

THE CONCENTRATION OF COMBINATION ANTIMICROBIAL ANTI-INFLAMMATORY EYE DROPS IN RABBIT AQUEOUS HUMOR FOLLOWING TOPICAL ADMINISTRATION

I. N. Okolov¹, C. A. Nikulin¹, T. K. Churakov¹, T. E. Morozova², N. V. Krivorotova², G. V. Karakashev², E. I. Savel'eva²

¹ Saint Petersburg's Federal State Budgetary Institution MNTK glaza Mikrokhirurgiya branch of Akkad. S. N. Fedorova Ministry of Health of Russia, St. Petersburg, Russia, Yaroslav Gashek St., 21, St. Petersburg, 192283, Russia

² Research institute of hygiene, professional pathology and ecology of the person FMBA, St. Petersburg, Russia

SUMMARY

Aim was to research objective consisted in an assessment of penetration of an antibacterial preparation of ciprofloxacin and a corticosteroid preparation of dexamethasone as a part of Kombinil Duo's combined medicinal form in moisture of the forward camera of an eye in experiment, and also in detection of the minimum overwhelming concentration (MOC) of ciprofloxacin in VPKG for the most frequent causative agents of eye infections.

Material and methods. In experiment on seven adult individuals of rabbits of breed the Chinchilla (fourteen eyes) eye drops Kombinil Duo, containing in quality of the main substance ciprofloxacin (3 mg/ml) and dexamethasone (1 mg/ml) are tested. Determination of concentration of the main substances of studied preparations in moisture of the eye camera carried out a method of a highly effective liquid chromatography (VEZhH) in combination with mass and spectrometer detecting (MS) on the liquid Shimadzu LC-20AB chromatograph with the mass and selective detector Shimadzu LCMS-2010EV (ESI).

Results and conclusion. The maximum concentration of ciprofloxacin in moisture of the forward camera of rabbits eye made 0,25 mkg/ml, and minimum — 0,06 mkg/ml, average concentration — $0,13 \pm 0,06$ mkg/ml. The anti-inflammatory component presented by dexamethasone in a studied eye form, is defined in the range from 0,14 to 0,63 mkg/ml. Average concentration of dexamethasone equaled $0,24 \pm 0,12$ mkg/ml. The obtained data testify that average concentration of ciprofloxacin in VPKG exceeds average MPK90 for strains the grampolozhitelnykh of bacteria which are potential activators of postoperative infectious complications: metitsillinchuvstvitelny golden стафилококки (MPK90-0,06 mkg/ml) and ftorkhinolonchuvstvitelny koagulazonegativny стафилококки (mkg/ml MPK90—0,05). The revealed concentration are sufficient for minimization of risk of development of postoperative infectious complications. In a pilot study it is shown that at an instillation in a conjunctival bag of rabbits of a complex preparation of Kombinil-Duo in moisture of the

forward camera of an eye concentration of antibacterial means (ciprofloxacin) sufficient for suppression of the main activators causing postoperative complications with ensuring anti-inflammatory action (at the expense of dexamethasone) is created. The obtained data can be used in clinical practice for correction of schemes on treatment and prevention of inflammatory diseases of eyes.

Keywords:

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INTRODUCTION

Postoperative pharmacotherapy of modern cataract surgery includes different medications, generally monodrugs (anti-inflammatory and antibacterial). Topical glucocorticoids are commonly used in ophthalmology and underlie anti-inflammatory treatment [1, 2]. Aseptic inflammation is a well-known response to surgical trauma, however, post-operative infectious complications concern the surgeons. They can be prevented by antibiotics belonging to aminoglycosides and fluoroquinolones which exhibit broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria and good pharmacokinetics [3-5]. To optimize pharmacodynamics and pharmacokinetics and to minimize side-effects, combination drugs containing glucocorticoids (dexamethasone) and antibiotics were developed. Currently, various combination drugs which contain dexamethasone in combination with antimicrobial agents such as aminoglycosides (framycitin, neomycin, gentamycin, tobramycin), fluoroquinolones (ciprofloxacin, norfloxacin, ofloxacin, gatifloxacin, moxifloxacin), chloramphenicol, polymyxin and gramicidin, are available on global pharmaceutical market. The data on their use in ophthalmology are available in domestic and international literature [2, 6-8]. The primary goal of combined therapy in ophthalmic surgery is to prevent post-op inflammation and infectious complications. Simultaneous use of two medications in a single formulation increases the accuracy of dosing and decreases “wash-out” effect. Besides, combined eye drops are comfortable for patients and improve medication compliance. It is of great importance in elderly persons. Considering that inflammation has a variety of clinical forms, it can be difficult for an ophthalmologist to determine who and when should be treated with a combination drug and when this medication should be withdrawn. In fact, rather few ophthalmic disorders require combination drugs which contain corticosteroid and antibacterial agent. Despite the established efficacy of combined drugs in ophthalmology, various side-effects are possible, especially in case of their long-term use. A combination of antibiotic and steroid may be undesirable as tobramycin is toxic to corneal epithelium unlike fluoroquinolones which are less toxic [9]. Second-generation fluoroquinolone (e.g., ciprofloxacin) which contains in Combinil Duo® eye drops has a broad-spectrum antibacterial activity against Gram-positive and Gram-negative microbes. Ciprofloxacin

is also highly effective against *P. aeruginosa* strains. Currently, the only one fluoroquinolone and dexamethasone combination is approved in Russian Federation, i.e., Combinil Duo® eye drops (ciprofloxacin 0.3% and dexamethasone 0.1%).

AIM

The aim of this study was to analyze the penetration of antimicrobial (ciprofloxacin) and corticosteroid (dexamethasone) in a single formulation of combined eye drops (Combinil Duo) into anterior chamber aqueous humor (ACAH) and to determine ciprofloxacin minimum inhibitory concentration (MIC) for the most common agents that cause eye infections.

MATERIALS AND METHODS

Combinil Duo® eye drops containing ciprofloxacin (3 mg/ml) and dexamethasone as active components were tested. 7 adult chinchilla rabbits weighing on average 2.0 ± 0.5 kg (14 eyes) were included in the study. Combinil Duo® (2 drops) was instilled in both eyes every 3 hours during one day. ACAH samples (10-50 μ l) were collected with insulin syringe 30 min following the last instillation. The samples were frozen and stored at -20°C . The concentrations of active components in ACAH were measured by high-performance liquid chromatography–mass spectrometry (HPLC–MS) using Shimadzu LC-20AB HPLC System (column Agilent Extend with a 15 cm length, 2.1-mm internal diameter, particle size 5 μ m) and Shimadzu LCMS-2010EV (ESI) mass-spectrometer. Quantitative evaluation was performed by absolute calibration method. Statistical analysis was performed using standard software Statistica 8.0 (StatSoft).

RESULTS

Active components levels in Combinil Duo® eye drops were measured. Nominal concentrations of ciprofloxacin and dexamethasone fully match the concentrations claimed in the instruction (see Table 1). Combinil Duo® eye drops with the same batch number were used in the study.

Table 1. Active components measurement in Combinil Duo® eye drops

Eye drops	Active components content, percentage of claimed in the instruction
CombinilDuo®	Ciprofloxacin – 100%

	Dexamethasone – 100%
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Ciprofloxacin and dexamethasone levels in ACAH following Combinil Duo® eye drops instillations are presented in Table 2. Analysis of ACAH samples (n=14) following Combinil Duo® eye drops instillations revealed that maximum, minimum and average concentrations of ciprofloxacin were 0.25 µg/ml, 0.06 µg/ml and 0.13±0.06 µg/ml, respectively. Maximum, minimum and average concentrations of dexamethasone were 0.63 µg/ml, 0.14 µg/ml and 0.24±0.12 µg/ml, respectively.

Table 2. Concentrations of ciprofloxacin and dexamethasone (active components of Combinil Duo®) in ACAH

Sample No.	Ciprofloxacin concentration, µg/ml	Dexamethasone concentration, µg/ml
1	0.06	0.19
2	0.08	0.14
3	0.23	0.29
4	0.13	0.18
5	0.09	0.27
6	0.09	0.16
7	0.25	0.63
8	0.08	0.14
9	0.23	0.29

10	0.13	0.18
11	0.09	0.27
12	0.11	0.16
13	0.13	0.23
14	0.15	0.34
M ± m	0.13 ± 0.06	0.24 ± 0.12

DISCUSSION

The study of eye drops pharmacokinetics and pharmacodynamics is certainly of theoretical and practical importance, particularly for the evaluation of their efficacy. Previously, therapeutic concentrations of third- and fourth-generation quinolones in cataract patients ACAH before phacoemulsification were measured by HPLC-MS [10]. Considering that inflammatory eye diseases (including that of infectious origin) attract a great interest, we attempted to assess and to compare the penetration of Combiniil Duo® active components (ciprofloxacin and dexamethasone) into rabbit ACAH. The data on intraocular penetration of combination drugs containing steroids and antimicrobials into human ACAH are almost absent. The majority of pharmacokinetics studies in ophthalmology was performed on rabbits with intravenous or subconjunctival drugs administration [11]. The anatomy and physiology of animal visual analyzer differ from that of human. This must be considered when analyzing the results of pharmacokinetics animal studies as they can differ from clinical data. Eye drops can penetrate into ACAH through the cornea. Corneal epithelium is permeable for hydrophilic agents whereas stroma is a barrier for hydrophobic agents. Eye drops penetrability depends on drug molecular weight. The presence of adjuvants which are different by their chemical composition can affect the level of active components penetrating into ACAH as well. Ciprofloxacin contained in Combiniil Duo® eye drops has broad-spectrum antibacterial and bactericidal activity against Gram-positive and Gram-negative microbes. According to international investigators, ciprofloxacin level in rabbit ACAH following intravenous and subconjunctival administration was 0.0595 and 0.094 µg/ml, respectively [11]. The concentration of ciprofloxacin in the vitreous was 0.08-0.32 µg/ml [12]. Our results show

that ciprofloxacin level in rabbit ACAH is 0.13 ± 0.06 µg/ml. The data obtained indicate that the average concentration of ciprofloxacin in rabbit ACAH exceeds MIC90 for Gram-positive microbes which are the potential causative agents of infectious complications, i.e., methicillin-resistant *S. aureus* (MIC90=0.06 µg/ml) and fluoroquinolone-sensitive coagulase-negative staphylococci (MIC90=0.05 µg/ml). The comparison of experimental findings on ciprofloxacin level in rabbit ACAH and literary data on MIC90 values for endophthalmitis causative agents revealed that these concentrations are sufficient to minimize the risk of post-operative infectious complications. Dexamethasone level which provides local anti-inflammatory action was 0.24 ± 0.12 µg/ml. Anti-inflammatory agents containing in monodrugs and combined eye drops have different penetrability through the cornea into anterior chamber [14, 15, 16].

CONCLUSIONS

It was demonstrated that Combinil Duo® instillations provide the concentration of antibacterial medication (ciprofloxacin) and anti-inflammatory drug (dexamethasone) which are sufficient to prevent infectious inflammatory complications. These data can be applied in clinical practice to update the algorithms of inflammatory eye diseases prevention and treatment.

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