

Colorectal microbicide design: triple combinations of reverse transcriptase inhibitors are optimal against HIV-1 in tissue explants

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Objective: Receptive anal intercourse in both men and women is associated with the highest probability for sexual acquisition of HIV infection. As part of a strategy to develop an effective rectal microbicide, we performed an ex-vivo preclinical evaluation to determine the efficacy and limitation of multiple combinations of reverse transcriptase inhibitors (RTIs).

Design: A nucleotide, PMPA (tenofovir), a nucleoside, FTC (emtricitabine), RTIs and two nonnucleoside RTIs, UC781 and TMC120 (dapivirine), were used in double, triple and quadruple combinations against a panel of CCR5-using and CXCR4-using clade B HIV-1 isolates and against RTI-escape variants.

Methods: Indicator cells and colorectal tissue explants were used to assess antiviral activity of drug combinations.

Results: All combinations inhibited the isolates tested in a cellular model and in colorectal explants and produced, for at least one of the compounds, a change in the dose–response curve. Double and triple combinations incrementally augmented activity, even against RTI-escape mutants, whereas quadruple combinations conferred little further advantage.

Conclusion: The colorectal explant model may be used to identify the best candidate molecules and their combinations at the preclinical stage. Furthermore, this study demonstrates that combinations based on RTIs with different HIV-1 inhibitory mechanisms have potential as colorectal microbicides.

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Introduction

Receptive anal intercourse (RAI) between serodiscordant couples in both men and women is associated with the highest probability of sexual HIV transmission [1–4]. The rectal mucosa has a single-cell columnar epithelium, easily damaged during RAI, and contains an abundance of highly activated target cells for HIV infection [5–7].

Moreover, a recent study has shown that multivariant transmission is significantly more likely in RAI compared with vaginal intercourse [8]. Taken together, these factors underscore the importance of developing protective modalities for this route of transmission. Microbicides, based on preventing viral transmission at the mucosal portal of entry, have been investigated primarily for vaginal application with less emphasis being given to

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either rectal safety or efficacy. Recently, the field has adopted antiretroviral-based microbicides as having the best chance of efficacy in clinical trials [9,10]. Indeed, the recent promising results from the CAPRISA 004 trial showing the effectiveness and safety of tenofovir [a nucleotide reverse transcriptase inhibitor (NRTI)] when administered vaginally in a gel formulation [11] have delivered proof of principle for this approach. So far, only two safety and acceptability phase I rectal microbicide trials have been performed using vaginal gel formulations of UC781, a nonnucleoside reverse transcriptase inhibitor (NNRTI) [12], or 1% tenofovir [13]. Efficacy of the rectal antiretroviral microbicide approach has been demonstrated in a study on macaques wherein locally administered tenofovir gel protected macaques against simian immunodeficiency virus (SIV) rectal challenge [14]. The strategy of mucosal applications of antiretrovirals as a microbicide will, however, need to take into account the increasing prevalence of strains resistant to antiretroviral drugs. It is estimated that in the developed world, 10–20% of new infections are caused by antiretroviral-resistant isolates harboring mutations that confer resistance to at least one of the three main types of antiretroviral drugs [15–22]. Previously, we have shown that NRTI–NNRTI and NNRTI–NNRTI combinations show promise as colorectal microbicides using modeling *ex vivo* [23]. Here, we significantly extend these studies to include another NRTI, FTC (also known as emtricitabine) enabling assessment of the antiviral activity of double, triple and quadruple combinations of PMPA and FTC (NRTIs) and two NNRTIs, UC781 and TMC120 (dapivirine), all currently in different phase I/II HIV prevention trials. The potency of combinations was tested against a panel of clade B wild-type viruses and against RTI-escape mutants using cellular and colorectal explant cultures.

Methods

Reverse transcriptase inhibitor drugs

PMPA (tenofovir) and FTC (emtricitabine) were donated by Gilead Sciences Inc. (Foster City, California, USA), UC781 by Biosyn Inc. (Huntington Valley, Pennsylvania, USA) and TMC120 (dapivirine) by the International Partnership for Microbicides (IPM) (Silver Spring, Maryland, USA). Drugs were used at non-cytotoxic concentrations determined by MTT viability.

Cells and viruses

TZM-bl cells [24–26] were grown in Dulbecco's Minimal Essential Medium (DMEM) (Sigma-Aldrich Inc., St Louis, Missouri, USA) containing 10% fetal calf serum (FCS), 2 mmol/l L-glutamine and antibiotics (100 U of penicillin/ml, 100 µg of streptomycin/ml).

A panel of HIV-1 clade B viruses consisted of X4-using RF, IIIB and R5-using BaL and YU.2. Full-length,

replication-competent proviral HIV-1 clone, pYU2 [27,28] was provided by the NIH AIDS Research & Reference Reagent Program (<http://www.aidsreagent.org/>), as well as the RTI-resistant HIV-1 clones: A17, highly resistant to UC781 with K103N and Y181C in the RT domain (mutations clinically associated with virological failure), which was obtained by culture in the presence of increasing concentrations of a NNRTI [29]; 71361–1 with a single-point mutation K65R conferring resistance to PMPA and 8415–2 with K65R and M184V point mutations conferring full resistance to FTC but partially restoring PMPA sensitivity [30]. The latter two clones were obtained from ligation of RT cDNAs extracted from infected individuals undergoing therapy into an RT-deleted vector. Plasmids were transfected into 293FT cells and all virus isolates were passaged through activated peripheral blood mononuclear cells [31] for 11 days.

Tissue explants

Surgically resected specimens of colorectal tissue were collected at St George's Hospital, London, UK, after receiving signed informed consent from all patients who were undergoing rectocele repair and colectomy for colorectal cancer [23]. Tissues were collected under protocols approved by the Local Research Ethics Committee. On arrival in the laboratory, muscle was stripped from the resected tissue, which was then cut into 2–3 mm³ explants comprising both epithelial and muscularis mucosae [32]. Colorectal explants were maintained with DMEM containing 10% FCS, 2 mmol/l L-glutamine and antibiotics (100 U of penicillin/ml, 100 µg of streptomycin/ml, 80 µg of gentamicin/ml).

Infectivity and inhibition assays

Inhibition assays were performed using a standardized amount of virus culture supernatant normalized for infectivity. Cells or tissue explants were incubated with serial dilutions of drugs, either singly or in mixtures combined at equipotent concentrations [23], for 1 h at 37°C before addition of virus. Alternatively, virus (10^3 TCID₅₀) was added to tissue explants for 2 h and then explants were washed four times with PBS before transferring onto gelfoam rafts (Welbeck Pharmaceuticals, UK) as described previously [32]. On days 3, 7, 11 and 15, approximately two-third of the supernatant were harvested and explants were re-fed with fresh media. The extent of virus replication was determined in TZM-bl cells by luciferase quantification of cell lysates (Promega, Madison, Wisconsin, USA) and in tissue explants by measuring the p24 antigen concentration in supernatants (HIV-1 p24 ELISA, AIDS Vaccine Program, National Cancer Institute, Frederick, Massachusetts, USA), as described previously [23].

Statistical and mathematical analysis

Fifty percent inhibitory concentration (IC₅₀) values were calculated from sigmoid curve fits (GraphPad

Prism, La Jolla, California, USA). All IC_{50} data presented fulfill the criterion of R^2 more than 0.7.

Results

FTC–nucleotide reverse transcriptase inhibitor or FTC–nonnucleotide reverse transcriptase inhibitor combinations are more active than individual drugs

To evaluate the effectiveness of FTC as a potential component of a rectal microbicide, it was first tested alone on TZM-bl cells against a panel of R5 and X4-using viruses and compared with the activity of PMPA, UC781 and TMC120. FTC inhibited the panel of clade B isolates with IC_{50} ranging from 369.84 to 602.65 nmol/l. The average IC_{50} of PMPA was found to be 14-fold higher than that of FTC; therefore, for dual combination evaluation, FTC and PMPA were combined at a ratio of 1:14. Likewise, equipotent combinations of FTC–UC781 and FTC–TMC120 were found to be 24:1 and 292:1, respectively. All combinations resulted in a reduction in IC_{50} for each compound (Table 1). Based on these results, the inhibitory activities of the double combinations were tested in the colorectal explant model against the R5-using isolates BaL and YU.2. A positive shift (to the right) in the dose–response curve for all mixed compounds was seen (Fig. 1a–c for BaL and data not shown) with a reduction in the IC_{50} of each drug when used in combination, in comparison with the activity of the drug alone (Table 2).

Next, the effect of inclusion of FTC in dual combinations was assessed on TZM-bl cells against two NRTI-resistant HIV-1 isolates, 71361-1 and 8415-2 and an NNRTI-resistant isolate, A17. The three dual drug combinations were active against all escape-mutants tested (Table S1, <http://links.lww.com/QAD/A160>) showing that combining two drugs maintains the viral inhibition even if resistance emerges against one of the drugs employed in the combination. However, with the FTC-fully resistant

isolate, 8415-2, there was no actual increase in potency with the FTC–UC781 and the FTC–TMC120 dual combinations and the dose–response curve of the mixture reflected the activity of UC781 or TMC120, the more potent drug, in the combination; with only a slight increase in the IC_{50} value of UC781 and TMC120, respectively (Table S1, <http://links.lww.com/QAD/A160>).

Double-drug combinations were then tested in colorectal explants against A17, as the other two RTI-resistant mutants were unfit for replication in explants. An increase in inhibitory activity was seen in the dose–response curves for all the drugs used in double combination (Fig. 2a–c). The IC_{50} of each individual drug decreased when mixed (Table S2, <http://links.lww.com/QAD/A160>) and the combination also reached higher levels of inhibition overall.

Triple combinations of reverse transcriptase inhibitors are more active than double combinations

Next, we assessed whether the increases in activity obtained with double-drug combinations could be further enhanced with triple combinations. The four RTIs were mixed, maintaining a constant proportion of concentrations based on equipotency for each individual drug measured by IC_{50} in TZM-bl cells. Thus, the ratios of 4167:12:1 for PMPA–UC781–TMC120, 292:12:1 for FTC–UC781–TMC120, 24:347:1 for FTC–PMPA–UC781 and 292:4167:1 for FTC–PMPA–TMC120 were used. For all the compounds included in the mixture and against all the wild-type isolates tested, the dose–response curves showed that the triple combinations were more potent than individual compounds alone (data not shown). The proportionate reduction (percentage) in IC_{50} obtained with the triple combinations was higher than for at least one of the double combinations tested (Table 1).

Interestingly, NRTI–NNRTI–NNRTI combinations were more potent than NRTI–NRTI–NNRTI combinations. TMC120 showed the strongest increase in

Table 1. Reductions in the IC_{50} of drugs used in combination in TZM-bl cells.

Combination	Average percentage reduction in the IC_{50} of the following drug (drug A) in double, triple or quadruple combinations ^a			
	FTC	PMPA	UC781	TMC120
FTC + PMPA	65.23 ± 10.91	32.23 ± 15.90		
FTC + UC781	66.84 ± 7.29		41.20 ± 5.11	
FTC + TMC120	66.22 ± 6.54			44.35 ± 9.38
PMPA + UC781 + TMC120		76.72 ± 8.49	63.20 ± 9.77	85.44 ± 2.02
FTC + UC781 + TMC120	86.79 ± 4.14		44.78 ± 11.96	70.58 ± 10.01
FTC + PMPA + UC781	89.75 ± 2.09	71.50 ± 10.06	56.93 ± 16.21	
FTC + PMPA + TMC120	92.97 ± 2.82	81.62 ± 6.95		34.73 ± 23.06
FTC + PMPA + UC781 + TMC120	91.62 ± 3.18	79.39 ± 15.35	78.81 ± 6.86	80.18 ± 2.84

^aThe data are the mean ± SD obtained against panel a viruses of the percentage of reduction in the IC_{50} of each compound when used in combination was calculated as follows: $\{1 - [IC_{50}(\text{drug A in combination})/IC_{50}(\text{drug A alone})]\} \times 100$.

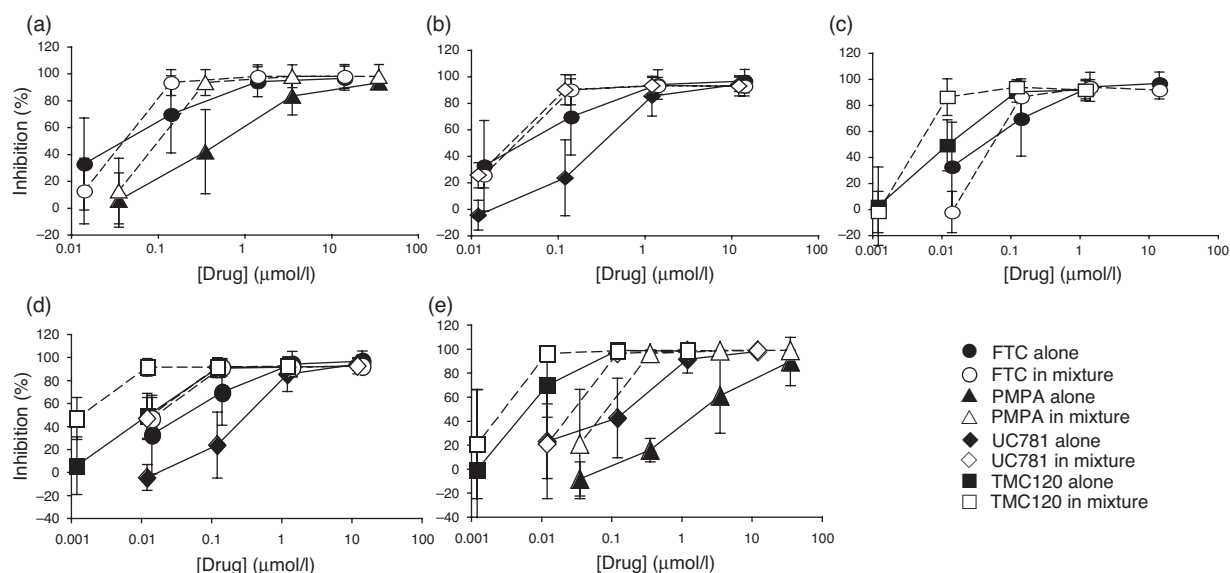


Fig. 1. Activity of FTC, PMPA, UC781 and TMC120 in dual and triple combinations against BaL in colorectal explants. Colorectal explants were treated for 1 h in the presence or absence of FTC and/or PMPA (a), FTC and/or UC781 (b), FTC and/or TMC120 (c), FTC, UC781 and/or TMC120 (d), or PMPA, UC781 and/or TMC120 (e). BaL was added for 2 h before four washes with PBS. Explants were then transferred to gelfoam rafts and cultured for 15 days. The concentrations of p24 in the harvested supernatants were quantified by ELISA and the extent of inhibition by each compound or combination was calculated. The percentage of inhibition was normalized relative to the p24 values obtained for explants not exposed to virus (0% infectivity) and for explants infected with virus in the absence of compound (100% infectivity). Data are means (\pm standard deviations) from three independent experiments performed in triplicate.

activity with an average IC_{50} reduction of 85.44% when combined with both PMPA and UC781 at the same time and a 70.58% reduction in the triple combination with FTC and UC781. In contrast, a reduction of only 34.73% was seen when TMC120 was combined with the NRTIs PMPA and FTC (Table 1). Thus, the NRTI–NNRTI–NNRTI combinations of non-formulated FTC, PMPA, UC781 and TMC120 studied were more active than any of the drugs tested individually or in double combinations, in TZM-bl cells.

We next assessed the activity of the more potent triple NRTI–NNRTI–NNRTI combinations in colorectal explants. Triple combinations were more potent than any of the compounds tested alone as shown by a large shift in

the dose–response curve against R5 isolates (Fig. 1d and e for BaL and data not shown). On average, the decrease in IC_{50} values (Table 2) was greater than that seen with double combinations in this model. Interestingly, the increase in the activity of UC781 in triple combinations was greater when tested in the explant model than when measured in TZM-bl cells with an average reduction in IC_{50} of 88% in colorectal explants compared with 54% in TZM-bl cells (Tables 1 and 2).

Characterization of the inhibitory activity of the NRTI–NNRTI–NNRTI combinations against RTI-escape mutants was first carried out in the TZM-bl model in order to include the mutants that were not fit for replication in the explants. A17 was resistant to UC781,

Table 2. Reductions in the IC_{50} of drugs used in combination in colorectal explants.

Combination	Average percentage reduction in the IC_{50} of the following drug (drug A) in double, triple or quadruple combinations ^a			
	FTC	PMPA	UC781	TMC120
FTC + PMPA	42.81 \pm 36.86	93.13 \pm 1.15		
FTC + UC781	61.36 \pm 16.20		84.34 \pm 15.00	
FTC + TMC120	53.96 \pm 0.85			81.95 \pm 12.55
PMPA + UC781 + TMC120		95.79 \pm 3.85	86.37 \pm 2.13	74.26 \pm 7.05
FTC + UC781 + TMC120	81.75 \pm 0.31		92.46 \pm 5.92	90.86 \pm 2.16
FTC + PMPA + UC781 + TMC120	61.78 \pm 10.03	94.47 \pm 3.05	85.59 \pm 13.23	78.82 \pm 8.12

^aThe data are the mean \pm SD obtained against BaL and YU.2 of the percentage of reduction in the IC_{50} of each compound when used in combination was calculated as follows: $\{1 - [IC_{50}(\text{drug A in combination})/IC_{50}(\text{drug A alone})]\} \times 100$.

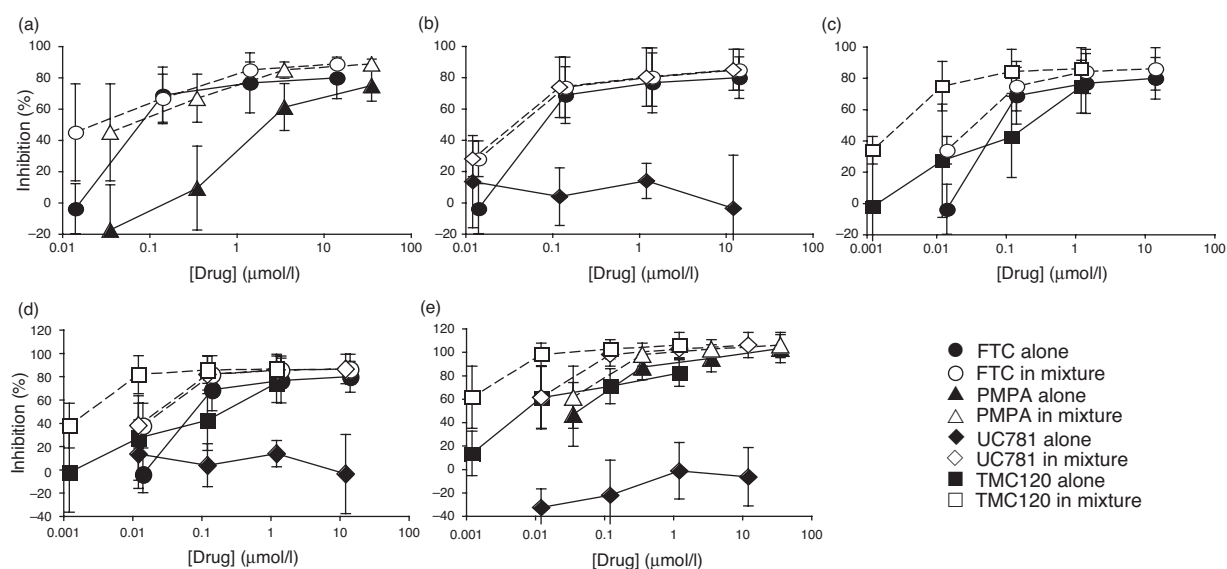


Fig. 2. Dual and triple combinations of FTC, PMPA, UC781 and/or TMC120 in colorectal explants are more active against A17 than the drugs alone. Colorectal explants were treated for 1 h in the presence or absence of FTC and/or PMPA (a), FTC and/or UC781 (b), FTC and/or TMC120 (c), FTC, UC781 and/or TMC120 (d) or PMPA, UC781 and/or TMC120 (e). A17 was added for 2 h and then the explants were washed four times with PBS and transferred to gelfoam rafts. Explants were kept in culture for 15 days. The concentrations of p24 in the harvested supernatants were quantified by ELISA and the extent of inhibition by each compound or combination was calculated. The percentage of inhibition was normalized relative to the p24 values obtained for explants not exposed to virus (0% infectivity) and for explants infected with virus in the absence of compound (100% infectivity). Data are means (\pm standard deviations) from three independent experiments performed in triplicate.

partially resistant to TMC120 and, as expected, fully sensitive to PMPA and FTC, and therefore, the triple combinations of PMPA or FTC with UC781 and TMC120 were active against this virus and the IC_{50} values for PMPA and FTC were reduced by 40.69 and 79.82%, respectively (Table S1, <http://links.lww.com/QAD/A160>). These combinations of RTIs were also able to inhibit 71361-1 mutant partially resistant to PMPA, up to 84% with a reduction of 56.29% in the PMPA IC_{50} (Table S1, <http://links.lww.com/QAD/A160>). 71361-1 was slightly resistant to FTC; however, the triple combination FTC-UC781-TMC120 inhibited this isolate up to 95% with the range of concentrations tested (Table S1, <http://links.lww.com/QAD/A160>). As 8415-2 mutant fully resistant to FTC was fully sensitive to TMC120 and UC781, both triple combinations were active against this isolate (Table S1, <http://links.lww.com/QAD/A160>). The PMPA-UC781-TMC120 triple combination was more active than any of the drugs titrated alone. However, there was no actual increase in potency with the FTC-UC781-TMC120 combination. The dose-response curve of the mixture reflected the activity of TMC120, the more potent drug, in the combination, with only a slight increase in the IC_{50} value of TMC120 (Table S1, <http://links.lww.com/QAD/A160>).

The inhibitory activities of NRTI-NNRTI-NNRTI combinations were then tested on the colorectal explant model against the NNRTI-escape mutant A17. The

dose-response curves corresponding to triple combinations showed an increased activity of all four compounds (Fig. 2d and e). With these combinations, not only a decrease in the IC_{50} for each individual compound was observed but also higher levels of inhibition were reached (up to 99% within the concentrations tested). The increase in the activity of each drug in both triple combinations tested was similar (Table S2, <http://links.lww.com/QAD/A160>).

Taken together, these data demonstrate the greater inhibitory potential of triple combinations as rectal microbicides compared with double combinations (NRTI-NRTI, NRTI-NNRTI or NNRTI-NNRTI) against multiple strains of HIV-1. Importantly, triple combinations may confer greater protection against RTI-resistant HIV-1 variants that may arise in HIV-infected partners of uninfected individuals.

Reverse transcriptase inhibitor quadruple combination confers little further advantage

The observation that combining drugs increases their individual activity led us to study whether there was a limit to how much further the antiviral activity could be augmented by mixing a higher number of compounds. The four drugs were combined using the same ratios as used for the triple combinations. For all compounds, the quadruple combination, in TZM-bl cells, showed a positive change in the dose-response curve involving a reduction in the IC_{50} values compared with those

obtained for individual compounds (Table 1). However, comparison of the reduction in IC_{50} for each drug when used in a triple or in a quadruple combination failed to show appreciable differences. Likewise, in the colorectal explant model, the shift of activity of the dose–response curves (Fig. 3a) and the reduction in the IC_{50} values (Table 2) were, again, not appreciably different than for any of the triple combinations.

These results were next confirmed against RTI-resistant isolates. In TZM-bl cells, for all three RTI-escape mutants, overall no further increase in activity was detected with quadruple combination compared with any of the triple combinations tested above. Only the NRTIs, FTC and PMPA, were more active against A17 when combined with the two NNRTIs (Table S1, <http://links.lww.com/QAD/A160>). In colorectal explants, the dose–response curve corresponding to the quadruple combination against A17 showed an increased activity for all the compounds used in the mixture (Fig. 3b), but this was similar to the potency measured with the triple combination (Table S2, <http://links.lww.com/QAD/A160>). Only PMPA was more active in the quadruple combination, with a 97.97% reduction in the IC_{50} compared with a 71.9% reduction obtained by the triple combination.

Discussion

Recently, increasing interest has been placed on the development of effective antiretroviral-based colorectal microbicides. The first colorectal efficacy study in macaques [14] and the two phase I clinical trials in humans [12,13] used single antiretroviral drugs. However, it is likely that more complex formulations will be required to realize the full potential of this approach.

These formulations will have to follow the guiding principles of microbicides design being effective, well tolerated, user friendly and affordable [33]. Taken together, our results confirm the potential of RTI combinations in the design of effective colorectal microbicides against R5-tropic and X4-tropic viruses and against RTI-escape mutants, which are increasingly prevalent in HIV-infected populations. Moreover, with increasing deployment of ART in developing countries, it is likely that the global prevalence of RTI-escape mutants will continue to increase. In-vivo evaluation of RTI combinations using the SIV macaque model has been reported for a formulation of PMPA and FTC administered intravaginally, which demonstrated complete protection against vaginal challenge with SIV [38]. This approach, however, is very expensive, allows only a limited number of combinations to be evaluated and is limited by the sensitivity of SIV-RT to drug action. The ex-vivo explant model combined with the TZM-bl cells used here potentially provides a powerful preclinical step to identify optimum development strategies, taking into account a range of virus isolates, RTI-escape mutants and multiple combinations of drugs. Histological analysis has shown that colorectal explants remained viable for more than 10 days with progressive loss of architecture, but maintenance of CD4 : CD8 T cells ratios [32]. There is currently a paucity of data regarding preservation of functions such as immune competence and mucus secretion for colorectal explants; however, conservation of function has been described in other tissue explants [34]. A limitation of the explant system is gauging an appropriate viral inoculum size. In this and previous studies [13,14,23,32,35], infection of explants has been performed with virus at 10^3 TCID₅₀ representing a relatively low multiplicity of infections in terms of total target cell population within tissue. However, this non-polarized system will not fully model infections *in vivo*. Validation of the explant model with respect to

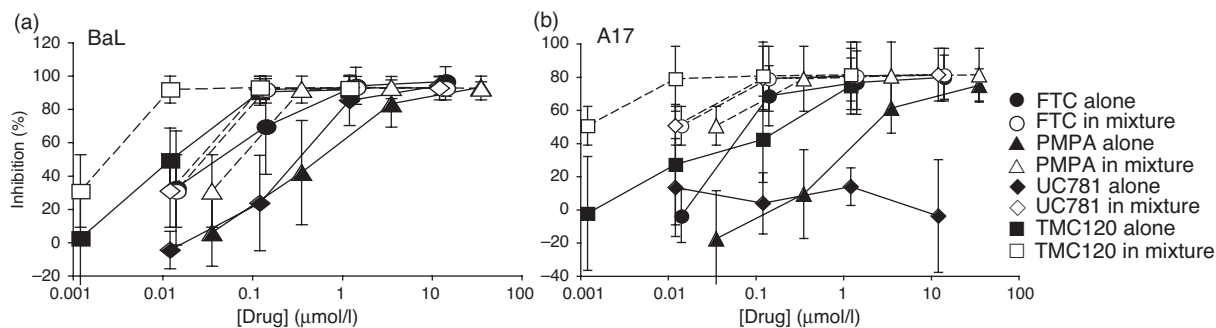


Fig. 3. Activity of quadruple combination of FTC, PMPA, UC781 and TMC120 in colorectal explants against BaL and A17. Colorectal explants were treated for 1 h in the presence or absence of FTC, PMPA, UC781 and TMC120 alone or mixed. BaL (a) or A17 (b) was added for 2 h before washing four times with PBS. Explants were then transferred on gelfoam rafts and cultured for 15 days. The levels of p24 in the harvested supernatants were quantified by ELISA and the extent of inhibition by each compound or combination of compounds was calculated. The percentage of inhibition was normalized relative to the p24 values obtained for explants not exposed to virus (0% infectivity) and for explants infected with virus in the absence of compound (100% infectivity). Data are the means (\pm standard deviations) from three independent experiments performed in triplicate.

intracellular drug distribution and concentration is not yet available. Moreover, ex-vivo modeling does not take into account modulation of drug availability that may occur *in vivo*, for example, due to protein binding. The study reported here has addressed infection with cell-free virus in a non-polarized system; however, cell-free virus may transcytose and virus-infected cells transmigrate across the epithelium [36] and further studies are needed to determine the potential of antiretrovirals to prevent infection by such mechanisms. Our results have significantly extended our earlier studies using dual drug combinations [23] to show the potential of FTC as a rectal microbicide candidate both in single use and more importantly in combination with other RTIs and we have defined an upper limit to gain of function.

Notably, the increase in inhibitory activity of drugs used in dual combination against escape mutants was reflected not only in the decrease in IC_{50} of each drug compared with the drug tested alone, but there was also increase in the inhibition reached. For example, dual combination of FTC and PMPA inhibited infection with the mutant partially resistant to PMPA and with the FTC-escape mutant by more than 85% (data not shown). The increase in activity seen with triple combinations compared with dual combinations tested in this study also applied to the dual combinations tested in the previous study [23], underscoring the potential for gain of activity. Hence, any one RTI can increase potency when combined with other RTIs, but this increase has a limit as shown when all four drugs were combined. This threshold could be due to multiple reasons. Some RTIs have been described not only to inhibit RT but also to affect other steps of the viral replication cycle, like viral polyprotein processing by protease in the case of the NNRTI TMC120 [37]. Furthermore, the antiviral potency of RTIs depends on the length of the template copied, the number of sites where the drugs might exert their effect and the presence within a viral population of viruses with different sensitivities to the drugs [38–40]. For example, different levels of activity have been detected when combining two NRTIs, zidovudine and zalcitabine, depending on the proportion of enzyme versus template:primer used [40]. The fractional inhibitory concentration approach used in our study has limitations, as only conventional IC_{50} ratios have been used and not a full combinatorial matrix due to technical restrictions related to tissue availability.

Although gain of function and, in particular, activity against RTI-escape variants is an important feature of rectal microbicide design, many other factors have to be taken into account, not least of which is safety. It was therefore encouraging to find that none of the drugs tested in combination over their entire range of activity were intrinsically cytotoxic to indicator cells or to colorectal explants that more closely represent the *in vivo* environment; however, in practical application, drugs

would be formulated in a delivery vehicle such as a gel. Taking into account functional and immunological differences between colorectal and vaginal tracts, it may be necessary to develop compartment-specific formulations. Rohan *et al.* [41] have shown that a tenofovir gel formulation, despite being non-toxic toward vaginal flora or ectocervical and colorectal explants, induced transient epithelial disruption in both tissue models probably due to the hyperosmolar nature of the formulation. Likewise, a recent phase 1 placebo-controlled trial concluded that rectal dosing with a vaginal formulation of 1% tenofovir was neither entirely well tolerated nor fully acceptable [13]. Hence, careful consideration will have to be taken regarding osmolarity of formulations and, in the case of drug combinations, compatibility of the vehicle to the individual components. The explant model has an important role to play in this regard.

Clearly, there is a need to balance the net gain in antiviral activity by using more drug combinations against formulation complexity and cost. antiretroviral-based therapy has led to the emergence of resistant isolates to these drugs. In the context of the prophylactic use of antiretrovirals formulated as microbicides, it is essential that formulations effectively confer protection against spreading of infection with such variants, as modeled in the present study. Significantly, emergence of drug resistance can reduce viral replication fitness in patients [42–46] and colorectal explants [23]. This study demonstrates promising potential of antiretroviral combinations as colorectal microbicides, even if there is a theoretical possibility that the prophylactic use of antiretroviral-based microbicides could select for emergence of escape mutants. This would seem to be unlikely in virus-naive individuals but could be an issue for HIV-infected individuals either using a microbicide, unaware of their HIV status, or for individuals using a microbicide in an attempt to reduce their chance of transmitting virus. Further pharmacokinetic studies and clinical trials will provide important data in this regard.

These issues notwithstanding, this study has provided further experimental evidence for the potential of combining RTIs as effective colorectal microbicides. An upper effective limit of three RTIs has been defined in the ex-vivo model. Further studies are now required to investigate combinations that include multiple types of drugs (RTIs, entry/fusion inhibitors, protease inhibitors and integrase inhibitors) and compartment-specific formulations enabling the optimal design of an efficient antiretroviral-based microbicide.

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C.H. performed all the experiments, analyzed the data and wrote the manuscript. M.C. and I.McG. were subrecipient principal investigators and contributed in editing the manuscript. P.A. was overall program director, provided oversight and edited the manuscript. R.J.S. supervised the experimental work and data analysis and contributed to the manuscript. We thank Naomi Armanasco, Mr Haggar, Mr Melville and the Colorectal Surgery Team, St George's Hospital, London, UK, for their assistance in obtaining human colorectal tissue.

Conflicts of interest

There are no conflicts of interest.

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