

Tear concentrations of azithromycin following topical administration of a single dose of azithromycin 0.5%, 1.0%, and 1.5% eyedrops (T1225) in healthy volunteers

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PURPOSE. To evaluate azithromycin tear concentrations after one drop of T1225 0.5%, 1.0%, and 1.5% eyedrops.

METHODS. In this randomized, double-masked study, 91 healthy volunteers received one drop into each eye of T1225 0.5% (n=23), T1225 1.0% (n=38), or T1225 1.5% (n=38). Azithromycin tear concentrations were measured by HPLC-MS at seven time points for 24 hours. Tolerability was evaluated.

RESULTS. T1225 1.0% and 1.5% had similar pharmacokinetic profiles. After a post-instillation peak (167 to 178 mg/L after 10 minutes), mean concentrations remained above 7 mg/L for 24 hours (except for T1225 1% at H24). A delayed increase of the azithromycin mean tear concentration might be explained by the known late azithromycin release from tissues after storage in cells. Areas under inhibitory curve (AUCs) of T1225 1.0% and 1.5% were higher than AUCs of T1225 0.5% and ranged between 47 and 90. The three T1225 concentrations were safe for the ocular surface.

CONCLUSIONS. Once daily instillation of T1225 1.0% and 1.5% was shown to reach an AUC markedly above the required threshold for an antibacterial activity against Gram-positive bacteria (25–35). These results suggest that a BID instillation is more likely to ensure antimicrobial activity against Gram-negative bacteria (threshold >100). (*Eur J Ophthalmol* 2008; 18: 13-20)

KEY WORDS. Area under the inhibitory curve (AUC), Azithromycin eyedrops, Minimal inhibitory concentration (MIC), Tear pharmacokinetics/pharmacodynamics, T1225

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INTRODUCTION

Azithromycin belongs to the second generation of macrolides (azalide). Its antimicrobial activity is comparable to that of time dependent bactericidal antimicrobial agents and the best predictive pharmacokinetic surrogate marker is the area under the inhibitory curve (AUC). Azithromycin has a number of unique pharmacokinetic properties which distinguish it from other antibiotics (1-8). Azithromycin is characterized by a long elimination half-life and notably high and sustained concentrations in

cells. Because of this long elimination half-life, low-frequency and short-treatment courses are justified and have been used for the treatment of respiratory tract infections since the 1990s (8, 9). In addition to its well-adapted spectrum of activity, its intracellular efficacy makes azithromycin effective against intracellular bacteria such as *Chlamydia trachomatis*. This is why its use had been expanded into the field of ophthalmology for the treatment of bacterial keratoconjunctivitis due to endemic trachoma (10). Efficacy of oral azithromycin is now well-established and is supported by the pharmacokinetic pro-

file noted in the tears and in the conjunctiva in humans (11, 12).

The potential usefulness of a topical azithromycin formulation was suggested during the first meeting of the WHO Alliance for the global elimination of trachoma (13). Based on the azithromycin spectrum of antimicrobial activity, this topical formulation is also expected to be effective in the treatment of bacterial purulent conjunctivitis.

In this context, a stable formulation of azithromycin eyedrops packaged in single-dose units was developed: T1225 eyedrops. Pharmacokinetic analysis in rabbits confirmed the availability of azithromycin in tears, conjunctiva, and cornea after a single instillation and twice daily instillations for 3 days of azithromycin 1.5% (data submitted to Current Eye Research). Two previous double-blind, vehicle-controlled, clinical trials in healthy volunteers have already demonstrated the good tolerability of T1225 0.5%, 1.0%, and 1.5% eyedrops (unpublished data). The aim of the present pharmacokinetic study was to determine which dosage provided the higher azithromycin tear concentration in healthy volunteers, 24 hours after a single administration. In addition, data were collected in order to determine a suitable daily dosing regimen for T1225.

METHODS

Study design

This was a phase I, exploratory, single-center, double-masked, randomized study performed in three parallel groups.

Subjects and treatment

Subjects were healthy volunteers aged from 18 to 45 years, with no relevant ocular abnormalities. All subjects attended a selection visit (day -5 to day -1), an inclusion visit (day 0), and a 24-hour follow-up after T1225 instillation until the final visit (day 1).

Each of the subjects' eyes was randomized to receive azithromycin 0.5%, 1.0%, or 1.5% eyedrops (T1225, single-dose unit, Laboratoires Théa, France). A second randomization was performed within each treatment group in order to attribute the time for tear sampling. A total of 182 tear samplings were performed at the seven time points, as follows: 6 samplings per time for T1225 0.5% (42

eyes); 10 samplings per time for T1225 1.0% and 1.5% (2×70 eyes).

This study was conducted in accordance with Good Clinical Practice, applicable guidelines, the Declaration of Helsinki, and local regulations. Ethics committee approvals were obtained prior to enrolling any subject. Written informed consent was obtained from each subject.

Determination of azithromycin concentrations and pharmacokinetic parameters

Collection of tear sampling. Tear samples were collected using the strips that are usually used to perform the Schirmer test. The strip was hooked in the lower conjunctival sac, over the temporal one-third of the lower eyelid margin, for 5 minutes or until the tears covered 10 mm of the strip. Each Schirmer strip was weighed before and after tear sampling.

Analysis of tear samples

The analysis was performed according to Good Laboratory Practice (Iris Pharma, France). Azithromycin was extracted from Schirmer strips in 1.5 mL polypropylene tubes by adding 1 mL ether and by vortexing 30 seconds. Tubes were then shaken for 30 minutes at 150 REV using an end-to-end stirrer then centrifuged for 10 minutes at 2000 *g*. The supernatant was evaporated at 40 °C under nitrogen atmosphere. The pellet was then diluted in 100 µL of mobile phase and 20 µL were injected into the column of a high-performance liquid chromatography coupled with a mass spectrometry detector (HPLC-MS). The validated values were 0.17 µg/g of tears for the limit of quantification (LOQ) and 0.007 µg/g of tears for the limit of detection (LOD). The repeatability ranged from 0.53 to 1.72% (coefficient of variability [CV]) and the intermediate precision ranged from 1.71 to 5.25% (CV).

Azithromycin concentrations were measured at 0.17 hours (H0.17), H0.5, H2, H4, H8, H12, and H24 in tear samplings, ensuring the determination of the maximum concentration (C_{max}) and the concentration 24 hours after instillation (C_{24h}).

The following pharmacokinetic parameters were calculated: area under the curve from 0 to 24 hours (AUC_{0-24h}) and elimination half-life ($T_{1/2}$). In order to further estimate pharmacokinetic/pharmacodynamic surrogate markers such as areas under the inhibitory curve (AUCs) = AUC_{0-24h}/MIC for reference minimal inhibitory concentrations

(MICs) and the time for which the concentration exceeds the MIC ($T > MIC$), we decided to choose 0.5 mg/L and 4 mg/L MIC as reference values. Indeed, the MICs for chlamydial bacteria are always inferior to 0.5 mg/L in the literature, and the 4 mg/L reference MIC defines intermediary susceptibility for most bacteria in the NCCLS guidelines (14) and the threshold for bacterial resistance in the French guidelines (15).

Safety assessment

The ocular examination was performed during the selection visit, and on day 1. Acceptability of the treatment was assessed by the investigator and by the subject. Ocular and systemic adverse events (AEs) were reported.

Statistical analysis

The pharmacokinetic profiles were established in each treatment group, using 95% CIs on the mean for each sampling time point. The program WinNonlin® pro version 3.2 (Pharsight corporation, Mountain View, CA) was used to perform the pharmacokinetic analysis and to calculate the parameters for each treatment group, using a non-compartmental analysis method.

A first analysis was performed using the mean concentration values calculated with all available individual values. A second analysis was performed excluding the more extreme individual values, i.e., outliers. Outliers were removed after identification by a statistical program designed for that purpose (Student *t*-test, comparison of the

suspected abnormal values to the mean of the other values of the sample, $p=0.05$).

RESULTS

Subjects

Ninety-one subjects were randomized to receive one drop of T1225 0.5%, 1.0%, or 1.5% with a single dose unit: 21 subjects (42 tear samples) in the T1225 0.5% treatment group, 34 (68 tear samples) in the T1225 1.0% group, and 36 (72 tear samples) in the T1225 1.5% group.

The mean age \pm SD was 24.8 ± 5.4 , ranging from 18.7 to 42.8 years old. The gender ratio was well balanced: 43 men (47%) and 48 women (53%).

Pharmacokinetic results

The azithromycin mean \pm SD/median tear concentrations in the treated eyes are presented in Table I. Mean tear concentrations appear higher in the 1.0 and 1.5% T1225 groups. In those groups, all mean concentrations, except one value at H24 (1.4 ± 1.9), remain higher than 7 mg/L, i.e., higher than the reference MICs (4 mg/L).

Despite the variability, the azithromycin mean tear concentrations showed some trends. The higher values were noted H0.17 after instillation (Tab. I): 98 ± 147 $\mu\text{g/g}$ for the T1225 0.5%, 167 ± 211 $\mu\text{g/g}$ for the T1225 1.0%, and 178 ± 426 $\mu\text{g/g}$ for the T1225 1.5%. A time-related decrease for the three used concentrations was observed

TABLE I - OCULAR PHARMACOKINETICS: AZITHROMYCIN MEAN/MEDIAN TEAR CONCENTRATIONS (PP population)

Time point	Azithromycin tear concentrations ($\mu\text{g/g}$ of tears), mean \pm SD								
	T1225 0.5%			T1225 1.0%			T1225 1.5%		
	Mean \pm SD	Median	N	Mean \pm SD	Median	N	Mean \pm SD	Median	N
H0.17	98.1 ± 147.4	32.5	6	166.6 ± 210.6	20.7	9	178.4 ± 426.3	16.5	11
H0.5	30.9 ± 41.3	17.3	6	154.6 ± 256.0	62.6	10	55.9 ± 74.7	20.8	10
H2	4.9 ± 6.1	2.5	6	22.9 ± 23.9	13.9	10	40.5 ± 47.1	25.0	10
H4	1.0 ± 1.1	0.6	6	8.3 ± 7.6	7.1	10	7.5 ± 10.18	4.1	10
H8	7.2 ± 8.5	3.7	6	97.1 ± 281.7	3.4	9	9.7 ± 20.04	4.7	11
H12	0.8 ± 0.7	0.7	6	117.6 ± 363.8	1.5	10	9.7 ± 10.2	4.4	10
H24	1.5 ± 2.1	0.5	6	1.4 ± 1.9	0.8	10	70.2 ± 217.2	1.0	10

PP = Per protocol; SD = Standard deviation; N = Number of samples (number of eyes)

from H0.17 to H4. Then, the values fluctuated, with an increase, maximal at H8 for the T1225 0.5%, at H12 for T1225 1.0%, and at H24 for T1225 1.5%. This delayed increase of the azithromycin mean tear concentration might be explained by the known late azithromycin release from tissues after initial storage in cells.

AUC_{0-24h}, T_{1/2}, C_{max}, and C_{24h} were analyzed using the mean concentration values calculated with all available individual values (Tab. II).

As all the other pharmacokinetic parameters, the AUIC appeared to be dose-related for both reference values of MIC. For a MIC of 0.5 mg/L, AUIC was 216.40 for the T1225 0.5% treatment group, 516.46 for the T1225 1.0% treatment group, and 725.34 for the T1225 1.5% treatment group. For a MIC of 4.0 mg/L, which corresponds to the most unfavorable conditions, AUIC was 27.05 for the T1225 0.5% treatment group, 64.55 for the T1225 1.0%

treatment group, and 90.66 for the T1225 1.5% treatment group. The T>MIC for a MIC of 0.5 mg/L appeared to be similar for the three azithromycin doses (24 hours), whereas the T>MIC is proportional to the administrated dose for a MIC of 4.0 mg/L. When using T1225 1.5% eyedrops, there was a longer, incalculable T>MIC at H24.

An additional analysis performed after exclusion of the outlying values (Tab. III) confirmed that T1225 0.5% eyedrops gave AUIC (AUIC=136.66) markedly lower than T1225 1.0% (AUIC=375.94) and T1225 1.5% (AUIC=393.30). Moreover, when excluding the outlying values, the difference between T1225 1.0% and T1225 1.5% appeared to be low (AUIC difference=17.36).

After one instillation of T1225 1.0% or 1.5% eyedrops, the AUIC for a MIC of 4 mg/L was between 47 and 90, with or without outlying values.

TABLE II - OCULAR PHARMACOKINETIC PARAMETERS IN THE TEAR SAMPLES (PP population with outlying values)

Parameter	AZM 0.5%, n = 42 samples	AZM 1.0%, n = 68 samples	AZM 1.5%, n = 72 samples
AUC0-24h (µg/g.h)*	108.20	258.23	362.67
t1/2 (h)	16.17	9.22	15.67
Cmax ± SD (µg/g)	98.09±147.44	166.56±210.59	178.34±426.32
C24h ± SD (µg/g)	1.46±2.10	1.43±1.87	70.21±217.20

*Note that the AUC0-24h was calculated without standard deviation, as the whole PK (all time points included) was not performed using samplings in a same subject (only one time point for each eye of each subject).

PP = Per protocol; AZM = Azithromycin; N = Number of sampling (number of eyes); AUC0-24h = Area under the curve from 0 to 24 hours; t1/2 = Elimination half-life; Cmax = Maximum concentration; C24h = Concentration 24 hours after instillation

TABLE III - AUIC AND T>MIC IN THE TEAR SAMPLES (PP POPULATION WITH AND WITHOUT OUTLYING VALUES)

Parameter	AZM 0.5% Outlying values		AZM 1.0% Outlying values		AZM 1.5% Outlying values	
	With	Without	With	Without	With	Without
Number of samples	42	37	68	61	72	65
AUIC for						
MIC = 0.5 mg/L	216.40	136.66	516.46	375.94	725.34	393.30
MIC = 4 mg/L	27.05	17.08	64.55	46.97	90.66	49.16
T>MIC (h) for						
MIC = 0.5 mg/L	24	24	24	24	24	24
MIC = 4 mg/L	3	0.75	8	7	NC*	7

*Not calculable since, due to the outlying values, AZM mean concentration at H24 was higher than the values observed until Min 30.

AUIC = Area under the inhibitory curve (AUC0-24h/MIC); MIC = Minimal inhibitory concentration; T>MIC = Time for which the concentration exceeds the MIC; PP = Per protocol; AZM = Azithromycin

Safety results

No serious AEs were reported and none of them led to premature discontinuation. Among the 91 subjects, only 2 subjects reported objective ocular signs: one subject in T1225 1.0% group (mild bilateral watering, palpebral edema, and folliculo-papillary conjunctivitis) and one subject in T1225 1.5% group (conjunctival hyperemia). Nasal passage of the product was experienced by 2 subjects (5%) for T1225 1.0% and 2 subjects (5%) for T1225 1.5%. All of the AEs were resolved at day 0. The acceptability of the treatments was similar in the three treatment groups: rated by the investigator as satisfactory in 97–100% of subjects.

DISCUSSION

The strategy most recently recommended by the WHO for the treatment of trachoma is a single oral dose of azithromycin (20 mg/kg in children or 1 g in adults). However, systemic use of azithromycin can be diverted from its initial target during campaign against trachoma. That is why a strong demand was expressed for the development of a topical formulation of azithromycin during the first meeting of the WHO Alliance for the global elimination of trachoma. Azithromycin is characterized by a long elimination half-life and notably high and sustained concentrations in cells. The high affinity of this drug for tissue is due to the presence of two basic tertiary amine groups, which give its amphiphilic properties (16). In vitro and in vivo models have demonstrated that azithromycin is taken up, transported, and released at the sites of infection by phagocytic cells such as polymorphonuclear neutrophils and macrophages. In addition, uptake is not saturable (17). Topical azithromycin is expected to be effective in the management of trachomatous conjunctivitis and purulent conjunctivitis, since according to the MICs, most bacteria causing conjunctival infections are susceptible to azithromycin. The MICs are determined in vitro and reflect the intrinsic antibacterial potency of the agent. Although this activity can be modified in vivo, the MIC provides a target concentration to be achieved or exceeded in vivo to ensure clinical success in case of systemic use (17). Regarding *C trachomatis*, a high in vitro azithromycin activity has been noted: MIC₉₀ is always ≤0.25 mg/L (18, 19). Regarding nonchlamydial conjunctival infections, studies reported also a good in vitro azithromycin activity.

The usually causative Gram-positive bacteria (3, 20) are *Staphylococcus epidermidis* (MIC₉₀ ranged between 0.63 and 2 mg/L) (21, 22), *Staphylococcus aureus* (MIC₉₀ ranged between 1 and 4 mg/L) (9, 22), and Streptococci, especially *Streptococcus pneumoniae* (MIC₉₀ ranged between 0.05 and 4 mg/L) (8, 9, 21, 22). The most common Gram-negative organism (23-25) is *Haemophilus influenzae* (MIC₉₀ ranged between 0.39 and 4 mg/L) (8, 9, 26). In children (27, 28), *Haemophilus influenzae* and *Streptococcus pneumoniae* are commonly isolated; and in newborns, *Neisseria gonorrhoea* (MIC₉₀ ranged between 10.05 to 0.5 mg/L) (8, 19, 21, 22, 26) and *C trachomatis* are often transmitted from the genital tractus of the mother.

It should be highlighted that these breakpoints (MICs) have been determined for systemic uses of this antibiotic. Consequently they are not totally applicable to the field of ophthalmology (29). Indeed, local concentrations reached after topical route are usually much higher than those reached by systemic route (30). In addition, physicochemical parameters, such as acidic pH and CO₂, have been hypothesized to alter the activity of azithromycin (31, 32). Today, no eyedrops with a pharmacokinetic profile similar to that of T1225 are available to hypothesize an appropriate dosing regimen to be tested in patients. Therefore, preliminary pharmacokinetic/pharmacodynamic data were to be obtained in healthy volunteers in order to limit the risk of treating the target children with a dosing regimen having insufficient antibacterial activity.

Results in our study showed a high variability in the pharmacokinetic data. Such variability is widely described when using antibiotics and other therapeutic classes in ophthalmology (33-39). This variability may be explained by the difficulties in sample collection. Quantities of tear samples are small and the presence of reflex tearing can interfere with the sample collection. Delayed increases of the azithromycin mean tear concentration led to late fluctuations of the variables and may be explained by a secondary release of azithromycin from a potential reservoir. This reservoir effect due to ions trapping properties may lead to a random release of azithromycin from cells, depending on the death occurrence of cells at different stages of maturation.

Our clinical study aimed to choose the most relevant T1225 concentration among the three tested concentrations and to provide preliminary data in order to simulate a suitable daily dosing regimen for T1225.

Regarding the choice of the T1225 concentration, all pharmacokinetic results were consistent and showed that

the T1225 0.5% dose reached azithromycin tear concentrations obviously lower than those obtained with both the higher concentrations of T1225 (1.0% and 1.5%). In the T1225 0.5% group, AUC was markedly inferior when compared to the 1.0% and 1.5% groups. Since the safety profiles of the three T1225 concentrations were similar, our clinical trial showed that both T1225 1.0% and T1225 1.5% should be favored to T1225 0.5% for further clinical development. However, the fluctuations of azithromycin concentrations in the tears appeared to be quite high and to occur randomly with no precise schedule. Consequently, no differences between T1225 1.0% and T1225 1.5% could be highlighted, especially after the exclusion of the outlying values. Therefore, no choice could be made between the concentration of T1225 1.0% and 1.5% on the basis of the present clinical trial.

Regarding the simulation of dosing regimen, the noted delayed fluctuations of the azithromycin mean tear concentration prevented any modeling as initially planned, and favored an analysis based on a surrogate pharmacokinetic/pharmacodynamic parameter. Azithromycin has a relatively slow, time-dependent bactericidal activity and an *in vivo* prolonged postantibiotic effect (40, 41). However, clinical data show that the AUC is the most predictive pharmacokinetic/pharmacodynamic parameter of therapeutic success of this antibiotic for systemic use (5, 40, 42–46). After one instillation of T1225 1.0% or 1.5% eyedrops, the AUC for a MIC of 4 mg/L, which corresponds to the most unfavorable conditions, was between 47 and 90, with or without outlying values. Therefore, once daily instillation of T1225 1.0% and 1.5% was shown to reach an AUC markedly above the required threshold for antibacterial activity against Gram-positive bacteria. A twice daily instillation was thus thought to provide the required antibacterial activity against most Gram-positive bacteria (threshold = 25–35) (46) and Gram-negative bacteria (threshold >100) (44, 46).

Based on these results, T1225 1.0% and 1.5% eyedrops instilled at a dosage regimen of twice daily should be the subject of further clinical trials in order to find the accepted threshold for Gram-positive and Gram-negative bacterial remission. This study highlighted that T1225 instilled twice daily for 3 days was efficacious and safe for the treatment of trachoma (47) and purulent bacterial conjunctivitis (48). The use of MIC references determined in healthy volunteers (experimental conditions) remains questionable. The experimental conditions of this study were different from those observed in daily practice when

treating conjunctival infections. In addition, MIC references were chosen according to the NCCLS standards. However, in the absence of any pharmacokinetic data on a topical azithromycin formulation, using those references was the first mandatory step to estimate the ocular pharmacokinetic/pharmacodynamic of azithromycin.

CONCLUSIONS

Safety results showed that the three azithromycin concentrations, instilled once in each eye, appeared to be safe and well tolerated by the ocular surface. The AUC appeared to be dose-related for both reference values of MIC. T1225 1.0% and 1.5% eyedrops had similar pharmacokinetic profiles. For both concentrations, a twice-daily dosage regimen was suggested in order to reach the accepted threshold for a favorable outcome in Gram-positive as well as Gram-negative bacteria.

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