SCIENTIFIC REPORT

A double application approach to ophthalmia neonatorum prophylaxis

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Aims: To investigate if a second drop of 2.5% povidone-iodine ophthalmic solution placed within the first postnatal day would achieve better prophylaxis against ophthalmia neonatorum than a single drop applied at birth.

Methods: A masked, prospective, controlled trial was conducted over a 2 year period in a Kenyan hospital. Randomisation was achieved by alternating weeks of one or two eye drop application to both eyes. All 719 neonates received one drop of the povidone-iodine solution to both eyes at birth, while 317 received a second drop at hospital discharge or 24 (SD 4) hours after delivery, whichever was first. All infants developing conjunctivitis within a month after birth underwent microbiological analysis using Gram and Giemsa stains, direct fluorescent antibody assay for *Chlamydia trachomatis*, and culture.

Results: Of the neonates receiving the one eye drop application, 18.4% returned with a red eye with discharge, 4.0% had organisms found on the initial smear, and 8.2% had a positive culture. The corresponding proportions for the multidrop group were 24.3%, 4.7%, and 10.4%. Of those returning with an inflamed eye, there were no cases of *Neisseria gonorrhoeae*, 4.2% in the single dose group and 3.9% in the double dose group were positive for *C trachomatis*, and 5.4% and 6.5% respectively for *Staphylococcus aureus*. At discharge, the eyelid oedema score of the double dose group was mildly greater than the single dose group (1.4 (0.67) *v* 1.2 (0.73), p=0.0002). There was no statistically significant difference between the groups in any other category.

Conclusion: There is no advantage to administering povidone-iodine prophylaxis against ophthalmia neonatorum twice in the first postnatal day over a single application at birth.

One of the greatest advances in the history of preventing blindness was the initiation of prophylaxis to prevent ophthalmia neonatorum. In the 19th century, the single greatest cause of blindness in European infants was gonorrhoeal conjunctivitis and keratitis acquired at birth. In 1881, Crede introduced the application of 2% silver nitrate ophthalmic solution to the eyes of neonates.[1] By this method, he reduced the incidence of this disease from 8% to 0.3%.

Since the time of Crede, efforts to further improve prophylaxis have focused on the use of medications other than silver nitrate. While silver nitrate has a very broad antimicrobial spectrum, there have been questions about its effectiveness against *Chlamydia*, which is now the most common cause of ophthalmia neonatorum in developed countries. In addition, toxic conjunctivitis is much more commonly seen with silver nitrate use than with other medications.[2] In many countries, other drugs used for this prophylactic purpose include erythromycin, tetracycline, and gentamicin. In 1995, we reported the use of a 2.5% povidone-iodine solution for ophthalmia neonatorum prophylaxis in more than 3000 neonates in Kenya.[3] Povidone-iodine was found to be more effective than erythromycin or silver nitrate for prophylaxis and was less toxic.

Despite this increased effectiveness of povidone-iodine, we were disturbed that the residual frequency of ophthalmia neonatorum in Kenya was still 13%. Since our definition of infectious ophthalmia neonatorum incorporated all cases that began within 1 month of birth and were culture positive, we may have included some infants who actually acquired the infection at home after delivery. We were especially suspicious of those infections caused by staphylococci. Yet, even if we excluded the staphylococci induced cases, we were still left with an incidence of about 8%.

We hypothesised that some pathogenic micro-organisms might have escaped contact with the povidone-iodine eye drop placed within minutes of birth because of the oedematous conjunctivas and eyelids commonly seen in newborns, especially in Africa. In an effort to further increase the effectiveness of the povidone-iodine prophylaxis, we considered a different strategy. We wished to determine if the incidence of ophthalmia neonatorum could be further reduced if povidone-iodine were applied to the eyes twice in the first postnatal day. A second eye drop instilled later in the day, when much of the oedema has subsided, might destroy more pathogens and further reduce the possibility of developing ophthalmia neonatorum.

PATIENTS AND METHODS

The project protocol was reviewed and approved by the human subjects committee of the Research and Education Institute at the Harbor-UCLA Medical Center and the Ministry of Health of Kenya. All babies born at the Presbyterian Church of East Africa Hospital in Kikuyu, Kenya, from January 2000 through October 2001 were candidates for the study. Infants were excluded if they had any obvious ocular malformations, the mother had received antibiotics during the last month of pregnancy, or the mother was unable to bring the infant back to the hospital in the event that conjunctivitis developed. Only babies born by a vaginal delivery were studied, since we have previously proved that the eyes of babies delivered by caesarean section are nearly always sterile.[4]

Informed verbal consent was obtained from each child's mother.[5] All infants in the study received one drop of a 2.5% povidone-iodine solution in both eyes within 20 minutes of birth, after the eyes and face had been cleaned. On alternate weeks, all enrolled babies born in that week received a second eye drop of the same solution to each eye. The second eye drop was administered at hospital discharge or 24 (SD 4) hours after delivery, whichever was first. Thus, each infant was assigned to a dosing schedule according to the week of

birth. This allocation system was easier to use in a busy maternity unit in a developing country, resulting in less confusion and possible misallocation, than a strict randomisation of infants. Data intake on all enrolled infants included birth date, birth weight (g), post-conceptual age at birth (weeks), sex, documented prenatal care, maternal vaginitis, cervicitis, or maternal illnesses and/or treatments during pregnancy, time after delivery when povidone-iodine was applied (minutes at first application, hours at second), problems with drug instillation, age at hospital discharge (hours), and eyelid swelling at time of hospital discharge (0–3, none, mild, moderate, severe). Each mother was shown pictures of inflamed eyes and instructed to return to the hospital with her infant if the child's eye began to have a discharge or became red within a month of birth. The instructions were the same regardless of the group assignment. For those infants returning with conjunctivitis, medical evaluation of their infection was made in the following categories: the number of days after birth, and after hospitalisation, in which their eye(s) became red; eyelid swelling, conjunctival redness, conjunctival swelling, and conjunctival discharge (scored 0–3, none, mild, moderate, severe). Infants returning with conjunctivitis were taken to the clinical laboratory of Nairobi Hospital for microbiological analysis of the inflamed eyes. Because the cultures were plated at the laboratory, no transport media were necessary. Conjunctival specimens obtained for detection of bacteria were inoculated immediately onto plates containing blood agar and Thayer-Martin medium. The inocula were streaked across the plates to promote isolation of the bacteria and the plates were incubated in an atmosphere of 5–10% carbon dioxide at 35℃ for 24–48 hours. Cultured organisms were identified by standard procedures. Two smears were stained with Gram and Giemsa stains, and observed for *C trachomatis* inclusion bodies and bacteria. For direct fluorescent antibody assay for *C trachomatis*, specimens were collected, fixed, stained with fluorescein conjugated monoclonal antibody (Syva Microtrak, Palo Alto, CA, USA), and observed for elementary bodies. The laboratory technicians had no knowledge of which prophylactic ocular medication dose was given to any infant.

To exclude cases of chemical conjunctivitis, we considered in the statistical analysis only those infants who returned at or after 48 hours of birth. While this approach may have excluded true cases of gonorrhoeal conjunctivitis, no cultures obtained within 48 hours of birth demonstrated *Neisseria gonorrhoeae*.

The population size necessary to attain an 80% power by a one sided test and to reduce the incidence of this disease by half (based on our previous Kenyan study) would require 286 babies per arm.[3] A one sided test was utilised since we were only interested in determining if a reduction of the bacterial flora resulted from the application of the second eye drop.

Logistic regression was used to determine the effect of the medication dosing on the risk of infection. Infants with conjunctivitis were grouped according to the results of the bacteriological culture and direct fluorescent antibody assay as having infections with *C trachomatis*, all bacteria under study, coagulase negative staphylococci, *Staphylococcus aureus*, or *N gonorrhoeae*. Infants were considered to have non-infectious conjunctivitis if no organisms were cultured. Each group was compared with each other and with the group of infants with no inflammation. The model included sex and the presence or absence of prenatal care, maternal vaginal and/or cervical infection, as covariates.

RESULTS

Of the 719 newborns studied, 351 were female. By alternation, 402 received the 2.5% povidone eye drop only at birth while 317 received a second eye drop before discharge. There was no significant difference between the groups for birth weight, post-conceptional age at birth, sex, documented prenatal care, presence of maternal vaginitis or cervicitis, or other infections or treatments during pregnancy, time after delivery when the first eye drop was applied, age at discharge, or age the subjects with inflamed eyes returned (Table 1).

Table 1 Demographic data for each group (mean (SD))

Characteristic	Single dose group	Double dose group
Birth weight (g)	3117 (401)	3153 (420)
Post-conceptional age (weeks)	39.9 (0.8)	39.9 (0.8)
Proportion male	49.8%	53.0%
Maternal prenatal care	97.5%	98.4%
Maternal vaginitis or cervicitis	5.5%	5.0%
Minutes after birth, first drop	6.1 (2.9)	6.1 (3.3)
Hours after birth, second drop	–	25.5 (3.6)
Age at discharge (hours)	25.1 (8.7)	25.8 (6.4)
Age at return (days)*	3.5 (2.3)	3.4 (1.9)
Total	402	317

*Subjects returned only if a parent determined that an eye became red and/or had discharge ⩾48 hours of age.

Of infants in the single dose arm, 18.4% were returned to the centre by a parent (from 48 hours to 1 month after birth) who felt the eye was either red, had a discharge, or both. In the double dose group, 24.3% did so. At the initial bacterial smear using Gram stain, there was no significant difference between the groups either in types of micro-organisms seen or in the overall frequency of a positive smear (Table 2). Similarly, the bacterial culture results showed no difference between the groups whether for specific organisms or for the overall rate of positive cultures (Table 3). The frequency of positive immunofluorescence for *C trachomatis* in each group was also similar. The overall infection rate in this study was 2.1% of all neonates.

At the time of discharge, the eyelid oedema score of the double dose group was mildly, but significantly greater than the single dose group (scores 1.4.(67) *v* 1.2.(73), p = 0.0002). There were no differences between the two groups for other indicators of inflammation (conjunctival redness, swelling, and discharge).

Despite adequate statistical power, there was no significant difference for microbial growth between the groups for any comparison.

DISCUSSION

In certain regions of the world, such as East Africa and South East Asia, ophthalmia neonatorum remains a major problem. Many hospitals in those areas are now using 2.5% povidone-iodine ophthalmic solution for prophylaxis based on the

Table 2 Findings of initial smear (number of returning patients after 48 hours of age (%))

Gram stain results	Single dose group	Double dose group
Gram positive cocci	14 (18.9)	11 (14.3)
Gram positive diplococci	1 (1.3)	0
Gram negative bacilli	1 (1.3)	2 (2.6)
Gram negative cocci	0	1 (1.3)
Gram negative diplococci	0	1 (1.3)
Gram positive cocci and yeast	0	0
Yeast	0	0
No organisms seen	58 (78.4)	62 (80.5)
Total	74 (100)	77 (100)

Table 3 Results of bacterial culture and chlamydia immunofluorescence tests (number of returning patients after 48 hours of age (%))

Results	Single dose group	Double dose group
Enterobacter cloacae	0	0
Escherichia coli	0	1(1.3)
Staphylococcus aureus	4 (5.4)	5 (6.5)
Pneumococcus	1(1.4)	0
Citrobacter kojeri	0	1 (1.3)
Pantoea agglomerans	0	1 (1.3)
Candida spp.	0	0
Methicillin resistant *Staph aureus*	1(1.4)	0
Total with growth	6 (8.1)	8 (10.4)
Chlamydia positive	3 (4.1)	3 (3.9)
No growth	68 (91.9)	69 (89.6)

report of our first Kenyan trial.[3] In that study, povidone-iodine was significantly more effective than erythromycin or silver nitrate for prophylaxis and was less toxic. However, the group that received the povidone-iodine solution still had an infection rate that far exceeds the incidence seen in industrialised countries–13% (8% if staphylococci, possibly acquired postnatally, are excluded). We wondered if a second eye drop placed around the time of discharge or at 24 hours after birth would further reduce the infection rate. That effect was not realised in this study, despite adequate statistical power.

How can an infection develop despite the use of povidone-iodine prophylaxis? One possibility is insensitivity of the micro-organism to povidone-iodine. However, at least in vitro, essentially all micro-organisms are susceptible to povidone-iodine. This sensitivity includes *Chlamydia*, *Neisseria gonorrhoea*, and *Staphylococcus* species.[6] Another possibility is that the 2.5% concentration of the solution used in this study was too dilute; yet povidone-iodine has been shown effective at a concentration as low as 0.1%.[7] Additionally, the 2.5% concentration was certainly effective in our first Kenyan study.[3] A third possibility is that there may have been insufficient contact time or no contact at all. It is possible that the conjunctival crypts may have protected some micro-organisms from contact with the povidone-iodine molecules, and thus permitted a subsequent infection. Indeed, many newborns, especially in Africa, have a very oedematous conjunctiva which may aid in that protection. Since the oedema largely resolves by 24 hours, we hoped that a second eye drop administered at that time would enhance the effect. Our data, however, show that this second drop did not augment the prophylaxis. On the negative side, the additional povidone-iodine eye drop did cause a mild increase in eyelid oedema ($p = 0.0002$).

Since supplementary eye drops of povidone-iodine did not provide additional benefit, other approaches can be explored. One possibility is to place two eye drops at birth—povidone-iodine and another effective medication. This strategy, however, would be more expensive than using povidone-iodine alone. Another possibility is to use a different form of povidone-iodine, such as a gel or an ointment to increase contact time.

We found that of those undergoing culture and *Chlamydia* testing, 93.2% of the single dose and 94.8% of the double dose group were negative. Since these infants developed inflammatory signs after 48 post natal hours and generally before 7 postnatal days, the timing would be a bit late for most cases of toxic conjunctivitis and too early for most cases of viral conjunctivitis. We believe that a number of these cases may have been brought in by parents perhaps overconcerned by an uninfected but irritated eye.

It is interesting to note that the overall infection rate in this study (2.1%) is far less than our previous study in Kenya (13.1%) using the same concentration of povidone-iodine.[3] A small part of this difference may be attributed to our excluding babies returning within 48 hours of birth in this study, but not in the first Kenyan study. This approach was adopted to minimise the inclusion of cases of chemical conjunctivitis. However, the nearly 1% infection rate of *Chlamydia* found in this study even after povidone-iodine prophylaxis is still of concern. Yet, these data are encouraging given the potentially blinding effects of ophthalmia neonatorum, especially gonococcal. This study found no cases of *Neisseria* induced ophthalmia neonatorum in over 700 births in Kenya following povidone-iodine prophylaxis. Previous studies in Kenya have found an incidence of gonococcal conjunctivitis as high as 6.4% when prophylaxis was not used.[8] Indeed, our own Kenyan data from 1995 found a 0.8% incidence of gonorrhoeal conjunctivitis even following povidone-iodine prophylaxis. This lack of infection, especially when considering that our microbiological techniques were very similar to that used in our previous Kenyan study, suggests a decrease in the overall frequency of gonorrhoea in Kenya. We feel that the decrease is due to the increased availability of prenatal care at which time maternal infections would be treated. In 1995, only 13% of the mothers had received prenatal care.[3] Babies born to mothers with vaginitis had a relative risk of developing ophthalmia neonatorum in general and gonococcal ophthalmia, in particular of 5.1 and 24.9 times that of the other infants, respectively ($p<0.002$).[9] By 2001, the proportion of mothers receiving prenatal care had increased to about 98% in the same hospital (Table 1). While prophylaxis against ophthalmia neonatorum should continue, this investigation strongly suggests that prenatal care should be vigorously promoted in developing countries.

Our data should be considered in light of some limitations of the study. Alternation was chosen as the method to allocate patients to each treatment arm. We felt that an alternation system would result in less confusion and possible misallocation in a busy maternity unit in a developing country than a strict randomisation of infants. Confounding with this method of assignment would be minimal since the groups were similar regarding demographic variables and microbiological results. Another potential issue is that we cannot be sure that each child who developed a red eye with discharge returned to the study centre. There is no reason to consider, however, that children in either group would be more likely to return.

In conclusion, this study supports the original notion of Crede in 1881 that a single drop of an effective medication given at birth is the best way to prevent the development of ophthalmia neonatorum. A second eye drop of povidone-iodine solution given approximately 24 hours later in this study was of no further benefit.

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