Pharmacokinetics of the protein microbicide 5P12-RANTES in sheep following single dose vaginal gel administration


Published in:
Antimicrobial Agents and Chemotherapy

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2017 ASM. This work is made available online in accordance with the publisher’s policies. Please refer to any applicable terms of use of the publisher.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Download date:02. Jul. 2019
Pharmacokinetics of the protein microbicide 5P12-RANTES in sheep following single dose vaginal gel administration

John W. McBridea, Nicola Diasb, David Camerona, Robin E. Offordc, Oliver Hartleycd, P. Boyda, Vicky L. Kettable, R. Karl Malcolmd

School of Pharmacy, Queen’s University Belfast, Belfast, UKa; Envigo, Cambridgeshire, UKb; Mintaka Foundation for Medical Research, Geneva, Switzerlandc; Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerlandd

# Address correspondence to Karl Malcolm, k.malcolm@qub.ac.uk

Running head: Pharmacokinetics of 5P12-RANTES vaginal gel in sheep

Keywords: vaginal microbicide, antiretroviral, CCR5 receptor inhibitor; HIV prevention, HIV/AIDS
Abstract

SP12-RANTES, a chemokine analogue that potently blocks the HIV CCR5 co-receptor, is being developed as both a vaginal and rectal microbicide for prevention of sexual transmission of HIV.

Here, we report the first pharmacokinetic data for SP12-RANTES, following single dose vaginal gel administration in sheep. Aqueous gel formulations containing low (1.24 mg/mL), intermediate (6.16 mg/mL) and high (32.0 mg/mL; suspension-type gel) concentrations of SP12-RANTES were assessed via rheology, syringeability and in vitro release testing. Following vaginal gel administration in sheep, SP12-RANTES concentrations were measured in vaginal fluid, vaginal tissue and serum over a 96-h period. All gels showed non-Newtonian pseudoplastic behaviour, with high concentration gels exhibiting greater viscosity and cohesive structure. In in vitro release testing, >90% SP12-RANTES was released from the low and intermediate gels after 72 h. For the high concentration gel, ~50% SP12-RANTES was detected, attributed to protein denaturation during lyophilisation and/or subsequent solvation of the protein within the gel matrix. In sheep, SP12-RANTES concentrations in vaginal fluid, tissue and serum increased in a dose dependent manner. Highest concentrations were measured in fluid (10^5–10^7 ng/mL) followed by tissue (10^4–10^6 ng/mL) – both several orders of magnitude above reported half maximal inhibitory concentrations – and lowest in serum (< 10^5 ng/mL). The SP12-RANTES pharmacokinetic data is similar to that reported previously for other candidate microbicides. These data, coupled with SP12-RANTES’s picomolar potency, its strong barrier to resistance, and full protection observed in a rhesus macaque vaginal challenge model, support the continued development of SP12-RANTES as a microbicide.
In the continued absence of an effective vaccine against HIV, development of vaginally-administered microbicide products remains a priority biomedical strategy aimed at addressing the urgent and yet unmet need for reducing women’s risk of sexually-transmitted infection with HIV, particularly in developing countries (1, 2). Small-molecule antiretrovirals – most notably dapivirine (3–10), tenofovir (11–15) and tenofovir disoproxil fumarate (14, 16–19) – are currently the primary focus for the microbicide field, offering relatively low costs for synthesis of the active pharmaceutical ingredient, a diverse range of practical formulation options, and potent antiviral activity targeted at the reverse transcription step in the HIV replication cycle. However, there is a strong rationale for the development of new antiviral agents for HIV prevention that overcome some of the disadvantages associated with these lead candidate, small molecule, antiretroviral microbicides, including the potential emergence of resistant viruses and their post-entry mechanism of action (20–22). By comparison, many of the larger molecular weight biopharmaceutical compounds that have been or are being considered as vaginal microbicides – including cyanovirin-N (23–25), Griffithsin (26, 27), 5P12-RANTES (28, 29), T-1249 peptide (30), retrocyclin RC-101 (31) and monoclonal antibodies (32–34) – generally exhibit greater antiviral potency than current small molecule antiretrovirals (at least in in vitro models) and usually act prior to viral entry into the host cell by either directly targeting the free virus or by blocking cell receptors (35–38).

PSC-RANTES is a highly potent CCR5-inhibiting protein and an analogue of the natural CCR5 chemokine ligand RANTES (39). PSC-RANTES interaction with the CCR5 receptor leads to
intracellular sequestration of the receptor and prevention of HIV binding and infection (40).

Although PSC-RANTES has previously shown full protection in a macaque challenge study (41), it requires expensive chemical synthesis (28). 5P12-RANTES, by comparison, is a fully recombinant analogue of PSC-RANTES that was first identified using a modified phage display selection strategy (42). Similar to PSC-RANTES, 5P12-RANTES exhibits picomolar anti-HIV potency and afforded complete protection against SHIV infection in a rhesus vaginal challenge study (29, 42).

Notably, in that challenge study, 5P12-RANTES was administered vaginally in a PBS solution 30 min prior to simian human immunodeficiency virus (SHIV) exposure. Clinical grade 5P12-RANTES can be produced to cGMP standards using low-cost production through industrial microbial fermentation methods commonly employed for proteins used in the food and detergent industries (42, 43). Furthermore, this analogue inhibits the CCR5 receptor without inducting receptor internalization or signal activation (42). This is particularly important from a clinical perspective as CCR5 activation can induce inflammation, a risk factor linked to enhanced susceptibility to HIV infection (44).

As part of our ongoing efforts towards developing sustained release vaginal formulations for 5P12-RANTES (including new vaginal ring strategies), we report here for the first time the pharmacokinetics of vaginally-administered 5P12-RANTES measured following single dose aqueous gel administration in the sheep model. The study is the first to report pharmacokinetic data for 5P12-RANTES.
Results

Visual appearance of gels

The low and intermediate concentration 5P12-RANTES gels were clear viscous gels, similar in visual appearance and viscosity to the placebo microbicide gel. The high concentration gel, in which 5P12-RANTES was deliberately incorporated above its solubility limit, was white in appearance due to the presence of suspended lyophilised 5P12-RANTES material.

Rheological and syringeability assessment of 5P12-RANTES gels

Rheograms of viscosity vs. shear rate are presented in Fig. 1A, and indicate non-Newtonian shear thinning pseudoplastic behaviour, meaning that the viscosity decreases with increasing shear rate. Apparent gel viscosity, as measured by application of the Power Law, also generally increased with increasing 5P12-RANTES concentration (Figure 1B). Significantly higher viscosity values were measured for high dose gels compared to blank, low and medium dose gels, attributed to the presence of both solid (i.e. dispersed lyophilisate) and dissolved 5P12-RANTES in the high dose gel and only dissolved 5P12-RANTES in the low and intermediate gels. Intermediate and low gels had a significantly greater viscosity than the blank gel, attributed to the pH of the 5P12-RANTES stock solution (6.4 mg/mL in 1.7 mM acetic acid; pH 4.0) weakly affecting the HEC gel.

Syringeability is a measure of the work required to expel a liquid-based or semi-solid formulation (including vaginal gels) from an applicator or through a syringe needle (Figure 1C) (45–50).
Syringeability values ranged from 107–130 N.mm and increased with 5P12-RANTES loading, although differences were mostly not significant (Figure 1D).

In vitro release of 5P12-RANTES

The in vitro cumulative release vs. time plots for 5P12-RANTES gel formulations into SVF are presented in Figure 2 and reveal the effect of initial 5P12-RANTES loading on drug release. The low and intermediate gels provided release of >90% of the original 5P12-RANTES loading after 72 h (4.6 and 32.6 mg, respectively). However, the high concentration gel provided only 50% release (64.4 mg), despite there being no gel residue visible (or dispersed 5P12-RANTES lyophilisate material) at the end of the release experiment.

Sheep pharmacokinetics

The three HEC gel formulations containing 5P12-RANTES showed no detrimental effects on the health of the animals. 5P12-RANTES was measured in all post-dose vaginal fluid samples (Figure 3a), with mean values steadily declining from a high of $10^5$–$10^7$ ng/g 1 h after dosing to $10^5$–$10^4$ ng/g 96 h after dosing. For most sampling timepoints, vaginal fluid concentrations of 5P12-RANTES increased with increasing 5P12-RANTES loading in the gel. (It should be noted that Weck-Cel vaginal sampling cannot differentiate between sampling of any gel resident in the vagina and sampling of the fluid itself.)

5P12-RANTES was also measured in all post-dose vaginal biopsy homogenate samples (Figure 3b); 12 h after gel dosing, mean 5P12-RANTES concentrations values in tissue were $1.75 \times 10^5$,
3.53 × 10^5 and 1.03 × 10^6 ng/g for the low, intermediate and high gels, respectively. At 96 h, mean tissue levels for the high concentration gel (the only gel formulation for which tissue biopsies were taken at this timepoint) had declined to 9.2 × 10^3 ng/g.

SP12 RANTES was detected in some, but not all, serum samples, at concentrations <70 ng/mL. Of the 36 serum samples taken for each gel formulation (4 sheep x 9 timepoints), 18, 13 and 4 samples in the low, intermediate and high gel groups, respectively, had SP12-RANTES concentrations below the lower limit of quantification (0.176 ng/mL). Plots of mean (n=4) SP12-RANTES concentration vs. time are presented in Figure 3c for the three gel formulations. For the most part, the profiles are similar, showing peak concentrations between 3 and 8 h after dosing and subsequently declining to ~0.2 ng/mL by the 96 h timepoint (Table 1). The serum concentrations measured for the intermediate and high concentration gels are similar, despite the higher SP12-RANTES loading in the high concentration gel.

Based on these pharmacokinetic profiles, the key pharmacokinetic parameters – C_{max}, t_{max} and AUC – are reported in Table 1. The C_{max} and AUC values in both serum and vaginal fluid increase with increasing dose. The relatively small and non-linear increase observed on comparing the high dose gel with the intermediate dose gel is due to the presence of solid SP12-RANTES in the high dose gel. Serum t_{max} values for SP12-RANTES, although variable, ranged from 1–96 h, and most commonly 4 h. In vaginal fluid, t_{max} values were also variable but mostly in the range 1 to 12 h post dosing. Finally, individual sheep PK profiles are presented in Figure 4.
Discussion

In this study, vaginally-administered 5P12-RANTES was measured following single dose aqueous gel administration in sheep. The pseudoplastic behaviour of the gel, and indeed the general magnitude of the viscosity values, is typical of gels intended for vaginal drug administration, as reported previously for aqueous hydroxyethylcellulose gels and non-aqueous silicone gels (50–57). From a clinical perspective, pseudoplasticity offers certain advantages and disadvantages. Gels will likely spread more easily at higher shear rates (during coitus) or following dilution with vaginal fluids or semen leading to increased distribution in the vaginal environment and enhanced tissue coverage (51). However, there is also an increased likelihood of leakage of the gel from the vagina (58–60). The syringeability values are higher than values reported previously for other water-based vaginal gels (e.g. 35–67 N.mm for rheologically structured vehicles (46, 49), and 4.4 N.mm for Replens™ vaginal moisturiser (49)), although the test method used in this study involved testing expulsion of a greater quantity of gel (4 g vs. 3 g) through a greater plunger distance (50 mm vs. 30 mm) and the use of a commercial plastic vaginal applicator rather than a general purpose disposable plastic syringe. All things considered, the syringeability values measured translate into ease and practicality of gel product administration, as verified by the animal handlers.

In the in vitro release testing study, a relatively low release/recovery value (~50%) for 5P12-RANTES was noted for the high dose gel, compared to >90% release from the low and intermediate gels. We attribute this low recovery to the rendering of the 5P12-RANTES inactive...
following the lyophilisation process and/or the subsequent dissolution of the lyophilisate in the

*in vitro* release medium.

5P12-RANTES has previously been shown to be stable by *in vitro* cell fusion assay (i) when

exposed to elevated temperatures (up to $55^\circ$C in water for 24 h and $40^\circ$C for 1 week), (ii) at normal

vaginal pH for 24 h, (iii) and when incubated with human cervicovaginal lavage or human semen

at $37^\circ$C for 24 h (39). The data presented here suggest for the first time that 5P12-RANTES is also

sufficiently stable *in vivo* to allow detection and measurement of the protein by Weck-Cel

sampling of sheep vaginal fluid, as reported previously for various small molecule HIV

microbicides (61–64). However, as previously noted for the *in vitro* fusion assay (39), protein

modifications that may affect 5P12-RANTES activity can not be completely excluded due to using

ELISA and more appropriate analytical techniques will be required when undertaking further

preclinical development. Only a small number of studies describing vaginal delivery of peptides

or proteins, and particularly systemic absorption, are reported in the literature (65–69).

5P12-RANTES has previously been shown to fully protect against SHIV infection in a rhesus

macaque vaginal challenge model following vaginal administration of 4 mL of a 1 mmol/L 5P12-

RANTES solution in PBS (29). For reference, 1 mmol/L = 7.90 mg/mL based on a molecular

weight of 7904.8 g/mol for 5P12-RANTES, and therefore the overall dose administered in the

macaque challenge study was 31.6 mg (compared to the 24.7 mg dose administered in this

study using the intermediate concentration gel). In that challenge study, inoculation with 300

TCID<sub>50</sub> SHIV162P3 occurred 30 min after gel dosing. Based on our pharmacokinetic data in

Commented [JM1]: Response 16
sheep which used the same dosing volume of 5P12-RANTES gel, and assuming similar
distribution and drug absorption characteristics between the species, we estimate 5P12-
RANTES concentrations in the protected macaques at the time of challenge of ~10^7 ng/g in
vaginal fluid, ~10^5 ng/g in vaginal tissue, and ~ 1 ng/mL in serum.

The 5P12-RANTES concentrations measured here in the three biological compartments in sheep
are, in fact, similar in magnitude to those measured for other microbicide candidates
administered to women by vaginal gel (60, 70–72). Clearly, the inclusion of additional dispersed
5P12-RANTES in the high concentration gel beyond the solubility limit achieved with the
intermediate gel does not drastically contribute to increased absorption of 5P12-RANTES.
However, the high dose formulation might give rise to sustained release due to a depot-type
effect following vaginal administration of the gel. The PK data does seem to support the
hypothesis that lyophilized 5P12-RANTES contained within the gel leads to extended
pharmacokinetic exposure as indicated with vaginal fluid at the 72 and 96 h time points, despite
the limited recovery of 5P12-RANTES from the lyophilized material in vitro. Further analysis
reveals that the t_max values are greater compared with those usually measured for small
molecule antiretrovirals, such as maraviroc (73), and indicate that 5P12-RANTES is less well
absorbed by the systemic compartment. This is not surprising given the relatively large
molecular weight and greater hydrophilic character of 5P12-RANTES. Overall, these serum data
demonstrate that solubilised 5P12-RANTES within the gel formulations is capable of being
absorbed into the systemic compartment and that serum concentrations are significantly lower
than vaginal fluid and tissue levels, as is preferred for any locally acting HIV microbicide.
Following previous data demonstrating that vaginal administration of SP12-RANTES offered full protection in a macaque vaginal challenge model (29) and that SP12-RANTES is biologically stable (39, 74), the SP12-RANTES pharmacokinetic data presented here in sheep is encouraging and supports continued development of SP12-RANTES as a low-cost, stable, highly potent, HIV microbicide candidate.
Materials and Methods

Ethics

The study was carried out in compliance with applicable sections of the United Kingdom Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012. The study complied with the applicable sections of the Codes of Practice for the Housing and Care of Animals used in Scientific Procedures, and the Humane Killing of Animals under Schedule 1 of the Act.

Materials

A solution of recombinant 5P12-RANTES, 7.9 kDa, (6.4 mg/mL in 1.7 mM acetic acid; pH 4.0) produced as described previously[43] was supplied to Queen’s University Belfast (Belfast, UK) by collaborators at the Mintaka Foundation for Medical Research (Geneva, Switzerland). Pharmaceutical grade hydroxyethylcellulose (Natrosol® 250HX Pharm; HEC) and glycerol were obtained from Ashland UK Limited (Manchester, UK). Sorbic acid (preservative), sodium chloride, potassium hydroxide, calcium hydroxide, lactic acid, acetic acid, glucose, and bovine serum albumin (BSA) (most for preparation of simulated vaginal fluid) were purchased from Sigma-Aldrich (Gillingham, UK) [75]. Urea was obtained from VWR International (UK). Polyethylene vaginal applicators (pre-filled design; capped; volume 5.5 cm³) were purchased from HTI Plastics (Lincoln, Nebraska, USA).

Preparation of 5P12-RANTES loaded HEC gels

The composition of the 5P12-RANTES gel formulations was based on the Universal Placebo gel that has previously been developed as a control gel for clinical testing of vaginal microbicides [4].
Separate gel batches were prepared for *in vitro* and pharmacokinetic testing. For each batch of intermediate concentration 5P12-RANTES gel (6.18 mg/mL), 0.1% w/w sorbic acid (0.0250 g), 0.85% w/w sodium chloride (0.2125 g), and 2.7% w/w HEC (0.6750 g) were added to 25 mL of the 5P12-RANTES stock solution and then mixed in a SpeedMixer™ (2 x 30 s at 3000 rpm; DAC 150 FVZ-K, Hauschild, Germany). The resulting gel was allowed to fully hydrate overnight at 4–6°C. For the low concentration gel (1.24 mg/mL), the stock 5P12-RANTES solution was first diluted 1:4 with Type 1 water (Millipore Direct-Q 3 UV Ultrapure Water System, Watford, UK), before addition of 0.1% w/w sorbic acid, 0.85% w/w sodium chloride, and 2.7% w/w HEC. For manufacture of the high concentration gel, 0.1% w/w BSA was first added to the stock 5P12-RANTES solution and 20 mL aliquots of the resulting solution lyophilised (AdVantage Pro BenchTop Freeze Dryer, VirTis. Gardiner, NY, USA) in individual petri dishes of known weight. The freeze-drying procedure was as follows; material was frozen by cooling from 5 to −40°C over 1 h, with additional freeze of 2 h. The condenser was set to -50°C with initial chamber pressure of 50 mTorr. The shelf was then raised to 20°C over 27 h with simultaneous increase in chamber pressure as required. The required weight of the resulting lyophilisate was added slowly with gently with gentle manual mixing to ultrapure Type 1 water containing 0.1% w/w sorbic acid, 0.85% sodium chloride, and 2.7% w/w HEC, speedmixed (2 x 30 s at 3000 rpm) and hydrated overnight at 4–6°C to produce the final gel formulation with a 5P12-RANTES concentration of 32.0 mg/mL. Gel formulations were adjusted to pH 4.5 ± 0.2 and stored at 4–6°C immediately after preparation.
Gels were filled into single-use, disposable, screw-top, plastic vaginal applicators. Each gel was mixed (SpeedMixer™; 30 s, 3000 rpm), transferred to a syringe, and 4.00 g gel was slowly and carefully dispensed into each applicator to avoid incorporation of air bubbles. Applicators were then sealed with the screw top. For the sheep study gels were shipped to the animal testing facility under controlled temperature conditions (2–8°C).

**Rheological and syringeability assessment of gels**

The rheological behaviour of the gels was assessed by continuous flow rheometry using an AR 2000 Rheometer fitted with a 40-mm diameter stainless steel parallel plate (TA Instruments, USA). Gel samples were applied to the base plate and the steel parallel plate was lowered to a gap distance of 1000 µm. Excess gel was removed before the test. Testing was performed at 37°C in continuous ramp mode from 0.01–100 s⁻¹. Gel viscosity was determined using the Power Law applied to the linear portion of the resulting log-log plot of viscosity vs. shear rate.

Gel syringeability was assessed by measuring the work required to expel 4.0 g of 5P12-RANTES-loaded HEC gels from plastic vaginal applicators using a Texture Analyser (TA-XT2, Stable Microsystems, UK) fitted with a texture profile analysis (TPA) probe. Applicators were secured vertically and the probe was lowered to 5.0 mm above the barrel plunger. In compression mode, the probe was programmed to move at a rate of 2.0 mm/s through 50 mm upon contact with the plunger (1.0 mm/s before contact, with a trigger force of 0.025 N). The work was calculated by measuring the area under the resultant force-distance plot.
In vitro dissolution testing of 5P12-RANTES gels

Simulated vaginal fluid (SVF), modified to include an increased BSA concentration (0.1% w/w) to reduce adsorption of 5P12-RANTES to glassware, was prepared according to previously described methods (75, 76). The SVF was filtered immediately after preparation (sterile Millex-GS Syringe Filter Unit, mixed cellulose ester membrane, Millipore, UK) and stored for a maximum of 3 days at 4–6°C prior to use.

Each gel (4.0 g) was syringed into a sealed glass flask containing SVF (25 mL) and stored in a shaking orbital incubator (Infors HT AGCH-4103; 37°C, 60 rpm, 25 mm throw). A 1.0 mL sample was taken periodically and replaced with an equivalent volume of fresh SVF. Samples were carefully taken from the top of the SVF volume to avoid disturbing or sampling any residual gel sample. Samples were subsequently stored at −20°C prior to analysis by enzyme-linked immunosorbent assay (ELISA).

The concentration of 5P12-RANTES in the SVF release medium was measured by ELISA using the Human CCL5/RANTES ELISA kit (R&D Systems, UK, cat no. DRN00B). Samples were analysed per the manufacturer’s instructions after appropriate dilutions were made to ensure concentrations fell within the 0.002–2 ng/mL range of the kit. Absorbance was measured at 450 nm using a microplate reader (Enspire Multimode Plate Reader, PerkinElmer, USA) and the optical density at 570 nm was subtracted to correct for plate imperfections. The standard curve was transformed to a four-parameter logistic (4-PL) curve-fit and sample concentrations were calculated relative to it. Concentration readings were subsequently multiplied by the appropriate dilution factor.
Sheep pharmacokinetic study

Pharmacokinetic evaluation of the three 5P12-RANTES gel formulations following single dose vaginal administration was conducted at Envigo (Huntingdon, UK) using approximately 3-year old Welsh Mule ewes as the test species. Sheep were housed in indoor pens with wheat straw bedding. The animals were provided with natural light, supplemented with overhead fluorescent lighting as necessary, and full fresh air. The animals grazed on ewe and lamb pencil pelleted diet, with good quality meadow hay *ad libitum* and unrestricted water supply. A total of four sheep were used in the study. Initially, each sheep received a single dose of the low concentration gel, followed by periodic sample collection over a 96-h period, and a subsequent 7-day wash out. The intermediate and high concentration gels were then tested according to the same general schedule. Each animal was restrained in a comfortable standing position on the day of gel administration and a 4.0 g dose administered using a pre-filled plastic vaginal applicator. Clinical condition, body weight, and food consumption were recorded during the study. Blood and vaginal fluid was sampled at multiple time points during all three phases. Blood samples were drawn from the jugular vein and incubated for a minimum of 60 min at room temperature to facilitate clotting. Subsequently, the samples were centrifuged (1500 g, 15 min) to separate serum, and then divided and stored at −20°C in polypropylene tubes. Vaginal fluid was sampled using a pre-weighed Weck-Cel sponge that was again weighed after fluid uptake. The sponge was held in place in the vagina for 1 min and stored at −20°C in borosilicate glass tubes. Vaginal tissue biopsies (5 mm diameter) were sampled from the dorsal aspect of the vagina under local anaesthetic (isoflurane/oxygen used where necessary) once per animal in the low and
intermediate gel experiments (12 h post gel application), and twice in the high concentration gel experiment (12 and 96 h post gel application). Analgesia was administered 30 min prior to vaginal biopsy collection. Samples were rinsed with RPMI 1640 and weighed prior to freezing at −70°C.

Frozen samples were submitted for analysis to the Envigo’s Department of Biomarkers, Bioanalysis and Clinical Sciences (Immunoassay). Briefly, all tissue samples were homogenised in homogenisation buffer (PBS + 2% Triton X-100 + protease inhibitor cocktail) at 4°C and kept on wet ice at all times. Tissue were placed in a minimum of 3 mL of buffer and the ratio of buffer:sample weight was recorded. Samples were homogenated (GentleMACS Dissociator) and centrifuged at 4,566 g for 10 min. Supernatant was collected into tubes (Watson labelled PP) and placed on wet ice or stored at −70°C. Analysis was performed using a Human CCL5/RANTES ELISA kit, according to the manufacturer’s instructions.

Funding

This study was completed as part of a research project entitled ‘Chemokine-based microbicides: a pathway from a first-in-human study towards Phase 2/3 and licensure’ funded by the Wellcome Trust, UK.

Transparency declarations

O.H. is the inventor on a patent concerning 5P12-RANTES. It is held by the Mintaka Foundation for Medical Research, a foundation registered in Geneva, Switzerland. O.H. and R.E.O. are

Commented [JM5]: Response 9

Commented [JM6]: Response 8
cofounders of the Mintaka Foundation, with the roles of Chief Scientific Officer and Chief Executive Officer, respectively. Other authors have nothing to declare.

**Author contributions**

R.K.M., J.M., V.K., R.E.O, P.B and O.H conceived the idea for the study and analysis. J.M. manufactured the gels and tested their in vitro performance, under supervision from R.K.M., P.B and V.K. N.D. and D.C. supervised completion of the sheep pharmacokinetic study. Data analysis was performed by J.M., R.K.M, D.C. and N.D., and the pharmacokinetic study report was drafted by N.D. and D.C., with input from R.K.M. and J.M. R.K.M. and J.M. drafted the manuscript. All authors provided critical reading that further developed the manuscript.

**Disclaimer**

The views expressed in this publication are those of the authors and not necessarily those of the Wellcome Trust.
References


23


grade production of the HIV inhibitor 5P12-RANTES in Pichia pastoris. Protein Expr Purif


Suspensions for intravenous (IV) injection: a review of development, preclinical and

46. Curran RM, Donnelly L, Morrow RJ, Fraser C, Andrews G, Cranage M, Malcolm RK,
Shattock RJ, Woolfson a D. 2009. Vaginal delivery of the recombinant HIV-1 clade-C
trimeric gp140 envelope protein CNS4gp140 within novel rheologically structured

47. Senyiğit ZA, Karavana SY, Eraç B, Gürsel O, Limoncu MH, Baloğlu E. 2014. Evaluation of
chitosan based vaginal bioadhesive gel formulations for antifungal drugs. Acta Pharm
64:139–56.

48. Karavana SY, Rençbe S, Şenyiğit ZA, Baloğlu E. 2012. A New In-Situ Gel Formulation of

49. Andrews GP, Donnelly L, Jones DS, Curran RM, Morrow RJ, Woolfson AD, Malcolm RK.
2009. Characterization of the rheological, mucoadhesive, and drug release properties of
highly structured gel platforms for intravaginal drug delivery. Biomacromolecules
10:2427–2435.


56. Owen DH, Peters JJ, Katz DF. 2001. Comparison of the rheological properties of


Figure 1. Characterisation of hydroxyethylcellulose (HEC) gels containing low dose: 1.24 mg/ml 5P12-RANTES; intermediate dose: 6.18 mg/ml 5P12-RANTES; high dose: 32 mg/ml 5P12-RANTES.

(a) Viscosity of gel formulations determined by continuous flow rheology and (b) through application of the Power Law was to determine apparent viscosity. Results displayed as mean + SEM, n=3. Significance determined by one-way ANOVA post hoc pairwise Tukey’s test, *P<0.05,
Setup for syringeability testing using a texture profile analyser (TA-XT2) and a vaginal applicator filled with gel which was employed to determine the work required (N.mm) to expel 4 g of gel, n=3, mean + SEM.
Figure 2. Cumulative release of 5P12-RANTES from hydroxyethylcellulose (HEC) gels containing a low (1.24 mg/g), intermediate (6.18 mg/g) or high (32.0 mg/g) dose of the CCR5 inhibiting peptide, as detected by ELISA. Gels were incubated in simulated vaginal fluid, 37°C, throughout the study, n=3, mean ± SEM.
Figure 3. Pharmacokinetic concentrations of 5P12-RANTES in (a) vaginal fluid, (b) vaginal tissue, and (c) serum of sheep following single dose vaginal administration of hydroxyethylcellulose (HEC) gels containing low (1.24 mg/g), intermediate (6.18 mg/g) and high (32.0 mg/mL) concentrations of 5P12-RANTES. Plot symbols represent mean + standard deviation of four replicates.
Figure 4. 5P12-RANTES pharmacokinetic profiles for individual sheep following single dose vaginal administration of hydroxyethylcellulose (HEC) gels containing low (1.24 mg/mL), intermediate (6.18 mg/g) and high (32.0 mg/mL) concentrations of SP12-RANTES. Plot symbols represent mean + standard deviation of four replicates.
Table 1. Description of 5P12-RANTES gel formulations and the concentrations measured in sheep vaginal fluid and serum vaginal following single dose vaginal gel administration. \( C_{\text{max}} \) (maximum serum concentration) and \( \text{AUC} \) (area under the curve) data are presented as means ± standard deviations of four replicates; \( t_{\text{max}} \) data (time to reach \( C_{\text{max}} \)) are presented as median and range of four replicates.

<table>
<thead>
<tr>
<th>SP12-RANTES conc. in gel (total SP12-RANTES dose administered)</th>
<th>Vaginal fluid</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( C_{\text{max}} ) (µg/g)</td>
<td>( t_{\text{max}} ) (h)</td>
</tr>
<tr>
<td>1.24 mg/g (4.94 mg)</td>
<td>869 ± 167</td>
<td>8.0, 1–12</td>
</tr>
<tr>
<td>6.18 mg/g (24.7 mg)</td>
<td>7920 ± 7890</td>
<td>3.5, 1–12</td>
</tr>
<tr>
<td>32.0 mg/g (128 mg)</td>
<td>12800 ± 17300</td>
<td>7.0, 1–12</td>
</tr>
</tbody>
</table>