Original article

Safety of tenofovir gel, a vaginal microbicide, in South African women: results of the CAPRISA 004 Trial

David C Sokal[1], Quarraisha Abdool Karim[2,3], Sengeziwe Sibeko[2], Nonhlanhla Yende-Zuma[2], Leila E Mansoor[2], Cheryl Baxter[2], Anneke Grobler[2], Janet Frolich[2], Ayesha BM Kharsany[2], Nomsa Miya[2#], Koleka Mlisana[2], Silvia Maarshalk[2], Salim S Abdool Karim[2,3]*

[1]FHI 360, Clinical Sciences Department, Durham, NC, USA
[2]CAPRISA – Centre for the AIDS Programme of Research in South Africa, University of KwaZulu-Natal, Durban, South Africa
[3]Department of Epidemiology, Columbia University, New York, NY, USA

*Corresponding author e-mail: abdoolq2@ukzn.ac.za
[#]Deceased

Background: Tenofovir gel, used vaginally before and after coitus, reduced women's acquisition of HIV by 39%. This is a safety assessment of tenofovir gel, including renal, bone, gastrointestinal, genital and haematological parameters.
Methods: In the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004, a double-blind, randomized placebo-controlled trial, 445 of the 889 eligibly enrolled women were assigned to tenofovir gel. All participants were advised to use the gel vaginally only, with one dose of gel within 12 h before and a second dose as soon as possible after sex, with no more than two doses in 24 h. Clinical and laboratory safety data were collected at monthly and quarterly visits, respectively. Genital assessments were undertaken at enrolment and quarterly thereafter, or as indicated.
Results: Women assigned to tenofovir gel were exposed to an average monthly vaginal dose of 240 mg of tenofovir (six applications). In total, six women, three in each group, had mild creatinine elevations, all of which occurred in July/August 2008. The incidence of anaemia was 3.5 and 3.8 per 100 women-years in tenofovir and placebo groups, respectively (*P*=0.80). Of the six women (four tenofovir and two placebo) experiencing bone fractures, none were associated with abnormal phosphate or calcium values. The proportion of women with diarrhoea was higher in the tenofovir gel group (17% versus 11%; *P*=0.026). There was no significant increase of any genital adverse event in the tenofovir group.
Conclusions: No significant renal, haematological, genital or bone effects were associated with the use of tenofovir gel. Aside from a puzzling increase in diarrhoea, tenofovir gel has an excellent safety profile.

Introduction

Women are disproportionately affected by the AIDS epidemic in Africa, the region with the highest global burden of HIV infection [1]. The Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 trial demonstrated that the use of an antiretroviral drug (tenofovir) in the form of a vaginal gel used before and after coitus can prevent HIV infection in women [2], with a 39% overall reduction (95% CI 6, 60), and a 54% reduction in women who used the gel consistently. Tenofovir gel has the potential, if used widely and consistently, to significantly alter the course of the HIV epidemic in Southern Africa [3].

Pharmacokinetic studies have shown that tenofovir gel leads to minimal systemic absorption, barely reaching the level of detection, with systemic (serum) levels about 100-fold lower than those achieved following oral administration of 300 mg, the usual daily dose used for AIDS treatment [4]. Although it is unlikely that long-term prophylactic use of tenofovir gel might lead to systemic toxicities, it would be irresponsible not to conduct a thorough safety analysis.

The most common side effect noted following daily administration of oral tenofovir has been nausea [5]. More recently, concerns about severe renal toxicity following daily oral administration of tenofovir, including cases of acute renal failure [6,7] and Fanconi syndrome leading to hypophosphataemia [8,9], have emerged. There have also been reports of decreased bone mineral density, which could potentially increase the risk of bone fractures [10,11]. However, serious adverse events

attributed to tenofovir have generally occurred in individuals with advanced AIDS and have not been seen in HIV-uninfected individuals.

It is not known whether any of the safety concerns observed from oral administration might be applicable to the long-term intermittent use of tenofovir gel. It is also not known whether there are systemic or local safety concerns related to the intermittent use of tenofovir gel that have not been observed from oral administration. For example, in the initial analysis of the CAPRISA 004 trial [2], we found an association between tenofovir use and diarrhoea. By contrast, controlled studies of oral administration of tenofovir have not found a consistent increased risk of diarrhoea [12].

The purpose of this study was to undertake an assessment of the systemic and local safety of intermittently applied tenofovir gel, including renal, bone, gastrointestinal, genital and haematological safety parameters.

Methods

Study design and study population

CAPRISA 004, a two-arm, double-blind, randomized placebo-controlled trial conducted from May 2007 to March 2010, assessed the safety and effectiveness of tenofovir gel in preventing HIV infection in women [2]. Women with renal disease, based on calculated creatinine clearance of less than 50 ml/min, were excluded. The final study population included a total of 889 eligibly enrolled women between 18 and 40 years of age at two sites: the CAPRISA Vulindlela Clinic in a rural community about 150 km west of Durban in the KwaZulu-Natal midlands and the CAPRISA eThekwini Clinic in central Durban. Ineligible women who had been co-enrolled in another microbicide study or who had been in another microbicide study in the previous 12 months were excluded [13]. The participants were followed for an average of 18 months (range 12–30).

Dosing regimen

Women were instructed to apply one dose of gel within 12 h before and a second dose as soon as possible after sex, but no more than two doses within 24 h, known as the BAT24 regimen. BAT24 stands for one dose before sex and one dose after sex; no more than two doses in 24 h. Because each gel insertion was 4 ml of 1% tenofovir gel, this dosage regimen should deliver 80 mg of tenofovir per day of use.

Safety monitoring

At each monthly visit, post-enrolment, information on adverse events was collected and pregnancy, HIV tests and physical and pelvic examinations were performed as indicated. Study gel was temporarily withheld if a woman became pregnant or if a study clinician suspected that an adverse event might be related to a clinical complaint. In the absence of a complaint, study staff conducted quarterly pelvic examinations and took blood samples at 3, 12 and 24 months, and at study exit, in order to evaluate haematological parameters and blood chemistries, including a complete blood count, liver function, renal function and serum electrolytes.

An internal Protocol and Safety Review Team, which included an independent clinician, reviewed new and cumulative adverse events approximately every 3 months. This team, which remained blind to study gel assignment throughout the trial, also reviewed each serious adverse event in detail. During the trial, that is, before unblinding, each serious adverse event was classified as related, probably related, possibly related, probably not related or not related. For the purpose of reporting to regulatory bodies, 'probably related' was considered related. During the trial, safety was also reviewed by an independent Data and Safety Monitoring Board.

Safety markers and grading

Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 9.1 (International Federation of Pharmaceutical Manufacturers and Associations [IFPMA], Geneva, Switzerland) [14] and were summarized in frequency tables by body system and treatment group, including both the number of events and the number of women with one or more of each type of event.

Adverse events were graded for severity using the 2004 Division of AIDS Table for Grading Adult and Pediatric Adverse Events [15]. For laboratory data, the planned safety analyses included two comparisons of the proportions of women experiencing any new or worsening abnormal results (grades 1–4), or any new or worsening severe abnormalities (grades 3 and 4) on or before their discontinuation date. The subgroup analyses on hepatic adverse events in HBV carriers have not been included here because they been reported elsewhere [2].

This analysis of the potential systemic effects of tenofovir gel includes a detailed assessment of renal, haematological, bone and gastrointestinal effects. For evaluation of renal function, creatinine and electrolyte elevations were assessed. Decreased creatinine clearance, defined as <80 ml/min per the local laboratory normal values for people aged 40 years or less, was calculated using the method of Cockcroft and Gault [16]. Haematological effects were assessed by decreases in haemoglobin, indicating potential anaemia, increases in white blood count and platelet count. Bone-related adverse events were based on abnormal calcium or phosphate levels and incidence of fractures. For these assays, we used a well-established diagnostic laboratory in South Africa, which had established their own

local normal values where appropriate, and quality control systems to monitor performance.

Gastrointestinal effects of tenofovir gel were examined by tabulating diarrhoeal events by calendar time in order to explore potential associations between external or environmental factors and by assessing the effect of increasing gel use on diarrhoeal disease occurrence.

Statistical analyses

Women-years were calculated from randomization to termination. We conducted an intent-to-treat analysis and excluded participants who never received study product or never provided any safety data. Additionally, the dose–response relationships for significant findings were investigated, that is, reported gel use and increased cases of diarrhoea.

A Poisson distribution was assumed for CIs of incidence rates. Incidence rates were compared using the Z-test. If a laboratory finding or an adverse event increased in severity from baseline, it was counted as an adverse event during the study. Adverse events were graded for severity using the 2004 National Institutes for Health Division of AIDS (DAIDS) Table for Grading Adult and Pediatric Adverse Events [15]. A χ^2 test for trend was done to compare adherence and diarrhoea reported. All reported *P*-values are two-sided and all CIs are 95%. We compared frequencies of genital and other adverse events experienced by 1% or more of the participants without adjusting for multiple comparisons. The statistical analysis was performed using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Ethics and regulatory oversight

The trial (NCT00441298) was approved by the University of KwaZulu-Natal's Biomedical Research Ethics Committee (E111/06), FHI360's Protection of Human Subjects Committee (9946) and the South African Medicines Control Council (20060835).

Results

Enrolment

A total of 889 women were eligibly enrolled (Figure 1). The number of renal, haematological, bone and gastrointestinal abnormalities were similar at baseline in the tenofovir and placebo groups (Table 1).

Exposure to tenofovir

The average monthly vaginal dose, based on the average use of six applicators, was 240 mg. Most women (96%) used 11 applicators or less, equivalent to 440 mg, each month, and the highest reported average monthly dosing was 30 applications, that is 1,200 mg. The overall safety of tenofovir gel use, based on data available until 1 May 2010, has been reported previously [2] and has not changed materially in this final safety analysis. In brief, the rates of severe or any adverse events were similar in the tenofovir and placebo gel groups, and the majority of participants in both groups did not have adverse events of a severity higher than mild or moderate severity (88.1% in the tenofovir gel group versus 85.8% in the placebo group).

Renal effects

Three women had mildly raised (grade 1) creatinine levels at enrolment (Figure 1); however, because their creatinine clearance was >50 ml/min they met the eligibility criteria. These three women did not experience any worsening in their creatinine clearance; in fact, their creatinine levels were normal on repeat testing. During follow-up there were three women, two in the tenofovir group and one in the placebo group, who had mild (grade 1) creatinine elevations, ranging from 103 to 111 μmol/l (Table 1). Study gel use was not discontinued in these women, and repeat test results were normal for all. A review of the six raised creatinine tests by calendar date showed that all of the elevated values, including elevations at enrolment, occurred within a 1-month period between 30 July 2008 and 25 August 2008. This prompted a review of the laboratory procedures and quality control records, which revealed that the calibrator lot for that month had a 10% positive bias; hence, these creatinine elevations were, therefore, most likely due to laboratory error.

Abnormal sodium and potassium levels were similar between the tenofovir and placebo gel arms. Overall, there were 85 elevated sodium levels recorded during the study: 44 in the tenofovir arm and 41 in the placebo arm (Table 1). One participant in each study arm experienced a severe (grade 3) increase in sodium (Table 1). The numbers of abnormal potassium levels were also similar between the tenofovir and placebo groups (Table 1). Only one participant in the placebo arm experienced a severe elevation of potassium during the study (Table 1).

Haematological-related abnormalities

Abnormal haemoglobin levels were documented for 51 women at enrolment; 25 in the tenofovir and 26 in the placebo groups, respectively (Figure 1). During follow-up there were a further 25 women in the tenofovir group and 26 women in the placebo group who experienced decreased haemoglobin levels (Table 1).

Incidence rates of any abnormal haemoglobin levels during follow-up (Table 1) were similar in the tenofovir and placebo groups: 3.51 (95% CI 2.27, 5.18) and 3.76 (95% CI 2.46, 5.51) per 100 women-years, respectively (*P*=0.80). The incidence rates of abnormal platelet levels in the tenofovir and placebo groups were

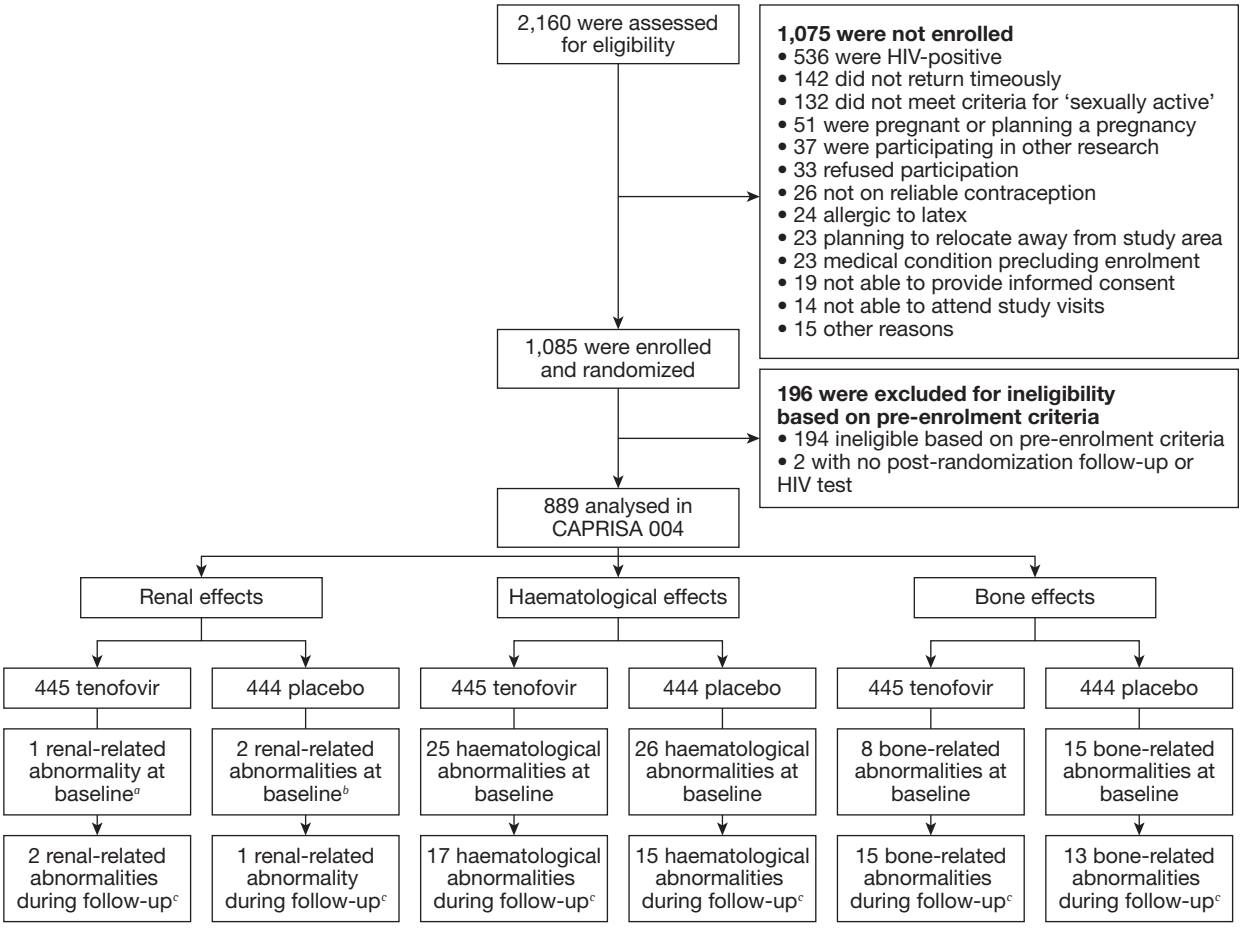
also similar during follow-up: 0.28 (95% CI 0.03, 1.01) versus 0.14 (95% CI 0, 0.81; *P*=0.59; Table 1).

Bone-related abnormalities

Abnormal calcium levels were documented for 23 women at enrolment: 8 in the tenofovir and 15 in the placebo groups, respectively (Figure 1). During follow-up there were 16 women in the tenofovir group and 14 women in the placebo group experiencing abnormal calcium levels (Table 1). Decreased phosphate levels were documented in 23 and 14 women at enrolment and 62 and 54 women during follow-up in the tenofovir and placebo groups, respectively (Table 1).

During follow-up, seven fractures were reported from six women. In total, four women were assigned to the tenofovir group and two were assigned to the placebo group. Women in the tenofovir group experienced similar rates of fractures compared with women in the placebo group; incidence of fractures was 0.70 (95% CI 0.23, 1.64) in the tenofovir group and 0.29 (95% CI 0.04, 1.05) in the placebo group (*P*=0.29; Table 1). Although clinicians graded two fractures in the tenofovir group as severe events, one woman had multiple fractures in a motor vehicle accident and one had a fracture of the left foot and ankle while jogging, none of the fractures were considered pathological fractures that were caused by a pathological process other than trauma, so none would be classified as grade 3 or 4 by DAIDS criteria. There was no relationship between calcium and phosphorus levels and occurrence of fractures. None of the fractures were considered to be related to gel use.

Figure 1. Selected renal, haematological and bone-related laboratory abnormalities at baseline and after 30 months of follow-up



Selected renal (creatinine), haematological (anaemia) and bone-related (hypo- or hyper-calcaemia) laboratory abnormalities at baseline and after 30 months of follow-up in the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 tenofovir gel trial by study arm. [a]Grade 1 abnormality with a calculated creatinine clearance of 79 ml/min. [b]Grade 1 abnormalities with a calculated creatinine clearance of 90 and 93 ml/min. [c]Excluding participants with baseline abnormality.

Table 1. Incidence rate of renal, haematological, bone and gastrointestinal-related abnormalities[a]

Type of abnormality	Tenofovir: Abnormalities at enrolment, *n*	Tenofovir: Any new adverse event during follow-up, events/participants	Tenofovir: Incidence per 100 women-years (95% CI)	Placebo: Abnormalities at enrolment, *n*	Placebo: Any new adverse event during follow-up, events/participants	Placebo: Incidence per 100 women-years (95% CI)	*P*-value[b]
Incidence rate of abnormalities, all grades[a]							
Renal							
Creatinine	1	2/2	0.28 (0.03, 1.01)	2	1/1	0.14 (0, 0.81)	0.59
Sodium	28	44/43	6.17 (4.49, 8.29)	21	41/40	5.93 (4.26, 8.05)	0.85
Potassium	66	86/83	12.07 (9.65, 14.90)	56	73/71	10.56 (8.28, 13.28)	0.4
Haematological							
Haemoglobin	25	25/24	3.51 (2.27, 5.18)	26	26/24	3.76 (2.46, 5.51)	0.8
White blood count	0	0	0	1	1/1	0.14 (0, 0.81)	–
Platelet count	5	2/2	0.28 (0.03, 1.01)	1	1/1	0.14 (0, 0.81)	0.59
Bone							
Calcium	8	16/15	2.25 (1.28, 3.64)	15	14/13	2.02 (1.10, 3.40)	0.78
Phosphate	23	62/56	8.7 (6.67, 11.15)	14	54/50	7.81 (5.87, 10.19)	0.56
Fractures	0	5/4	0.7 (0.23, 1.64)	0	2/2	0.29 (0.04, 1.05)	0.29
Gastrointestinal							
Albumin	10	51/46	7.16 (5.33, 9.41)	15	40/39	5.79 (4.13, 7.88)	0.31
Bilirubin total	9	21/19	2.95 (1.82, 4.50)	4	20/19	2.89 (1.77, 4.47)	0.95
Diarrhoea[c]	0	90/74	12.63 (10.16, 15.52)	0	66/50	9.55 (7.39, 12.15)	0.08
Nausea	0	4/4	0.56 (0.15, 1.44)	0	5/5	0.72 (0.23, 1.69)	0.71
Incidence rate of grade 3 or 4 abnormalities[c]							
Renal							
Creatinine	0	0	0	0	0	0	–
Sodium	0	1/1	0.14 (0, 0.78)	1	1/1	0.14 (0, 0.81)	0.98
Potassium	0	0	0	1	1/1	0.14 (0, 0.81)	–
Haematological							
Haemoglobin	3	5/5	0.7 (0.23, 1.64)	3	4/4	0.58 (0.16, 1.48)	0.77
White blood count	0	0	0	0	0	0	–
Platelet count	2	1/1	0.14 (0, 0.78)	0	0	0	–
Bone							
Calcium	0	0	0	–	0	0	–
Phosphate	2	11/11	1.54 (0.77, 2.76)	2	10/10	1.45 (0.69, 2.66)	0.88
Gastrointestinal							
Albumin	0	0	0	1	0	0	–
Bilirubin total	0	0	0	0	1/1	0.14 (0, 0.81)	–

[a]Grades 1, 2, 3 and 4. [b]*P*-values comparing incidences. [c]Diarrhoea refers to enteritis, diarrhoea, diarrhoea haemorrhagic, diarrhoea infectious, dysentery and gastroenteritis, and excludes two cases of gastroesophageal reflux, which were included in a previously published analysis. All cases of diarrhoea and fractures throughout study are included in the analysis. These data are updated and differ slightly from numbers previously reported.

Table 2. Genital adverse events occurring in >1% of the study population

System organ class	Preferred term	Tenofovir (*n*=445)		Placebo (*n*=444)		*P*-value[a]
		Events, *n*	Women, *n* (%)	Events, *n*	Women, *n* (%)	
Infections and infestations	Cervicitis	44	39 (8.8)	39	32 (7.2)	0.4581
	Herpes simplex	7	7 (1.6)	10	9 (2.0)	0.6257
	Pelvic inflammatory disease	13	13 (2.9)	15	14 (3.2)	0.8481
	Sexually transmitted disease	6	6 (1.3)	11	10 (2.3)	0.3276
	Urinary tract infection	135	100 (22.5)	120	93 (20.9)	0.6256
	Vaginal candidiasis	156	114 (25.6)	187	130 (29.3)	0.2298
Reproductive system disorders	Cervical discharge	5	5 (1.1)	8	8 (1.8)	0.4202
	Coital bleeding	2	2 (0.4)	7	6 (1.4)	0.1775
	Dysmenorrhoea	5	5 (1.1)	11	10 (2.3)	0.206
	Genital discharge	35	32 (7.2)	39	30 (6.8)	0.8954
	Genital haemorrhage	5	4 (0.9)	4	4 (0.9)	1
	Genital pruritus female	19	16 (3.6)	31	29 (6.5)	0.048
	Genital rash	5	5 (1.1)	4	3 (0.7)	0.7253
	Genital ulceration	15	15 (3.4)	13	12 (2.7)	0.6966
	Metrorrhagia	13	12 (2.7)	33	30 (6.8)	0.0044
	Vaginal discharge	203	156 (35.1)	239	156 (35.1)	1
	Vaginal haemorrhage	6	6 (1.3)	3	3 (0.7)	0.5056

[a]*P*-values comparing proportions.

Gastrointestinal abnormalities

Among the 445 women in the tenofovir group, 17% (74/445) had one or more episodes of diarrhoea compared with 11% (50/444) of women in the placebo group (P=0.026). To further explore the association of tenofovir gel with diarrhoea, a dose–response relationship between increasing tenofovir gel use and episodes of reported diarrhoea was investigated. There was a slight but non-significant trend towards an increasing incidence of diarrhoea with more tenofovir gel use (χ^2 for trend P=0.06 for the tenofovir group). As gel use increased, 12%, 20% and 19% of women using a median of <5, 5–9 or >9 applicators per month reported one or more episodes of diarrhoea, respectively. There was no evidence of a trend in diarrhoea by gel usage in the placebo gel group.

There is a possibility that the diarrhoea could be attributed to rectal use of the gel; however, this is not possible to ascertain because we did not explicitly collect data on rectal use of the gel. In a nested, case-control substudy exploring women's experiences of gel use with open-ended questions, there were no reports of rectal use of gel (MacQueen, FHI360, Durham, NC, USA, personal communication). The study methodology and partial results have been reported [17].

Reported cases of diarrhoea fluctuated throughout the study, but the seasonal patterns and peaks were similar in the tenofovir and placebo groups (Figure 2). Although clinicians did not consider any of the cases of diarrhoea as severe or life-threatening events, three cases in the tenofovir group and eight cases in the placebo group were classified as 'bloody diarrhoea', which would meet the DAIDS definition of a grade 3 event. All cases of diarrhoea were reported to have been resolved by the end of the study.

Genital adverse events

We observed similar rates of the most common vaginal disorders, such as vaginal discharge, vaginal candidiasis and urinary tract infections, in both study groups (Table 2). In terms of infectious disorders, no significant differences between groups were observed. By contrast, there were significantly fewer reports of metrorrhagia (P=0.004) and genital pruritis (P=0.048) in the tenofovir group compared with the placebo group.

Other adverse events

Rates of the most common disorders, for example, influenza and headache, were similar in both groups.

In summary, the combined numbers of cases of enteritis, diarrhoea, haemorrhagic diarrhoea, infectious diarrhoea, dysentery and gastroenteritis were significantly increased in the tenofovir group. Furthermore, otitis media was significantly increased in the tenofovir group (10 cases) compared with the placebo group (one case; P=0.01; Table 3); the possibility that this is a chance occurrence cannot be ruled out.

Serious adverse events

A total of 44 serious adverse events, including two deaths, were reported during the CAPRISA 004 trial, and none were judged to be related to gel use. Four of those events occurred in participants who were excluded from the main analysis and the primary study results. Of the four, three of the excluded women had been ineligibly

Table 3. Adverse events[a] occurring in >1% of the study population

System organ class	Preferred term	Tenofovir (*n*=445)		Placebo (*n*=444)		
		Events, *n*	Women, *n* (%)	Events, *n*	Women, *n* (%)	*P*-value[b]
Blood and lymphatic system disorders	Anaemia	7	6 (1.3)	4	4 (0.9)	0.7525
	Neutropenia	5	5 (1.1)	7	6 (1.4)	0.7729
Ear and labyrinth disorders	Ear pain	4	4 (0.9)	6	5 (1.1)	0.7525
Eye disorders	Conjunctivitis	26	20 (4.5)	15	14 (3.2)	0.3822
	Conjunctivitis allergic	12	12 (2.7)	13	12 (2.7)	1
Gastrointestinal disorders	Abdominal pain	41	37 (8.3)	42	35 (7.9)	0.9023
	Abdominal pain lower	31	30 (6.7)	20	18 (4.1)	0.1017
	Constipation	13	13 (2.9)	15	13 (2.9)	1
	Diarrhoea	56	46 (10.3)	46	33 (7.4)	0.1569
	Dyspepsia	8	6 (1.3)	4	4 (0.9)	0.7525
	Gastritis	12	11 (2.5)	5	5 (1.1)	0.206
	Gingival pain	6	5 (1.1)	3	3 (0.7)	0.7253
	Haemorrhoids	7	6 (1.3)	9	9 (2.0)	0.4503
	Nausea	4	4 (0.9)	5	5 (1.1)	0.7525
	Toothache	16	16 (3.6)	11	11 (2.5)	0.435
General disorders and administration site conditions	Pain	17	15 (3.4)	8	8 (1.8)	0.2041
Infections and infestations	Abscess limb	7	7 (1.6)	2	2 (0.5)	0.1775
	Acarodermatitis	4	4 (0.9)	5	5 (1.1)	0.7525
	Acute tonsillitis	4	4 (0.9)	5	5 (1.1)	0.7525
	Body tinea	4	4 (0.9)	9	9 (2.0)	0.1761
	Bronchitis	29	26 (5.8)	29	24 (5.4)	0.8844
	Dysentery	3	3 (0.7)	7	7 (1.6)	0.2237
	Fungal infection	6	6 (1.3)	3	3 (0.7)	0.5056
	Gastroenteritis	26	24 (5.4)	10	10 (2.3)	0.0217
	Helminthic infection	13	11 (2.5)	5	5 (1.1)	0.206
	Influenza	365	216 (48.5)	314	220 (49.5)	0.7886
	Lower respiratory tract infection	20	18 (4.0)	9	9 (2.0)	0.1164
	Otitis media	20	17 (3.8)	21	19 (4.3)	0.7375
	Otitis media acute	10	10 (2.2)	1	1 (0.2)	0.0112
	Pharyngitis	30	29 (6.5)	27	25 (5.6)	0.6739
	Pulmonary tuberculosis	7	7 (1.6)	3	3 (0.7)	0.341
	Sinusitis	48	32 (7.2)	44	37 (8.3)	0.5338
	Subcutaneous abscess	7	7 (1.6)	5	5 (1.1)	0.7729
	Tinea infection	10	10 (2.2)	8	7 (1.6)	0.6257
	Tinea versicolour	12	10 (2.2)	13	9 (2.0)	1
	Tonsillitis	45	36 (8.1)	40	36 (8.1)	1
	Upper respiratory tract infection	29	26 (5.8)	25	16 (3.6)	0.1541
Injury, poisoning and procedural complications	Joint sprain	8	8 (1.8)	2	2 (0.5)	0.1074
	Limb injury	9	8 (1.8)	2	2 (0.5)	0.1074
	Skin laceration	8	7 (1.6)	9	8 (1.8)	0.8018
Investigations	Alanine aminotransferase increased	10	10 (2.2)	9	9 (2.0)	1
	Aspartate aminotransferase increased	4	3 (0.7)	5	5 (1.1)	0.5056
	Blood alkaline phosphatase increased	5	5 (1.1)	4	4 (0.9)	1
	Blood lactate dehydrogenase increased	8	8 (1.8)	4	4 (0.9)	0.3844
	Blood phosphorus decreased	12	11 (2.5)	16	15 (3.4)	0.435
	Blood potassium decreased	6	6 (1.3)	6	6 (1.4)	1
	Blood sodium decreased	6	6 (1.3)	3	3 (0.7)	0.5056
	γ-Glutamyl transferase increased	5	5 (1.1)	6	6 (1.4)	0.7729
	Haemoglobin decreased	5	5 (1.1)	4	4 (0.9)	1

[a]Excluding genital adverse events and death. [b]*P*-value comparing proportions.

Table 3. Continued

System organ class	Preferred term	Tenofovir (*n*=445) Events, *n*	Tenofovir (*n*=445) Women, *n* (%)	Placebo (*n*=444) Events, *n*	Placebo (*n*=444) Women, *n* (%)	*P*-value[b]
Metabolism and nutrition disorders	Decreased appetite	8	7 (1.6)	8	8 (1.8)	0.8018
	Hypokalaemia	7	7 (1.6)	8	8 (1.8)	0.8018
Musculoskeletal and connective tissue disorders	Arthralgia	20	16 (3.6)	20	20 (4.5)	0.5023
	Back pain	28	24 (5.4)	27	25 (5.6)	0.8844
	Pain in extremity	19	17 (3.8)	15	13 (2.9)	0.5781
Nervous system disorders	Dizziness	8	8 (1.8)	5	5 (1.1)	0.5782
	Headache	126	93 (20.9)	133	102 (23.0)	0.4667
	Tension headache	4	4 (0.9)	5	5 (1.1)	0.7525
Breast disorders	Breast pain	5	5 (1.1)	5	5 (1.1)	1
Respiratory, thoracic and mediastinal disorders	Cough	39	36 (8.1)	40	34 (7.7)	0.901
	Nasal congestion	31	24 (5.4)	17	15 (3.4)	0.1896
	Pharyngolaryngeal pain	8	8 (1.8)	4	4 (0.9)	0.3844
Skin and subcutaneous tissue disorders	Acne	5	5 (1.1)	6	5 (1.1)	1
	Dermatitis allergic	7	7 (1.6)	8	8 (1.8)	0.8018
	Rash	21	20 (4.5)	17	15 (3.4)	0.4908

enrolled. One participant, who died post-enrolment after being struck by lightning, was excluded because she lacked a post-randomization HIV test. The other death occurred in the placebo group. The participant died at home of unknown causes after a brief illness, including bloody diarrhoea, headache and leg pain. At her last study visit, she had had discordant HIV rapid test results. On the death certificate her cause of death was listed as pneumonia, but no autopsy was carried out.

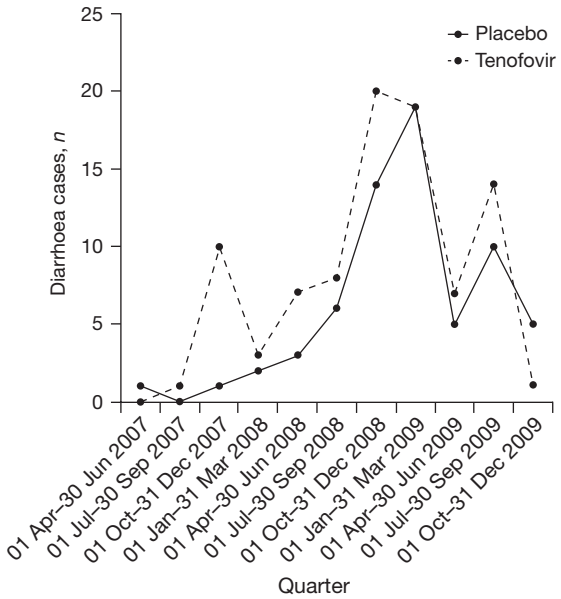
The most common causes of serious adverse events were related to hospitalization for pregnancy or pregnancy complications, including hospitalizations for six caesarean sections, three spontaneous abortions and two elective pregnancy terminations.

Discussion

We found no safety concerns emanating from the use of tenofovir gel. With regard to the most important safety concern with tenofovir use, we found no evidence to suggest any renal toxicity from use of tenofovir gel, and we did not discontinue any women due to concerns about compromised renal function. It should be noted that women who had an estimated creatinine clearance of <50 ml/min at screening were not enrolled in the trial.

Similarly, we found no evidence of haematological or bone-related toxicity. Adverse events in these two categories were similar in the tenofovir gel group compared with the placebo gel group. It is difficult to hypothesize a plausible physiological mechanism for the apparent association between diarrhoea and tenofovir vaginal gel, but a local effect might be possible; a primate study showed that tenofovir levels in the rectum are elevated after vaginal administration, perhaps due to diffusion of the drug through venous channels in the perineum [18].

Figure 2. Number of diarrhoea cases by quarter



Given the low systemic tenofovir exposure from vaginal dosing, it is not surprising that we did not find any renal effects. As noted by Mayer *et al.* [19] after a 40 mg vaginal dose, the 'median maximum systemic tenofovir concentration for all subjects (3.4 ng/ml) corresponded to approximately 1% of the maximum and 7% of the minimum blood concentrations…' compared with the usual 300 mg oral dose. Even for women who used tenofovir gel multiple times per week, systemic exposure would be low.

In a recent report of a placebo-controlled study of 300 mg of a daily oral dose of tenofovir plus 200 mg of emtricitabine (Truvada) for HIV prevention in men [20], there was a non-significant finding of increased creatinine elevations in the tenofovir group, 26 versus 15 elevations (P=0.08). Seven men in the tenofovir group and three in the placebo group were taken off the study product (without unblinding) due to concern about creatinine elevations. Four subjects subsequently resumed taking tenofovir and did not have a recurrence of creatinine elevation.

In a similar placebo-controlled study among heterosexuals that was halted after demonstrating significant protection against HIV, the Partners PrEP Study, no safety concerns were raised for participants who received oral tenofovir [21]. Given the safety of oral tenofovir for HIV prevention, it is unlikely that topical tenofovir, which remains largely localized in the genital tract, would have any adverse systemic effects.

The VOICE study (Vaginal and Oral Interventions to Control the Epidemic) recently halted the tenofovir gel arm because the study failed to demonstrate effectiveness against HIV when tenofovir gel was prescribed for once-daily application, but researchers emphasized that there were no concerns about safety [22].

In conclusion, coitally linked, intra-vaginal use of tenofovir gel in this trial was not associated with harmful effects on kidney function, haematological or bone parameters and was not associated with any increase in genital adverse events. The association with diarrhoea is intriguing and merits further research.

Acknowledgements

We pay tribute to the women who participated in this trial; their dedication and commitment made this study possible. We thank the Health Department of the City of Durban, the traditional leadership of Vulindlela, specifically Nkosi Sondelani Zondi and Nkosi Nsikayezwe Zondi, and members of the Community Advisory Boards at the Vulindlela and eThekwini Research Clinics. The CAPRISA 004 Protocol Safety Review team included Luvuyo Mtongana and Zaheen Omar. We thank them for the contributions in monitoring participant safety during the trial. The authors also thank Kavita Nanda, Charles Morrison and Kate MacQueen and all of FHI 360 for their helpful review of the manuscript. MedDRA® trademark is owned by IFPMA on behalf of ICH.

Disclosure statement

QAK was co-principal investigator of the HIV Prevention Trials Network, which conducted the HPTN 052 trial of treatment for prevention. SSAK is an executive committee member of the Microbicide Trials Network, which is undertaking the VOICE trial of oral and topical PrEP. DCS is a co-inventor of a vaginal device that might improve delivery of vaginal microbicides. The remaining authors declare no competing interests.

References

1. UNAIDS. UNAIDS Report on the global AIDS Epidemic 2010. Geneva: Joint United Nations Programme on HIV/AIDS. (Updated 11 February 2011. Accessed 11 February 2011.) Available from http://www.unaids.org/globalreport/
2. Abdool Karim Q, Abdool Karim SS, Frohlich JA, *et al*. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* 2010; **329:**1168–1174.
3. Williams BG, Abdool Karim SS, Abdool Karim Q, Gouws E. Epidemiological impact of tenofovir gel on the HIV epidemic in South Africa. *J Acquir Immune Defic Syndr* 2011; **58:** 207-210.
4. Abdool Karim SS, Kashuba A, Werner L, Abdool Karim Q. Drug concentrations following topical and oral antiretroviral pre-exposure prophylaxis: implications for HIV prevention in women. *Lancet* 2011; **378:**279–281.
5. Viread (tenofovir disoproxil fumarate). *Package insert* 2008. Gilead Sciences, Inc., Foster City, CA, USA.
6. Nelson MR, Katlama C, Montaner J, *et al*. The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. *AIDS* 2007; **21:**1273–1281.
7. Herlitz LC, Mohan S, Stokes MB, Radhakrishnan J, D'Agati VD, Markowitz GS. Tenofovir nephrotoxicity: acute tubular necrosis with distinctive clinical, pathological, and mitochondrial abnormalities. *Kidney Int* 2010; **78:**1171–1177.
8. Parsonage MJ, Wilkins EG, Snowden N, Issa BG, Savage MW. The development of hypophosphataemic osteomalacia with myopathy in two patients with HIV infection receiving tenofovir therapy. *HIV Med* 2005; **6:**341–346.
9. Gupta SK. Tenofovir-associated Fanconi syndrome: review of the FDA adverse event reporting system. *AIDS Patient Care STDS* 2008; **22:**99–103.
10. Calmy A, Fux CA, Norris R, *et al*. Low bone mineral density, renal dysfunction, and fracture risk in HIV infection: a cross-sectional study. *J Infect Dis* 2009; **200:**1746–1754.
11. Mulligan K, Glidden D, Gonzales P, *et al*. Effects of FTC/TDF on bone mineral density in seronegative men from 4 continents: DEXA results of the Global iPrEx Study. *18th Conference of Retroviruses and Opportunistic Infections*. 27 February–2 March 2011, Boston, MA, USA. Abstract 94LB.
12. Gilead Sciences Inc. *Investigators brochure: tenofovir gel (GS-1278)* 2010. Foster City, CA, USA: California Gilead Sciences, Inc.
13. Abdool Karim Q, Kharsany AB, Naidoo K, *et al*. Co-enrollment in multiple HIV prevention trials – experiences from the CAPRISA 004 Tenofovir gel trial. *Contemp Clin Trials* 2011; **32:**333–338.
14. MedDRA®. Medical Dictionary for Regulatory Activities. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). (Updated 1 March 2013. Accessed 27 March 2013.) Available from http://www.meddramsso.com/
15. Division of AIDS. Table for grading adult and pediatric adverse events. NIAID DAIDS Regulatory Support Center (RSC). (Updated 14 March 2013. Accessed 27 March 2013.) Available from http://rsc.tech-res.com/safetyandpharmacovigilance/gradingtables.aspx
16. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16:**31–41.

17. MacQueen K, van Loggerenberg F, Weaver M, *et al*. Behavioral recall accuracy in a microbicide trial: effects of psychological stress, serostatus, and timing of interview. *M2010 Microbicides Conference: Building Bridges in HIV Prevention*. 22–25 May 2010, Pittsburgh, PA, USA. Abstract 408.

18. Nuttall J, Kashuba A, Wang R, *et al*. The pharmacokinetics of tenofovir following intravaginal and intrarectal administration of tenofovir gel to rhesus macaques in a microbicide trial. *M2010 Microbicides Conference*. 22–25 May 2010, Pittsburgh, PA, USA. Abstract 40.

19. Mayer KH, Maslankowski L, Gai F, *et al*. Safety and tolerability of vaginal tenofovir gel in abstinent and sexually active HIV-infected and uninfected women. *AIDS* 2006; **20:**543–551.

20. Grant RM, Lama JR, Anderson PL, *et al*. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N Engl J Med* 2010; **363:**2587–2599.

21. Baeten JM, Donnell D, Ndase P, *et al*. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. *New Engl J Med* 2012; **367:**399–410.

22. Marrazzo J, Ramjee G, Nair G, *et al*. Pre-exposure prophylaxis for HIV in women: daily oral tenofovir, oral tenofovir/emtricitabine, or vaginal tenofovir gel in the VOICE Study (MTN 003). *Conference on Retroviruses and Opportunistic Infections*. 3–6 March 2013, Atlanta, GA, USA. Abstract 26LB.

Accepted 6 July 2012; published online 23 August 2012