Intraocular Pharmacokinetics of Povidone-Iodine and Its Effects on Experimental *Staphylococcus epidermidis* Endophthalmitis

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**PURPOSE.** The purpose of this study was to investigate the pharmacokinetics and safety of intravitreal povidone-iodine (PVI) and its efficacy against experimental *Staphylococcus epidermidis* endophthalmitis.

**METHODS.** In phase I, forty New Zealand white rabbits were divided into groups I and II and received intravitreal 0.1% and 0.3% PVI, respectively. Electroretinography (ERG) and histologic examinations were conducted at baseline, 6, and 12 hours. Half-life was determined using high-performance liquid chromatography. In phase II, after the induction of *S. epidermidis* endophthalmitis, 0.1% and 0.3% PVI were injected intravitreally once in groups A and B and three times every second day in groups C and D (n = 10 in each group). Electroretinographs, histologic examinations, and vitreous cultures were conducted on day 14.

**RESULTS.** Electroretinography and histologic examinations did not reveal any notable retinal damage in phase I in either group. Half-lives were 3.27 and 3.58 hours in groups I and II, respectively. In phase II, all groups demonstrated marked improvement, compared to controls. Bacterial growth was found in four eyes in group A (20, 60, 60, and 70 colony forming units [CFU]) and in three eyes in group B (20, 40, and 60 CFU) but not in those belonging to groups C and D at day 14. Retinal damage with lymphocyte infiltration in the inner retinal layers was more common in groups A and B than in groups C and D.

**CONCLUSIONS.** Half-life of PVI was approximately 3 hours in the vitreous. Repeated injection of intraocular PVI, even at low concentrations, is most likely to be effective for the treatment of bacterial endophthalmitis.

**Keywords:** endophthalmitis, povidone-iodine, *Staphylococcus epidermidis*
Intraocular Povidone-Iodine

However, the pharmacokinetics of PVI and the efficacy of repeated PVI injections have not yet been studied.

The aim of this study was to evaluate the pharmacokinetics of PVI in vitreous humor and its effect on *S. epidermidis* endophthalmitis in rabbit eyes, using single and repeated injections.

**METHODS**

**Animal Model and Experimental Design**

Eighty New Zealand white rabbits (Covance, Princeton, NJ, USA), each weighing approximately 2 kg, were used. All procedures were performed in accordance with Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The study was approved by the Pusan National University Yangsan Hospital institutional review board (certification PNUYH 2014-019/24 February 2014).

This study consisted of two phases as follows: Phase I was conducted to elucidate the pharmacokinetics and safety of PVI in the eye, whereas phase II was conducted to determine the efficacy of intravitreal PVI in the treatment of *S. epidermidis* endophthalmitis. Animals with media opacity that obscured fundoscopic examination or ocular disease were excluded at baseline.

**Phase I Study**

Forty rabbits were divided into two groups (n = 20 in each group). Group I included rabbits receiving intravitreal 0.1% PVI (0.1 mg/0.1 mL) injections, whereas group II rabbits received 0.3% PVI (0.3 mg/0.1 mL) injections. Povidone-iodine was intravitreally injected into the right eye. All eyes underwent slit-lamp and indirect fundoscopic examinations at baseline, and then 0.5, 1, 2, 6, and 12 hours after PVI injection. At each time point, three rabbits were killed and their vitreous humors were collected after examination. Povidone-iodine concentration in the vitreous humor was calculated using high-performance liquid chromatography (HPLC). Pharmacokinetic analysis of ocular drug concentration-time data was performed using noncompartmental and compartmental analyses. At baseline, 6, and 12 hours after PVI injection, electroretinography (ERG) was conducted in five rabbits in each group.

**Phase II Study**

Forty rabbits were divided into four groups (n = 10 in each group). All rabbits received 50 colony-forming units (CFU) of *S. epidermidis* intravitreally in both eyes. When signs of endophthalmitis, including conjunctival injection, chemosis, flare in the anterior chamber, and decreased red reflex, were observed, PVI was administered in the vitreous cavity after vitreous aspiration. Group A received a single injection of 0.1% PVI and group B a single injection of 0.3% PVI. Groups C and D received intravitreal 0.1% and 0.3% PVI, respectively, 3 times every second day. Fellow eyes receiving sham injection were controls. Slit lamp and fundoscopic examinations were performed daily during the first week and every other day in the second week. Electroretinography, histologic examination, and vitreous cultures for *S. epidermidis* were conducted at day 14.

**Intravitreal Injection**

The procedure was performed under sterile conditions, using an operating microscope for visualization. All injection procedures were performed as follows: Rabbits were anesthetized with intramuscular injection of 25 mg/mL tiletamine plus zolazepam (Zoletil, Virbac, France), and ventilated room air. Pupils were fully dilated with 2.5% phenylephrine and 1% cyclopentolate hydrochloride. After instillation of 0.5% proparacaine for topical anesthesia, intravitreal injections were performed using a 30-gauge needle attached to a tuberculin syringe. Anterior chamber paracentesis using a 26-gauge needle was performed prior to intravitreal injection to avoid increased intraocular pressures and to minimize vitreous reflux after injection. The needle was inserted bevel up and 2-mm posterior to the limbus; it was advanced toward the center of the vitreous until the tip could be viewed with an operating microscope. A volume of 0.1 mL was slowly injected. Arterial pulsation around the optic disk was checked using the indirect ophthalmoscope.

**Determination of Total Iodine in Eye Samples by High-Performance Liquid Chromatography**

Vitreous samples were stored at −20°C until used and were allowed to thaw to room temperature before processing. Samples (200 µL) were combined with 50 µL sodium thiosulfate solution (100 µg/mL) in each tube and then mixed by vortexing for 1 minute. Two-hundred fifty microliters of methanol were added to each sample to precipitate the protein by vortexing for 2 minutes. The mixture was centrifuged at 2500g for 10 minutes to remove any precipitated material. The supernatant was assayed by HPLC (Gilson, Middleton, WI, USA). A reverse-phase capcell-pak C$_{18}$ column (100 × 4.6 mm; pore size, 5 µm) was used. The mobile phase consisted of a mixture of methanol and water (30:70, v/v) with 6 mmol/L tetrabutylammonium bromide (TBA.Br) as the ion-pair reagent. Flow rate was set at 1.0 mL/min, and the detection wavelength was 223 nm. Curve standards were prepared by dissolving KI in mobile phase. The amount of iodide in the samples was determined from the peak area correlated with the standard curve. The chromatographic run time for each analysis was 12 minutes.

**Pharmacologic Data Analysis**

Pharmacokinetic analysis of ocular drug concentration-time data was performed using noncompartmental and compartmental analysis (WinNonlin version 3.1 software; Pharsight, Mountain View, CA, USA). Noncompartmental analysis was performed to calculate the following pharmacokinetic parameters: total area under the plasma concentration-time curve from time zero to time infinity (AUC), time-averaged total body clearance (CL), apparent volume of distribution at a steady state ($V_{ss}$), and terminal half-life ($t_{1/2}$). Compartmental analysis was performed to estimate the compartmental volume of distribution ($V_d$), maximal elimination rate ($V_{max}$), and Michaelis-Menten constant ($K_m$) by fitting the data to the following equation that describes a one-compartment model with Michaelis-Menten elimination kinetics:

$$\frac{dC}{dt} = \frac{V_{max}/V_d 	imes C}{K_m + C},$$

where $C$ and $t$ are mean PVI concentration and time, respectively.

**Electroretinography**

ERG responses were recorded using a commercial ERG system (Verice Science version 6.0 EDI; Electro-Diagnostic, Inc., Redwood City, CA, USA). Rabbits were placed in a dark room for 1 hour. Pupils were dilated by instillation of 2.5% phenylephrine and 1% cyclopentolate hydrochloride. Refer-
Figure 1. Mean concentration-time profiles of groups I (eyes receiving 0.1% PVI) and II (eyes receiving 0.3% PVI) in the vitreous of rabbits. After intravitreal PVI injection, the mean PVI concentration decreased with a nonlinear decay pattern in both groups, and then it changed to 1-exponential linear decay after 2 hours. Vertical bars represent standard deviations.

Histologic Examination

The anterior segment was removed, and a full-thickness specimen including the retina and sclera was obtained at the same distance below the optic disc. Tissues were prefixed with 2.5% glutaraldehyde (at 4°C, phosphate buffer, pH 7.2) and were postfixed with 1% osmium tetroxide in the same buffer. Materials were dehydrated with a series of graded ethyl alcohol and were embedded in epoxy resin (Epon 812 mixture). These materials were stained with 1% toluidine blue for light microscopy.

Bacterial Preparation and Quantification

The S. epidermidis strain was incubated overnight in mannitol salt agar (MSA; Difco, Hunt Valley, MD, USA) at 37°C. The culture was grown until its concentration reached approximately 50 CFU per mL. A 26-gauge needle was used to inject 50 CFU of S. epidermidis (0.1 mL suspension with a concentration of 500 CFU/mL) directly into the vitreous cavity via the pars plana, 2 mm posterior to the limbus. Quantitative and qualitative vitreous culture was performed at day 14 in the phase II study. Vitreous humors (0.05 mL) were obtained in a sterile manner using a 24-gauge needle with a 1.0-mL syringe. The specimen was placed on MSA and spread over the agar surface with a sterile bent glass rod. After overnight incubation at 35°C, the number of colonies yielded per plate was expressed as CFU per milliliter.

Statistical Analysis

Data are means ± SD. Amplitudes and implicit times of a-waves and b-waves in ERG were measured and compared between the tested eyes, using the Mann-Whitney U test. All analysis was conducted using SPSS software version 18.0 (SPSS, Inc., Chicago, IL, USA). A P value of <0.05 was considered statistically significant.

RESULTS

Phase I Study

Clinical Examination. Drug-induced ocular complications such as retinal hemorrhage, retinal detachment, optic atrophy, retinal ischemia or infarction, cataracts, corneal opacity, or severe intraocular inflammation were absent in both groups.

Pharmacokinetic Analysis. The mean concentration-time profiles of groups I and II in the vitreous humor are shown in Figure 1. Relevant pharmacokinetic parameters are listed in the Table. As shown in Figure 1, nonlinear decay in the log concentration profiles of PVI was observed during the initial 2 hours. In the noncompartmental analysis, the dose increase from 0.1% to 0.3% resulted in an overproportional increase of AUC; the dose-normalized AUC values of groups I and II were 0.222 and 0.478 h/mL, respectively. Moreover, CL and Vss tended to decrease as the dose increased (Table). These results indicated that vitreous PVI exhibited dose-dependent (nonlinear) pharmacokinetics after ocular injection. Thus, nonlinear Michaelis-Menten kinetics were used to describe the elimination process in the compartmental analysis. The t1/2 values of the 0.1% and 0.3% PVI in the vitreous were 3.27 and 3.58 hours, respectively. The PVI concentration data was well described by the one-compartment model with single Michaelis-Menten elimination kinetics, and the Vd, Vmax, and Km values were successfully estimated as shown in the Table.

Electroretinography. Before and after PVI injection, there were no significant changes in the mean values of a- and b-wave amplitudes at baseline, 6, and 12 hours in groups I and II (Fig. 2).

Phase II Study

Clinical Examination and Microbiological Results. All eyes receiving 50 CFU of S. epidermidis displayed obvious signs of endophthalmitis (hyperemic conjunctiva, chemosis, miosis, and vitreous haze) within 24 to 48 hours. Endophthalmitis was confirmed to detect S. epidermidis in the aspirated...
vitreous samples. After PVI injection, all four groups demonstrated improvement of conjunctival hyperemia, chemosis, and vitreous inflammation, compared to control eyes. Of 10 eyes, 8 eyes in groups A and B had moderate vitreous opacity. Mild vitreous opacity was observed in all eyes of groups C and D. Control eyes showed manifestations of severe bacterial endophthalmitis including chemosis and severe vitreous opacity at day 14 (Fig. 4).

Of 10 eyes, 4 eyes (receiving 20, 60, 60, and 70 CFU) and 3 eyes (receiving 20, 40, and 60 CFU) demonstrated S. epidermidis growth in vitreous samples in groups A and B at day 14, respectively. However, there was no bacterial growth in groups C and D. The control group had bacterial growth of 5,500 ± 550 CFU (average) in all eyes.

Electroretinography. Figure 5 shows representative ERG waves at day 14. Electroretinography waves were not recordable in all eyes of the control group. There were statistically significant differences in amplitudes of the a-wave at −5 to 0 dB between eyes given a single PVI injection (groups A and B) and eyes that received repeated PVI injections (groups C and D). The b-waves in groups A and B showed a significant decrease in amplitudes at all intensities, compared to groups C and D (Fig. 6).

Histologic Examination. Groups A and B had more prominent retinal damage, including lymphocyte infiltration in the inner retinal layers, than that in Groups C and D, which showed minimal inflammatory change (Fig. 7). Control eyes demonstrated severe inner retinal damage with destructive tissue changes.

**DISCUSSION**

Intravitreal use of PVI was first reported by Whitacre and Crokett. In their study, PVI at concentrations of 0.05 to 0.5% did not produce any notable retinal damage, although one eye had mild vitritis after 0.5% PVI injection. However, 5.0% PVI induced severe retinal damage in all eyes. They concluded that PVI concentrations between 0.05% and 0.5% would be tolerable in the eyes. Trost et al. confirmed a safety threshold of 0.1% PVI for S. epidermidis endophthalmitis, and concluded that intracocular PVI was not a useful treatment for bacterial endophthalmitis. However, this result could mostly be attributable to the single injection as well as low concentration (0.1%). This hypothesis was supported by the work of Brozou et al., who showed that S. epidermidis endophthalmitis improved after a 0.2% PVI injection.

Based on previous results, we selected 0.1% and 0.3% concentrations of PVI for evaluation in this study. Electroretinographs and histologic examination of the retina confirmed that both 0.1% and 0.3% PVI were tolerable in the phase I study.
study. Considering the $t_{1/2}$ determined in the phase I study, we administrated PVI into the vitreous every second day for repeated injections in the phase II study.

Well-known limitations of intraocular drug use include the short duration of action owing to its use at a low concentration to achieve efficacy and avoid toxicity; the efficacy of drug is directly related to the concentration achieved, which also correlates with the degree of tissue damage. Thus, repeated injections are a common method to treat intraocular disease, infection, and inflammation. For example, the regular injection of antivascular endothelial growth factor agent is widely accepted for the treatment of wet age-related macular degeneration and diabetic macular edema.\textsuperscript{12–15}

In cases of endophthalmitis, the standard care is intravitreal drug administration with or without surgical treatment.\textsuperscript{16} Repeated injections of antibiotics are occasionally administered in cases that do not fully recover after a single injection of the drug. In such cases, efficacy and safety have been proven in previous studies.\textsuperscript{17,18} Thus far, the half-life of various antimicrobial and antifungal agents have been reported to be from 0.5 to 30 hours.\textsuperscript{19} The present study showed that single use of 0.1\% PVI did not have any beneficial effect on endophthalmitis, which correlates with the results of previous studies, whereas

**FIGURE 4.** Representative views show anterior and posterior segments at day 14 in the phase II study. (A) Repeated 0.1\% PVI injections in the eye show white conjunctiva, minimal vitreous opacity, and small retinal hemorrhage. No bacterial growth was detected in the vitreous samples. (B) Eye receiving a single injection of 0.3\% intravitreal PVI demonstrated mild conjunctival injection, moderate vitreous opacity, and retinal hemorrhage (\textit{white arrows} are retinal hemorrhage). Sixty CFUs of \textit{S. epidermidis} was cultured in the vitreous. (C) The control eye receiving a sham injection had moderate conjunctival injection and severe vitreous opacity that obscured the optic disc. More than 5000 CFU \textit{S. epidermidis} were observed in the vitreous.

**FIGURE 5.** Electroretinogram (ERG) waves from eyes with \textit{S. epidermidis} endophthalmitis 14 days after PVI injection in the phase II study. (A) Electroretinography shows nearly normal waves in the eyes without bacteria. (B) Decreased waves were detected in eyes with bacterial growth. (C) Control eyes (sham injection) did not have waves at all decibels.
repeated injections of 0.1% PVI were found to be effective. Moreover, repeated injections achieved better results in the vitreous culture for bacterial growth, ERG, and histology than a single PVI injection with high concentration (0.3%). Both repeated injection of 0.1% and 0.3% PVI did not differ in the treatment of S. epidermidis endophthalmitis. Our results demonstrated that PVI could have a beneficial effect in treating bacterial endophthalmitis by using repeated injections of its 3-hour half-life, even at a low concentration. Moreover, it is well known that PVI has an additional advantage as a broad-spectrum agent against microorganisms, compared to antibiotics.

There are some limitations to this study. First, it is based on an animal model and the study was designed to investigate short-term changes. Response to intraocular infections and subsequent treatment in rabbits may differ from that in humans. Thus, our results from this experimental animal model may not reflect the clinical course in humans directly. Second, this study could lack sufficient power to detect the beneficial effects of PVI on bacterial counts in the vitreous due to the small number of animals used. A larger number of animals tested could generate a different outcome. Finally, it is most likely that similar results would not be observed if we altered the types of bacteria, size of the inoculation, and timing of the treatment.

In conclusion, PVI has a 3-hour half-life in the vitreous. Both 0.1% and 0.3% PVI can be tolerated in rabbit eyes. Repeat injections of intravitreal PVI could be effective for the treatment of S. epidermidis endophthalmitis, even at a low concentration. Further investigation of intraocular PVI use should be conducted for the treatment of various pathogens causing endophthalmitis.

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