

Intravitreal injections primary prevention: a case-control study

F. AVOGARO, A. FLORIDO, A. CALANDRI, M. MALVASI, E.M. VINGOLO

Department of Sense Organs, Faculty of Medicine and Odontology, Sapienza University, Rome, Italy

Abstract. – OBJECTIVE: Intravitreal injections (IVI) of therapeutic substances are one of the most common procedures in ophthalmology and, for sure, the most feared complication of them is endophthalmitis. Nowadays, a precise prophylactic protocol does not exist to avoid these infections, and the role of new antiseptic drops is an interesting field of research in this regard. In this article we are going to discuss the tolerability and the efficacy of a new antiseptic drop based on a solution of hexamidine diisethionate 0.05% (Keratosept®; Bruschetti Srl, Genoa, Italy).

PATIENTS AND METHODS: This was a single-center, case-control study, comparing the *in vivo* effect of hexamidine diisethionate 0.05% with povidone iodine 0.6% solution during IVI program. Ocular bacterial flora composition was analyzed with a conjunctival swab on day 0. After injection patients underwent antibacterial prophylaxis with Keratosept for 3 days or povidone iodine 0.6%. A second conjunctival swab was collected on day 4 and patients were asked to fulfill a questionnaire based on the OS-Di model, to investigate the ocular tolerability of the drug administered.

RESULTS: Efficacy was tested on 50 patients, 25 of whom received hexamidine diisethionate 0.05% drops and the other 25 received povidone iodine 0.6% solution drops, 100 total conjunctival swabs, 18 positive swabs before and 9 after treatment for the first group and 13 before and 5 after for the second one. Tolerability was tested on 104 patients, 55 underwent Keratosept therapy and 49 povidone iodine one.

CONCLUSIONS: Keratosept demonstrated a good efficacy profile with better tolerability against povidone iodine in the analyzed sample.

Key Words:

Intravitreal injections, Endophthalmitis, Antibiotics, Antiseptic.

Introduction

Intravitreal injections (IVI) of therapeutic substances are one of the most common procedures in

ophthalmology. Intravitreal injections are useful tools for various retinal diseases. It has been proven how anti-VEGF therapy can successfully improve visual acuity in chorioretinal diseases such as diabetic retinopathy (DR), exudative age-related macular disease (ARMD) and retinal vein occlusion (RVO). Nowadays, it is one of the most common surgery procedures and, as all surgeries, is associated with potential complications, such as cataract, IOP elevation, hemorrhages and ocular inflammation, retinal vascular occlusion, retinal vasculitis and injection-related endophthalmitis (IRE)¹. For sure, the most feared complication is endophthalmitis with an incidence of 0.042% to 0.075% after IVI procedures². It is associated with a poor visual prognosis even with prompt diagnosis, and intravitreal antibiotics administration or vitrectomy. Moreover, endophthalmitis post IVI has shown to have worse VA improvement after treatment, compared to those following cataract surgery³. It is well known that in most of the cases, these infections are held by ocular bacterial flora⁴. After cultures, the most common species that have been isolated belong to GRAM + (*Staphylococcus spp.*, *Streptococcus spp.*, *Corynebacterium spp.*, and *Propionibacterium*); among GRAM –, the most isolated are *Haemophilus* and *Neisseria*. The normal ocular microbiota has a defensive role in these pathogenic bacteria, preventing their proliferation and colonization^{5,6}. Intravitreal injections have a low incidence of infections but are associated with a small chance of endophthalmitis. The rate of serious infective complications is lower than 1%⁷. There have been many improvements of the procedural techniques adopted in primary preventions before and after the injection itself. Prophylactic measures such as antiseptic drops, gloves and masks have all been proposed to prevent post-injection endophthalmitis, and there remain significant variants in protocols. Patel et al⁸ demonstrated that the impact of face mask use is not significant on endophthalmitis incidence

after IVI. This was confirmed by Naguib et al⁹ since the implementation of universal masking during COVID-19 did not show changes in the rate of post-intravitreal endophthalmitis. Among the variety of interventions to reduce the risk of infection, PVI antiseptics stands out as the most strongly supported by evidence¹⁰. Due to the continuous increasing number of intravitreal injections, the incidence IRE are likely going to arise. To date, the prophylactic use of topical antibiotics before intravitreal injections has been reported in many large series to even increase the rate of endophthalmitis¹¹. For this reason, the most effective choice has proven to be antiseptic drops¹². Many studies^{13,14} proved *in vitro* an effective antibacterial activity of povidone iodine 0.6% against *S. Epidermidis*, *S. Aureus*, *P. Aeruginosa* and some *Candida spp.* Hexamidine diisethionate 0.1% has been used in medicine since the 1950s as an antiseptic agent, and similarly to povidone iodine, it has proven to have rapid bactericidal efficacy, even at lower concentrations. Hexamidine diisethionate is a diamidine, an hydrosoluble cationic agent with activity against bacteria, fungi, yeasts, and free-living amoebae. Being positively charged, it can bind the negatively charged walls and membranes of bacteria, perturbing the binding sites. For these reasons, it already has a role in minor conjunctivitis, blepharitis and *Acanthamoeba* keratitis treatment even though not recommended as monotherapy¹⁵. An *in vitro* study conducted by Pinna et al¹⁶ showed the ability of hexamidine diisethionate 0.05% to kill the organisms tested (*S. Aureus*, *S. Epidermidis*, *Candida spp.*, *P. Aeruginosa*) with different timing (poorly effective against *P. Aeruginosa*).

In this article, we are going to discuss the tolerability and the efficacy of a new antiseptic drop based on a solution of hexamidine diisethionate 0.05%, which has been shown to be suitable for prophylaxis of infections related to IVI¹⁶.

Patients and Methods

This was a single-center, case-control study, comparing the *in vivo* effect of hexamidine diisethionate 0.05% in polyvinyl alcohol 1.25% vehicle (Keratosept[®]; Bruschettoni Srl, Genoa, Italy) with povidone iodine 0.6% solution on the composition of the resident microbial flora in patients undergoing IVI. The study was conducted at the Ophthalmology department of A. Fiorini Hospital in Terracina (LT). Patients' afferent to

the Terracina Hospital were enrolled between September 2021 and June 2022 if they presented the following inclusion criteria: age more than 18 years, indication for IVT therapy and ability to understand and sign the informed consent form. Patients were excluded if presenting ongoing eye infections, commonly used contact lenses, had a recent history of eye surgery, presented history of allergy to the drugs, had been using eye topical and/or systemic antibiotics within the past 3 months, or if they were using eye drops. After inclusion in the study, the patient's ocular bacterial flora composition was analyzed by collecting a conjunctival swab on day 0. After having performed the first sampling, patients underwent IVT; in accordance with the most recent recommendations, eyelids and periorcular skin area was disinfected with povidone iodine 10% and an ophthalmic solution containing povidone iodine 5% was instilled in the eye subjected to IVT¹⁷. After injection patients were randomly assigned to an antibacterial prophylaxis with Keratosept[®] or iodopovidone 0.6% for 3 days. A second conjunctival swab was collected on day 4 and patients were asked to fulfill a questionnaire based on the OSDI model, to investigate the ocular tolerability of the drug administered. The conjunctival swab samples were sent to the microbiology laboratory at the A. Fiorini Hospital of Terracina and seeded in blood agar, chocolate agar and blue eosin agar methylene and incubated at 37°C for 48 h. At the end of the incubation period Gram stain and identification of microorganisms was performed. The primary endpoint of this research was to evaluate any change in the composition of the resident microbial flora and any reduction or increase in conjunctival bacterial load in patients treated with hexamidine diisethionate 0.05% in polyvinyl alcohol 1.25% vehicle (Keratosept[®]; Bruschettoni Srl, Genoa, Italy) compared to those treated with povidone iodine 0.6%. Secondary endpoints were the evaluation of any differences in the incidence of infective complications in the 2 groups and any differences in the tolerability of the drugs by the patients.

Statistical Analysis

All data from the study was analyzed through SPSS Statistics 28 (IBM Corp., Armonk, NY, USA). The efficacy of two drops among the sample subjects was analyzed through analysis of covariance (ANCOVA) test ($p=0.535$). In addition, the effective reduction of bacterial charge of the two samples was verified through

McNemar Change test (p -values of 0.008 and 0.004). The twelve ordinal variables of the test based on the OSDi items were analyzed through a median nonparametric test and Mann-Whitney U test ($p < 0.001$).

Results

The comparison of the efficacy of the two drops was tested on 50 patients, 25 of whom received hexamidine diisethionate 0.05% drops and the other 25 received povidone iodine 0.6% solution drops. The total amount of conjunctival swabs was 100, 31 of whom were positive to common bacteria before drops administration and 14 were positive five days after the antiseptic therapy. Pre-treatment positive swabs were 18 for the hexamidine diisethionate group and 13 for the povidone iodine group. After the therapy, 9 swabs from the first group and 5 from the second group remained positive. The distribution of the swabs and the bacteria found are shown in Table I. We accept the null hypothesis of the ANCOVA test being the significance of the test 0.535. There wasn't any statistically significant difference between pre- and post-drug administration swabs among hexamidine diisethionate and povidone iodine groups. The McNemar Change test excluded the null hypothesis for the bacterial charge reduction both for the iodopovidone and Keratosept® samples with p -values of 0.008 and 0.004, respectively.

Tolerability of povidone iodine 0.6% ophthalmic solution vs. hexamidine diisethionate 0.05% solution was evaluated through OSDi test on a total of 127 subjects, 23 of whom were excluded due to an incomplete or incorrect test execution. A total of 104 OSDi questionnaires were considered for the statistical analysis, 49 patients received povidone iodine drops and 55 patients hexamidine diisethionate drops. We refused the null hypothesis in 10 of the 12 OSDi items (Table II). The total score of the test, considered a continuous variable, was evaluated through Mann-Whitney U nonparametric test. It suggested a significance ($p < 0.001$) of the differences between the mean values of OSDi scores between the two ophthalmic solutions (Figure 1).

Discussion

According to recent *in vitro* studies^{13,14} both iodopovidone 0.6% and Keratosept® were able to reduce the presence of the bacteria reported by the conjunctival swabs. *Staphylococcus Epidermidis* in particular, which is considered one of the main causes of post-surgical endophthalmitis. In addition, the comparison of the *in vivo* activity of these two drops showed no statistically significant difference. Lastly, Keratosept® demonstrated a better tolerability against iodopovidone 0.6% through OSDi test evaluation.

Table I. Pre- and post-treatment conjunctival swabs results.

	Hexamidine diisethionate 0.05%	Povidone iodine 0.6%
Total conjunctival swabs	50	50
Pre-treatment positive swabs	18	13
Isolated pre-treatment bacteria	<i>Methicillin-sensible Staph. epidermidis</i> <i>Methicillin-resistant Staph. epidermidis (MRSE)</i> <i>Methicillin-resistant Strept. haemolyticus</i>	<i>Methicillin-sensitive Staph. epidermidis</i> <i>Methicillin-resistant Staph. epidermidis (MRSE)</i>
Post-treatment positive swabs	9	5
Isolated post-treatment bacteria	<i>Methicillin-sensible Staph. epidermidis</i> <i>Methicillin-resistant Staph. epidermidis (MRSE)</i> <i>Methicillin-resistant Staph. haemolyticus</i>	<i>Methicillin-resistant Staph. epidermidis (MRSE)</i>

Table II. OSDi questionnaire variables Mann-Whitney hypothesis test, Keratosept vs. povidone Iodine score.

Null Hypothesis	Sig. ^{a,b}	Decision
1 The distribution of Eyes that are sensitive to light is the same across categories of Drop type	0.000	Reject the null hypothesis
2 The distribution of Eyes that feel gritty is the same across categories of Drop type	0.005	Reject the null hypothesis
3 The distribution of Painful or sore eyes is the same across categories of Drop type	0.092	Retain the null hypothesis
4 The distribution of Blurred vision is the same across categories of Drop type	0.001	Reject the null hypothesis
5 The distribution of Poor vision is the same across categories of Drop type	0.000	Reject the null hypothesis
6 The distribution of Reading is the same across categories of Drop type	0.000	Reject the null hypothesis
7 The distribution of Driving at night is the same across categories of Drop type	0.001	Reject the null hypothesis
8 The distribution of Working with a computer is the same across categories of Drop type	0.000	Reject the null hypothesis
9 The distribution of Watching TV is the same across categories of Drop type	0.000	Reject the null hypothesis
10 The distribution of Windy conditions is the same across categories of Drop type	0.000	Reject the null hypothesis
11 The distribution of Places or areas with low humidity is the same across categories of Drop type	0.058	Retain the null hypothesis
12 The distribution of Areas that are air conditioned is the same across categories of Drop type	0.003	Reject the null hypothesis

^aThe significance level is .050. ^bAsymptotic significance is displayed.

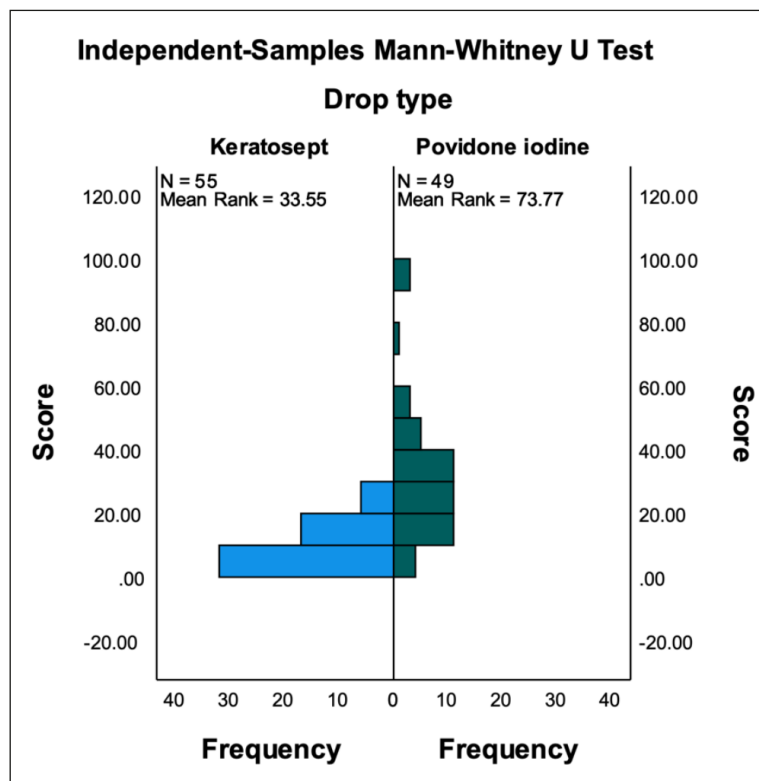


Figure 1. Frequencies distribution of OSDi scores, visually suggests the lower scores for Keratosept against povidone iodine solution, supported by test significance with $p < 0.00$.

Conclusions

All endpoints of the study were achieved. In the considered sample we accepted the null hypothesis. No statistically significant difference between cases, patients that received Keratosept® drops and patients (controls) that received iodopovidone 0.6% drops, was detected. The evaluated tolerability profile was good. Through the OSDi test in the analyzed sample, we found a statistically significant better tolerability of Keratosept® against iodopovidone 0.6% drops. Any side effect or complication was reported by patients or found during controls. In line with *in vitro* findings^{14,16}, our *in vivo* analysis suggested a similar efficacy profile in reducing the resident bacterial charge of the found pathogen agents. *S. Epidermidis* charge was reduced by both Keratosept and iodopovidone 0.6% solutions with better tolerability of the first one. Considering our data both drops could be judged useful in the reduction of *S. Epidermidis* conjunctival bacterial charge and, being this pathogen one of the main actors in the pathogenesis of post-surgical endophthalmitis¹⁸, they could be considered for the improvement of the prophylaxis against this severe infection with a better tolerability for Keratosept.

Although this study is preliminary and has some limitations, the correlation between the conjunctival bacterial charge reduction and the reduction of endophthalmitis incidence have to be demonstrated. Due to the small incidence of this infection, a large amount of data is necessary. We had small numbers, our sample was not selected randomly from the general population, and we did not have a single- or double-blind criteria of drug administration. Thus, perspective further studies are necessary. The incidence of post-surgical endophthalmitis is quite low but, considering the number of surgical procedures made yearly (e.g., IVI, Cataract surgery etc.), the absolute numbers of cases is not insignificant, also considering the severity and the high ocular morbidity of this condition. In conclusion post-surgical endophthalmitis is a concrete problem and the possibility to find and standardize a prophylaxis protocol to reduce its incidence could be an interesting research field. It has been found out that antibiotics are not a good option, and they are probably counterproductive¹⁹. However, the role of antiseptic drops has been poorly investigated and could be another interesting research field. In our study we tried to evaluate the *in vivo* activity of two antiseptic drops that demonstrated the same efficacy against the bacteria present in the patient's conjunctival swabs with a better tolerability for Keratosept®.

Authors' Contributions

Conceptualization, E.M.V. and F.A.; methodology, F.A.; software, F.A.; validation, E.M.V., M.M. and A.F.; formal analysis, F.A.; investigation, M.M.; resources, A.C.; data curation, A.C.; writing—original draft preparation, F.A.; writing—review and editing, A.F.; visualization, F.A.; supervision, E.M.V.; project administration, A.C.; All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Ethics Approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Ophthalmological Sciences department, Rome University “La Sapienza” in the session of 27 July 2021 No. 7.3.

Informed Consent

Informed consent was obtained from all subjects involved in the study.

Availability of Data and Materials

The data used or analyzed during the current study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

ORCID ID

Filippo Avogaro: 0000-0002-7475-5145
Antonio Florido: 0000-0003-4921-5830
Mariaelena Malvasi: 0000-0002-1190-9733
Enzo Maria Vingolo: 0000-0002-8363-5866

References

- 1) Singh R, Davoudi S, Ness S. Preventive factors, diagnosis, and management of injection-related endophthalmitis: a literature review. *Graefes Arch Clin Exp Ophthalmol* 2022; 260: 2399-2416.
- 2) Straňák Z, Veith M, Studený P, Penčák M. [The incidence of endophthalmitis after the application of intravitreal injections in FNKV with regard to various prophylactic antibiotic regimens]. *Cesk Slov Oftalmol* 2014; 70: 184-188.
- 3) Dar N, Pillar S, Friehmann A, Belkin A, Ofir S. Endophthalmitis after intravitreal injections versus

- cataract surgery: a 15-year cohort. *Int Ophthalmol* 2020; 40: 73-79.
- 4) Lu LJ, Liu J. Human Microbiota and Ophthalmic Disease. *Yale J Biol Med* 2016; 89: 325-330.
 - 5) Pleyer U, Baatz H. Antibacterial Protection of the Ocular Surface. *Ophthalmologica* 1997; 211: 2-8.
 - 6) Dave SB, Toma HS, Kim SJ. Ophthalmic Antibiotic Use and Multidrug-Resistant *Staphylococcus epidermidis*. *Ophthalmology* 2011; 118: 2035-2040.
 - 7) Kiss S, Dugel PU, Khanani AM, Broder MS, Chang E, Sun GH, Turpcu A. Endophthalmitis rates among patients receiving intravitreal anti-VEGF injections: a USA claims analysis. *Clin Ophthalmol* 2018; 12: 1625-1635.
 - 8) Patel SN, Hsu J, Sivalingam MD, Chiang A, Kaiser RS, Mehta S, Park CH, Regillo CD, Sivalingam A, Vander JF, Ho AC, Garg SJ, Wills Post-Injection Endophthalmitis (PIE) Study Group. The Impact of Physician Face Mask Use on Endophthalmitis After Intravitreal Anti-Vascular Endothelial Growth Factor Injections. *Am J Ophthalmol* 2021; 222: 194-201.
 - 9) Naguib MM, Ghauri S, Mukhopadhyay A, Scheffler AC. Endophthalmitis After Intravitreal Injections During the Covid-19 Pandemic with Implementation Of Universal Masking. *Retina* 2021; 41: 2208-2214.
 - 10) Patel SN, Gangaputra S, Sternberg P, Kim SJ. Prophylaxis measures for postinjection endophthalmitis. *Surv Ophthalmol* 2020; 65: 408-420.
 - 11) Hunyor AP, Merani R, Darbar A, Korobelnik JF, Lanzetta P, Okada AA. Topical antibiotics and intravitreal injections. *Acta Ophthalmol* 2018; 96: 435-441.
 - 12) Lau PE, Jenkins KS, Layton CJ. Current Evidence for the Prevention of Endophthalmitis in Anti-VEGF Intravitreal Injections. *J Ophthalmol* 2018; 2018: 8567912.
 - 13) Musumeci R, Bandello F, Martinelli M, Calaresu E, Cocuzza CE. In vitro bactericidal activity of 0.6% povidone-iodine eye drops formulation. *Eur J Ophthalmol* 2019; 29: 673-677.
 - 14) Pinna A, Donadu MG, Usai D, Dore S, D'Amico-Ricci G, Boscia F, Zanetti S. In vitro antimicrobial activity of a new ophthalmic solution containing povidone-iodine 0.6% IODIM®. *Acta Ophthalmol* 2020; 98: e178-e180.
 - 15) Grare M, Dibama HM, Lafosse S, Ribon A, Mourer M, Regnoul-de-Vains JB, Finance C, Duval RE. Cationic compounds with activity against multidrug-resistant bacteria: interest of a new compound compared with two older antiseptics, hexamidine and chlorhexidine. *Clin Microbiol Infect* 2010; 16: 432-438.
 - 16) Pinna A, Donadu MG, Usai D, Dore S, Boscia F, Zanetti S. In Vitro Antimicrobial Activity of a New Ophthalmic Solution Containing Hexamidine Diisethionate 0.05% (Keratosept). *Cornea* 2020; 39: 1415-1418.
 - 17) Fagan XJ, Al-Qureshi S. Intravitreal injections: a review of the evidence for best practice: Intravitreal injections: evidence for best practice. *Clin Experiment Ophthalmol* 2013; 41: 500-507.
 - 18) Ciulla TA, Starr MB, Masket S. Bacterial endophthalmitis prophylaxis for cataract surgery. *Ophthalmology* 2002; 109: 13-24.
 - 19) Menchini F, Toneatto G, Miele A, Donati S, Lanzetta P, Virgili G. Antibiotic prophylaxis for preventing endophthalmitis after intravitreal injection: a systematic review. *Eye* 2018; 32: 1423-1431.