In-vitro Susceptibility of Infective Keratitis Causative Bacteria to Topical Povidone Iodine

Abstract

Purpose: To evaluate the in-vitro susceptibility of bacterial isolates from the cases of corneal ulcer to povidone iodine.

Setting: Department of ophthalmology, and clinical microbiology research center, Shiraz University of Medical Sciences, Shiraz, Iran.

Patients and Methods: The in-vitro susceptibility and Minimum Inhibitory Concentration (MIC) of the bacteria isolated from the cornea of 50 cases of bacterial corneal ulcer to povidone iodine was determined by broth dilution method. Concentrations of 1%, 2%, 5% and 10% of povidone iodine were tested.

Results: Bacterial isolates included 50% S. epidermidis, 20% P. aerogenosa, 10% S. aureus, 4% Acinetobacter, 4% S. pneumoniae, 4% E-coli, 2% Proteous, 2% S. viridance, 2% Diphtheriod and 2% Bacillus. Povidone iodine bactericidal efficacy for gram positive bacteria was at least 82.36%, 91.2%, 100% and 100% in 1%, 2%, 5% and 10% concentrations after 5 minutes exposure time. The bactericidal efficacy of this agent over the gram negative bacteria was at least 62.5%, 87.5%, 100% and 100% in 1%, 2%, 5% and 10% concentrations after 5 minutes exposure time, respectively.

Conclusion: Povidone iodine was shown to have a broad spectrum in-vitro bactericidal efficacy in cases of bacterial keratitis. If further in-vivo studies confirm the efficacy of this agent over the bacterial keratitis, it would be suggested as a broad spectrum medication for the treatment of these cases.

Keywords: MIC, Minimum Inhibitory Concentration

Introduction

Bacterial keratitis is a serious ocular emergency. Numerous microorganisms on the lid margin and in the normal uninfected conjunctival fornices provide a source of potentially pathogenic bacteria to the cornea. Without proper treatment it can impair vision or progress to the loss of the eye.

The increasing number of the corneal surgeries and widespread use of cosmetic contact lenses are accompanied by the increasing rate of the infective keratitis. Accurate determination of the infective microorganisms as well as the choice of an effective agent for treatment are important responsibilities of the ophthalmologist. The therapeutic plan includes rapid eradication of the viable bacteria from the cornea and the suppression of the inflammatory response, by initiation a specific antibacterial agent. Attempts to treat these infections by antibiotics might not be uniformly successful. Resistant bacterial isolates to the antibiotics have been found and many of the new types of the antibiotics may not be available especially in the
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developing countries. Povidone iodine has been proved to be an effective agent in prophylaxis of the postoperative endophthalmitis and treatment of the neonatal conjunctivitis. In addition, in vitro studies have shown potent fungicidal efficacy of this agent over the fungi isolated from the fungal corneal ulcers. This study was designed to evaluate the bactericidal efficacy of various concentrations of this agent over the bacteria isolated from the bacterial keratitis cases in Khalili Hospital Shiraz University, a major referral center in the south of Iran.

Patients and methods
During a period between June 2001 and March 2002, 50 cases of culture proven bacterial keratitis who were referred to Khalili Hospital emergency service were enrolled in this study. The patients’ complete history and ocular physical examination including the size, shape, depth and drawing of the ulcer were recorded. Under sterile condition after the instillation of one drop of tetracain 0.5% (Sina Darou Co.), and without using any periocular disinfectant, corneal scraping was performed for each patient. The scraping was performed by No. 15 Bard Parker blade from the depth and active edges of the ulcer.

The specimen was directly transferred to culture plates including blood agar, chocolate agar and sabouraud’s dextrose agar and incubated in 25°C for 48 hours. Direct smears for gram and, giemsa stains from the specimens were also prepared. After identification of the bacterial isolates by the routine microbiology methods in vitro susceptibility was checked by MIC method for the 1%, 2%, 5% and 10% concentrations of the povidone iodine. We used and compared two types of povidone iodines made in Iran and UK.

MIC method: Different concentrations (1%, 2%, 5%, 10%) of each type of povidone iodine were added to tubes containing TBS (Trypsinase Soya broth) media. Then colonies of each type of bacteria were transferred to each tube. For each type of bacteria 10 tubes were determined: eight tubes containing 1%, 2%, 5% and 10% concentrations of each Iran or UK made povidone iodines, one control negative containing only povidone iodine without bacteria and one control positive containing bacteria without povidone iodine. After exposure time of 5 minutes and one hour, a sample from each tube was taken and transferred over the culture plate and incubated in 37°C for 24 hours. The least concentration of the povidone iodine which could inhibit the growth of the bacteria is known as MIC.

Results
Total of 50 cases of culture proven bacterial keratitis were reviewed. 31 patients were male and 19 female. The age range was 15-80 years (mean 56.08 years). Thirty Three Percent of them had previous history of systemic and/or topical antibiotic consumption prior to referral. The size of the ulcer varied from 1 × 2 to 10 × 10 mm. Extension to the limbus was seen in 4 cases (8%). Hypopyon was seen in 19 cases (38%) and melting of the cornea in 14 cases (28%). Regarding the predisposing factors, 47% of cases had some degree of blepharitis and 41% dry eye, 12% trichiasis, 6% entropion or ectropion, 22% history of recent ocular surgery (cataract surgery, PKP, pterygium) or wearing contact lens and 4% diabetes mellitus. The most common bacterial isolate was S. epidermidis. The frequency of different bacterial isolates is summarized in Figure 1.

![Figure 1: Distribution of Bacterial Isolates in 50 Cases of Bacterial Keratitis](image-url)

The Results of susceptibility test after 5 and 60 minutes for both UK and Iran made povidone iodine solutions in various dilutions of 1.2.5 and 10 percent are summarized in Tables 1-4. These Tables show the percentage of the tubes which became culture negative (bactericidal efficacy) with each
concentration at two different exposure time.

**Table-1: Bactericidal Efficacy of UK Made Povidone Iodine Over the Gram Positive Bacteria.**

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<thead>
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<th>Concentration</th>
<th>Exposure time:</th>
<th>5 Minutes</th>
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<tr>
<td>1%</td>
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<td>82.36%</td>
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<td>2%</td>
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**Table-2: Bactericidal Efficacy of Iran Made Povidone Iodine Over the Gram Positive Bacteria.**

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<th>Concentration</th>
<th>Exposure time:</th>
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**Table-3: Bactericidal Efficacy of UK Made Povidone Iodine Over the Gram Negative Bacteria.**

<table>
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<th>Concentration</th>
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<tbody>
<tr>
<td>1%</td>
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<td>62.5%</td>
<td>87.5%</td>
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<tr>
<td>2%</td>
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<td>87.5%</td>
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**Table-4: Bactericidal Efficacy of Iran Made Povidone Iodine Over the Gram Negative Bacteria.**

<table>
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<th>Concentration</th>
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<th>1 Hour</th>
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<tr>
<td>1%</td>
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<td>62.5%</td>
<td>93.7%</td>
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The results showed that povidone iodine had a broad-spectrum bactericidal activity and no statistical difference was seen between the two brands of povidone iodine solutions. The difference between 1% and 5% of povidone iodine was statistically significant (P<0.05 Statistical Analysis was done using Fisher’s exact test).

**Discussion**

The external eye and the surrounding tissues harbor bacteria throughout the life. The organisms isolated from the conjunctival sac usually are similar to those found on periorcular skin. Less frequently, normal bacterial flora of the gastrointestinal tract are isolated from the conjunctival sac of the healthy eye. Infrequently, streptococci and a number of anaerobic bacteria invade the conjunctiva. In case of decreased local or systemic immunity or damaged corneal epithelium, the inhabitant bacteria of the lid margin or the conjunctiva may cause infective keratitis. The most frequent bacteria isolated in our cases of bacterial keratitis were *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*. This result is similar to the reports from many other studies. Recently, multi resistant species of the staph coagulase negative have been reported. There is also an increasing number of reports about the resistance of the *Pseudomonas aeruginosa* isolates of the infective keratitis to the broad spectrum antibiotics such as ciprofloxacin and ceftazidime. Streptococci and non-aerogenic pseudomonas are the most resistant isolates of the bacterial keratitis to the ciprofloxacin and ceftazidime which are the most effective antibiotics used in the treatment of corneal ulcer. The use of antibiotics in the treatment of infections of the external eye is appealing. The universal use of antibiotics could be specially justified in the developing countries, where the cost and availability of the antibiotics limit their use. Povidone iodine would seem to be an ideal agent for such cases. It is indicated for preoperative disinfection of the preocular region and irrigation of the ocular surface and has been reported significantly to reduce the incidence of endophthalmitis after intraocular surgery. It is a combination of povidone (polyvinyl pyrrolidone) and iodine. Povidone is a water soluble polymer of high colloidal and osmotic pressure which acts as a surface agent and readily combines and leaves the remaining 70%-90% of free iodine that is available for the antimicrobial activity. It is a broad spectrum microbicidal with efficacy against bacteria, viruses
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and fungi. There has been reports of the use of this agent for treatment of corneal ulcer in human eye. Hale used it in conjunction with antibiotic treatment in four cases of pseudomonas corneal ulcer. Kadam applied povidone iodine directly onto the corneal ulcers in 35 patients, 30 of whom being bacterial. Schuerman and Vidic described favorable results in an uncontrolled trial of 40 cases of conjunctivitis or keratoconjunctivitis. Bacterial culture was positive in 28 patients on the onset of the disease, but only three remained positive after 3 days of treatment with topical povidone iodine solution. The patients well tolerated the drop. The corneal toxicity and epithelial healing of the rabbit eyes with povidone iodine was studied by York et al.

Concentration of 5% of povidone iodine showed only slightly more irritation than gentamicin. The rate of healing of cornea epithelium one day less in the model with the use of povidone iodine. Michalova et al. investigated the effect of povidone iodine on the rabbit p.aerogena keratitis, by applying hourly topical drops of povidone iodine. The clinical appearance and microbiological quantification did not show therapeutic efficacy. In our study we used the broth dilution method to determine the bactericidal effect of povidone iodine. It is the most reliable method to determine the bactericidal sensitivity test. Povidone iodine showed acceptable efficacy against bacterial isolates of the corneal ulcer in our patients. Our results showed 100% bactericidal efficacy with effect usage of 5% concentrations of povidone iodine and 62.5% to 100% efficacy in using 1% and 2% concentrations against both gram positive and gram negative bacteria. The bactericidal efficacy of 5% and 10% povidone iodine was even higher than antibiotics in our study. However, the bactericidal efficacy of povidone iodine was not statistically different from fluoroquinolines.

There are also other factors which influence the results of the treatment of the infective keratitis, including the time that the drug remains in contact with cornea, the penetration and bioavailability of the drug. It is also influenced by the virulence of the causative microorganism. The results of this study and the previous related reports provide a promising background for in vivo investigations regarding the therapeutic efficacy of the povidone iodine both in the animal models of the bacterial keratitis and in clinical practice.

Acknowledgment
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