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Evaluation of povidone-iodine as a disinfectant solution for contact lenses: Antimicrobial activity and cytotoxicity for corneal epithelial cells

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Abstract

Povidone-iodine (PVP-I) possesses broad-spectrum antimicrobial activity and is used clinically as a disinfectant. We evaluated the disinfectant properties and safety of PVP-I for use as a contact lens solution. The concentrations of PVP-I required to reduce the number of *Staphylococcus aureus* or *Candida albicans* by 3 log units were lower than were those of hydrogen peroxide, polyhexamethylene biguanide (PHMB), and benzalkonium chloride (BAK). The cytotoxicity of PVP-I for cultured human corneal epithelial (HCE) cells was less than that of the other three agents. The safety margin for PVP-I was thus greatest among the tested compounds. PVP-I appears suited for use as a contact lens disinfectant.

Keywords: Soft contact lens; Disinfection; Povidone-iodine; Hydrogen peroxide; Multipurpose solution; Safety margin

1. Introduction

Cleaning and disinfection of contact lenses are essential for their safe wearing [1]. Disinfection is especially important for soft contact lenses in order to avoid lens-related infection of the cornea [2–5]. The incidence of microbial keratitis induced by wearing of contact lenses is 3.5–14.0 cases per 10,000 individuals for daily-wear hydrogel lenses and 20.0–144.6 cases per 10,000 individuals for extended-wear hydrogel lenses [6–8]. Although the frequency of this condition is relatively low, it is sight threatening and can be avoided by proper lens handling and management [9]. The heat-based system was the first method approved for disinfection of soft contact lenses and has remained the most efficient [10,11]. This system has disadvantages, however, including its induction both of lens deformation and of deposition of denatured proteins [12]. To avoid such problems associated with heat, a chemical disinfection system based on hydrogen peroxide was introduced [13]. Although hydrogen peroxide is effective against a broad range of microbes, neutralization is required to avoid serious insults to the ocular surface. This neutralization step is a disadvantage in terms of compliance [14,15]. Multipurpose disinfectant solutions, which contain cleaning, rinsing, and disinfectant agents in a single solution, were developed to resolve such problems. Currently, multipurpose disinfectant solutions are used widely, but the antimicrobial activity of these solutions is not as great as that of heat- or hydrogen peroxide-based disinfection systems [16–18]. The development of a new disinfection system with an increased and broader spectrum antimicrobial activity, with a reduced impact on contact lens materials, and with a low cytotoxicity at the ocular surface is thus desirable to ensure the comfort and safety of soft contact lenses.

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Povidone-iodine (PVP-I) is an iodinated polyvinyl polymer that has been used as a topical antiseptic to prevent infections of the skin or mucous membranes during surgery [19,20]. PVP-I possesses broad-spectrum antimicrobial activity against bacteria, yeasts, molds, other fungi, *Acanthamoeba*, and certain viruses [21–24], but it has a low toxicity to human cells and tissues [25]. On the basis of its low toxicity at the ocular surface, a 5% solution of PVP-I has been applied as an antiseptic before intraocular surgery and has been shown to markedly reduce the abundance of the conjunctival bacterial flora [26–29]. No toxic effects of such irrigation on the ocular surface were observed.

To evaluate the efficacy of PVP-I as a contact lens disinfectant, we have now examined both its antimicrobial activity against the bacterium *Staphylococcus aureus* and the fungus *Candida albicans* as well as its cytotoxicity toward cultured human corneal epithelial (HCE) cells. PVP-I was compared in these tests with hydrogen peroxide and the major chemical components of multipurpose solutions, polyhexamethylene biguanide (PHMB) and benzalkonium chloride (BAK).

2. Methods

2.1. Growth of microorganisms

S. aureus (Institute for Fermentation at Osaka (IFO) 13276) was grown on soybean-casein digest agar (Nihon Pharmaceutical, Kanagawa, Japan) at 35 °C for 18–24 h, and *C. albicans* (IFO1594) was cultured on Sabouraud dextrose agar (Becton Dickinson, Franklin Lakes, NJ) at 25 °C for 42–48 h. The microbial cells were harvested with Dulbecco's phosphate-buffered saline (PBS), and the cell suspension was transferred to a sterile syringe attached to a sterile absorbent cotton pad and was centrifuged at 2000 rpm for 10 min at 25 °C. The pellet was resuspended in PBS, and the optical density at 660 nm of the resulting suspension was measured with a single-beam spectrophotometer (U-2000A; Hitachi, Tokyo, Japan). The final inoculum was adjusted to a density of 1×10^7 to 1×10^8 colony-forming units (CFU) per milliliter.

2.2. Culture of a human corneal epithelial cell line

An HCE cell line that had been transformed with a simian virus 40-adenovirus recombinant vector was kindly provided by Araki-Sasaki et al. [30]. The cells were cultured in supplemented hormone epithelial medium (SHEM), consisting of Dulbecco's modified Eagle's medium–Ham's F12 (50:50, v/v) (Invitrogen, Carlsbad, CA) supplemented with 15% heat-inactivated fetal bovine serum (Invitrogen), human recombinant insulin (5 μg/ml) (Nacalai Tesque, Kyoto, Japan), cholera toxin (0.1 μg/ml) (Sigma–Aldrich, St. Louis, MO), human recombinant epidermal growth factor (10 ng/ml) (Sigma–Aldrich), 0.5% dimethyl sulfoxide (Katayama Chemical Industries, Tokyo, Japan), and gentamicin (40 μg/ml).

2.3. Measurement of antimicrobial activity

PVP-I (BASF Japan, Tokyo, Japan), hydrogen peroxide (Nipponperoxide, Kanagawa, Japan), PHMB (Sanyo Chemical Industries, Kyoto, Japan), and BAK (Tokyo Kasei Kogyo, Tokyo, Japan) were tested for antimicrobial activity against challenge organisms based on the current ISO guidelines [31,32]. PVP-I (0.05–50,000 ppm), hydrogen peroxide (3–300,000 ppm), PHMB (0.1–1000 ppm), and BAK (0.2–2000 ppm) at various concentrations in PBS (16 ml) were placed individually in test tubes, and 0.16 ml of a suspension of *S. aureus* (log[mean number of organisms ± S.D.]: 5.23 ± 0.058 for PVP-I, 5.47 ± 0.416 for hydrogen peroxide, 5.40 ± 0.300 for PHMB, 5.17 ± 0.321 for BAK) or *C. albicans* (log[mean number of organisms ± S.D.]: 5.83 ± 0.058 for PVP-I, 5.80 ± 0 for hydrogen peroxide, 5.97 ± 0.058 for PHMB, 5.87 ± 0.231 for BAK) was inoculated into the tubes at time zero. The tubes were incubated at 25 °C for 30 s–24 h; at various times, 1 ml of each solution was removed and added to 9 ml of neutralizing solution (sodium sulfite [Kishida Chemical, Osaka, Japan] for PVP-I; catalase [Nagase ChemteX, Osaka, Japan] for hydrogen peroxide; D/E Neutralizing Broth [Becton Dickinson] for PHMB and BAK) and incubated for 10 min at room temperature. Portions (1 ml) of the mixtures were removed and serially diluted with PBS, and 1 ml of each dilution was then plated on culture agar (soybean-casein digest agar for *S. aureus*; glucose-peptone digest agar for *C. albicans*) in triplicate. The cultures were incubated for 5 days at 35 °C for *S. aureus* and at 25 °C for *C. albicans*, after which the numbers of colonies were counted and used to determine the log reduction in CFU per milliliter. According to the ISO 14729 guidelines [13], a reduction in live *S. aureus* of ≥99.9% (3 log units) and a reduction in live *C. albicans* of ≥90% (1 log unit) represent significant disinfectant activity. However, we determined the concentrations of each agent required to reduce the number of live *S. aureus* and *C. albicans* by 3 log units.

2.4. Evaluation of cytotoxicity

Cytotoxicity of each disinfectant was examined by staining with neutral red, which is incorporated into the lysosomes of viable cells [33]. HCE cells were cultured under 5% CO_2 for 3 days at 37 °C in 48-well culture plates (Coster, Corning, NY) at a density of 2×10^4 cells per well in SHEM. The medium was then removed, and the subconfluent cells were washed twice with SHEM before incubation for 5 s–30 min at 37 °C in the CO_2 incubator with various concentrations of PVP-I (10–50,000 ppm), hydrogen peroxide (30–30,000 ppm), PHMB (1–10,000 ppm), or