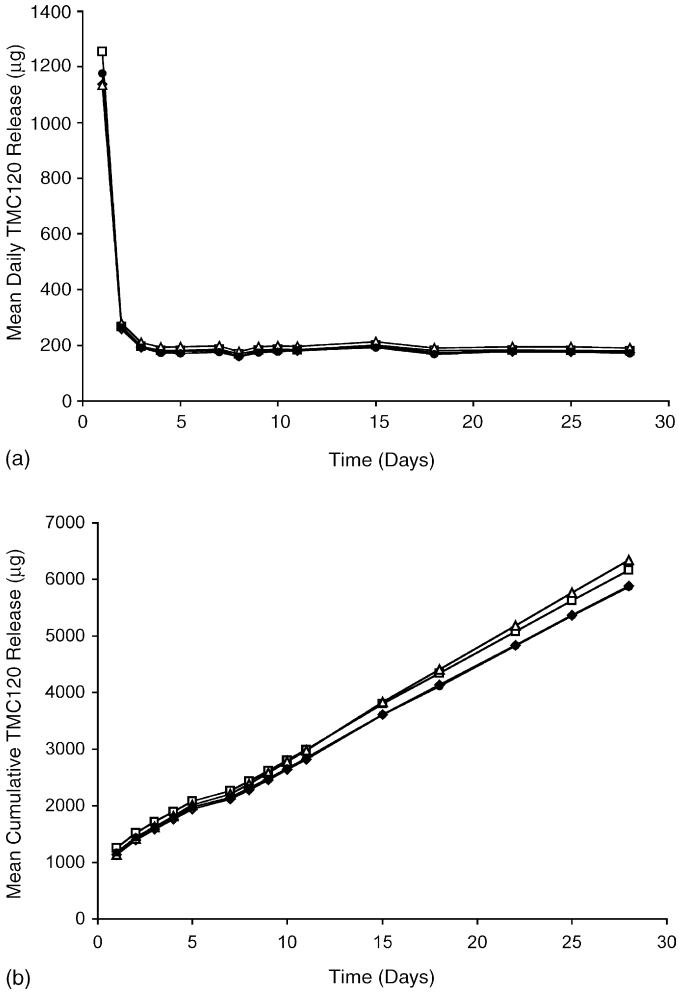

Fig. 4. Mean *in vitro* daily (a) and cumulative (b) release of TMC 120 from reservoir-type silicone vaginal rings containing various PDMS loadings: (●) 0% (w/w), (◊) 1.0% (w/w), (□) 5.0% (w/w), and (△) 10.0% (w/w) (Formulations 1, 7, 8 and 9, respectively).

3. Results and discussion

3.1. In vitro release of TMC 120 from silicone reservoir vaginal rings

Daily and cumulative release versus time profiles describing the release of TMC 120 from the non-excipient-loaded vaginal rings (Formulations 1, 2 and 3, Table 1) are presented in Fig. 2. The rings differed in the amount of mechanical filler in the silicone elastomer and the relative core/sheath dimensions. Each profile depicts an initial burst followed by a constant daily release phase. The burst is a common feature of reservoir-type IVRs and is attributed to enhanced dissolution of the active within the core of the ring under the high temperature conditions associated with curing, and its subsequent diffusion into and deposition within the sheath layer on cooling to ambient temperature. The presence of active in the sheath effectively reduces the diffusional pathway of the diffusing molecules during the initial release period. Thereafter, when the excess active in the sheath layer has been depleted, the diffusional pathway for all molecules is restored to that defined by the sheath thickness, and the release profile adopts the conventional steady-state profile. For Formulations 1–3, the magnitude of the burst, quantified by extrapolating the linear portion of the cumulative release versus time profile until it intercepts the *y*-axis, is shown to depend both upon the filler concentration and the relative core/sheath dimensions (Table 2). The larger burst observed with decreased filler content is attributed to the enhanced ability of the sheath layer, on account of its larger silicone/filler ratio, to dissolve TMC 120 from the core during the elevated temperature manufacturing process. It is also apparent that the magnitude of the Day 1 release is between 7 and 10 times larger for the 4.5 mm core rings (Formulations 1 and 2) compared with the 2.0 mm core ring (Formulation 3), a consequence of the larger drug loading in the former ring coupled with a smaller sheath volume into which the TMC 120 may permeate during manufacture.

The constant daily release rates observed after the initial burst (Fig. 2) are indicative of zero-order kinetics, i.e. release rate is independent of drug concentration in the IVR. In fact, for a given core/sheath ratio, the only influence of increasing drug loading should be to prolong the duration of constant daily release. The magnitude of the constant daily release rate is directly related to the IVR sheath thickness, as predicted on the basis of the following equation describing the diffusion-controlled membrane

Table 2
Summary of the release data for the various vaginal ring formulations

Form no.	Equation of line of best fit	R^2 coefficient	Release rate (μg/day)	Burst (μg)
1	$y = 173.7x + 989.5$	0.998	173.7	989.6
2	$y = 184.5x + 1577.2$	0.998	184.5	1577.2
3	$y = 80.6x + 72.9$	0.998	80.6	72.9
4	$y = 173.4x + 1372.9$	0.998	173.4	1372.9
5	$y = 171.1x + 3164.2$	0.999	171.1	3164.2
6	$y = 166.5x + 5853.1$	0.999	166.5	5853.1
7	$y = 176.1x + 944.7$	0.998	176.1	944.7
8	$y = 182.1x + 1052.1$	0.998	182.1	1052.1
9	$y = 193.0x + 919.7$	0.998	193.0	919.7
10	$y = 171.6x + 1033.5$	0.998	171.6	1033.5
11	$y = 190.5x + 725.0$	0.997	190.5	725.01
12	$y = 205.4x + 620.6$	0.997	205.5	620.7
13	$y = 255.3x + 56.7$	0.994	255.3	56.7

permeation process (Chien, 1992) and discussed in detail by Malcolm (2003):

$$Q = D_{SIL} C_{SIL} t / h$$

where Q is the cumulative release per unit area, D_{SIL} the apparent diffusion coefficient of TMC 120 in the silicone elastomer, C_{SIL} the solubility of TMC 120 in the silicone elastomer, h the IVR sheath thickness, and t is time. A comparison of the release rates observed for the 2 and 4.5 mm core rings containing 22% (w/w) filler (174 and 81 μg/day, Formulations 1 and 3, respectively, Table 2) confirms the direct relationship between release (Q/t) and sheath thickness (h). The small increase in release rate associated with decreasing the filler concentration to 10% (w/w) (184 μg/day, Formulation 2) is the consequence of a less tortuous diffusional pathway for the TMC 120 molecules.

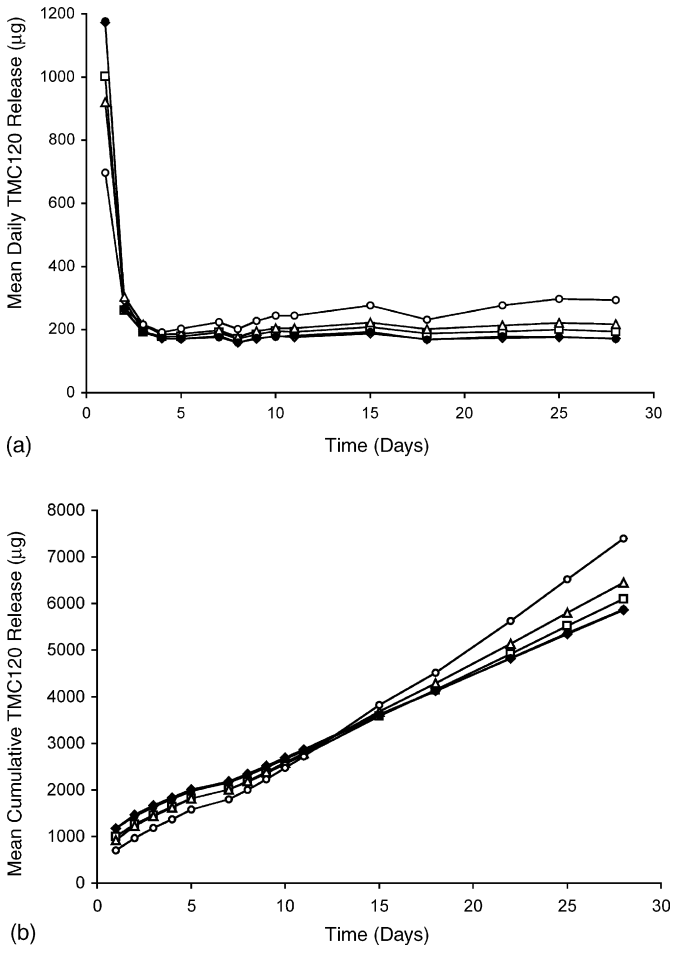

Fig. 5. Mean *in vitro* daily (a) and cumulative (b) release of TMC 120 from reservoir-type silicone vaginal rings containing various lactose loadings: (●) 0% (w/w), (◊) 1.0% (w/w), (□) 5.0% (w/w), (△) 10.0% (w/w) and (◯) 20.0% (w/w) (Formulations 1, 10, 11, 12 and 13, respectively).

In order to investigate the influence on TMC 120 release of incorporating excipients into the IVR formulations, three pharmaceutically acceptable vaginal excipients were selected. Isopropyl myristate (IPM) and poly(dimethylsiloxane) (PDMS) represent relatively hydrophobic, low and high molecular weight liquid excipients, while lactose represents a relatively hydrophilic, low molecular weight solid excipient. The liquid excipients were incorporated into both the core and the sheath compartments of 4.5 mm core, 22% (w/w) filler IVRs (i.e. Formulation 1) at 1, 5 and 10% (w/w) concentrations, while the solid excipient was also added at 20% (w/w). It proved difficult to incorporate more than 10% (w/w) of the liquid excipients into the IVRs without dramatically inhibiting the elastomer curing reaction and the mechanical integrity of the resulting rings.

The rationale for incorporating IPM and PDMS into the IVR formulations was that such hydrophobic liquids might enhance the permeation rate of TMC 120, either through increased solubility of the hydrophobic TMC 120 molecule in the silicone elastomer or by providing a more open network structure with a concomitant increase in the TMC 120 diffusion rate. The daily and cumulative release profiles for IVRs containing IPM and PDMS are shown in Figs. 3 and 4, respectively, while the release rates and burst data for each formulation are provided in Table 2. Although the addition of increasing amounts of IPM to the IVRs produced a proportional increase in the Day 1 burst (Fig. 3) ($y = 353x + 1136$, $R^2 = 0.9976$) and prolonged the duration of the burst from 2 days (Formulation 1) to 5 days (Formulation 6), it did not significantly influence the magnitude of the subsequent steady-state release rate (Table 2). The large burst effect observed with increasing IPM concentration may be undesirable as the elevated levels of TMC 120 in the vagina may promote systemic uptake of the microbicide. On the other hand, an enhanced

burst might be useful in establishing an initial concentration of the microbicide in the vagina.

The increased burst and unchanged steady-state release rate are consistent with a system wherein a proportion of the TMC 120 dispersed within the core dissolves in the IPM, and then co-diffuses through the sheath in the solvated state before being released. However, once the concentration of IPM is depleted in the IVR (up to 5 days) the release rate is dictated by the conventional permeation of TMC 120 through the silicone matrix (mediated by solubility of TMC 120 within the silicone elastomer only), thereby generating similar release rates as the non-excipient IVR. Additional evidence for this explanation is provided later by the weight-change analysis (Fig. 7), where the post-release mass of the IVRs decreased with increasing initial IPM loading.

By comparison, the PDMS-loaded VR formulations had minimal effect both the burst effect and the release rate (Fig. 4 and Table 2) compared with the non-excipient core-type ring. It is therefore considered that TMC 120 has similar solubility in the PDMS liquid as in the silicone elastomer network, since, from the perspective of a small molecule such as TMC 120, the molecular environments are very similar. It is also clear that the incorporation of liquid PDMS into the crosslinked elastomer network did not provide a more 'open' network structure leading to enhanced release rate.

Dimethyl silicone elastomers are hydrophobic materials with excellent water-repellent characteristics. However, the hydrophobic properties may be substantially modified through the addition of hydrophilic substances into the elastomer mix prior to crosslinking/molding. This approach is particularly useful in enhancing the release of hydrophilic drugs, and is mediated by permitted water uptake and the subsequent formation of aqueous pockets/channels in the elastomer through which a water-soluble drug molecule may diffuse and be released (Carelli and Di Colo, 1983; Hseih and Chien, 1985; Di Colo, 1992; Rehula and Di Colo, 1993; Kajihara et al., 2003). The hydrophilic excipient selected for this study was lactose, a simple, low molecular weight sugar. The incorporation of lactose into the IVRs had the surprising effect of decreasing the burst while also increasing the release rate in a concentration-dependent manner (Fig. 5 and Table 2). The reduction in the burst parameter (Table 2) may be attributed to the uptake/release of the isopropanol/water dissolution media into the lactose-loaded external surface layers of the VR, thereby competitively inhibiting the release of TMC 120. By Day 3, however, all the lactose-loaded rings were releasing similar daily amounts of TMC 120. Thereafter, the release rates were noted to increase linearly with increasing lactose loading ($R^2 = 0.989$) (Table 2). This trend may result from the dissolution of lactose particles from the surface layers of the ring to produce solvent-filled pockets/channels in the silicone elastomer, which would in effect provide an increased surface area for release of TMC 120 from the rings.

3.2. Post-release weight of vaginal rings

The highly hydrophobic nature of conventional dimethyl-silicone elastomers ensures that they have limited water absorption/permeation ability. However, the addition of various hydrophilic agents to silicone elastomer formulations has been shown to improve water uptake and, in the case of hydrophilic drug molecules, enhance release rates (Riggs et al., 1997).

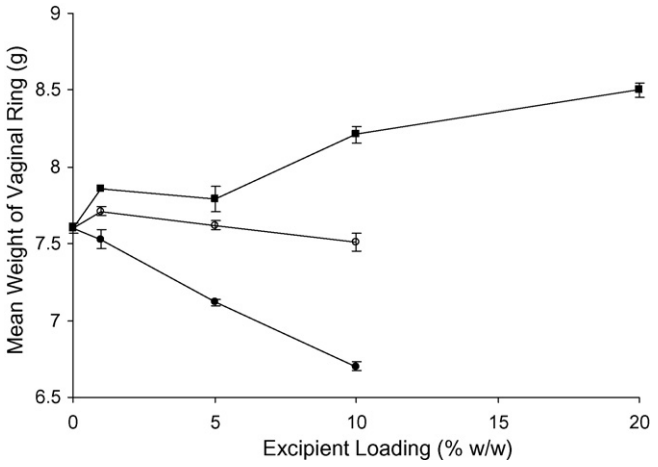

Fig. 6. Mean weight of vaginal ring formulations after 28-day release period: (●) IPM, (◯) PDMS and (■) lactose.

Conventional silicone IVRs containing no excipient (Formulations 1–3) produced no significant weight changes during the release period. The post-release weight of IVR formulations containing various loadings of IPM, PDMS and lactose are presented in Fig. 6. In all cases, the extent of the weight changes suggest that they are attributed to weight gain/loss resulting from inclusion of the excipient into the IVR formulation rather than release of the drug itself.

IPM-loaded rings showed a marked decrease in weight proportional to the loading, verifying that IPM is released from the IVRs into the release medium. Moreover, the observed weight decreases confirm that all of the initial IPM content has been released from the rings during the course of the release study, a fact consistent with the relatively low molecular weight of IPM (MW 270.5 g/mol) and its highly hydrophobic character (estimated log $P = 7.17$).

For the PDMS-loaded rings, a similar decrease in weight was observed, correlating with increasing PDMS loading. However, in this case the weight loss is an order of magnitude less than that observed with IPM, presumably due to the fact that the polymer chains of PDMS interpenetrate with those of the silicone elastomer network, thereby inhibiting diffusion and subsequent release of PDMS from the IVRs.

The lactose-loaded IVRs showed an increase in ring weight as a function of excipient loading, as a result of absorption of the release medium. α-Lactose monohydrate dissolves to the extent of approx. 25 g/100 ml in water.

3.3. Mechanical testing of vaginal rings

In addition to their potential for modifying drug release rate, there is also the possibility that inclusion of excipients into IVR formulations may adversely influence their mechanical properties. The mechanical properties of an IVR must ensure optimal vaginal compatibility and user acceptability. If

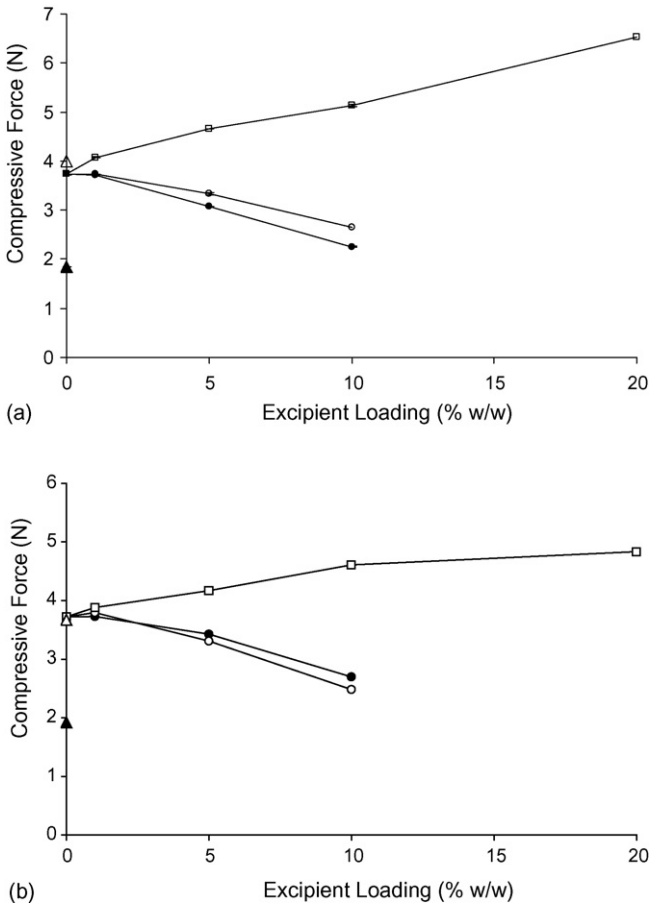

Fig. 7. Influence of excipient type and concentration on the: (a) pre- and (b) post-release compressional mechanical properties of TMC 120-loaded, reservoir-type, silicone vaginal rings prior to release: (●) IPM, (◯) PDMS, (□) lactose, (▲) 10% (w/w) filler vaginal ring and (△) 2 mm core vaginal ring.

the mechanical strength is too low, the ring could either be expulsed from the vagina or be prone to rupture. If the mechanical strength is too high, the inflexibility of the device could cause irritation/ulceration of the vaginal tissue. It was, therefore, necessary to assess the mechanical characteristics of the rings before and after release, since both incorporation of such excipients into the rings and the subsequent release/uptake of drug/excipients/release medium are likely to influence their mechanical characteristics. The compressional strength of each VR formulation was evaluated on a specially modified Texture Analyser, and the results are presented graphically in Fig. 7.

Before release, the compression strength of the IVRs increased linearly with increasing percentage of the solid excipient (lactose) and decreased linearly with increasing percentage of liquid excipients (PDMS, IPM). Both trends are readily explained in terms of the physical state of the excipient, whereby solid excipients impart rigidity to the silicone elastomer (in much the same way as mechanical fillers do), while liquid excipients 'dilute' the silicone network. IVRs manufactured from 10% (w/w) filled silicone elastomer (no excipient, Formulation 2) were observed to have approximately half the mechanical strength of those prepared from 22% (w/w) filled silicone elastomer (Formulations 1 and 3).

After release, only the IVR containing lactose displayed significant differences in mechanical properties compared to those determined before the release period. IVRs containing this solid excipient maintained an approximately linear increase in compressional force with increasing percentage of excipient. However, the magnitude of the post-release compression force was decreased at each concentration of excipient relative to those measured before release. This reduction is most probably caused by: (i) the dissolution and subsequent release of lactose from the surface layers of IVR matrix, thereby reducing the overall solid content, and (ii) the uptake of liquid release medium, as discussed earlier.

Despite the considerable changes in mechanical properties caused by incorporation of excipients into the IVRs, the compressional forces reported are still regarded as acceptable in relation to their potential use *in vivo*, based on previous experience with intravaginal ring systems.

3.4. Predicted vaginal concentrations of TMC 120 required to provide protection

It is possible to predict, based on published EC_{50} values for TMC 120 (Herrewege et al., 2004) and assuming *in vivo* release rates are similar to those observed *in vitro*, whether or not TMC 120 released from an IVR formulation might be effective in preventing vaginal transmission of HIV. A conservative upper limit for the combined volume of semen plus vaginal fluid is 16 ml, comprising 8 ml of vaginal fluid and 8 ml of semen (Chia et al., 1998; Owen and Katz, 1999). TMC 120 *in vitro* release rates observed in this study ranged from 13 to 255 μg/day (Table 2), equivalent to approximately 1.6×10^{-9} to 3.2×10^{-8} mol/h. Thus, within 1 h of applying the IVR, and assuming no net gain or loss of vaginal/seminal fluid, the concentrations of TMC 120 would be established in the 0.1–2.0 μM range. Although these calculations do not take into account: (i) the complex dynamics of fluid gain and loss from the vagina (particularly during and after intercourse), (ii) the possibility of forming vaginal tissue depots of TMC 120, or (iii) the potential for systemic uptake of TMC 120 through continuous use of the ring, they do suggest that IVR delivery systems have the potential to provide long-term maintenance of TMC 120 levels within the micro/nanomolar effective concentration range postulated on the basis of *in vitro* HIV cellular data.

4. Conclusions

This study establishes the ability to modify the burst effect and the daily release rate associated with *in vitro* release of the lead vaginal microbicide TMC 120 from reservoir-type silicone vaginal rings through incorporation of pharmaceutically acceptable vaginal excipients having a diversity of physicochemical characteristics. A particular significance of the study lies in the fact that such substances could be added to IVR formulations to provide a secondary function beyond that of modifying the drug release characteristics. Importantly, the observed *in vitro* TMC 120 release rates from all of the IVR formulations are within the nano/micromolar range required to prevent HIV infection.

Simple modification of the ring design, by changing the TMC 120 core length or the sheath thickness, may also be useful in further optimising release rates for effective *in vivo* protection.

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