Reexamining Ophthalmic Drugs, Safety and Tolerability in Phase 1 Clinical Trials

Patricia del Carmen Muñoz Villegas (patriciamuv@gmail.com)
Laboratorios Sophia https://orcid.org/0000-0002-7559-1218
Andrea Anaid Navarro-Sánchez
Laboratorios Sophia
Alejandra Sánchez-Ríos
Laboratorios Sophia
Oscar Olvera-Montaño
Laboratorios Sophia
Leopoldo Martin Baiza-Durán
Laboratorios Sophia

Research article

Keywords: Healthy volunteer, Phase 1 trial, Safety, Tolerability, ophthalmic drug.

Posted Date: September 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-63599/v1

Abstract

Background: The purpose of this study was to evaluate the safety and tolerability profile of drugs used for treating common eye disorders when applied to normal healthy volunteers (NHV) as explored in phase 1 trials.

Methods: A total of 166 NHV were identified in six phase 1 trials. These included the exposure to lubricant (n=88), hypotensive (n=48) or antibiotic (n=30) ophthalmic drugs, examined in a retrospective analysis. The primary endpoints were visual comfort, assessed by the ocular comfort index (OCI); and safety, evaluated through laboratory evaluations, vital signs, visual acuity (VA), intraocular pressure (IOP), lissamine green and fluorescein staining, conjunctival hyperemia, chemosis, and the incidence of adverse events (AE). Other measured parameters included discomfort (assessed by burning, itching and foreign body sensation) and conjunctival impression cytology.

Results: Compared to baseline, 72.3%, 39.6% and 66.7% of participants (for lubricant, hypotensive and antibiotic treatments, respectively), improved their OCI score by their final visit (p=0.001). As expected for NHV, laboratory evaluations and vital signs were within normal ranges in 88% of NHV, with no significant differences observed between treatments. Similar results were found for VA, corneal and conjunctival staining and chemosis. IOP decreased significantly in the hypotensive agents group (p=0.001), trace to mild hyperemia was reported in 20.7%, 26% and 5.2% of NHV in each group (p=0.006). Additionally, lubricant and hypotensive investigational drugs (ID) had a lower risk of incidence of AE than approved drugs (AD) of these groups (OR 0.856, 95% CI [0.365, 1.999], and OR 0.636, 95% CI [0.096, 4.195], respectively). Meanwhile, on antibiotic drugs the risk for ID related AE was higher (OR 1.313, 95% CI [0.309, 5.583]).

Conclusions: Phase 1 trials are important in order to ensure the safety and tolerability of ophthalmic medications. This study demonstrates that NHV do not face a significant risk of harm in these studies, since 98% of the reported AE were mild, and all AE were resolved by the end of the study in which they appeared. Furthermore, it was also demonstrated that the instillation of ID is as safe and tolerable as that of AD in NHV.

Trial registration: The studies were registered at clinicaltrials.gov as follows: NCT04081610, NCT03524157, NCT03520348, NCT03966365, NCT03965052 and, NCT03519516.

Background

Clinical research can be divided into 4 phases, and the basic standards and technical requirements of each phase are strictly regulated. Phase 1 trials are performed on new drugs which have already cleared a previous pre-clinical safety and toxicology assessment [1]. During their participation in phase 1 trials, normal healthy volunteers (NHV) play an important role in identifying side effects of investigational drugs (ID) [2]. Any physical change, harmful or unpleasant reactions that a participant experiences while in such a trial, regardless of severity (mild, moderate or severe), is considered an adverse event (AE) rather than an “effect” because any detected symptoms may or may not be directly related to the experimental drug [2, 3]. In general, the overall risk of serious/severe AE has been estimated to be very low for NHV participating in phase 1 trials. Some common side effects such as headache, diarrhea, experiences of nausea, skin rashes, among others are frequently reported mild-AEs in trials evaluating systemic drugs [2, 4]. The phase 1 trials of ophthalmic medications included in this study varied in terms of the types of ID being tested, study procedures, and protocol designs. However, inclusion criteria generally matched or overlapped, including characteristics such as therapeutic area, and treatment-specific safety and tolerability parameters. They also included the evaluation of vital signs, laboratory tests, and matching protocol-defined parameters.

In order to present a wider view of NHV who have undergone ophthalmology trials, our study describes relevant elements of phase 1 clinical trials, including drugs used for common eye disorders: dry eye therapy, glaucoma and antibiotic agents [5]. Dry eye disease and glaucoma are two chronic eye diseases, with a broad spectrum of treatment options to manage both these conditions. Dry eye disease is characterized by instability of the tear film that can be due to either insufficient tear production or its poor quality [6]. The different stages of dry eye disease treatment have as common denominator the use of ocular lubricants, as recommended by the Tear Film and Ocular Surface Society’s Dry Eye Workshop (TFOS DEWS II). Lubricants are usually administered every 4 to 6 hours, but they can be used much more often following a “*pro re nata*” scheme. These variable dosage schemes and potentially frequent administration makes this type of medication’s safety profile important to know and analyze. Glaucoma is the second leading cause of blindness worldwide, and it is expected to affect as much as 79.6 million people by the end of this year; 74% of them with a diagnosis of primary open-angle glaucoma (POAG) [7]. Pharmacologic therapy continues to be the initial treatment for most patients with glaucoma. As the incidence of glaucoma increases with age, the patients often have numerous comorbidities and use various medications. Two or more drugs are often required for adequate mid- and long-term intraocular pressure (IOP) control. The efficacy and safety of fixed combinations offer multiple benefits as compared to concomitant application of their individual active ingredients [8, 9]. Finally, ocular infections, whether the affect superficial structures like the conjunctiva or cornea, or compromise inner segments of the eye, may be caused by a diverse group of bacteria, virus and fungi. Bacterial

conjunctivitis in adults is most commonly caused by gram-positive microorganisms such as *Staphylococcus* and *Streptococcus pneumonia*; as well as some gram-negative strains like *Haemophilus influenzae* y *Moraxella catarrhalis* [10, 11]. Widely used antibiotic eye drops possess a broad spectrum of action, and are effective against most ocular bacterial pathogens, however, the frequency of eye drop administration depends almost exclusively on the severity of the infection and its clinical presentation. Even though topically administered antibiotics are much less likely to cause AEs than those administered systemically, and their absorption generally appears under detectable limits, it is important to assess their safety and tolerability [5].

Furthermore, the results obtained in ophthalmic phase 1 clinical trials may provide valuable information in regards of the potential adherence to be expected for a topically applied solution. As has been stated earlier in this document, there are many ophthalmological diagnoses that require a specific dosage of a topically applied medication, including cases of multiple daily instillations for a long period of time. In order to continue protecting users from possible serious and irreversible consequences of an undertreated pathology, safety and tolerability profiles of common ophthalmic drugs must be studied.

The purpose of this study was to evaluate the tolerability, visual comfort and safety profile after ophthalmic drugs instillation (ID and approved drugs [AD]) in NHV enrolled in phase 1 clinical trials.

Methods

Study design

The design of this study was based on previous similar published works [12 – 14]. A retrospective analysis of the former phase 1 clinical trials executed by Laboratorios Sophia, S.A. de C.V. was performed. Six studies met the criteria to be considered in the analysis, including a prospective registration on clinicaltrials.gov, and whether the trial was completed as planned, with a median duration of 8.5 (7 to 10) day. The studies included were randomized, parallel, prospective, controlled, single-center trials. The studies were registered at clinicaltrials.gov as follows; NCT04081610, NCT03524157, NCT03520348, NCT03966365, NCT03965052 and, NCT03519516. They were conducted in six centers in Mexico after an ethics committee for each trial study reviewed and approved said study's protocol and its corresponding informed consent form (see **Declarations section**). All studies were conducted in compliance with the Declaration of Helsinki and in accordance with Good Clinical Practices Standards. All volunteers that participated provided written and signed informed consent. NHV were recruited between July 2017 and October 2019, see **Table 1**. This study adheres to CONSORT guidelines.

Subjects

Inclusion criteria included normal healthy male or female volunteers (aged 18 – 45 years), visual acuity of 20/30 or better, IOP $\geq$ 10 and $\leq$ 21, normal vital signs, normal laboratory evaluation with 20% of margin. In the case of women of childbearing age, a birth control method was necessary, because pregnancy/breastfeeding or high childbearing potential without a birth control method before inclusion were considered exclusion criteria. Other exclusion criteria were the prevalent use of topical ocular drugs, trial participation <90 days before signing informed consent, current pharmacological medication or herbology treatments (that may affect the study's outcomes) by any other route of administration and being a contact lens user. For more information about inclusion and exclusion criteria see **Supporting Information**.

Treatment and Evaluations

One hundred and sixty-six NHV were randomized in a 1:1 ratio to receive either a lubricant (ID: n=38 or AD: n=50), a hypotensive drug (ID: n=24 or AD: n=24), or an antibiotic drug (ID: n=15 or AD, n=15) through computer software randomization numbers (SAS Institute, Inc, Cary, NC, USA).The posology and experimental time of each trial are represented in **Table 1**.

For the analysis of the variables described during the ophthalmological exploration (VA, IOP, ocular surface staining, ocular symptomatology, and conjunctival impression cytology [CIC]), each eye was considered an individual case [15].

Primary Endpoints

Visual comfort: Ocular surface irritation was examined using the Ocular Comfort Index (OCI). The OCI questionnaire is suitable for assessing the impact of ocular surface disease and changes in severity to design therapeutic strategies. Only the extreme responses (0, never; 6, always) were labeled, in order to minimize the effects of differences in subjective interpretation [16].

Safety assessments: Safety was evaluated through changes on specified laboratory evaluations of interest including liver-associated enzymes (LAE), vital signs, visual acuity (VA), intraocular pressure (IOP), fluorescein and lissamine green corneal and conjunctival staining,

conjunctival hyperemia, chemosis and incidence of adverse events. The laboratory evaluations included glucose, creatinine, hematic cytometry, and LAE (AST, ALT, direct bilirubin (conjugated) and total bilirubin). To pool the data from different studies, LAE levels were converted to multiples of the upper limit of normal value (ULN) [17]. The measured vital signs were the heart and respiratory rate (HR and RR, respectively), and systolic and diastolic blood pressure (SBP and DBP, respectively). Potential safety signals for most laboratory evaluations were identified based on values that fell outside of normal reference ranges in Mexican population. The VA was determined with a Snellen chart. The IOP was measured using a calibrated Goldmann applanation tonometer. The ocular surface was evaluated with a slit lamp, aided by fluorescein and green lissamine staining (GLS). Surface dye staining was classified in a scale from 0 to V in accordance with the percentage of affected area (Oxford scale). Changes in conjunctival hyperemia were assayed with the Efron scale and incidence of chemosis was recorded. For AE evaluation, any method used in the clinical trials to elicit participant/reported AE, such as a diary, checklist, memory aid, etc., whether applied face-to-face or otherwise were considered. AEs occurring during each clinical protocol were recorded and included in this analysis.

Secondary Endpoints:

NHV under lubricants and antibiotic trials were questioned about their satisfaction with their respective treatment (discomfort), and it was measured through the ocular symptomatology post-installation determined by burning, itching and foreign body sensation (FBS) using a survey questionnaire. Additionally, only in trials involving lubricants goblet cells density and Nelson's grades were determined by CIC.

Data Analysis

Since this study was not designed to test a specific hypothesis, a sample size was not calculated based on statistical power calculations. We investigated the association of the type of treatment (lubricant, hypotensive or antibiotic drug) and their safety profile. In a second analysis, we stratified data by type of drugs: ID versus AD (pre-specified in each protocol). The categorization was based on the conclusion of the study (e.g. the ID was than safer as AD). Evaluations were performed in per-protocol population, established as a randomized participant with no major deviation from the protocol (PP; n=162 NHV). All the participants who were enrolled in each study were included in the AE analyses (intent-to treat population, ITT; n=166 NHV). The continuous variables were assessed using a general lineal model (GLM) multivariate analysis and repeated measures for data collected at three times. Tukey's comparisons were used when required for the post hoc analyses. The ordinal variables were analyzed using 2 x 2 contingency tables and the differences were calculated with Pearson Chi-square test or Fisher's exact test. For the AE analysis, a logistic regression was used to calculate odd ratio (OR) and 95% confidence interval (CI) for the association between determinants and studies. ORs were used to determine whether the ID exposure may be a risk factor for the incidence of AE, and to compare the magnitude of type of drug risk factor for that outcome as follows: OR=1, exposure does not affect odds of outcome; OR>1, exposure associated with higher odds of outcome, and OR<1, exposure associated with lower odds of outcome [18]. All data analyses were in SPSS 19.0 software for Windows (SPSS Inc., Chicago, IL, USA). A p-value ≤ 0.05 was considered significant.

Results

Characteristics of the participants: A total of 166 NHV, were enrolled, two subjects discontinued their participation because of either AE (ocular hypotonia and rhinitis), other for protocol deviations , and another due to poor adherence of indicated treatment (<80%). Therefore, 162 NHV completed their entire protocol without deviations up to the safety call, instilling ID or AD during a study from different therapeutic areas, including lubricants, hypotensive agents and antibiotics see **Figure 1**. There were no demographic or clinically relevant differences at baseline between treatment groups. Mean age ± standard deviation (SD) was 27.41 ± 6.6 years (range 18 – 45), 53.6% of the NHVs were female (Fisher exact test, p=0.641), see **Table 2**.

Primary Endpoints

Visual Comfort: Baseline OCI score (mean ± SD) was similar between lubricant and hypotensive treatment groups (see **Table 2**); meanwhile, the antibiotic group had a lower basal OCI score ($F_{(2,165)}$=11.760; p=0.0001). On the final visit, 72.3%, 39.6% and 66.7% of the NHV in each group improved their initial score (Pearson Chi-square test, p=0.001). The mean of change ± SD from baseline to final visit was -6.24 ± 12.5 for lubricant, 2.12 ± 10.9 for hypotensive and, -3.31 ± 9.8 for antibiotic group. The hypotensive group showed a significant increase in their score compared to lubricant (Tukey test, p=0.0001). Additionally, no significant differences between type of drug (ID or AD) were observed ($F_{(1,158)}$=0.011, p=0.924), also between-factor interaction (treatment x type of drug) was not significant (p=0.356), see **Table 3**.

Laboratory Evaluations: As expected for NHV, laboratory results for hematological and biochemical parameters were within normal ranges at baseline. We did, however, find statistically significant differences on HTC, HGB and MCV levels (all of them, under ULN). The hypotensive group levels were higher than that of lubricant and antibiotic groups (Tukey test, p<0.05). On the final visit, the MCV level was significantly different between treatments (p=0.0001), also we found differences between type of drug, on AST and total bilirubin (p<0.05), however

between-factor interaction (visit x treatment x type of drug) was not significant (p>0.05), see **Table 4**. ALT and AST elevations were observed in three subjects under lubricants and other on hypotensive study group (2.4%), from baseline to final visit (reported as an AE). Two subjects (AD) had values greater than 1.2 times ULN-AST. For ALT levels, two NHV had values 1.5 times higher, and other 2.6 times higher, meanwhile only one NHV in ID had one value greater than 1.02 times ULN during the treatment. LAE were resolved by the end of follow-up study period and no safety additional issues were raised.

Vital Signs: As expected for NHV vital signs parameters were within normal ranges at baseline. Relatively few vital signs values were statistically lower between treatments at baseline. The RR in hypotensive group ($F_{(2,164)}$=4.209; p=0.017), the SBP in antibiotic ($F_{(2,164)}$=3.446; p=0.034) and, the DBP in lubricant group ($F_{(2,164)}$=4.180; p=0.017) were consistently and significantly lower at the final visit (p-values: 0.001, 0.049 and, 0.006 respectively). However, the interactions between-factors (visit x treatment and visit x type of drug) were not significant (p>0.05), see **Table 5**. On final visit, only 1 (0.6%) NHV had high HR, 12 (7.3%) NHV had high SBP, and 3 (1.8%) NHV had high DBP. However, these findings were not reported as AEs.

Visual acuity (VA) and intraocular pressure (IOP): Baseline VA was similar between groups however, the mean value for the antibiotic group was lower compared with the other treatments ($F_{(2,302)}$=19.956, p=0.048). The type of drugs was different too (p=0.048), but the interaction (treatment x type of drug) was not significant (p=0.681). On the final visit, no significant differences between treatments, or types of drugs, were observed (p>0.05). The visit x treatment interaction was significant (p=0.016) but visit x type of drug and between-factors interactions were not significant (0.166 and 0.562, respectively), see **Table 3**.

Baseline IOP was similar between treatments and type of drug, without differences (p-values: 0.062 and 0.691, respectively). On the final visit, as expected, the IOP decreased significantly by 2.3 mmHg in the hypotensive agents group (p=0.0001), without differences between the type of drug (p=0.913). The visit x treatment interaction was significant (p=0.0001) but visit x type of drug and between-factors interactions were not significant (0.830 and 0.298, respectively), see **Table 3**.

Fluorescein and lissamine green staining: On the final visit, fluorescein staining was graded as absent (grade 0, Oxford scale) for 81.3% and as minimal-to-mild (grade I or II) for 18.7% of NHV exposed to lubricants. Meanwhile 89.6% and 94.8% were absent and 10.4% and 5.2% were grade minimal for hypotensive and antibiotic treatments, respectively (Pearson Chi-square test, p=0.088). No differences were observed between the factor type of drug on baseline and at the final visit (Pearson Chi-square test, p-values; 0.915 and 0.416). Similar percentages for LGS were observed on final visit, 85.3% was absent and 14.7% minimal-to-mild for lubricant, 88.5% absent and, 11.5% was minimal-to-mild for hypotensive, meanwhile 96.6% was absent and 3.4% minimal for antibiotic group (Pearson Chi-square test, p=0.051). No differences were observed between the factor type of drug on baseline and at the final visit (p-values; 0.257 and 0.053), see **Table 3**.

Conjunctival hyperemia and chemosis: Similar findings were observed in the analysis of conjunctival hyperemia; after the intervention time and compared with baseline, there was no significant improvement in all groups, It was graded as trace to mild hyperemia for lubricant (20.7%), hypotensive (26%) and antibiotic groups (5.2%) (Pearson Chi-square test, p=0.006). The type of drug was significant on baseline (p=0.022), but this finding was not retained in the final visit (Pearson Chi-square test, p=0.638). Finally, no participants presented chemosis before or after their respective treatment, see **Table 3**.

Adverse events: A total of 205 AE occurred in 59.6% of NHV (99/166). The hypotensive group had a higher incidence of AE than that the other treatments (Pearson Chi-square test, p=0.0001). A total of 65 AEs/39 NHV were reported for lubricants (46.2% for ID vs 53.8% in AD, p=0.781), 117 AE/43 NHV for hypotensive agents (47% for ID vs 53% in AD, p=0.705), and 23 AE/17 NHV (56.5% for ID vs 43.5% in AD, p=0.559) for antibiotic drugs. The most common class of reported AE were burning (30.7% [26.2%, 27.4%, and 60.9% in lubricant, hypotensive and antibiotic treatments respectively]), followed by conjunctival hyperemia (11.7% [19.7% and 4.3% in hypotensive and antibiotic treatments]), and itching (10.7% [15.4%, 6.8%, and 17.4% in lubricant, hypotensive and antibiotic treatments respectively]), see **Figure 2**. There was a total of 201 mild AE (98% of the total AE), 3 moderate (1.4%), and one serious AE (0.5%, ocular hypotonia), without differences between treatments (Pearson Chi-square test, p=0.667). Only 14.6% of the AE were deemed unrelated to the study medication. No deaths were reported. The logistic regression (used to calculate ORs), found that in lubricant trials, the ID had a lower risk of incidence of AE than AD, OR 0.856, 95% CI, [0.365, 1.999]. Similar results occurred in hypotensive drugs, OR 0.636, 95% CI, [0.096, 4.197], nevertheless for antibiotic drugs, the risk of incidence for ID was higher than AD, OR 1.313, 95% CI, [0.309, 5.583]. For all groups, no differences between the risk for occurrence of AE in ID versus AD were observed, OR 1.008, 95% CI, [0.541, 1.887].

Secondary endpoints

Burning, itching and FBS, were considered the parameters to evaluate discomfort. In all studies, lubricants and antibiotics were well tolerated. At the final visit, burning sensation was reported in 19.5% NHV for lubricants and 27.6% for the antibiotic group (Pearson Chi-square test, p=0.191). No significant differences were observed between types of drugs in each treatment (p>0.05). Findings were similar for

itching between treatments: 23.2% for lubricant, and 17.2% for antibiotic group reported itching at the final visit (Pearson Chi-square test, p=0.650). For each treatment, there were no differences between ID and AD (Pearson Chi-square test, p-values; 0.343 and 0.741). Additionally, only 13.4% of NHV exposed to lubricants reported FBS at the final visit (Fisher exact test, p=0.003), however, these findings were not statistically different in the analysis between types of drugs (Fisher exact test, p=1.000). Finally, compared to baseline, there was a significant increase in conjunctival goblet cell density (280.2 ± 140.3 cell/mm^2 vs 332.3 ± 126.7 cell/mm^2, p=0.001) by the final visit in the lubricants group. At baseline, 37.8% NHV were classified as having normal CIC grade-0 (classification of Nelson), by the final visit, 41.5% had grade 0, without significant differences (p=0.700). No significant differences were observed between type of drugs on baseline and final visit (Pearson Chi-square test, p-values; 0.957 and 0.533).

Discussion

Phase 1 trial participants are typically healthy volunteers who pass health screenings and have no identifiable medical conditions related to the investigative drugs [2]. Additionally, because these volunteers are in “good health”, they gain no direct medical benefit from research participation; also, they are typically recurring participants enrolling serially in phase 1 trials [19]. The main purpose for executing phase 1 trials is to evaluate a drug´s safety, with a low risk of originating numerous and/or severe AEs in unaffected parties. Participating in such trials requires every NHV to report any symptoms experienced during the study in order to identify all the adverse events potentially related to the ID [2]. In this case, the six trials included were comparative studies with the approved counterpart of each ID, therefore allowing the confirmation of previously described AE or the addition of newly discovered unfavorable related symptoms. More so, evaluating the tolerability of such ophthalmic products in NHV may translate in a clinically and statistically significant conjecture on how the symptoms associated to their application may affect the user’s adherence and consequently their efficacy once they are commercialized.

The OCI was selected to evaluate tolerability since it provides a valid measurement on the basis of Rasch analysis, more so than other questionnaires like the Ocular Surface Disease Index (OSDI) or McMonnies, However, all these tools have shown weak correlations with objective dry eye disease clinical tests like VA, tear break-up time or corneal fluorescein staining [20]. Because of this, it is advisable to the use both subjective and objective markers as endpoints in clinical trials for ophthalmic lubricants. On the other hand, topical hypotensive agents, of any drug families available today, characterize themselves for giving rise to ocular surface symptoms in a more prevalent and severe fashion than other ophthalmic products [21]. This study confirms this presumption as shown by the decrease in OCI scores by the final visit of NHVs in the hypotensive agents group, being this decrease significantly lesser for them in comparison to those in the antibiotics and lubricants groups (39.6%, 66.7% and 72.3%, respectively).

For other variables regarding ocular surface evaluation, such as hyperemia, NHVs exposed to hypotensive agents and lubricants did not present a statistically significant difference (26% and 20.7%, respectively), whereas the antibiotic group presented a lower incidence (5.2%). Both fluorescein and lissamine green staining did not show a difference when comparing basal and final visits for any type of medications.

A total of 205 AE in 99 NHV (59.6%; 99/166) were reported. For the hypotensive agents group 117 AE were present in 43 NHV (98.3% considered mild), a significantly greater number than that of lubricants and antibiotics. (p = 0.0001) As shown in Fig. 2, for every group of medications there was no difference between ID and AD groups (lubricants, p = 0.781; hypotensive agents, p = 0.705; antibiotics, p = 0.559). However, it is relevant to point out that for the ocular hypotensive agents group, all the studied variables were also considered AEs; whereas for the lubricants and antibiotics groups the AEs and the findings of the ophthalmological explorations were analyzed separately in the original trials. It is also worth mentioning that none of the total moderate (3) AEs were considered related to the use of the studied ophthalmic products. Concerning the only reported severe AE, as expected with the hypotensive agents, the IOP decreased significantly in one NHV who experienced a decrease of ~ 8 mmHg. However, by the final follow up visit, this parameter had normalized, and no safety additional issues had been raised. Similarly, decreased in IOP has been observed in other phase 1 trials where NHV receiving hypotensive agents like beta-blockers or prostaglandin analogues also reported a significant IOP drop [22, 23].

The vital signs are fundamental to assess any drug’s general safety profile because they are objective measurements of essential physiological functions. Even though the systemic effects of ophthalmic solutions are rare, some compounds have reported to cause systemic alterations after absorption into the bloodstream, particularly those used to lower de IOP since their mechanism of action may influence receptors in the cardiovascular and pulmonary systems. Blood pressure, heart and respiratory frequencies were measured. Basal values were normal, and by the final visit there were no clinical or statistically significant differences, observing only a slight raise in HR, SBP and DBP (0.6%, 7.3%, and 1.8%, respectively). Despite the reported potential variations of vital signs after instillation, NHV exposed to hypotensive agents presented an increase of DBP equivalent to a nonsignificant p = 0.006, while the rest of parameters being either equal or lower than those belonging to the other two study groups.

Regarding the laboratory evaluations, some participants were considered to suffer transaminitis and were considered to present an AE for such results. However, it is important to mention that none of those NHV had any other related signs or symptoms, and that all cases remitted within the 7-day follow-up without clinical relevance. Furthermore, a slight increment of ALT (10% above de ULN) and AST or bilirubin (20% above the ULN) has been reported in the literature as acceptable as long as no other signs or symptoms of apparent disease are present [24]. It has also been reported that ALT elevation above the ULN can occur in participants of phase 1 trials with no history of significant disease being treated only with placebo. Out of the volunteers receiving placebo for 14 days, 20% reported at least one value between one and two times the ULN value, and some even obtained values higher than twice the ULN. The probability of having an increased value raises with repeated measurements [17]. Such increments in healthy participants can be explained by genetic polymorphisms, intraindividual short-term (1–7 days) and long-term variations, weight, body mass index, age, sex, physical activity and maintained calorie intake [17, 24–26]. Hence, the results of transaminases in phase 1 trials should be analyzed and interpreted carefully to avoid misdiagnosed hepatotoxicity [17].

Finally, goblet cell density was evaluated for the lubricant group, and an increase, though statistically significant, was observed. This increase coincides with reports portrayed in other studies and may translate in a clinically meaningful enhancement.

Summarizing, all the data analyzed in this study, from the subjective OCI questionnaire to the objective evaluation of ophthalmological variables, systemic examinations and laboratory results, ascertains that ophthalmic medications from three different pharmaceutical families are safe and tolerable when applied on the ocular surface of healthy volunteers. No new adverse events were detected beyond those previously described in literature for each group; however, for both lubricants and hypotensive agents, the investigative drugs proved to have a lesser risk to produce adverse events, in comparison to their approved counterparts.

This study had several limitations. First, the data presented belonged to NHV under pharmacological treatment, but no participants assigned to placebo were included. This means that interpretations of safety and tolerability may be biased [2, 4]. A second limitation is enrollment of Mexican population from the same city, not having a random sample from overall Mexican population. However, considering the ethnical background of Mexicans in general, it is not expected to have a great variation of phenotypes since the habitants ancestry is fairly homogeneous [27]. Thirdly, due to ethical reasons, the trials included in this study were executed following a single dose/concentration schedule, in order to reduce the unnecessary risk of possible harm, especially since the active ingredients are known compounds and their posology has already been stablished. However, despite these limitations, the overall strength of our statistical analyses and the data management allowed a mitigation of these risk perceptions.

Conclusion

In conclusion, phase 1 clinical trials of ophthalmologic medications provide meaningful information with a small risk of yielding any severe or long-lasting adverse events. Furthermore, it was also demonstrated that the instillation of ID is as safe and tolerable as that of AD in NHV. Safety and tolerability profiling can not only protect future patients of deleterious local or systemic signs or symptoms induced by ophthalmic drugs, but also assess the tolerability associated with them and therefore their potential effectiveness both through their actions on the ocular surface and the prospective adherence for medium and long treatment periods.

Abbreviations

AD, approved drug; AE, adverse events; ALT, alanine transaminase; AST, aspartate transaminase; CIC, conjunctival impression cytology; DBP, diastolic blood pressure; FBS, foreign body sensation; GLS, green lissamine staining; HCT, hematocrit; HGB, hemoglobin; ID, investigational drug; IOP, intraocular pressure; LAE, liver-associated enzymes; NHV, normal healthy volunteer; OCI, ocular comfort index; RBC, red blood cell count; SBP, systolic blood pressure; ULN, upper limit of normal value; VA, visual acuity; WCB, white cell count.

Declarations

Ethics approval and Consent to Participate: The studies' protocols were approved by their respective Institutional Review Boards, as follows: Comité de Ética en Investigación Hospital Real San José; Comité de Investigación Instituto Jalisciense de Investigación Clínica S.A. de C.V.; Comité de Ética en Investigación Centro Hospitalario Vicor, S.A. de C.V., CHG Hospitales; and Comité de Ética en Investigación de la Unidad de Bioequivalencia, S. de R.L. de C.V. All volunteers that participated provided written and signed informed consent.

Consent for publication: Not applicable.

Availability of data: The datasets generated and/or analysed during the current study are available in the Open Science Framework (https://osf.io) repository, as DOI 10.17605/OSF.IO/GZU6J.

Competing Interest: The authors declare that they have no other competing interest in this work.

Funding: This study was sponsored by Laboratorios Sophia, S.A. de C.V. (Zapopan, Jalisco, Mexico). The sponsor provided support in the form of salaries for authors (MVP, NSA, SRA, OMO, and BDL), this does not alter our adherence to the Good Publication Practice guidelines for pharmaceutical companies (GPP3) polices on sharing data and materials.

Authors' contribution: MVP, NSA, and SRA wrote the manuscript with support from OMO. MVP analysed and interpreted the data. NSA and SRA contributed to the design and implementation of the research. OMO and BDL supervised the project. All authors read and approved the final manuscript.

Acknowledgments: The authors thank Dr. Alfredo Gazca (Biomedical Research G&L, S. de R.L. de C.V.), Dr. Mariana Díaz (Private office), Dr. Anel De Luca (Centro Oftalmológico San Ángel), Dr. Manuel Zepeda (Unidad Clínica de Bioequivalencia, S. de R.L. de C.V.), Dr. José Navarro (Private Office), and Dr. Miguel Padilla (Private Office).

References

1. Wei Y, Li H, Wang H, Zhang S, Sun Y. Pychological status of volunteers in phase I clinical trial assessed by symptom chechlist 90 (SCL-90) and Eysenck personality questionnaire (EPQ). Med Sci Monit, 2018;24:4968-73.
2. McManus L, Fisher JA. To report or not to report: exploring healthy volunteers' rationales for disclosing adverse events in phase I drug trials. AJOB Empir Bioeth, 2018;9(2):82-90.
3. Edwards IR, Aronson JK. Adverse drug reactions: definitions, diagnosisi, and management. Lancet, 2000;356(9237):1255-9.
4. Johnson RA, Rid A, Emanuel E, Wendler D. Risk of phase I research with healthy participants: a systematic review. Clin Trials, 2016;13(2):149-60.
5. Drugs for some common eye disorders. JAMA, 2020;323(5):470-71.
6. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye Workshop (2007). Ocul Surf, 2007;5(2):93-107.
7. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol, 2006;90(3):262-7.
8. Holló G, Topouzis F, Fechtner RD. Fixed-combination intraocular pressure-lowering therapy for glaucoma and ocular hypertension: advantages in clinical practice. Expert Opin Pharmacother, 2014;15(12):1737-47.
9. Gómez-Aguayo F, Paczka JA, Leñero-Córdova R, et al. A phase III randomized clinical trial of a 0.5% timolol + 0.2% brimonidine + 2.0% dorzolamide fixed combination, preservative-free ophthalmic solution vs 0.5% timolol + 0.2% brimonidine + 2.0% dorzolamide fixed combination in patients with controlled POAG. Ophthalmol Ther, 2018;7(1):145-56.
10. Hutnik C, Mohammad-Shahi MH. Bacterial conjunctivitis. Clin Ophthalmol, 2010;4:1451-7.
11. Seal DV, Barrett SP, McGill JI. Aetiology and treatment of acute bacterial infection of the external eye. Br J Ophthalmol, 1982;66(6):357-60.
12. Cai Z, Christianson AM, Ståhle L, Keisu M. Reexamining transaminase elevation in phase I clinical trials: the importance of baseline and change from baseline. Eur J Clin Pharmacol, 2009;65,(10):1025-35.
13. van den Bogert CA, Souverein PC, Brekelmans CTM, et al. Non-publication is common among phase 1, single-center, not prospectively registered, or early terminated clinical drug trials. PLoS ONE, 2016;11(12):e0167709.
14. Allen EN, Chandler CI, Mandimika N, et al. Eliciting adverse effects data from participants in clinical trials. Cochrane Database Syst Rev, 2018; 1(1): MR000039.
15. Sullivan BD, Crews LA, Messmer EM, et al. Correlations between commonly used objetive signs and symptoms for the diagnosis of dry eye disease: clinical implications. Acta Ophthalmol, 20914;92(2):161-6.
16. Johnson ME, Murphy PJ. Measurement of ocular surface irritation on a linear interval scale with the ocular comfort index. Invest Ophthalmol Vis Sci, 2007;48(10):4451-8.
17. Rosenzweig P, Miget N, Brohier S. Transaminase elevation on placebo during phase I trials: prevalence and significance. Br J Clin Pharmacol, 1999;48(1):19-23.
18. Szumilas M. Explaining odds ratios. J Can Acad Child Adolesc Psychiatry, 2010;19(3):227-29.
19. Walker RL, Cottingham MD, Fisher JA. Serial participation and the ethics of phase 1 healthy volunteer research. J Med Philos, 2018;43(1):83-114.

20. McAlinden C, Gao R, Wang Q, et al. Rasch analysis of three dry eye questionnaires and correlates with objetive clinical tests. Ocul Surf, 2017;15(2):202-10.
21. Zhang X, Vadoothker, Munir WM, Saeedi O. Ocular surface disease and glaucoma medications: a clinical approach. Eye Contact Lens, 2020;45(1):11-18.
22. Mottow-Lippa LS, Lippa EA, Naidoff MA, et al. 008% timolol ophthalmic solution. A minimal-effect dose in a normal volunteer model. Arch Ophthalmol, 1990;108(1):61-4.
23. Sponsel WE, Mensah J, Kiel JW, et al. Effects of latanoprost and timolol-XE on hydrodynamics in the normal eye. Am J Ophthalmol, 2000;130(2):151-9.
24. Breithaupt-Groegler K, Coch C, Coenen M, et al. Who is a 'healthy subject'?-consensus results on pivotal eligibility criteria for clinical trials. Eur J Clin Pharmacol, 2017;73(4):409-16.
25. Piton A, Poynard T, Imbert-Bismut F, et al. Factors Associated With Serum Alanine Transaminase Activity in Healthy Subjects: Consequences for the Definition of Normal Values, for Selection of Blood Donors, and for Patients With Chronic Hepatitis C. MULTIVIRC Group. Hepatology 1998;27(5):1213-9.
26. Pacifico L, Ferraro F, Bonci E, et al. Upper limit of normal for alanine aminotransferase: Quo vadis?. Clinica Chimica Acta, 2013;422:29-39.
27. Romero-Hidalgo S, Ochoa-Leyva A, Garcíarrubio A, et al. Demografic history and biologically relevant genetic variation of Native Mexicans inferred from whole-genome sequencing. Nat Commun, 2017;8(1):1005.

Tables

Table 1
Characteristics of studies

NCT number	Treatment	Study design	FPFV – LPLV	Enrolled (n)	Treatment duration	Posology	Visits
NCT04081610	• SH-PF; PRO-037 • SH-PF; Lagricel® Ofteno (LOF)	Randomized, opened, parallel, controlled	09/19/19–10/23/19	34	7 days	1 drop QID, both eyes	2 (BV, FV)
NCT03524157	• SH/CS-PF; PRO-087 • Xanthan gum/CS-PF; Xyel® Ofteno (XOF) • PEG/PG; Systane® Ultra	Randomized, double-blind, parallel, controlled	07/18/17–02/21/18	30	10 days	1 drop QID, both eyes	5 (SV, BV, V1–2, FV)
NCT03520348	• Dexpanthenol; PRO-167 • Dexpanthenol; Corneregel®	Randomized, double-blind, parallel, controlled	10/12/17–05/22/18	24	10 days	QID, right eye (approximately 1 centimeter)	4 (SV, BV, V1, FV)
NCT03966365	• Timolol/Brimonidine/Dorzolamide PF-fixed combination; PRO-122 • Timolol/Brimonidine/Dorzolamide fixed combination; Krytantek® Ofteno (KOF)	Randomized, double-blind, parallel, controlled	05/03/19–07/02/19	24	7 days	1 drop BID, both eyes	4 (SV, BV, V1, FV)
NCT03965052	• Travoprost; PRO-179 • Travoprost; travatan®	Randomized, double-blind, parallel, controlled	04/24/19–07/02/19	24	10 days	1 drop QID (at night), both eyes	4 (SV, BV, V1, FV)
NCT03519516	• Levofloxacin; PRO-174 • Ciprofloxacin; Sophixin® Ofteno (SOF)	Randomized, double-blind, parallel, controlled	02/19/18–05/14/18	30	7 days	- Days 1 and 2: 1 drop, 8 daily applications (every 2 hours), both eyes -Days 3–7: 1 drop QID (every 4 hours), both eyes	4 (SV, BV, V1, FV)

Abbreviatures: BID, twice a day; BV, basal visit; CS, chondroitin sulfate; FPFV, first patient-first visit; FV, final visit; LPLV, last patient-last visit; PEG, polyethylene glycol 400; PF, preservative-free; PG, propylene glycol; QID, four times a day ;SH, sodium hyaluronate; SV, screening visit.

Investigational drugs: PRO-037, PRO-087, PRO-167, PRO-122, PRO-179, and PRO-174, by Laboratorios Sophia, S.A. de C.V., Zapopan, Jal, Mexico.

Approved drugs: Lagricel® Ofteno, Xyel® Ofteno, Krytantek® Ofteno, and Sophixin® Ofteno by Laboratorios Sophia, S.A. de C.V., Zapopan, Jal, Mexico, Systane® Ultra, Alcon Laboratories, Inc., Fort Worth, TX, USA, Corneregel®, Bausch & Lomb, Inc., Berlin, Germany, Travatan®, Alcon Laboratories, Inc., Fort Worth, TX, USA.

Table 2
Initial characteristics of each group (n = 166 NHV; 308 eyes)

	Lubricant	Hypotensive	Antibiotic
Female / Male[a], %	53.4 / 46.6	47.9 / 52.1	63.3 / 36.7
Age[b], year ± SD	28.44 ± 6.4	26.21 ± 6.1	26.30 ± 7.6
OCI[b], score ± SD	26.98 ± 11.6	24.91 ± 9.2	15.63 ± 12.3*
IOP[b], mmHg ± SD	13.17 ± 1.9	13.81 ± 2.4	12.62 ± 1.5
VA[b], Snellen ± SD	20.85 ± 2.4	21.95 ± 3.5*	20.33 ± 1.6
Conjunctival hyperemia grade 0[a], n (%)	98 (64.5)	81 (84.4)	60 (100)*
LGS grade 0[a], n (%)	119 (78.3)	83 (86.5)	60 (100)*
Fluorescein staining grade 0[a], n (%)	116 (76.3)	90 (93.8)	60 (100)*

Abbreviatures: IOP, intraocular pressure; LGS, lissamine green staining; NHV, normal healthy volunteer; OCI, ocular comfort index; SD, standard deviation; VA, visual acuity.

[a]Chi square test, [b]ANOVA, between treatments, *p < 0.05.

Table 3
Ophthalmological exploration at final visit (n = 164, NHV; 304 eyes)

	Lubricant	Hypotensive	Antibiotic
OCI[b], score ± SD	20.40 ± 11.1	27.03 ± 10.8	12.85 ± 11.8*
IOP[b], mmHg ± SD	12.70 ± 1.6	11.53 ± 2.1*	12.60 ± 1.4
VA[b], Snellen ± SD	20.53 ± 1.9	21.07 ± 2.9	20.66 ± 2.2
Conjunctival hyperemia grade 0[a], n (%)	119 (79.3)	71 (74.0)	55 (94.8)*
LGS grade 0[a], n (%)	128 (85.3)	85 (88.5)	56 (96.6)
Fluorescein staining grade 0[a], n (%)	122 (81.3)	86 (89.6)	55 (94.8)*

Abbreviatures: IOP, intraocular pressure; LGS, lissamine green staining; NHV, normal healthy volunteer; OCI, ocular comfort index; SD, standard deviation; VA, visual acuity.

[a]Chi square test, [b]ANOVA, between treatments, *p < 0.05.

Table 4
Hematological and biochemical parameters

Treatment	Type of drug	HTC, % (40–54)	HGB, g/dL (12–18)	RBC, M/Ul (4.5–6.5)	MCV, fL (84–104)	WBC, mi/Ul (4–11)	Platelet, mi/Ul (150–400)
		Mean ± Standard deviation					
Baseline hematological parameters							
Lubricants	ID	42.44 ± 3.9	13.83 ± 1.4	4.66 ± 0.4	91.24 ± 3.3	6.74 ± 1.4	253.45 ± 44.6
(n = 53)	AD	43.76 ± 4.0	14.48 ± 1.6	4.83 ± 0.4	90.70 ± 3.9	6.51 ± 1.3	254.42 ± 51.6
Hypotensive	ID	45.55 ± 3.9	14.74 ± 1.3	4.89 ± 0.5	93.28 ± 4.6	7.09 ± 2.5	240.29 ± 54.2
(n = 48)	AD	46.93 ± 4.8	15.20 ± 1.5	4.95 ± 0.5	94.96 ± 3.8	7.13 ± 1.7	241.50 ± 39.8
Antibiotic	ID	43.63 ± 3.9	14.21 ± 1.3	4.77 ± 0.5	91.67 ± 3.1	6.19 ± 1.4	254.00 ± 43.9
(n = 30)	AD	42.51 ± 4.8	13.85 ± 1.8	4.63 ± 0.7	92.34 ± 4.2	6.28 ± 1.7	257.67 ± 49.3
Final hematological parameters							
Lubricants	ID	42.19 ± 3.8	13.75 ± 1.4	4.64 ± 0.4	91.17 ± 3.7	6.63 ± 1.5	249.00 ± 39.3
(n = 52)	AD	43.66 ± 4.0	14.31 ± 1.5	4.81 ± 0.4	90.89 ± 4.1	6.57 ± 1.4	252.60 ± 48.0
Hypotensive	ID	45.58 ± 4.2	14.76 ± 1.4	4.92 ± 0.5	92.87 ± 4.1	6.83 ± 1.6	254.04 ± 52.0
(n = 46)	AD	46.01 ± 4.2	14.99 ± 1.3	4.89 ± 0.4	94.23 ± 3.8	6.61 ± 1.6	261.04 ± 53.8
Antibiotic	ID	44.21 ± 4.0	14.17 ± 1.3	4.78 ± 0.5	92.71 ± 3.0	6.14 ± 1.6	253.13 ± 38.1
(n = 29)	AD	43.05 ± 4.5	13.85 ± 1.7	4.64 ± 0.6	93.11 ± 3.9	6.24 ± 1.6	239.14 ± 30.7
p^1		0.884	0.250	0.276	0.0001	0.345	0.103
p^2		0.213	0.154	0.296	0.532	0.544	0.780
Treatment	Type of drug	Glucose, mg/dL (70–100)	Creatinine, mg/dL (0.7–1.3)	AST, U/L (0–38)	ALT, U/L (0–41)	TB, mg/dL (0–1.1)	DB, mg/dL (0–0.3)
		Mean ± Standard deviation					
Baseline biochemical parameters							
Lubricants	ID	81.05 ± 6.8	0.87 ± 0.2	19.91 ± 6.5	19.23 ± 8.4	0.57 ± 0.2	0.14 ± 0.1
(n = 53)	AD	83.48 ± 7.6	0.85 ± 0.1	20.48 ± 4.0	21.41 ± 9.5	0.66 ± 0.2	0.15 ± 0.1
Hypotensive	ID	85.83 ± 7.2	0.82 ± 0.2	18.57 ± 4.1	20.96 ± 10.5	0.67 ± 0.2	0.13 ± 0.0
(n = 48)	AD	85.91 ± 6.9	0.81 ± 0.2	17.17 ± 5.9	16.57 ± 7.9	0.65 ± 0.2	0.12 ± 0.0
Antibiotic	ID	86.54 ± 6.9	0.85 ± 0.1	18.93 ± 6.6	21.00 ± 7.9	0.67 ± 0.3	0.15 ± 0.1
(n = 30)	AD	81.33 ± 7.3	0.83 ± 0.1	17.93 ± 7.2	17.80 ± 9.0	0.68 ± 0.2	0.17 ± 0.1
Final biochemical parameters							
Lubricants	ID	82.18 ± 6.4	0.88 ± 0.1	17.55 ± 3.9	20.82 ± 11.5	0.62 ± 0.2	0.14 ± 0.1
(n = 52)	AD	84.14 ± 7.4	0.85 ± 0.1	20.70 ± 6.9	25.17 ± 18.6	0.59 ± 0.2	0.14 ± 0.1
Hypotensive	ID	89.92 ± 8.6	0.82 ± 0.2	18.08 ± 4.7	18.67 ± 10.2	0.68 ± 0.3	0.12 ± 0.1
(n = 46)	AD	87.39 ± 6.4	0.80 ± 0.1	20.17 ± 7.1	19.25 ± 10.6	0.69 ± 0.3	0.14 ± 0.1
Antibiotic	ID	87.07 ± 6.7	0.85 ± 0.1	19.07 ± 5.9	20.27 ± 6.1	0.67 ± 0.2	0.15 ± 0.1
(n = 29)	AD	80.86 ± 7.0	0.84 ± 0.1	18.93 ± 6.5	17.29 ± 7.8	0.64 ± 0.2	0.17 ± 0.1
p^1		0.753	0.942	0.134	0.321	0.703	0.548

Treatment	Type of drug	HTC, % (40–54)	HGB, g/dL (12–18)	RBC, M/Ul (4.5–6.5)	MCV, fL (84–104)	WBC, mi/Ul (4–11)	Platelet, mi/Ul (150–400)
		Mean ± Standard deviation					
p^2		0.657	0.414	0.011	0.337	0.044	0.848

Abbreviatures: AD, approved drug; ALT, alanine transferase; AST, aspartate transferase; DB, direct (conjugated) bilirubin; GLM, general linear model; HGB, hemoglobin; HTC, hematocrit; ID, investigational drug; MCV, mean corpuscular volume; RBC, red blood cell; TB, total bilirubin; WBC, white blood cell.

GLM multivariate, Plots: p^1 for visit x treatment, p^2 for visit x type of drug.

Table 5
Vital Signs

Treatment	Type of drug	HR, beats/min (60–100)	RR, breaths/min (12–20)	SBP, mmHg (90–120)	DBP, mmHg (60–80)
		Mean ± Standard deviation			
Baseline					
Lubricant	ID	75.19 ± 6.4	16.92 ± 3.1	111.89 ± 11.0	70.14 ± 6.1
	AD	72.63 ± 8.3	16.43 ± 2.8	115.47 ± 8.6	71.29 ± 5.3
Hypotensive	ID	75.17 ± 6.4	16.08 ± 1.3	112.54 ± 8.0	74.08 ± 5.6
	AD	73.21 ± 7.9	15.92 ± 1.1	110.67 ± 7.3	72.83 ± 4.7
Antibiotic	ID	70.86 ± 6.6	17.93 ± 1.7	109.29 ± 5.1	70.71 ± 4.8
	AD	70.53 ± 4.0	17.33 ± 1.7	109.00 ± 7.6	71.67 ± 5.9
Final					
Lubricant	ID	74.59 ± 8.6	16.89 ± 2.7	112.00 ± 10.9	69.24 ± 5.9
	AD	74.90 ± 10.7	16.20 ± 2.9	115.18 ± 9.6	70.98 ± 5.8
Hypotensive	ID	74.25 ± 9.3	15.96 ± 1.2	111.05 ± 8.9	74.29 ± 5.1
	AD	76.46 ± 7.5	15.71 ± 1.3	111.21 ± 7.8	72.46 ± 4.2
Antibiotic	ID	71.57 ± 7.7	18.36 ± 1.7	109.29 ± 7.0	72.50 ± 5.8
	AD	70.93 ± 5.2	17.73 ± 2.0	109.33 ± 8.0	71.33 ± 5.2
p^1		0.597	0.445	0.932	0.532
p^2		0.155	0.780	0.619	0.463

Abbreviatures: AD, approved drug; DBP, diastolic blood pressure; GLM, general linear model; HR, heart rate; ID, investigational drug; RR, respiratory rate; SBP, systolic blood pressure.

GLM repeated measures, p^1 for visit x treatment, p^2 for visit x type of drug. For lubricant: n = 86; hypotensive: n = 48, and antibiotic: n = 29.

Figures

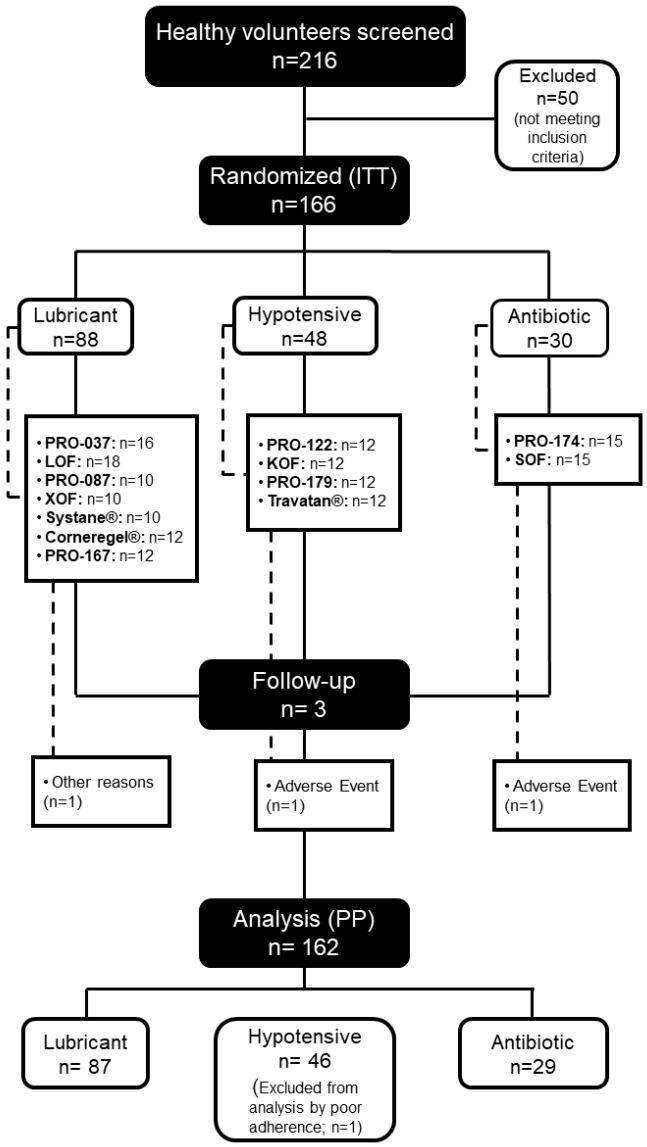

Figure 1

Study flow diagram, summary of NHV search and selection.

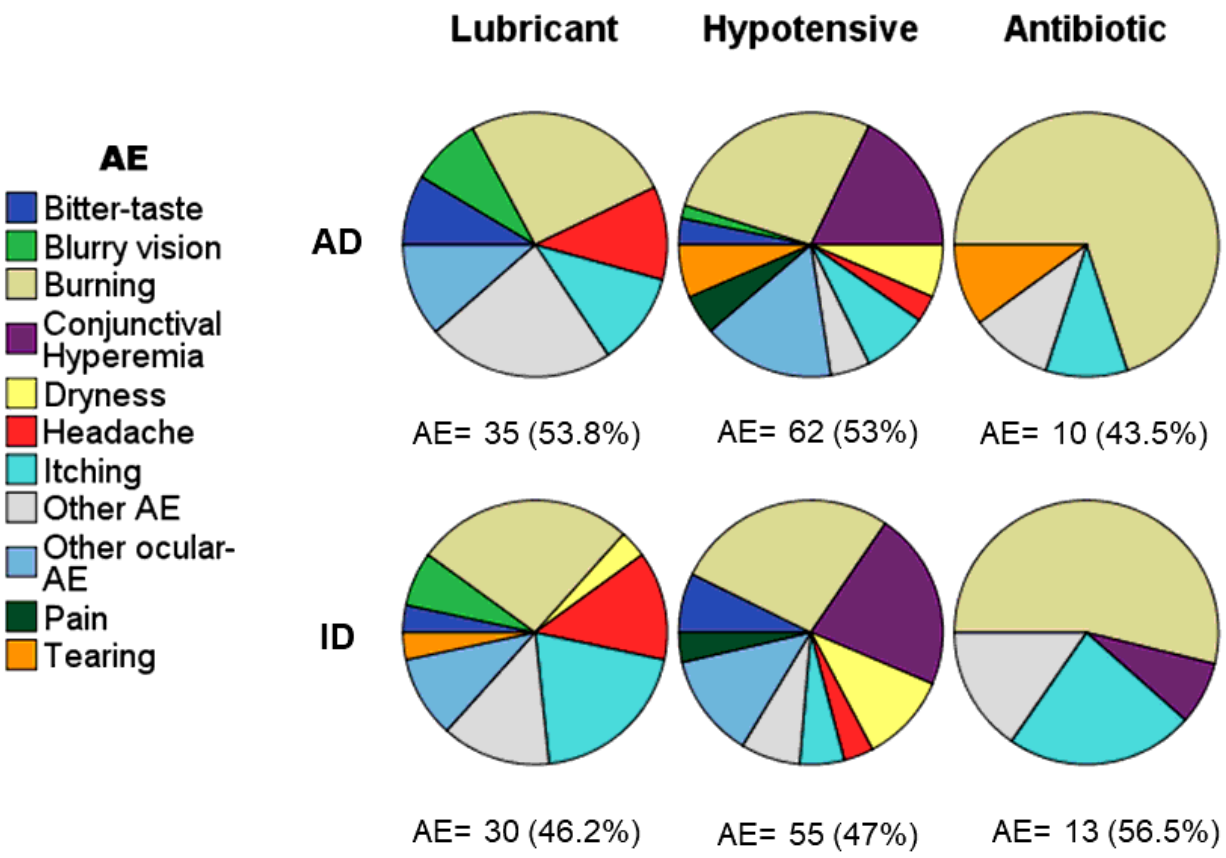

Figure 2

Incidence of adverse events (AE) in ITT population for approved drugs (AD, upper polar graphs) and investigational drugs (ID, lower polar graphs). A total of 205 AE was presented in 59.6% NHV. For another AE the incidence was <2%. Presence of AE in hypotensive drugs > lubricant and antibiotic drugs, Chi square test, p=0.0001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CONSORT2010ChecklistBOPHD2001036.doc
- Additionalfile1BMCOph.docx