

Long-term, controlled release of the HIV microbicide TMC120 from silicone elastomer vaginal rings

R. Karl Malcolm*, A. David Woolfson, Clare F. Toner,
Ryan J. Morrow and Stephen D. McCullagh

School of Pharmacy, Medical Biology Centre, Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

Received 21 June 2005; returned 12 August 2005; revised 17 August 2005; accepted 17 August 2005

Objectives: The feasibility of providing prolonged and controlled release of the experimental non-nucleoside reverse transcriptase inhibitor TMC120 from a silicone vaginal ring in quantities sufficient to maintain a vaginal concentration offering protection against heterosexual HIV transmission was investigated.

Methods: Core-type, silicone elastomer vaginal rings containing TMC120 were manufactured, and *in vitro* release studies performed under sink conditions. The experimental release data, as determined by HPLC, were correlated with estimates of vaginal TMC120 concentrations required to inhibit HIV replication.

Results: Continuous, zero-order release of TMC120 from core-type vaginal rings was observed *in vitro* over a 71 day period, equivalent to 136 µg/day. The release rate is predicted to maintain vaginal concentrations of the antiretroviral in the range of several orders of magnitude in excess of reported HIV inhibitory concentration values.

Conclusions: Continuous and prolonged zero-order release of TMC120 from a silicone vaginal ring device at quantities predicted to prevent HIV infection was observed.

Keywords: antiviral, diffusion, drug release, zero order

Introduction

Given the devastating effects of the HIV/AIDS epidemic, especially within the developing world, and the continuing difficulties in developing an effective HIV vaccine, there is a clear scientific rationale for developing alternative strategies to prevent HIV infection. The most promising strategy currently being pursued to prevent heterosexually acquired HIV is that of vaginal microbicides.^{1,2} These are chemical substances that, when formulated appropriately and applied to the vagina before intercourse, have the potential to either prevent or reduce the risk of HIV transmission through a number of well-established mechanisms.^{1,2}

As of June 2005, 15 HIV microbicide candidates, including the potent experimental non-nucleoside reverse transcriptase inhibitor (NNRTI) TMC120,^{3–5} were undergoing clinical trials.⁶ Most of these compounds are being evaluated in conventional semi-solid gel formulations designed to provide a single dose of the

microbicidal agent, applied immediately before intercourse. The potential for developing controlled release formulations for long-term administration of vaginal microbicides has only recently been considered,² and may overcome many of the compliance, acceptability and efficacy issues associated with single-dose, gel-based products.⁷

Several *in vitro* and animal model studies have demonstrated that TMC120 is a potent NNRTI that effectively prevents both cell-free and cell-associated HIV infection at concentrations in the nanomolar range.^{3–5} In an *in vitro* model using monocyte-derived dendritic cells and autologous CD4+ T cell co-cultures designed to mimic sustained antiretroviral delivery, TMC120 blocked primary infection with monotropic HIV-1 Ba-L at a 0.01 µM concentration and secondary cultures at 0.1 µM.³ In addition, the low cytotoxicity and high therapeutic index of TMC120 make it an ideal candidate for development as an HIV vaginal microbicide. Currently, microbicidal gel formulations of TMC120 are being tested in Phase 1 trials.

*Corresponding author. Tel: +44-28-9097-2319; Fax: +44-28-9024-7794; E-mail: k.malcolm@qub.ac.uk

The emergence of resistance to NNRTIs is well documented. Although initial studies have demonstrated that TMC120 is active against both wild-type and drug-resistant strains of HIV, the whole issue of resistance, particularly in long-term use as a prophylactic vaginal microbicide, will need to be closely monitored in subsequent clinical investigations.

In this study, we report for the first time the prolonged and controlled release of a lead candidate vaginal microbicide, TMC120, from a silicone elastomer vaginal ring. Vaginal rings have already been commercialized for the sustained release of steroids for hormone replacement therapy (Femring®, Estring®) and contraception (Nuvaring®).⁷

Materials and methods

Materials

TMC120 was kindly provided by Tibotec-virco (Mechelen, Belgium). MED6382 medical grade silicone elastomer was supplied by Warner Chilcott UK. Tetrapropyl orthosilicate (95%) and tin(II) 2-ethylhexanoate (95%) were purchased from Sigma–Aldrich (Gillingham, UK). Isopropanol (99.8%) and HPLC-grade acetonitrile were purchased from Riedel-de Haën and ultra-pure water was obtained using an Elga Purelab Maxima system.

Manufacture of core-type silicone vaginal rings loaded with TMC120

Core-type vaginal rings (also known as ‘reservoir-type’ rings) containing 400 mg of TMC120 in a full-length silicone elastomer core and encapsulated with a non-medicated silicone elastomer sheath layer were manufactured on a laboratory-scale, ring-making machine according to a standard method already described in the literature.⁸ The rings had the following characteristics: 5.5 mm core cross-sectional diameter; 9.0 mm ring cross-sectional diameter; 55.0 mm overall ring diameter; 10.0 (±0.2) g mean weight of rings.

In vitro release studies

Each TMC120-loaded vaginal ring was suspended in a stoppered 250 mL conical flask containing 200 mL of a 1:1 mixture of isopropanol/water (to ensure sink conditions), and the flasks were placed in an orbital (60 rpm; 32 mm orbital diameter) shaking incubator at 37°C (Sanyo Gallenkamp IOX400.XX2.C). Sampling followed by complete replacement of the release medium was performed every day for the first 14 days and at regular intervals thereafter.

Quantification of release of TMC120 using HPLC analysis

The amount of TMC120 released from the rings was quantified using reversed-phase HPLC with ultraviolet detection (Waters Breeze HPLC system; Phenomenex Synergi 4μ Fusion-RP80 column 4.6 mm i.d.×150 mm; temp. 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; 10 μL injection volume; TMC120 retention time 2.9 min). A linear calibration plot for TMC120 was obtained over the range 0.1–100 μg/mL ($r^2 = 0.999$).

Results and discussion

The daily amounts of TMC120 released from the vaginal rings over the initial 14 days of the release study are presented in Figure 1. The

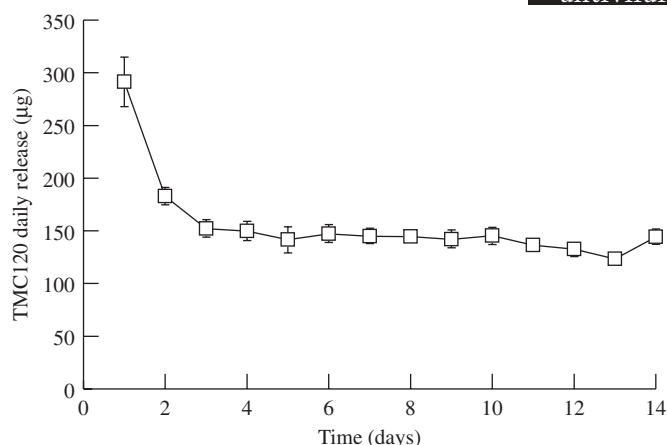


Figure 1. *In vitro* daily release of TMC120 from silicone, core-type vaginal rings over the initial 14 days.

release profile clearly shows a burst phase (days 1 to 2) followed by a linear phase (days 3 to 14). The burst effect has been observed previously in core-type vaginal rings and is a well-understood phenomenon attributed to drug solubility and manufacturing factors.⁸ The linear release phase describes a constant daily release (‘zero-order’ release kinetics) of ~140 μg/day resulting from the diffusion-controlled permeation of silicone-solubilized TMC120 molecules from the drug-loaded core, through the non-medicated silicone sheath layer of the ring, and into the surrounding release medium. The linear controlled release profile was maintained over the 71 days of the study, as demonstrated in the cumulative release profile (Figure 2), providing an average daily release of 136 μg/day ($R^2 = 0.9995$) as determined by linear regression. The y-intercept value of the line regression equation ($c = 0.2444$ mg; Figure 2) provides a quantitative measure of the magnitude of the initial burst effect. Given that only 2.5% (10 mg) of the total 400 mg TMC120 loading was released over 71 days, and by extrapolating the release profile to longer time periods, it is predicted that this constant daily release rate of TMC120 from a single ring device would easily be maintained for 1 year, and theoretically for up to 4 years (based on 50% total drug release).

Based on upper limits for the volumes of cervicovaginal fluid (8 mL)⁹ and semen (8 mL),¹⁰ and assuming that the *in vivo* release rate of TMC120 from a vaginal ring is similar to the ring-controlled (i.e. diffusion-controlled) release rate observed *in vitro* (136 μg/day, 5.7 μg/h or 1.7×10^{-8} mol/h), then the concentrations of TMC120 in the combined fluids within the vagina are calculated to be quickly established within the nanomolar/micromolar range required to prevent HIV infection (0.1 μM at 10 min, 1.1 μM at 1 h, 13.2 μM at 12 h and 26.4 μM at 24 h after initial ring placement). Of course, the calculations do not take into account the complex dynamics of fluid gain and loss from the vagina, or the possibility of forming tissue depots of TMC120 through continuous use of the ring. However, the results clearly demonstrate the potential for providing long-term and continuous protection against HIV infection in the form of a female-controlled vaginal ring device. Furthermore, the TMC120 release rate from the vaginal ring may be substantially varied through simple modification of the ring dimensions, e.g. varying the TMC120-core length and/or sheath layer thickness.

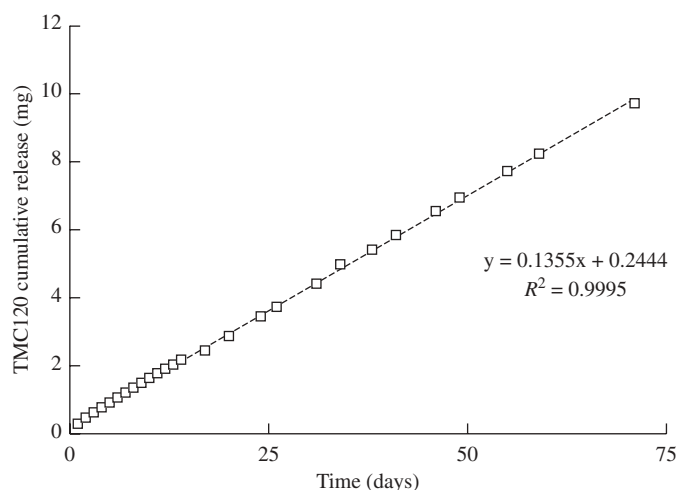


Figure 2. *In vitro* cumulative release of TMC120 from silicone, core-type vaginal rings over 71 days.

Acknowledgements

The assistance of Dr Jens Van Roey (Tibotec Pharmaceuticals Ltd) in establishing the vaginal concentrations of TMC120 required to afford protection is gratefully acknowledged. We acknowledge Warner Chilcott (UK) Ltd for the kind supply of polymers and equipment. The financial support of Tibotec Pharmaceuticals Ltd and the International Partnership for Microbicides is also acknowledged.

Transparency declarations

R. K. M. and A. D. W. have received research funds from Tibotec Pharmaceuticals Ltd and The International Partnership

for Microbicides. They also serve on the Scientific Advisory Board of the International Partnership for Microbicides.

References

1. Malcolm K, Woolfson D, Toner C *et al.* Vaginal microbicides for the prevention of HIV transmission. In: Harding SE, ed. *Biotechnology and Genetic Engineering Reviews*. Dorset: Intercept Ltd, 2004; 81–121.
2. Moore JP, Shattock RJ. Preventing HIV-1 sexual transmission—not sexy enough science, or no benefit to the bottom line? *J Antimicrob Chemother* 2003; **52**: 890–2.
3. Herrewewe YV, Michiels J, Van Roey J *et al.* In vitro evaluation of nonnucleoside reverse transcriptase inhibitors UC-781 and TMC120-R147681 as human immunodeficiency virus microbicides. *Antimicrob Agents Chemother* 2004; **48**: 337–9.
4. Herrewewe YV, Vanham G, Michiels J *et al.* A series of diaryl-triazines and diarylpyrimidines are highly potent nonnucleoside reverse transcriptase inhibitors with possible applications as microbicides. *Antimicrob Agents Chemother* 2004; **48**: 3684–9.
5. Di Fabio S, Van Roey J, Giannini G *et al.* Inhibition of vaginal transmission of HIV-1 in hu-SCID mice by the non-nucleoside reverse transcriptase inhibitor TMC120 in a gel formulation. *AIDS* 2003; **17**: 1597–604.
6. Alliance for Microbicide Development. *Microbicide Research and Development Database*. <http://secure.microbicide.org/NetReports/ClinicalTrialsOngoingByProduct.aspx> (13 June 2005, date last accessed).
7. Woolfson AD, Malcolm RK, Gallagher R. Drug delivery by the intravaginal route. *Crit Rev Ther Drug Carrier Syst* 2000; **17**: 509–55.
8. Woolfson AD, Malcolm RK, Gallagher RJ. Design of a silicone reservoir intravaginal ring for the delivery of oxybutynin. *J Controlled Release* 2003; **91**: 465–76.
9. Owen DH, Katz DF. A vaginal fluid simulant. *Contraception* 1999; **59**: 91–5.
10. Chia SE, Tay SK, Lim ST. What constitutes a normal seminal analysis? Semen parameters of 243 fertile men. *Hum Reprod* 1998; **13**: 3394–8.