Assessment of ocular irritation and tear fluid drug levels following administration of antibiotics suspended in a self-emulsifying eye drops

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INTRODUCTION

Our project focuses on the use of SEO (isotropic mixtures of oil and surfactant) for substances that are unstable in an aqueous environment, in particular antibiotics. Eye drops with cefuroxime sodium (CEF) and vancomycin hydrochloride (VAN) were prepared using SEO as a carrier. These antibiotics are freely soluble in water but insoluble in SEO, so the suspension type formulation was obtained. Upon contact with tear fluid SEO emulsifies, while the suspended antibiotic immediately dissolves [1].

Studies have already proven the long-term stability of both CEF and VAN in SEO, which was 2 years at a temperature of 40°C while in an aqueous solution a complete decomposition of CEF was observed after 7 days, and the VAN content dropped to 41% of its initial value after one month [2].

Our preliminary irritation test conducted on rabbit eyeballs classified SEO placebo as practically non-irritating and SEO-CEF as minimally irritating [4]. These results provided the basis for continuing the research. Therefore, the aim of our study was to perform a comprehensive *in vivo* irritation test on porcine eyeballs, accompanied by an evaluation of the antibiotic concentration in the eye, in particular in tear fluid.

MATERIALS AND METHODS

2.1. Preparation of eye drops

Micronized (<25 μm) CEF, VAN (5% w/w) and anhydrous sodium citrate (2%) were suspended in a self-emulsifying oil (SEO) system consisting of Miglyol and Tween 20 (5%). The dispersions were subjected to a high-pressure homogenization. The homogeneity of all formulations was verified microscopically.

2.2. Application of eye drops

The study was performed with a permission of 2nd Local Ethics Committee for Animal Testing in Warsaw nr WAW2/093/2025. Healthy pigs were treated with one drop of tested formulation to each eyeball, three times a day for three consecutive days. Six groups of animals (n=10) received one of the following preparations: CEF-SEO, VAN-SEO, VAN-solution, CEF-solution (adjusted to isotonicity with NaCl), 0.9% NaCl and SEO-placebo.

2.3. Irritation assessment according to the Draize test

Before and 30 minutes after application, a visual inspection of the conjunctiva, cornea, and iris was performed. The observed changes were scored, with a maximum of 80 points for corneal changes, 20 points for conjunctival changes, and 10 points for iris changes, giving a total possible score of 110 points.

2.4. Analysis of CEF and VAN concentration in tear fluid

The tear fluid was collected using paper strips for the Schirmer test: 2 hours and 5 hours after the application. Afterwards, the strips were weighed and the mass was recorded. Extraction was performed for 30 min using 1 mL of HPLC mobile phase. The analysis of antibiotics in the extracts was conducted using HPLC isocratic method previously described [2]. Detection wavelength for CEF was 273 nm and for VAN 210 nm. Statistical differences were evaluated using the paired Student's T-test, with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

3.1. *In vivo* eye irritation test

The tolerance study performed 30 minutes after the administration of SEO-based eye drops confirmed the absence of any irritating effects in all tested formulations. Mild conjunctival hyperemia was the only ocular change observed after CEF-SEO or VAN-SEO administration (**Figure 1**). According to the Draize test assessment protocol, the maximum possible score is 110 points while for CEF-SEO it was 0.213±0.62 and for VAN-SEO: 0.188±0.59, classifying both as non-irritating according to the Kay and Calandra scale (<0.5).

The highest score, observed for Miglyol alone (0.406±0,84), was also within the non-irritating range. In most animals, irritation appeared only in one eye and usually on the first day after application, resolving before the next dose. Notably, the VAN-solution achieved a score of 0 in all assessments, while CEF-SEO proved less irritating than the

currently used CEF-solution. **Figure 1** presents the results obtained during the study. Each geometric symbol corresponds to a specific formulation, while the number inside indicates the number of eyeballs in which the changes were found (maximum score was not higher than 8).

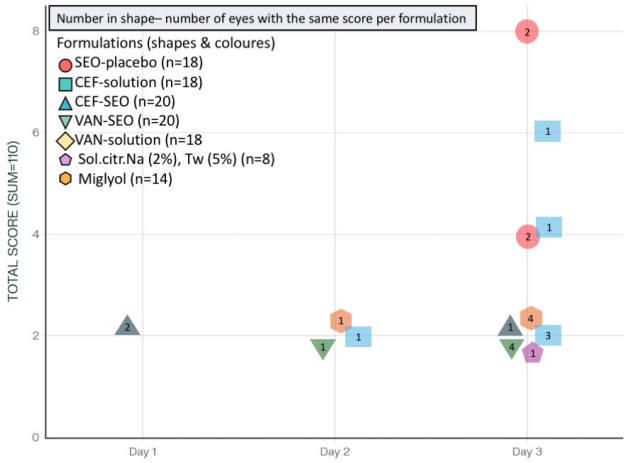

Figure 1. Number of eyes showing changes in conjunctiva depending on the type of formulation and days of application.

3.2. Antibiotic concentration in tear fluid

According to the literature, the time required for complete drug removal from the ocular surface is approximately 30 minutes. In this study, however, a substantial concentration of CEF was still detected in the conjunctival sac 5 hours after administration (**Table 1**). Interpretation of the results was limited by high variability and the fact that each strip represented a unique, non-repeatable sample. For example, in one pig receiving the CEF solution, concentrations after 5 hours reached 15.26 mg/mL in the left eye and 6.32 mg/mL in the right. Although the variability prevents definitive conclusions, the data indicate notably higher concentrations of CEF in tear fluid after CEF-solution administration (15.3 mg/mL vs. 1.53 mg/mL for CEF-SEO at 2 hours after application), what demonstrated different pharmacokinetics. Analysis of the tear fluid after administration of VAN is in progress.

CONCLUSION

The SEO formulations were well tolerated, and the results of the irritation test classified these innovative eye-drops as non-irritating. This represents a technological success, as the anhydrous SEO system enhances the stability of antibiotics unstable in aqueous environments and thus holds potential for future clinical use and patient application.

Large individual variability resulted in tear-fluid concentration values that should be interpreted carefully. However, based on the results obtained, it can be assumed that the tested CEF-solution and CEF-SEO exhibit different pharmacokinetic profiles after administration. In the next step of the project the amount of the antibiotics penetrating to the eye ball structures will be also analyzed what will allow to explain the lower concentration of CEF in the tear fluid when CEF-SEO was administered.

CEF CONCENTRATION IN TEAR FLUID (mg/mL)

	CEF-SOLUTION		Statistical significance (SS)	CEF-SEO	
TIME AFTER APPLICATION	Left eye (L)	Right eye (R)	(SOL VS SEO)	Left eye (L)	Right eye (R)
2 h after application	17.83	26.07	statistically significant	0.15	6.30
	7.66	4.51		0.56	0.13
	5.57	15.25		2.28	2.00
MEAN±SD	**10.35±5.36**	**15.28±8.80**		**1.00±0.92**	**2.81±2.58**
SS (L VS R)	statistically insignificant			statistically insignificant	
5 h after application	17.09	3.63	statistically significant	0.18	0.12
	7.19	6.49		0.27	0.83
	25.91	5.61		4.95	0.40
	5.77	8.13		0.18	0.65
	20.35	7.73		2.09	0.25
MEAN±SD	**15.26±7.72**	**6.32±1.61**		**1.54±1.86**	**0.45±0.26**
SS (L VS R)	statistically insignificant			statistically insignificant	

Table 1. Partial results of CEF concentration (mg/mL) in tear fluid marked 2h and 5h after application (paired T test p< 0.05).

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ACKNOWLEDGMENT

The research is funded by a Medical Research Agency grant nr 2024/ABM/03/KPO/ KPOD.07.07-IW.07-0046/24-00.