Original article

Safety and acceptability of vaginal disinfection with benzalkonium chloride in HIV infected pregnant women in west Africa: ANRS 049b phase II randomised, double blinded placebo controlled trial

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Objectives: To study the tolerance and acceptability in Africa of a perinatal intervention to prevent vertical HIV transmission using benzalkonium chloride disinfection.
Design: A randomised, double blinded phase II trial.
Setting: Prenatal care units in Abidjan (Côte d'Ivoire) and Bobo-Dioulasso (Burkina Faso).
Patients: Women accepting testing and counselling who were seropositive for HIV-1 and under 37 weeks of pregnancy were eligible. A total of 108 women (54 in each group) enrolled from November 1996 to April 1997, with their informed consent.
Intervention: Women self administered daily a vaginal suppository of 1% benzalkonium chloride or matched placebo from 36 weeks of pregnancy, and a single intrapartum dose. The neonate was bathed with 1% benzalkonium chloride solution or placebo within 30 minutes after birth.
Main outcome measures: Adverse events were recorded weekly, with a questionnaire and speculum examination in women through delivery, and examination of the neonate through day 30. The incidence of genital signs and symptoms in the women and cutaneous or ophthalmological events in newborns were compared between groups on an intent to treat basis.
Results: The median duration of prepartum treatment was 21 days (range 0–87 days). Compliance was 87% for prepartum and 69% for intrapartum treatment, and 88% for the neonatal bath, without differences between the two groups. In women, the most frequent event was leucorrhoea; the incidence of adverse events did not differ between treatment groups. In children, the incidence of dermatitis and conjunctivitis did not differ between the benzalkonium chloride and placebo groups (p=0.16 and p=0.29, respectively).
Conclusion: Vaginal disinfection with benzalkonium chloride is a feasible and well tolerated intervention in west Africa. Its efficacy in preventing vertical HIV transmission remains to be demonstrated.
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Keywords: microbicides; vertical transmission; Africa

Introduction

The human immunodeficiency virus (HIV) infection pandemic has been spreading for two decades, heterosexual transmission being predominant in Africa.[1] Mother to child transmission of HIV may occur in utero, intrapartum, and postnatally through breast feeding[2] with transmission rates ranging from 14% to 39%.[3] Various interventions have been designed and evaluated around the world to reduce mother to child transmission of HIV in developing countries, looking for effective, safe, affordable, and sustainable interventions.[4] The observation that the first born twin is at higher risk of HIV infection than the second one,[5] the demonstrated protective effect of elective caesarean delivery,[6] and the association between prolonged rupture of membranes and increased mother to child transmission[7] suggest that direct exposure of the fetus to HIV in the maternal genital tract before and during delivery could be an important route of vertical transmission.

For these reasons, vaginal microbicides have been considered as a potential intervention to decrease mother to child transmission of HIV. Vaginal microbicides may offer several advantages in resource poor settings, besides the fact that they are inexpensive. From a public health point of view, benefits may go beyond HIV infected women and their offspring, as the use of vaginal antiseptics has been demonstrated to prevent perinatal and postpartum bacterial infections regardless of HIV status.[8 9] They could therefore be potentially considered for use, even where HIV testing in pregnancy is not available. To date, a single study in Malawi, using chlorhexidine, showed no reduction in mother to child transmission of HIV type 1 (HIV-1) except in the context of prolonged rupture of membranes.[10] However, in this study, vaginal disinfection was performed at advanced stages of labour.

Potential candidates for vaginal disinfection have to inactivate HIV in vitro and in vivo, be well tolerated and be available for self administration by women. Benzalkonium chloride has been shown to inactivate HIV in vitro.[11 12] It has been used as a spermicide and as a topical microbicide for decades throughout the world. Benzalkonium chloride is not absorbed by mucosae and has therefore no systemic effect. When used routinely as a spermicide, it has been shown to have no local toxic effect.[13] However, the tolerance of benzalkonium chloride used repeatedly in HIV infected pregnant women and in newborns has not yet been evaluated. This is of particular concern because some microbicides have been shown to irritate human mucosae.[14]

We performed a phase II clinical trial with the following objectives: (1) to study the tolerance of vaginal disinfection by benzalkonium chloride vaginal capsules in HIV infected pregnant women and of bathing the newborn with benzalkonium chloride diluted solution; and (2) to assess the acceptability of this intervention in urban populations of west Africa. Because of the high incidence of lower genital tract signs and symptoms in these populations, a randomised, placebo controlled study design was required for this evaluation.

Material and methods

SETTING

The study was performed in neighbourhood maternal/infant care units in the cities of Abidjan (Côte d'Ivoire) and Bobo-Dioulasso (Burkina Faso). The Yopougon unit in Abidjan performs over 8000 deliveries each year, with an HIV seroprevalence of 14.8%, and the National Hospital and Farakan units in Bobo-Dioulasso perform over 2200 and 3800 deliveries each year, respectively, with seroprevalences of 9.2% and 10.6%.[15] Pregnant women from the two sites were mainly aged 18–29, one half were illiterate, most were housewives or vendors, and 85% were married or living maritally. These and other demographic characteristics of the populations have been described previously.[15]

ELIGIBILITY

HIV testing was offered to all pregnant women attending prenatal visits after individual pretest counselling. Women who accepted to be tested and returned to obtain their test result received individual post-test counselling. Women with a positive HIV-1 or dually reactive (HIV-1 + HIV-2) serodiagnosis were informed about the possibility of entering the trial as described elsewhere.[16]

HIV DIAGNOSIS

Serum samples were screened for HIV-1 antibodies using a commercial enzyme immunoassay (Genelavia, Pasteur, Paris, France). When serum samples were positive on immunoassay, the findings were confirmed by a commercial immunoenzymatic strip method using two synthetic peptides (Peptilav, Sanofi Diagnostics Pasteur, Paris) on the same sample. A second sample was then obtained from women with an HIV antibody positive test to verify their HIV status with a rapid test (Multispot, Pasteur, Paris) before enrolment in the trial.

ENROLMENT

HIV-1 positive or dually reactive pregnant women, aged 18–45 years, living within city boundaries, were offered to enter the trial, if their gestational age, assessed by ultrasound, clinical examination and date of last menstrual period, was less than 37 weeks. Written consent was obtained individually after the study objectives and content had been explained by a specially trained counsellor, in French or in a vernacular language.

At enrolment, women had a physical examination, CD4 and CD8 lymphocyte counts, sexually transmitted infections (STD) screening including direct examination for *Candida albicans*, *Trichomonas vaginalis*, and vaginal lactobacilli, and culture for gonococci and clue cells. Vaginal flora were classified I–IV; I: normal lactobacilli flora, II: predominant lactobacilli, III: rare lactobacilli; and IV: absence of lactobacilli. When a cervicovaginal infection (gonorrhoea, chlamydiae, candidiasis, trichomoniasis) was clinically diagnosed before inclusion, enrolment was suspended until it had been successfully treated and other inclusion criteria were still met.

TREATMENT

Double blind randomisation was performed using a pre-established computerised list drawn by the independent statistician. Ten blocked randomisation was used to assign eligible women to one of the study regimens. Sequentially numbered sealed drug packages containing the appropriate treatments identical in appearance were prepared by an independent central pharmacy according to the randomisation list.

The study protocol was approved by ethics committees in Burkina Faso, Côte d'Ivoire, and France and by the scientific committee of the ANRS.

Women were given vaginal capsules of either benzalkonium chloride (1% concentration) or placebo from 36 weeks of pregnancy until labour. Women were instructed to self administer one suppository every evening, by inserting it into the vagina with a finger. A blister pack containing treatment for 1 week was supplied, and renewed at the following visit, until delivery.

Another vaginal suppository (benzalkonium chloride or placebo) was administered at beginning of the delivery process in the maternity ward under supervision of the study team. Finally, the neonate was bathed with a benzalkonium chloride solution (1% concentration) or placebo within 30 minutes after birth, in the delivery room.

The trial drugs, vaginal capsules and bath powder were provided by Innothera (Paris, France). Benzalkonium chloride and placebo capsules differed only with respect to the presence or not of benzalkonium chloride (18.9 mg) in the internal phase, and were composed

Table 1 Recruitment of ANRS 049b trial Abidjan, Côte d'Ivoire, and Bobo-Dioulasso, Burkina Faso, 1996–7

	Abidjan	*Bobo-Dioulasso*
HIV-1 infected women returning for test result	189	138
Women eligible (n, %)	188 (99.5%)	133 (96.4%)
Women enrolled (n, %)	40 (21.3%)	68 (49.3%)
Reasons for non-enrolment		
No return for pre-inclusion/inclusion	95	40
Refusal to participate	7	8
Delivery before inclusion	22	14
Inclusion in another clinical trial (ANRS 049a)	24	3

of identical inert excipients for the internal phase (polyoxyethylene glycol, polyoxyethylene glycol 7 glyceryl cocoate, colloidal silicium, hydroxypropylcellulose, and silicone oil) and capsule itself (safety controlled gelatine, glycerine, and purified water). The capsule form is stable under room temperature, even under west African climates, and does not require refrigeration.

The solution was prepared from pouches containing 100 g of powder on site before each neonatal bath. Benzalkonium chloride and placebo powder both contained Xylitol so that the final solution had an identical colour.

FOLLOW UP

Women were followed weekly from enrolment through 8 days after delivery. For tolerance, women were asked about genital signs and symptoms, and examined clinically for genital irritation with a speculum examination with the naked eye. In Abidjan, the complete microbiological examination was repeated 8 days after enrolment. To assess the acceptability of the daily self administration of capsules, women were interviewed every 2 weeks using a standardised questionnaire on previous use of any vaginal route therapies, and on sensations of discomfort, smell, ease of vaginal insertion, symptoms in the partner. Medical care was offered free of charge for women and children until 18 months after delivery/birth. There was a specific counselling on breast feeding, HIV transmission, and infant nutrition as presented elsewhere.[17]

Table 2 Baseline characteristics of pregnant women included in the ANRS049b trial, Abidjan, Côte d'Ivoire and Bobo-Dioulasso, Burkina Faso, 1996–7

	Benzalkonium Chloride (n=54)		*Placebo (n=54)*		
	No	*%*	*No*	*%*	*p Value*
Marital status					0.75*
Single or widowed	14	25.9	11	20.4	
Common law union	9	16.7	11	20.4	
Married	31	57.4	32	59.2	
Education level					0.22*
None	29	53.7	21	38.9	
Primary (1–6 years)	17	31.5	19	35.2	
Secondary and higher	8	14.8	14	25.9	
Obstetric and gynaecological history					
Stillbirth	5	9.4	0	0	0.057†
Genital ulceration	10	18.5	11	20.7	0.77*
Vaginal discharge	32	59.3	33	61.1	0.84*
Genital erosion	15	27.7	16	29.6	0.83*
WHO staging at entry					0.08*
1	35	64.8	45	83.3	
2	13	24.7	7	12.9	
3	6	11.1	2	3.7	
Gynaecological examination at entry					
Perineal ulcers	3	5.6	3	5.6	0.66†
Condyloma acuminata	7	12.9	2	3.7	0.08†
Vaginal discharge	28	51.8	17	31.4	0.032*
Vaginal inflammation	1	1.8	1	1.8	0.99†
Vaginal ulcers	0	0	0	0	—
Cervical inflammation	4	7.4	3	5.6	0.5†
Cervical ulcers	0	0	4	7.4	0.11†
CD4 class (CD4+ $\times 10^6$/l)					0.27*
0–349	14	26.3	9	16.9	
350–499	15	28.3	10	18.8	
500–749	11	20.8	14	26.4	
⩾750	13	24.5	20	37.7	

*χ^2 test.
†Fisher's exact test.

MAIN OUTCOME MEASURES

Reproductive tract symptoms and signs, including genital ulcers, were recorded until delivery for women. Irritations of the skin, mucosae, or eyes were recorded from birth through 30 days for the neonates. Occurrence of adverse events was generally expressed as incidence rate per 100 person days. Comparisons between the treatment arms were performed on an intent to treat basis.

Compliance was estimated by dividing the number of days with treatment by the number of days between enrolment and delivery, according to the women's report and the count of the empty blisters.

STATISTICS

The trial was designed to observe a difference in the occurrence of genital ulcers from 5% in the placebo group to 20% in the treatment group, with an alpha type one error (two sided) of 010 and a power of 80%. χ^2 Test, Student's *t* test, and Fisher's exact test were used when appropriate to compare the treatment groups.

Results

RECRUITMENT AND BASELINE CHARACTERISTICS

In Abidjan, between 31 October 1996 and 19 February 1997, HIV testing and counselling was offered to 2091 women attending prenatal care and accepted by 1812 women, of whom 228 were seropositive for HIV-1 and HIV-2 were dually seropositive for HIV-1 and HIV-2, an overall seroprevalence of HIV-1 of 13.3%. Among 189 HIV-1 infected women who returned for their test result, 188 were eligible for enrolment, and 40 were enrolled. In Bobo-Dioulasso, between 26 September 1996 to 16 May 1997, HIV testing and counselling was offered to 1646 women, and accepted by 1538. HIV-1 seropositivity was detected in 136 women and dual HIV-1/HIV-2 seropositivity in two, and overall HIV-1 seroprevalence of 8.9%. Among 138 HIV-1 infected women who returned for their test result, 133 were eligible for enrolment and 68 were enrolled. In both centres, the principal reasons for non-enrolment were (1) failure to return for pre-enrolment or enrolment, (2) delivery before enrolment, and (3) refusal to participate, as described in table 1.

From September 1996 to May 1997, 108 women were included in the trial (40 in Abidjan and 68 in Bobo-Dioulasso). Participants were equally assigned to the benzalkonium chloride arm (n=54) and the placebo arm (n=54).

Baseline characteristics of the pregnant women enrolled in the trial are summarised in table 2. Women's mean age was 24.6 years (SD 5.5 years) in the benzalkonium chloride group

and 24.8 years (4.9 years) in the placebo group, which was not statistically different (p = 0.83). Mean parity did not differ between the benzalkonium chloride and placebo groups (1.8 (1.9) and 1.7 (1.8), respectively, p = 0.87). Education level and marital status were comparable in the two treatment groups. Women were also comparable for medical history and clinical HIV disease stage, according to the World Health Organisation (WHO) classification. There was a higher prevalence of vaginal discharge at entry among women receiving benzalkonium chloride, compared with the placebo group (p = 0.03). The mean CD4+ lymphocyte count tended to be higher in the placebo arm than in the benzalkonium chloride arm (534 $\times 10^6$/l, (267) versus 647 $\times 10^6$/l (320); p = 0.05) but distribution of CD4 count strata did not differ significantly between the two groups. Only 21.6% of the women overall presented with less than 350 CD4+ cells $\times 10^6$/l. Microbiological findings did not differ between the two groups at entry. Out of 54 women in each group, *Trichomonas vaginalis* infections were found in five women in the benzalkonium chloride group and six in the placebo group (p = 0.70), gonococcal infections in one in the benzalkonium chloride group and none in placebo group (p = 0.50), *Chlamydia trachomatis* in one woman in the benzalkonium chloride group and none in placebo group (p = 0.50), and fungal infections in 16 in the benzalkonium chloride and 19 in the placebo group (p = 0.53). The distribution of the lactobacilli flora did not differ between the two groups (p = 0.59).

ACCEPTABILITY

When interviewed on their history of administration of vaginal capsules, 33 out of 102 (32%) women followed until day 8 post partum had already used vaginally administered therapies to treat lower genital tract infections or discharge; they were equally distributed among the two treatment groups (p = 0.62). Among these, 12 women found the study capsules to be easier to use than vaginal suppositories they had used previously, 19 found no difference, and two found them to be less easy to use, with a similar distribution between treatment groups.

Table 3 Incidence density rates of tolerance outcomes in women until delivery (per 100 days of antenatal treatment) and in their newborns during the first month of follow up (per 100 days of follow up) Abidjan, Côte d'Ivoire and Bobo-Dioulasso, Burkina Faso, 1996–7

	Benzalkonium chloride (n=54)		*Placebo (n=54)*		
	No	*Incidence*	*No*	*Incidence*	*p Value*
Women:					
Genital ulceration	2	0.17	6	0.45	0.12
Vaginal pruritis	6	0.52	9	0.68	0.31
Burning sensation	4	0.35	2	0.15	0.18
Pelvic pain	10	0.87	9	0.68	0.30
Pain during intercourse	1	0.08	0	0	0.23
Leucorrhoea	23	2.0	19	1.4	0.14
	(n=55)		*(n=53)*		
Liveborn infants:					
Dermatitis	10	1.8	36	2.3	0.16
Conjunctivitis	11	0.73	9	0.56	0.29

Among the 69 women out of 102 (68%) who had never used vaginal capsules before, 25 (36%) found them easy to use and 44 (64%) were without an opinion. Three women (two in the benzalkonium chloride group and one in the placebo group) found it unacceptable because of the change of smell of vaginal secretions but did not stop the treatment. One woman in the placebo group reported that her partner experienced discomfort during intercourse.

COMPLIANCE

Women used vaginal capsules during a median period of 20 days in the benzalkonium chloride group (range 0–71 days) and 21 days in the placebo group (range 1–87 days) (p = 0.33). The proportion of the expected prepartum doses actually used was 89% in the benzalkonium chloride group versus 85% in the placebo group (p=0.43). Among the 105 women (97%) who delivered in a maternity ward, intrapartum capsules were administered in 63% and 74% of women in the benzalkonium chloride and placebo groups, respectively (p = 0.16). Women who did not receive the intrapartum treatment spent a significantly shorter time in the delivery ward (median time of 15 and 20 minutes in benzalkonium chloride and placebo groups, respectively) than women who received it (2 hours and 3 hours 40 minutes, respectively).

The benzalkonium chloride/placebo bath was given to 85% (47/55) and 91% (48/53) of the liveborn infants, respectively (p = 0.41). Overall, the uptake of the intervention was strictly comparable in the two arms.

PERINATAL OUTCOMES

Three stillbirths were observed in the placebo group and one in the benzalkonium chloride group. Four of 108 women gave birth to twins, two in each group. There were two neonatal deaths in the placebo group, both due to fetal distress in a context of prolonged labour.

TOLERANCE

There was no statistically significant difference in the incidence of adverse effects between the two groups of pregnant women (table 3). The most frequent events were leucorrhoea and pelvic pain, which occurred with an incidence of 2.0 and 0.87 per 100 days of antenatal treatment in the benzalkonium chloride group, and 1.4 and 0.68 in the placebo group, respectively. The difference was not statistically significant. Two women had to stop the treatment, one in the benzalkonium chloride group because of the appearance of cervical lesions and another in the placebo group because of cervical epithelial disruption.

Incidence density rates of tolerance outcomes in children were strictly comparable in the two groups (table 3). One hundred and eight liveborns were followed for this tolerance phase during an average period of 27 days (range 0–34 days). In children, dermatitis was the most frequent adverse effect, but was equally distributed in the two groups with an incidence of 1.8 per 100 days in the benzalkonium chloride group, and 2.3 in the placebo

Table 4 Distribution of vaginal lactobacilli flora before and after administration of benzalkonium chloride or placebo vaginal suppositories in Abidjan, Côte d'Ivoire, ANRS 049b trial, 1996–7

Flora at inclusion	*II*	*III*	*IV*	*Total*
Women in the placebo group (n=17)				
Flora at day 8 after inclusion				
II	1	1	0	2
III	0	0	0	0
IV	2	2	11	15
Total	3	3	11	17
Women in BC group (n=16)				
Flora at day 8 after inclusion				
II	4	0	0	4
III	0	1	0	1
IV	5	0	6	11
Total	9	1	6	16

group; p = 0.16). It occurred later in the benzalkonium chloride group than in the placebo group (21.8 days after birth on the average versus 15.3 days, p = 0.06). Incidence and timing of conjunctivitis were not significantly different in the two groups of neonates.

CHANGES IN VAGINAL FLORA BEFORE AND AFTER USE OF THE VAGINAL CAPSULES

The vaginal lactobacilli flora were examined at enrolment and after the first week of treatment in 33 consecutive women enrolled in Abidjan. Modifications in vaginal flora are shown in table 4. The proportion of class IV flora increased in the first week of treatment in both groups. The incidence of stage IV vaginal flora after 1 week of treatment did not differ between the two groups (5/10 in the benzalkonium chloride group and 4/6 in the placebo group, p=0.22).

Discussion

This randomised placebo controlled trial shows that benzalkonium chloride vaginal capsules are well tolerated by HIV infected pregnant women in west Africa, with a daily administration during a median of 3 weeks. A neonatal bath with the same antiseptic does not appear to have adverse effects in neonates. The feasibility and acceptability of these measures were good in the population studied. However, uptake of the intrapartum component of the intervention was lower than for the prenatal component. This was not because of home deliveries which accounted only for 3% of births in our sample, but because of the short time spent in the delivery ward as previously reported in Africa.[18]

The tolerance of benzalkonium chloride capsules in pregnant women appears better for long term use than in previous reports using nonoxynol-9 in non-pregnant sex workers.[19] Tolerance is comparable to that reported in pregnant women using chlorhexidine,[9] but benzalkonium chloride has the advantage of being available in vaginal capsule form, a presentation convenient for self administration. Vaginal suppositories are commonly used in most west African countries to treat lower genital tract infections. The capsule form has the additional advantage of not melting in warm climates, and therefore not requiring any form of refrigeration for storage.

In this trial, we did not evaluate the tolerance of benzalkonium chloride in the presence of STDs, as the study protocol excluded women who presented with an untreated STD. Because genital complaints and symptoms were frequently found in our study, syndromic treatment of STDs should be emphasised in this population,[20] especially if a benzalkonium chloride disinfection is to be applied at the end of pregnancy.

It has been recently shown in Kenya that shedding of HIV during pregnancy is associated with genital discharge and immunosuppression.[21] Thus, vaginal disinfection has the potential to decrease the local viral load in the genital tract and ultimately play a role in preventing transmission of HIV from mother to child. In the clinical trial evaluating vaginal disinfection with chlorhexidine in Malawi, no decrease in mother to child HIV transmission was observed.[9] However, the intervention was restricted to a limited application of a cotton swab soaked with chlorhexidine, a treatment which may have been too short to inactivate the virus and eliminate it from the birth canal. Furthermore, the rate of vertical HIV transmission appeared significantly decreased in the chlorhexidine group among women presenting with prolonged rupture of the membranes. These findings underscore the potential relevance of repeated administrations of virucides in late gestation, in order to decrease genital tract viral load before the onset of labour or rupture of the membranes. The present phase II trial was not designed to provide an estimate of the efficacy of the intervention in preventing vertical transmission.

In many settings where HIV testing and counselling is not available as a routine procedure for pregnant women, especially in rural areas, one of the major advantages of vaginal disinfection is that it may be offered as a low cost public health intervention to all pregnant women regardless of their HIV status. Indeed, vaginal disinfection has been shown to decrease perinatal bacterial infections and mortality in Malawi[9] as has been demonstrated in Sweden.[8]

As it is well tolerated, benzalkonium chloride may now be considered for efficacy trials in the reduction of mother to child transmission of HIV, possibly in combination with other interventions, such as short course antiretroviral prophylaxis, whose efficacy has been recently been demonstrated in developing countries.[22] In addition to targeting the reduction of mother to child transmission of HIV, particularly in African countries where HIV infection is highly prevalent, such an intervention should have wider public health benefits in reducing maternal and neonatal morbidity and mortality.

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