Plasma levels and systemic safety of 0.1% unpreserved Timolol maleate gel, 0.5% Timolol aqueous solution and 0.5% Timolol maleate gel

Concentration plasmatique et tolérance systémique d’un gel de maléate de timolol 0,1% non conservé, d’une solution aqueuse de timolol 0,5% et d’un gel de maléate de timolol 0,5%

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Summary
Purpose. — To compare systemic absorption of three formulations of timolol eye drops: 0.1% timolol maleate gel, 0.5% timolol aqueous solution, and 0.5% timolol maleate gel.
Methods. — This was a double cross-over phase I study. Cross-over 1: two weeks of 0.1% timolol gel once daily, followed by a 3-week wash-out period and then two weeks of 0.5% timolol aqueous solution twice a day (group 1) or the reverse (group 2). Cross-over 2: two weeks of 0.1% timolol gel once daily, followed by a 3-week wash-out period, and then two weeks of 0.5% timolol gel once daily (group 3) or the reverse (group 4). Subjects underwent tonometry, blood sampling, and heart rate and blood pressure assessments (during bicycle exercise and head-up tilt tests) before and after instillation at the beginning and end of each treatment period.
Results. — Forty-three healthy volunteers were randomized: 11 subjects in groups 1, 2, and 3, and 10 subjects in group 4. Areas under the concentration-time curve (AUC) values after administration of timolol 0.5% formulations were 15- to 38-fold higher than those seen after administration of timolol 0.1% gel. Maximum timolol concentrations after instillation of 0.1% gel are reduced by almost 90% compared to concentrations obtained after both 0.5% aqueous solution and 0.5% gel instillation. The AUC between 0 and 12 h post-administration were also reduced by up to 93 to 98%.

Keywords
β-Blockers; Cardiac side effects; Timolol; Timolol maleate gel

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MOTS CLÉS
β-Bloquant ;
Effets secondaires cardiovasculaires ;
Timolol ;
Gel de maléate de timolol

Introduction

Glaucoma is the second most common cause of blindness and low vision in the world [1]. Glaucoma is defined as a group of diseases that have certain common features causing optic nerve damage. The most common type of glaucoma is primary open-angle glaucoma (POAG), which accounts for approximately 75% of all cases [2]. Elevated intraocular pressure (IOP) is the main risk factor in glaucoma development and damage, and is the only treatable risk factor for POAG as IOP is modifiable. Timolol maleate is a non-specific beta-blocker, which is widely used as a standard medication to lower IOP, with proven efficacy and safety [3,4].

Currently, topical beta-blockers, and in particular timolol, are one of the first choices in medical management of POAG and ocular hypertension, beta-blockers are the second powerful class after prostaglandins. Although the incidence of side effects with timolol treatment is low, systemic side effects induced by topically-applied aqueous timolol cannot be ignored. Indeed, independent of its topical administration, its systemic absorption, added to its non-selective beta-adrenergic receptor antagonist properties, limit its use in patients with pre-existing cardiovascular or respiratory disease [5–9]. Reported side effects due to systemic absorption following ophthalmic administration of timolol include reduction in heart rate during exercise, bradycardia, nocturnal hypotension, bradycardia and bronchospasm in patients with reactive airway or chronic obstructive lung disease [10–16].

Many ophthalmic drugs are applied in high concentrations due to the low ocular bioavailability of ophthalmic solutions, but a previous study has shown that timolol has a high systemic bioavailability, with four-fifths of 0.5% timolol solution applied in the eye absorbed into the systemic circulation [17]. Timolol is currently approved for reduction of IOP in different concentrations (0.1%, 0.25% and 0.5%) in different dosage forms (aqueous solution, maleate gel). The high concentrations are assumed to be the cause of ocular and also systemic side effects [18] due to its high systemic bioavailability and a concentration of 0.2 ng/mL would seem to be the limit beyond which cardiologic systemic effects could appear [17]. Hydrogels have considerable potential to improve the safety profile of this very useful drug as they substantially increase the ocular absorption of timolol compared with conventional aqueous eye drop preparations. This increase in ocular bioavailability allows a reduction of both the timolol concentration and the frequency of administration, whilst maintaining efficacy [19,20]. The reduction of the dose and frequency of administration results in a reduction of the quantity of timolol absorbed into the bloodstream, thus significantly reducing the likelihood of systemic side effects compared with conventional aqueous timolol preparations. Indeed, studies using 0.5% timolol gel formulations and 0.5% timolol gel-forming solution have shown fewer systemic effects in terms of peak heart rate during exercise and pulmonary function compared to placebo or 0.5% timolol aqueous solution [21–24]. More recently, 0.1% timolol gels have proved to be clinically equipotent to 0.5% timolol in aqueous solution [19,20] with a reduced systemic absorption and fewer side effects [20].

The present study was designed to compare systemic absorption of 0.1% timolol maleate gel (once daily) to 0.5% timolol aqueous solution (twice daily) and to 0.5% timolol maleate gel (once daily).
Materials and methods

This study, approved by the ethics committee of Clermont-Ferrand, was conducted according to accepted standards of Good Clinical Practice (GCP), to specific guidelines if any, and in agreement with the Declaration of Helsinki (2004) and local regulations.

Written informed consent was obtained from all healthy volunteers. This was a phase I, monocentre, open, randomised, double cross-over (4 × 10 subjects expected) study:

- cross-over 1: two weeks of 0.1% timolol gel (Geltim LP, Laboratoires Théa), once daily followed by a 3-week wash-out period and then two weeks of 0.5% timolol aqueous solution (Timoptol, Merck Sharp Dohme-Chibret) twice a day (group 1) or the reverse (group 2);
- cross-over 2: two weeks of 0.1% timolol gel once daily, followed by a 3-week washout period, and then two weeks of 0.5% timolol gel (Timoptol LP, Merck Sharp Dohme-Chibret) once daily (group 3) or the reverse (group 4).

The treatment order was randomized according the four sequences described in Fig. 1. Patients instilled the products in both eyes.

Subjects attended five study visits: the Screening visit (D-14), the inclusion visit/dispensing visit 1 (Day 0), a follow-up visit 1 (Day 14 ± 3 days), a dispensing visit 2 (D35 ± 3 days) and a follow-up visit 2/final visit (Day 49 ± 3 days).

During the screening visit, subjects underwent a clinical systemic examination, an ECG, a thorough slit-lamp examination, best far corrected visual acuity evaluation, pachymetry, tonometry, a head-up tilt test (heart rate and blood pressure), a bicycle exercise test (heart rate and blood pressure) and inclusion and non-inclusion criteria were checked. For bicycle exercise tests, subjects were asked to produce a sub-maximal effort, i.e. reaching a heart rate of 80% of the age-predicted maximum heart rate. The designated target heart rate for each subject was to be determined after few minutes of warm-up at the screening visit for period 1 and after the washout period at D35 for period 2. The subject then performed the test at each visit until he reached his target workload and the cardiovascular parameters were measured during 30 seconds for the heart rate and two measurements for the blood pressure.

The dispensing and follow-up visits were divided in two steps: before instillation when tonometry (T0), blood sampling (T0), heart rate and blood pressure assessments both during head-up tilt test and bicycle exercise (only at dispensing visits) were realised, and after instillation when blood samples for pharmacokinetics (PK) and tonometry were carried out (at 10 and 30 minutes and, 1 h, 1 h 30, 4 h, 8 h, and 12 h after instillation), and heart rate and blood pressure were assessed during head-up tilt test and bicycle exercise 2 hours after instillation.

Furthermore, at each visit, subjects were questioned about adverse events.

In order to determine the plasma levels of timolol, blood samples (5 mL) were taken on Day 0, Day 14, Day 35 and Day 49, i.e. at the beginning and at the end of each period of treatment. Samplings were done through venous catheter before instillation and at 10′, 30′, 1 h, 1 h 30, 4 h, 8 h, and 12 h after administration of one drop of the study product. Timolol concentrations in plasma were analysed using rapid resolution liquid chromatography/tandem mass spectrometry (RRLC-MS/MS).

A descriptive analysis and statistical testing (non-parametric Koch approach) was performed for the bicycle exercise test, head-up tilt test, pharmacokinetic (PK) parameters—area under the curve between 0 and 12 h post-instillation (AUC(0–12h)) and maximal concentration (Cmax)—and tonometry variables. For the plasma concentrations, other PK parameters (tmax and t1/2) and evolution of IOP (change between the different time points and T0), only a descriptive analysis was performed.

Results

Demography

Sixty-seven subjects were screened for the study, 56 were selected, 43 were included and randomized into the four following groups:
• 11 subjects in group 1 (timolol 0.1% gel/0.5% aqueous solution);
• 11 subjects in group 2 (timolol 0.5% aqueous solution/0.1% gel);
• 11 subjects in group 3 (timolol 0.1% gel/0.5% gel);
• 10 subjects in group 4 (timolol 0.5% gel/0.1% gel)

The overall mean age was around 26 years in both cross-over, ranging from 19 to 42 years old. There were more males than females in cross-over 1 (59.1% vs 40.9%), and more females than males in cross-over 2 (33.3% vs 66.7%) (Table 1).

### Pharmacokinetics

In cross-over 1, Cmax was statistically significantly lower with timolol 0.1% gel than with timolol 0.5% aqueous solution after both the first instillation, and after two weeks of treatment ($P=0.0003$). After the first instillation, Cmax was 0.08 ng/mL with timolol 0.1% gel and 0.77 ng/mL with timolol 0.5% aqueous solution. After two weeks of treatment, Cmax was 0.11 ng/mL with timolol 0.1% gel and 0.87 ng/mL with timolol 0.5% aqueous solution (Table 2).

Similar results were observed in cross-over 2, with a significantly lower Cmax ($P=0.0004$) after the first instillation of timolol 0.1% gel (0.08 ng/mL) than after the first instillation of timolol 0.5% gel (0.67 ng/mL) and a significantly lower Cmax ($P=0.0004$) after two weeks of treatment with timolol 0.1% gel (0.11 ng/mL) than with timolol 0.5% gel (0.69 ng/mL).

Tmax was reached more rapidly with timolol 0.1% gel than with both timolol 0.5% formulations (Table 2).

Mean timolol plasma concentrations did not exceed 0.10 ng/mL after instillation of timolol 0.1% gel at either timepoint measured, but they reached 0.80 ng/mL and 0.66 ng/mL after two weeks of treatment with timolol 0.5% aqueous solution and 0.5% gel, respectively (Fig. 2). The AUC values were 15- to 38-fold higher after ocular administration of 0.5% preparation than after administration of 0.1% gel. Mean half-life ($t_{1/2}$) was approximately 3 h 30 after treatment with timolol 0.5% aqueous solution and approximately 4 h 30 after treatments with timolol 0.5% gel; $t_{1/2}$ was not calculated for timolol 0.1% gel because too many values were below the lower limit of quantification.

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### Table 1 Demographic characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Cross-over 1</th>
<th>Cross-over 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>26.3 (5.6)</td>
<td>25.8 (6.5)</td>
</tr>
<tr>
<td>Min—Max</td>
<td>20—38</td>
<td>19—42</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13 (59.1%)</td>
<td>7 (33.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>9 (40.9%)</td>
<td>14 (66.7%)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>22.1 (3.3)</td>
<td>22.1 (2.4)</td>
</tr>
<tr>
<td>Min—Max</td>
<td>16.9—27.8</td>
<td>18.7—27.5</td>
</tr>
</tbody>
</table>

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### Table 2 Pharmacokinetic parameters.

<table>
<thead>
<tr>
<th></th>
<th>Cross-over 1</th>
<th>Cross-over 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beginning of period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL) Mean (SD)</td>
<td>0.08 (0.09)</td>
<td>0.08 (0.12)</td>
</tr>
<tr>
<td>Tmax (min) Mean (SD)</td>
<td>23.68 (21.40)</td>
<td>11.11 (22.72)</td>
</tr>
<tr>
<td>$t_{1/2}$ (min) Mean (SD)</td>
<td>6.32 (9.55)</td>
<td>5.11 (10.05)</td>
</tr>
<tr>
<td><strong>AUC0—12h</strong> Mean (SD)</td>
<td>5.05 (16.81)</td>
<td>5.11 (10.05)</td>
</tr>
<tr>
<td><strong>End of period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL) Mean (SD)</td>
<td>0.11 (0.14)</td>
<td>0.11 (0.14)</td>
</tr>
<tr>
<td>Tmax (min) Mean (SD)</td>
<td>4.44 (5.11)</td>
<td>4.44 (5.11)</td>
</tr>
<tr>
<td>$t_{1/2}$ (min) Mean (SD)</td>
<td>5.84 (11.65)</td>
<td>5.11 (10.05)</td>
</tr>
<tr>
<td><strong>AUC0—12h</strong> Mean (SD)</td>
<td>5.84 (11.65)</td>
<td>5.84 (11.65)</td>
</tr>
</tbody>
</table>
Pharmacokinetic of 0.1% unpreserved Timolol maleate gel

Cardiologic parameters during exercise

The mean baseline for the 80% maximum heart rate during the exercise test was around 158 beats per minute (bpm) in the four treatment groups.

In the cross-over 1, the mean 80% maximum heart rate during exercise decreased to 117.8 bpm (SD 13.0) after treatment with timolol 0.1% gel and to 112.6 bpm (SD 6.9) after timolol 0.5% aqueous solution 2 h after the first instillation, and to 112.8 bpm (SD 11.3) and 111.4 bpm (SD 11.3), respectively, 2 h after the last instillation of the 2-week treatment. The mean drug-induced change in heart rate was not statistically significant between the two treatments.

In cross-over 2, the mean 80% maximum heart rate during exercise decreased to 118.9 bpm (SD 9.4) after treatment with timolol 0.1% gel and to 114.9 bpm (SD 13.4) after timolol 0.5% gel 2 h after the first instillation and to 119.4 bpm (SD 14.2) and 113.7 bpm (SD 11.2), respectively, 2 h after the last instillation of the 2-week treatment. The reduction in heart rate was statistically significantly smaller with timolol 0.1% gel at both the beginning and the end the treatment periods ($P = 0.0377$ and $P = 0.0422$).

There was no difference between treatments in the systolic and diastolic blood pressure during exercise.

Cardiologic parameters during head-up tilt test

There was no difference between timolol 0.1% gel and timolol 0.5% aqueous solution on cardiologic parameters (heart rate and blood pressure) after head-up tilt tests. However,
at the beginning of the treatment periods, there was a significantly smaller reduction in heart rate with timolol 0.1% gel compared to timolol 0.5% gel at 1 min ($P = 0.0190$) and 3 min ($P = 0.006$) after head-up.

There was no clinical difference in blood pressure between treatments after the head-up tilt test.

**IOP**

The change in IOP during the day was similar for all treatments. The IOP decreased after the first instillation of the products by approximately 2 mmHg in healthy volunteers in cross-over 1 and by approximately 1.5 mmHg in healthy volunteers in cross-over 2, after which time it remained stable (daily variation of ±1 mmHg). At the end of the 2-week treatment, IOP was stable and similar with the three treatments in both cross-overs (Fig. 3).

**Safety**

There was no notable change from baseline in any treatment group in the incidence or severity of conjunctival hyperaemia, chemosis, corneal staining punctuations or folliculo-papillary conjunctivitis, and also no notable change in break-up time or visual acuity. There were no statistically significant differences between the treatment groups in ocular signs at any timepoint.

Ocular adverse event (AE) were less frequent with timolol gel, (reported in less than 5% of patients) than with aqueous solution (in greater than 25% of patients) (Table 3). All ocular AE were related to treatment.

In 0.1% and 0.5% gel group, two patients reported systemic AE possibly related to treatment. These AE were headache and fatigue under 0.1% gel treatment and headache and dry mouth under timolol 0.5% gel treatment.
Pharmacokinetic absorption

Healthy volunteers, both male and female, were recruited at the local hospital. All volunteers were in good general health, without any sign of ocular disease, and had not used ophthalmic preparations for at least 2 weeks. The study was approved by the local ethics committee, and all volunteers gave written informed consent.

The study comprised two phases: an open-label phase followed by a randomised double-blind phase. In the open-label phase, all volunteers were given a single 0.1% timolol maleate gel instillation into the right eye under phase II

Discussion

This phase I, monocenter, open, randomised, double cross-over study was mainly designed to measure the systemic absorption of three formulations of timolol eye drops: 0.1% timolol maleate gel, 0.5% timolol aqueous solution or 0.5% timolol maleate gel. Cardiologic parameters (heart rate, blood pressure) and intraocular pressure, although in healthy volunteers, were also assessed during this study.

Timolol is known to be a non-selective beta-adrenoceptor antagonist and timolol maleate is often applied as an ophthalmic solution for glaucomas and ocular hypertension. By blocking the β2 receptors in the bronchioles, timolol may provoke bronchospasm and worsen chronic obstructive airways disease or asthma. In addition, many other side effects have been described, including depression, increased low-density cholesterol levels, hair loss, sexual impotence, fatigue, confusion, and disorientation [25]. Reducing the absorption and thus, the systemic concentration of timolol, may be important for reducing the occurrence of systemic adverse effects. Thus, a once-daily instillation of 0.1% timolol concentration as a single-dose-unit ophthalmic gel without preservative could be the best approach; efficacy of this product was already demonstrated in a precedent study [26]. The similar efficacy of IOP-lowering effect of such a formulation of 0.1% timolol gel compared to a 0.5% solution formulation was already shown both in patients [27] and in healthy volunteers [20].

Maximum plasmatic concentrations (Cmax) of timolol obtained after instillation of 0.1% maleate gel were reduced by almost 90% compared to concentrations obtained after both 0.5% aqueous solution and 0.5% gel instillation. Areas under the concentration-time curve (AUC) between 0 and 12 hours post-administration were also reduced by up to 93 to 98%. Furthermore, in all patients, 8 h after instillation of 0.1% maleate gel, timolol could not be quantified in plasma.

Thus, when patient would instil the following drop (24 h later), there is no more plasmatic timolol.

Surprisingly, there was a significant difference on the drug-induced change in heart rate (in peak heart rate during the exercise test and after head-up tilt test) between treatment with 0.5% timolol gel and 0.1% timolol gel but not between 0.5% aqueous solution and 0.1% timolol gel treatments although PK curves are similar between the both timolol 0.5% formulations.

Timolol is often prescribed as a long-term treatment, and as the present study is not based on a chronic treatment of the drug, safety conclusions on the different timolol formulations tested could be slightly modified when associated with a long-term treatment.

The IOP-lowering effect of 0.1% timolol maleate gel administered once daily was equivalent to that obtained with 0.5% timolol formulations used once or twice daily. As the subjects were healthy volunteers, we cannot draw direct conclusions as to the extent of IOP reduction in patients. Indeed, results on IOP are not reliable because they were obtained in young healthy volunteers while these treatments are usually prescribed in elderly people. However, this finding in healthy subjects is in agreement with those reported in other studies in patients and healthy volunteers [17,20,28].

Pharmacokinetic parameters showed important standard deviations, which reflects the pharmacokinetic sources of variability in inter-and intra-individual as influenced by many factors. Such variation is a well-known phenomenon in pharmacokinetic studies of the eye and may be explained by several factors:

• the volume of the anterior chamber is different in hyperopic, emmetropic, and myopic patients. Aqueous humour production varies with patient age and diurnal time. Thus, the turnover of the aqueous humour and the washout of timolol to bloodstream may differ considerably among individuals;

• the integrity of the corneal epithelium, with its rapid turnover, also varies and may influence the corneal penetration of timolol;

• tear production and composition differ with age and sex. This may influence the retention of eye drops in the conjunctival sac and washout of the drug;

• the total amount of timolol instilled per patient may vary slightly because of minor variations of the drop size.

Nevertheless, by comparing the similarities of pharmacokinetic results obtained in cross-over 1 and 2 for timolol 0.1% gel, we could conclude that our dosages are robust.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Number of patients with at least one ocular adverse event (AE).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Timolol 0.1% gel</td>
</tr>
<tr>
<td></td>
<td>n = 43</td>
</tr>
<tr>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>All AE</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td>Eye irritation</td>
<td>—</td>
</tr>
<tr>
<td>Eye pain</td>
<td>—</td>
</tr>
<tr>
<td>Photophobia</td>
<td>1</td>
</tr>
<tr>
<td>Vision blurred</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
</tr>
</tbody>
</table>

Tolerance

Global assessment of ocular tolerance was judged better in timolol gel 0.1% group in both cross-over: in cross-over 1, treatment was well tolerated in 84.2% of patients under timolol 0.1% gel compared to 73.7% under timolol aqueous solution 0.5%; in cross-over 2, treatment was well tolerated in 94.4% of patients under timolol 0.1% gel compared to 83.3% under timolol 0.5% gel.
Overall, this study and results already published [20] demonstrated that systemic concentrations are considerably lower following treatment with a timolol 0.1% gel formulation than following treatment with timolol 0.5%, even when administered once daily as a gel.

Disclosure of interest
D.R. and C.O. are employees of Laboratoires THEA. F.C. was the coordinator of this study. N.B., H.N. and C.D. were co-investigators.

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