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Original Article

Formulation and Clinical Evaluation of Povidone-Iodine Ophthalmic Drop

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Abstract

Ophthalmia neonatorum is generally defined as conjunctivitis occurring within one month of life. The sources of this infection are environmental organisms or the organisms colonized in the birth canal. Untreated infection can cause blindness, especially if the corresponding organisms are *Neisseria gonorrhoeae*, or *Chlamydia trachomatis.*

Povidone-iodine ophthalmic solution is an effective antibacterial agent with broad antibacterial and antiviral activity to which no bacterial resistance has been known. It is less expensive and less toxic than the agents currently used to prevent neonatal conjunctivitis. It turns the conjunctiva brown for a few minutes, a characteristic that can serve as an indicator of being properly applied. Because this preparation is not available in Iran, its formulation can be valuable.

In this study, the povidone-iodine ophthalmic solution was prepared in concentration of 2.5%, and then required control parameters such as pH, self-preservation effect, tonicity, sterility, and chemical stability were studied. In this clinical study, one drop of povidone-iodine ophthalmic solution was instilled in each eye of 475 neonates within 30 minutes of birth.

This study demonstrated that povidone-iodine in concentration of 2.5% is self-preservative against microbial contamination. The pH of solution was adjusted about 5 near to the pH of tear using sodium hydroxide 0.1 N and citric acid 0.5%, because in this pH povidone-iodine was more stable. Tonicity was measured according to an in vitro hemolytic method. Povidone-iodine 2.5% solution was packaged in amber color bottles, and after ensuring from its sterility, it was used in clinical study. Among the population studied, eye discharge was observed in 2.94 percent in comparison to the control group in which eye discharge was observed in 10.9 percent.

In conclusion because of availability, low cast, and good clinical results, a 2.5% ophthalmic solution of povidone-iodine is desirable to use as a prophylactic agent against ophthalmia neonatorum.

Keywords: Ophthalmia neonatorum; Povidone-iodine; Ophthalmic solution; Conjunctivitis.

Introduction

Conjunctivitis in the first month of life is known as ophthalmia neonatorum. Untreated infection of Ophthalmia neonatorum can cause blindness, especially if the offending organisms are *Neisseria gonorrhoeae* (1). Perhaps the greatest advances toward preventing blindness in neonate were accomplished in 1881. Karl S.F. Crede applied a 2% solution of silver nitrate to the eyes of neonates and reduced the incidence of ophthalmia neonatorum from 10% to 0.3% (2).

An ideal agent must be broad spectrum and cause less resistance to microorganisms, induce less toxic reaction, be easily administered and available at low cost, and be applicable worldwide. The choice of agent to prevent

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ophthalmia neonatorum has been controversial. Silver nitrate ophthalmic solution concentration was reduced to 1% in order to decrease the frequency of toxic conjunctivitis and was remained popular. However, as a result of a perceived ineffectiveness of silver nitrate against *Chlamydia* and other organisms and its tendency to cause toxic conjunctivitis, other medications such as erythromycin 0.5% and tetracycline 1.0% ointments, have been widely accepted, although yet not perfectly suitable (2).

Povidone-iodine ophthalmic solution in dose of one drop three times a day has been administered in the first postoperative week. It controls the increase in conjunctival bacterial colony-forming units (3).

Unlike the other agents currently used for prophylaxis against ophthalmia neonatorum, povidone-iodine is also effective against microorganisms other than bacteria. Povidone-iodine has been found to be potent against fungi in concentrations as low as 0.1% (4). Povidone-iodine is effective against *Neisseria gonorrhoeae,* in concentrations as low as 1%, it is effective against *Chlamydia trachomatis*, and in concentration of 0.5% or less, its antiviral spectrum includes the human immuno-deficiency virus and herpes simplex virus (1). According to properties of povidone-iodine, it is suitable for the prophylaxis and treatment of ophthalmia neonatorum. In their pilot study, Isenberg and his coworkers have shown that a 2.5% solution of povidone-iodine was not irritating to the sensitive eyes of neonates, whereas a 5.0% solution that they had used in previous studies occasionally produced some conjunctival hyperemia (1).

In contrast to silver nitrate and erythromycin, povidone-iodine has not been associated with true bacterial resistance. Povidone-iodine is highly effective against the bacteria found in the eye at the birth. Although silver nitrate, erythromycin and povidone-iodine reduce the number of colony forming units, povidone-iodine achieves the best level of statistical significance and erythromycin was shown to be the worst (2).

In Isenberg clinical study thyroid disorders did not develop in any of more than 3000 newborns that received a 2.5% ophthalmic solution of povidone-iodine (4).

Experimental

Materials

Povidone-iodine powder was obtained from Tolid Daru Pharm. Co., Iran. Citric acid, sodium chloride, sodium hydroxide, sodium thiosulfate, iodine, potassium iodide, Soybean-casein digest medium and Nutrient agar medium were from Merck, Germany. Sabouraud dextrose agar medium, Soybean-casein digest agar medium and Fluid thioglycolate medium were from Oxoid, UK.

Methods

For the preparation of povidone-iodine 2.5% solution, 2.5 g of povidone-iodine was dissolved in sterile distilled water and after adjusting of the pH, volume of solution was corrected up to 100 ml.

For adjusting of the pH, 5-11 ml of sodium hydroxide 0.1 N and 0.2 ml of citric acid 0.5% was added to povidone-iodine solution.

Antimicrobial preservative effectiveness test was performed according to USP 24 with following microorganisms: *Candida albicans* (ATCC No.10231), *Aspergillus niger* (ATCC No.16404), *Escherichia coli* (ATCC No. 8739), *Pseudomonas aeruginosa* (ATCC No. 9027), and *Staphylococcus aureus* (ATCC No.6538). Soybean-casein digest was used as culture medium.

Tonicity of the preparation was measured according to Reed and Yalkowsky hemolytic method in that the initial mixture of RBCs and ghosts was washed with normal saline in order to remove hemoglobin and interfering substances. The intact RBCs remaining after washing with normal saline were lysed with water and centrifuged. The supernatant layer was evaluated spectrophotometrically at 540 nm (6).

The Sterility test was carried out according to USP 24. *Bacillus subtilis* (ATCC No. 6633) and *Candida albicans* (ATCC No. 10231) were used as test microorganisms. Fluid thioglycollate medium and soybean-casein digest medium was used as culture media. Sodium thiosulfate was used to neutralize antimicrobial effect of povidone-iodine (9 ml sodium thiosulfate neutralized 10 ml povidone-iodine 2.5% solution with pH 5).

After ensuring the primary sterility test (sterility of media, growth promotion test, and inactiveness of antimicrobial effect) direct transfer sterility test was preformed according to USP 24.

The buffering capacity of povidone-iodine 2.5% solution was measured according to Van Slyke method (7) using sodium hydroxide and evaluation of pH as function of NaOH equivalent gram.

For the stability study, this preparation was packaged in plastic and amber glass containers, which were stored at room temperature. Samples were collected immediately and after 14, 30, 60 and 90 days after preparing of the solution. 20 ml of preparation was transferred to a beaker, and water was then added up to 30 ml. Each sample was immediately titrated with sodium thiosulfate 0.02 N solution. Starch paste was used as an indicator to distinguish the end point.

Clinical study of this preparation was performed in Oct. 2000 at Hafez Maternity Hospital in Shiraz. During this study all infants received one-drop of solution after cleaning the eyes and face. The drop was instilled within the first 30 minutes after delivery. Infants born at private hospital and did not receive any prophylactic agent, were considered as a control group.

Results

In antimicrobial preservative effectiveness study, solutions were observed at 7, 14, 21, and 28 days after incubation. No changes in appearance of tubes and no colony growth in each plate were observed. Thus no additional preservative is necessary to preserve the 2.5% povidone-iodine solution and it is a self-preservative product.

Results of tonicity study showed that 2.5% povidone-iodine solution according to Reed and Yalkowsky method was isotonic (Table 1 and Table 2).

Table 1. Absorbance of hemoglobin due to lysis of RBCs in variable ratios of blood with povidone-iodine solution and normal saline.

	Blood: Betadine			Blood: normal saline		
Ratio of mixture	1:1	2:1	3:1	1:1	2:1	3:1
sample 1	0.008	0.013	0.028	0.005	0.012	0.026
sample 2	0.010	0.069	0.106	0.015	0.076	0.011
sample 3	0.04	0.032	0.051	0.033	0.041	0.068
sample 4	0.002	0.024	0.075	0.005	0.033	0.089
Mean	0.015	0.034	0.065	0.014	0.0405	0.073
SD	0.017	.024	.033	0.019	0.027	0.036

Table 2. Statistical analysis of Reed and Yalkowsky test.

	Ratio of blood: povidone-iodine solution		
	1:1	2:1	3:1
Calculated Z	0. 46474	0.33282	0.33673

Maximum acceptable Z=1.959961

Sterility test depicted that this product is sterile, since no bacterial or fungal contamination was observed.

Buffer capacity of 2.5%povidone-iodine solution was 1.02×10^{-3}.

The stability data showed that povidone-iodine solution was stable for 97 and 364 days, in plastic and amber containers, respectively.

Clinical study (Table 3) demonstrated that among 475 neonate, after instillation of povidone-iodine solution, secretion was still observed in 14(2.94%) neonates among those 6 neonates (1.26%) had negative cultures and 8(1.68%) had positive cultures. Table 3 shows the distribution and percentages of infants with infectious and noninfectious conjunctivitis in the treatment group. In the control group, among 385 neonates who did not receive any prophylactic agents, secretion was observed in 42(10.90%) neonates, 9(2.33%) had negative and 33(8.57%) had positive cultures. Table 4 shows distribution and percentages of infants with infectious and noninfectious conjunctivitis in the control group.

Table 3: Distribution of conjunctivitis types in treatment group.

Cause of conjunctivitis	Frequency (Percent)
Staphylococcus epidermis	4 (0.84)
Staphylococcus aureus	3 (0.63)
Pseudomonas	1 (0.21)
Noninfectious conjunctivitis	6 (1.26)

Table 4: Distribution of conjunctivitis types in control group

Cause of conjunctivitis	Frequency (Percent)
Staphylococcus epidermis	15 (3.89)
Staphylococcus aureus	14 (3.63)
Enterobacter	2 (0.52)
Escherichia coli	2 (0.52)
Noninfectious conjunctivitis	9 (2.33)

Conclusion

Povidone-iodine ophthalmic solution is an effective antibacterial agent with broad antibacterial and antiviral spectrum and no bacteria resistance. It is less expensive and less toxic than the agents currently used to prevent neonatal conjunctivitis. Povidone-iodine turn's surface of the eye brown for a few minutes, a

characteristic that can serve as an indicator that it has been properly applied.

An ideal ophthalmic preparation should be formulated at a pH near the tear fluid pH value in which optimum stability is also achieved (8). For maximum stability, the pH of this product was adjusted to 5. In general, it is accepted that a low pH per se will not cause stinging or discomfort on instillation. When the overall pH of the tears, after instillation, change rapidly to pH 7.4, discomfort can be minimum. On the other hand, if the buffer capacity is sufficiently low, after instillation of product to eye, the pH can be adjusted tear and reached to optimum value (8). This product has a low buffer (1.02×10^{-3}) capacity that is appropriate to change immediately after mixing with tear fluid.

The results showed that povidone iodine 2.5% ophthalmic solution does not require an additional preservative.

An ophthalmic solution is considered isotonic when its tonicity equals to the tonicity of 0.9% sodium chloride solution. The eyes usually can tolerate different tonicities equivalent to 0.5% to 1.8% sodium chloride (8). Based on the results from tonicity study there was no significant difference between povidone-iodine 2.5% solution and normal saline, therefore, povidone-iodine 2.5% solution is isotonic.

According to the result of stability test, plastic container is not suitable for packaging and storage of povidone-iodine ophthalmic solution. According to USP24 and accepted limits for povidone-iodine solution, stability of product in amber glass container was calculated 35 months and in plastic container was evaluated 9 months.

The comparison of control group, with the experimental groups, showed significant difference between these groups (p 0.0001). Clinical study demonstrated that 2.5% povidone-iodine is a very useful for the prophylaxis of ophthalmia neonatorum. Organisms that were found in the neonate eyes after administration of povidone-iodine 2.5% solution were *Staphylococcus epidermis*, *Staphylococcus aureus* and *Pseudomonas* which were environmental organisms, that entered the neonates eyes after instillation of povidone-iodine drop.

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